# Paths to Innovation

## Discovering Recombinant DNA, Oncogenes, and Prions in One Medical School, Over One Decade

## Perspectives in Medical Humanities

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To my teachers, and to the students and
colleagues who became my teachers

# Paths to Innovation

## Discovering Recombinant DNA, Oncogenes, and Prions in One Medical School, Over One Decade

Henry R. Bourne

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Cover image: Detail of perspective cross-section of a laboratory in one of the Health Science Towers, drawn by an architect before the buildings were constructed at UCSF, in the mid-1960s. Courtesy of Department of History of Health Sciences, UCSF.

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More generally, I plead fervently for increased support of oral history projects in medicine and the life sciences. Despite the tendency of biomedical science to care most about discoveries that happened this week, correct guidance of its future will also require that we understand its past. Philanthropists, foundations, universities, the National Academy of Sciences, the National Institutes of Health, and anyone who cares about our future should contribute generously.

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# Introduction

On sunny afternoons forty or fifty years ago, a red-tailed hawk riding thermals over San Francisco would often witness a curious scene.[1] Two figures clamber up metal stairs to the roof of a tall white building, south of Golden Gate Park. The older man, clad in a white lab coat, places a hand on the other's shoulder and invites him to look around. In his dark suit and well-shined shoes, the younger figure—in those days, always a man—first looks west, to the Pacific Ocean, and then turns north, toward the red towers suspending the Golden Gate Bridge. Finally, to the east, he gazes over pastel houses, churches, and skyscrapers at the glorious blue of San Francisco Bay.

When the weather is right, different actors play the ritual over again, with similar costumes and gestures. A well-read hawk might guess he is witnessing a primordial temptation scene, but this Mephisto doesn't promise power over all the world, and the Faust figure rarely risks his immortal soul.

In prosaic reality, the white building was the teaching hospital of an academic medical center, the University of California at San Francisco (UCSF). The older man, a department chair or dean, was invoking the help of sun and blue sky—"recruiting weather"—to persuade yet another bright young academic to take a faculty job. Comparing UCSF to other institutions with open faculty positions, the younger man knew that open skies and distant horizons don't necessarily augur a wondrous future. Still, the rooftop prospect may have tempted him, if only because it appeared so far, in ambience or miles, from New York, Boston, or Baltimore.

Five decades later, we know what the future did bring. By the 1980s, a series of extraordinary events had transformed UCSF into a world leader in fundamental biological research and training, making it one of the best medical schools in the US and the world. UCSF scientists had revolutionized biology by discovering recombinant DNA, created a burgeoning biotechnology industry by exploiting the new technology to synthesize hormones in microorganisms, discovered the first mutant genes that trigger cancer, and found an entirely new and controversial mechanism for

transmission of disease by protein particles called prions. These discoveries changed biology and medicine forever, at UCSF and in the world at large, in a transformation surpassing any those earlier rooftop players might have imagined.

In the 1960s UCSF's health science campus occupied a row of four big buildings, including a fourteen-story teaching hospital, on the south side of grandly named Parnassus Avenue. In 1966, two 15-story glass-covered structures were added, to house laboratories of UCSF faculty. Patients, care-givers, and students bustled in and out of the busy but tranquil campus, in which the School of Medicine accounted for five sixths of the total staff, floor space, and budget. A good but not outstanding regional institution, the medical school delivered patient care of high quality. Biomedical research at UCSF and most other state-supported schools lagged far behind research at schools like Harvard, Columbia, and Johns Hopkins. Compared to UCSF, the top private schools competed much harder—and more successfully—to attract research grants from the National Institutes of Health (NIH).

For more than 50 years, UCSF had been split between San Francisco and UC Berkeley across the Bay. Hospital and clinical services were located in the city, while the medical school's first-year students and most of its basic science departments were located in Berkeley. Moving these departments to San Francisco in 1958 made it possible to change its name from "UC Medical School" to the School of Medicine at UCSF. (UCSF, precisely speaking, denotes the entire campus, comprising all four schools—Dentistry, Nursing, and Pharmacy, as well as Medicine.) Because the best scientists in these departments elected to stay in their former quarters at Berkeley, however, the research of those who did move to San Francisco proved more sleepy than exciting.

By 1960, scientists at UCSF's mother institution, UC Berkeley, were beginning to earn world-wide recognition—especially in physics and chemistry, but also in life sciences—and didn't think much of research at the medical center in San Francisco. One retired UCSF professor, then recently recruited to San Francisco, remembers a mid-60s stroll down Parnassus Avenue with Herbert Evans, a renowned physiologist and academic luminary who had stayed in Berkeley when the basic science departments came to San Francisco. Waving his cane at UCSF's buildings, Evans called them "skyscrapers inhabited exclusively by pygmies!"[2]

The seeds of transformation had already been planted, however, and the pygmy dynasty would fade away. The principal changes took place between 1969 and the early 1980s. Some were visible—UCSF built new buildings, attracted more students, assembled a busier, more comprehensive clinical enterprise, overflowed its parking facilities, and filled its corridors with hurrying humanity. Other changes, more pro-

found but invisible, grew out of the DNA revolution, biotechnology, and discovery of fundamentally new mechanisms of disease. UCSF's doctors made major contributions to developing new technologies for clinical care in many fields, including magnetic resonance imaging, organ transplantation, and treatment of infant respiratory distress syndrome. Medical students and graduate students were smarter and better prepared than in earlier years, not just more numerous. In the 1980s and after, UCSF led the US in garnering NIH grants, attracted first-class biologists from all over the world, congratulated its first Nobel Prize awardees, and supplied presidents or directors for the National Academy of Sciences, the Food and Drug Administration, the Center for Disease Control, the NIH, and departments and schools all over the US and the world.

How did this meteoric transformation come about? Why did it take place on this one campus, in little more than a single decade? Like other cause-and-effect questions in history, these are hard to answer. It is hard enough to identify the hidden well-springs of creativity in any individual artist, entrepreneur, political leader, or scientist. Compounding the mystery, UCSF's burst of creativity involved a constellation of disparate individuals asking separate scientific questions.

Did the sudden cascade of creative ideas and individuals erupt because of something special in the institution itself? Human organizations can stifle creativity, or kindle and nurture it. At different times in its history, the Roman Catholic Church has done both. How do institutions make creativity happen? To create new knowledge and invest their intellectual capital, advanced twenty-first century societies badly need to answer this question. We face challenges—international economic crises, climate change, needs for renewable sources of energy, the ravages of complex degenerative diseases, and many others—that cry out for creative solutions. Is it possible to construct institutions expressly designed to foster scientific creativity? Can we design a startup company that will generate and maintain creativity? Or perhaps a high school, an agricultural college, an institute, or a medical school? What essential elements are required for an institution to foster creativity? We don't really know how to frame the question, but it does seem reasonable to examine a case in which, by chance or by unconscious design, creativity suddenly thrived in a particular institution—as it did, rather unexpectedly, at UCSF in the 1970s.

My own qualification for writing about science at UCSF is that I know personally many of the individuals who played key roles in the institution's burst of creativity in the 1970s. I became a postdoctoral fellow there in 1969 and served thereafter as a faculty member and (from 1984 to 1993) as chair of a basic science department.[3] In addition, I enjoy an advantage not available to most professional historians—that

is, most of this history's protagonists are still alive.

When I began writing this book, I shared with most of my colleagues a straightforward view of what happened at UCSF in the 1970s. Like many good myths, the prevailing view highlighted a succession of brave and talented heroes, plus a single dastardly villain. The first hero was a pioneering scientist with a brilliant mind and fiery temperament. He was soon joined by a shrewd, charismatic physician, skilled in the magic of persuading others. Together, their determined magic defeated the villain and his allies, an antediluvian horde driven by the fear that scientific investigation would inevitably detract from teaching students and caring for patients. The heroes' eventual triumph allowed UCSF to attract an ambitious, hard-nosed, and visionary scientist who recruited a superb group of young scientists united by a common goal—to use the rapidly expanding knowledge of genetics and molecular biology to understand biology and treat disease. By 1982, when this last hero left the scene, UCSF scientists had completed an epochal series of discoveries, and the institution's basic science research matched that of any biomedical institution in the US or the world.

I bought into this creation myth long ago, and still think part of it is correct. The heroes, constituted of flesh and blood, put enormous skill and passion into transforming UCSF from a pedestrian regional medical school into a leading biomedical powerhouse, internationally recognized for its patient care, training of health professionals, and broad-ranging research, both in laboratories and in clinical settings. When I began this book, these heroic "Great Men" loomed for me as the aces, kings, queens, and jacks that always appear to dominate history's otherwise anonymous deck. Their actions, sometimes in moments of high drama, often in painstakingly small increments, explain many events I describe in this book.

Does UCSF's micro-version of the Great Man Theory of History (aka GMTH) also explain how UCSF became the cradle for scientific creativity that produced recombinant DNA, the biotech industry, oncogenes, and prions? As I began to explore the stories of the young scientists at UCSF whose discoveries disrupted the rules and changed the whole game of biology, it became clear that their stories form a separate saga, full of its own human drama and even heroism, which runs alongside the face card story and intersects it at several points. Realizing that the young discoverers themselves constituted a highly distinctive set of cards dealt to UCSF at the same time as the famous face cards, I found this second story more and more intriguing—and began to wonder what the face cards had to do with their discoveries.

Unlike the face cards, the discoverers arrived on the UCSF scene unheralded and obscure, but quietly became wild cards with unexpected transformative qualities. Realizing that a particular card's wildness can be independent of face cards in the

same hand, I began to question the GMTH's explanatory power. Even granting the face cards major roles in transforming UCSF, I still had to determine what had they to do with the transformative discoveries at UCSF in the 1970s. I set out to answer this question, and ask readers to join me in my quest. Along the way, I decided to place one central theme at the center of the saga, by focusing primarily on creative scientific discoveries and their relation to the institutional environment. As a result, we shall observe UCSF's face cards mainly as they interact with and influence the accomplishments of four remarkable wild card researchers. This has meant necessarily devoting less attention to the larger, more complex story of how UCSF became a leading venue for biomedical research and training—a fascinating tale that should attract a worthy teller.

This book tries to tell why both key wild and face card individuals came to San Francisco, what they dreamed of doing, how they felt about what they found and weighed their options, and what drove them, from day to day, to create a future no one foresaw. As their stories unfolded, one academic department rose to dominate basic science research at UCSF, while molecular analysis of genes gave birth to recombinant DNA, the biotech industry, oncogenes, and prions.

Taken together, I think these stories make it very unlikely that a simple version of the GMTH explains the burst of discovery that occurred at UCSF in the 1970s. But the burst of discovery did happen, in a short time and a very small space. As readers follow these individual scientists and academic leaders, I hope they will ask the following question: if face card leaders didn't engineer an institutional environment responsible for these discoveries, what did cause it? More generally, what combination of forces, acting in a limited space and a very short time, can endow an otherwise ordinary institution with the capacity to create extraordinary new knowledge? Answering those questions may tell us how to design institutions better able to nurture creative discovery in the future.

While you read and search for answers, I urge you to ruminate now and again about that rooftop temptation scene. If our bookish red-tailed hawk was mistaken in invoking Mephisto and Faust, what clues eluded him? What roles did those actors really play? Why did they come to San Francisco in the first place? What did they seek? After leaving the roof for lives in the buildings below, what did they find? Once you have answered those questions, join me in crafting a new drama, one that ends with a burst of creative innovation tomorrow, rather than forty years ago. When the curtain rises, who must take the stage? To achieve the actors' dreams, and ours, what must we all say to one another? What must we do?

Chapter One

# Nowhere to Go But Up!

## UCSF Consolidates, 1958-1964

In 1955, Richard J. (Dick) Havel looked at faculty positions at medical schools on the west coast. So far his San Francisco visit had confirmed his dim view of UC's medical school. After talking with several uninspiring older professors, now he had finally found a bright young assistant professor, "Izzy" (for Isidore) Edelman. In a tiny basement office, Havel spoke his mind.

"Why should I come here? This is a provincial western school. There's practically no research going on."

Edelman first countered that UCSF would soon build a "cardiovascular institute." Then, conceding a weak argument, he matched Havel's frankness: "Look at it this way, Dick. You've got nowhere to go but up!"[1]

Indeed, UCSF was very much a provincial medical school, with very few active researchers on its faculty. The institute Edelman mentioned was an unfinished floor of a new building, without scientists or a leader. That empty shell could have served as a perfect symbol for the combination of risk, doubt, and promise that disturbed and enticed Havel, Edelman, and others at UCSF in the 1950s and 1960s.

### UC Medical School

A medical school's development, like that of a person, depends on its origins, the trials and tribulations of its early life, external influences from social and economic events, and a unique, always changing internal flux of ideas, precepts, and temptations. UC's medical school may have appeared provincial to Dick Havel, but it delivered clinical care better than anyone would have predicted from its inauspicious birth in the raffish world of San Francisco after the Gold Rush. By the 1850s, the city's population had swelled from a few hundred (in 1846) to more than 60,000. Thousands died every year from injuries and infections, including cholera. The Great Sponge Case of 1856 hints at what medical care was like. Called to see a newspaper

editor who had been shot in the chest, Hugh Toland, a surgeon recently arrived from South Carolina, agreed with several other doctors that the wound should be plugged with a large sponge. One practitioner, R. Beverly Cole, strongly disagreed. When the patient died five days later, Cole averred that the sponge left in the wound caused his death. Toland's recommended treatment, Cole contended, was "gross malpractice." Instead, one newspaper opined, the doctors simply scared the patient to death.[2]

Now, 150 years later, UCSF's medical students attend lectures in Cole Hall and in Toland Hall, named for medical adversaries who learned to work together in a common cause. In 1864 Toland founded Toland Medical College in the oldest part of the city, near the docks that welcomed the huge influx of Gold Rushers in the mid-nineteenth century. It was a "proprietary" medical school—that is, a profit-making enterprise for Toland, its owner. Each of the eight students in its first class earned the MD degree by attending two four-month classes and paying $130. In 1873 Toland deeded the college to the University of California, founded five years earlier in Berkeley, across San Francisco Bay. With Beverly Cole as its first dean, the school was renamed the Medical Department of the University of California.

By 1898, crowding and inadequate facilities had forced the school to move to larger quarters in the western part of the city, overlooking Golden Gate Park. Built on the south side of Parnassus avenue, near the foot of Mount Sutro (named for the city's mayor, Adolph Sutro, who donated the land) the school's new facilities housed the Medical Department, with its small hospital and classes for teaching basic sciences, as well as UC's pharmacy and dentistry schools. In 1905 the medical school required that all successful applicants must have completed two years of college study—and consequently admitted a new entering class of only nine students, compared to 33 the year before.[3]

The next year, 1906, brought the great San Francisco earthquake. Although the main building on Parnassus was not damaged, loss of other buildings and overcrowding at the county hospital (on Potrero Hill, where San Francisco General Hospital is now) created a drastic need for more space. In response, the University moved first-year students and their instructors in Physiology, Anatomy, and Pathology back to Berkeley. The move cleared space for patient care and clinical training on Parnassus, but cleaved the medical school into two parts, separated by San Francisco Bay. For more than fifty years, basic sciences were taught in Berkeley, clinical medicine in San Francisco. The split followed a fault line that divides most medical schools—a line, that is, between basic biomedical sciences and researchers who seek to understand nature, on the one hand, and the practice of clinical medicine by doctors committed to treating diseased patients, on the other. Every medical school experiences persis-

tent tension along this fault line. This tension affected the life of every person in our story, sometimes positively, sometimes not.

From the beginning, the split between basic science in Berkeley and clinical medicine in San Francisco was widely deplored. In 1910, in a famous report that triggered reform of medical education throughout the US, Abraham Flexner lamented the division of UC's medical school into two parts, and in 1912 the university's Board of Regents announced their intention to reconsolidate the school—a reconsolidation not effected until 1958. Among the medical school's faculty, the long delay enhanced the respect of Berkeley-based preclinical faculty for so-called "pure" research (that is, research untainted by any requirement to produce a useful result), because they naturally identified with the larger campus nearby. These preclinical faculty members also became less interested in and even overtly hostile to teaching practical aspects of medical care. Herbert Evans, who became Professor of Anatomy in the medical school in 1914 and later deplored the pygmy-inhabited skyscrapers at Parnassus, was the medical school's first research star at Berkeley.[4] He isolated growth hormone, co-discovered and purified vitamin E, and advanced early knowledge of reproduction by studying the estrus cycle of rats. Eventually appointed to the chair of Anatomy, Evans did not deign to teach anatomy. Instead, he hired clinicians from outside the University to direct dissections, and underlined their dispensability by calling them "the hat-rack boys." (Several other stars of the medical school faculty at Berkeley also worked on basic aspects of endocrine hormones.[5] A very few prominent researchers on the Parnassus campus studied clinically important diseases.[6])

On the clinician-teachers in San Francisco, the long-lasting separation from their colleagues in Berkeley exerted a reciprocal effect. Increasingly, the clinicians criticized Berkeley's focus on basic science, insisting that doctors should learn only information immediately applicable to patients and disease. Perhaps in part for this reason, in 1910 a distinguished physiologist left the Physiology department in Berkeley to move to New York's Rockefeller Institute, one of the few bastions of "pure" research in the US. "There is as yet no room in a state university for pure research," he complained. "It may be done on the sly, but public pressure is against it."[7]

Obstacles to reconsolidating UC's medical school included the huge cost of new construction, the unwillingness of basic science faculty to leave Berkeley, and the clinicians' reciprocal desire not to lose their large patient population in San Francisco. In 1946, soon after World War II, years of backing and filling finally ended when the state legislature and the governor agreed to commit real money to build a large teaching hospital and medical science building at Parnassus.[8] In response, the Berkeley section of the University's Academic Senate promptly announced that reconsolidation

should take place on its own side of the bay. Herbert Evans predicted that a Regents' decision to consolidate UC Medical School on "a bleak, fog-ridden hillside" in San Francisco would irretrievably damage medicine in the state.

At almost the same time, Stanford University's School of Medicine debated terminating a similar split. Medical students had studied basic sciences at Stanford's campus in Palo Alto, thirty miles south of San Francisco, but completed most of their clinical training in San Francisco, where Stanford shared clinical facilities in city hospitals with the UC Medical School. Persuaded that its teaching and medical practice would increasingly depend on scientific discovery, in 1958 the school eventually moved its entire operation to a new facility in Palo Alto. Stanford's clinicians were divided, some staying with their patients in San Francisco, others enthusiastically joining their colleagues down on "the Farm."

As for the University of California, in 1949 the Regents unequivocally designated Parnassus as the site of the UC School of Medicine. In 1955 patients moved into the new 485-bed Moffitt Hospital (named for a former chair of the school's Department of Medicine) on the site. The adjoining Medical Sciences Building was completed a year later. By 1956, when Richard Havel joined the faculty in San Francisco, it was clear that the Departments of Physiology, Biochemistry, and Anatomy would move to Parnassus. They did so in 1958, almost simultaneously with Stanford's move in the opposite direction. Berkeley's research stars chose not to move, including Evans, who was no longer active in research. (Pathology had moved earlier, and Pharmacology and Bacteriology were already at Parnassus.)

## Clark Kerr and John Saunders

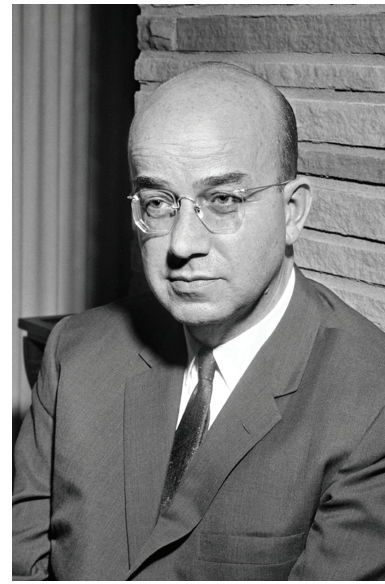
The 1950s and early 1960s were a time of contrast and contradiction. The economy rapidly recovered from the Great Depression, more people had better-paying jobs, and airplanes transported citizens and goods across the US in a few hours. But Senator Joseph McCarthy fomented fear of communism at home and abroad, conflict over racial integration spread through southern states, and a nuclear arms race appeared to threaten World War III. Touting a non-existent missile gap between Russia and the US, John Kennedy won the 1960 presidential election and his predecessor, Dwight Eisenhower, left office warning that the "military-industrial complex" could prove dangerous to the nation's future. Kennedy seemed to represent youth, change, and hope, but was assassinated in Dallas. Against this background of dramatic events and social and economic change, the medical school on Parnassus avenue would experience a quieter but nonetheless profound transformation.

Before his death in 1945, President Franklin Roosevelt had asked Vannevar

Bush, chief coordinator of scientific research in the US during the War, to devise a plan for the future of science in the US when the war was over. Published that same year, Bush's report, "Science, the Endless Frontier," called for government to support and coordinate massive investment in science, aimed at promoting three goals: national security (that is, maintaining military power), public welfare (new jobs and economic growth, based on creation of new products), and fighting disease. Implementation of Bush's program led to continuing national investment in development of weapons, new federal policies favoring technological advances in industry, formation of the National Science Foundation, and enormous growth of the National Institutes of Health.

Perhaps more than any other state, California took advantage of the new drive to create scientific knowledge. California's economy began to prosper mightily, partly by producing weapons for the military and partly by devising and adapting new technologies—e.g., the electronic industry's birth in the 1950s, just south of San Francisco, in a region that would become known as Silicon Valley—but also because rapid population growth and a flood of migrants from other states augmented the postwar baby boom. Together, prosperity and the increasing demand for college education triggered and shaped major changes in the University of California, beginning immediately after World War II. In response to the huge postwar influx of students supported by the GI Bill, UC Berkeley's enrollment rose to 27,500 in 1964, compared to 15,000 before the war.

Remarkably, increases in numbers were accompanied by substantial advances in academic quality, and both took place under the leadership of a man many consider the twentieth century's most influential figure in higher education. Clark Kerr, a professor of economics who studied relations between industry and labor, was appointed the first chancellor of UC Berkeley in 1952, and the University Regents chose to make him president of the entire University of California in 1958. A shrewd visionary, he showed a remarkable capacity for analyzing and handling complex conflicts, in negotiations between labor and management and in academia. As UC's president, Kerr presided over substantial expansions in student body and faculty on existing campuses, opened three new ones (Riverside, San Diego, and Santa Cruz), and completely reorganized the much more numerous campuses of California State University, as well as the state's Community Colleges.[9] His Godkin Lectures, published in 1963 as *The Uses of the University,* described the birth of the modern research university and predicted its future.[10] The idea that universities educate a privileged class to direct government and society, he wrote, was rapidly being replaced by the new "multiversity," charged with producing new knowledge. Instead of maintaining homogeneous



Clark Kerr, President of the University of California, 1958-1967. Photo taken at a press conference in 1965.

faculties and student bodies, multiversities would have to learn how to juggle shifting, competing priorities and pressures—political, social, economic, and scientific.

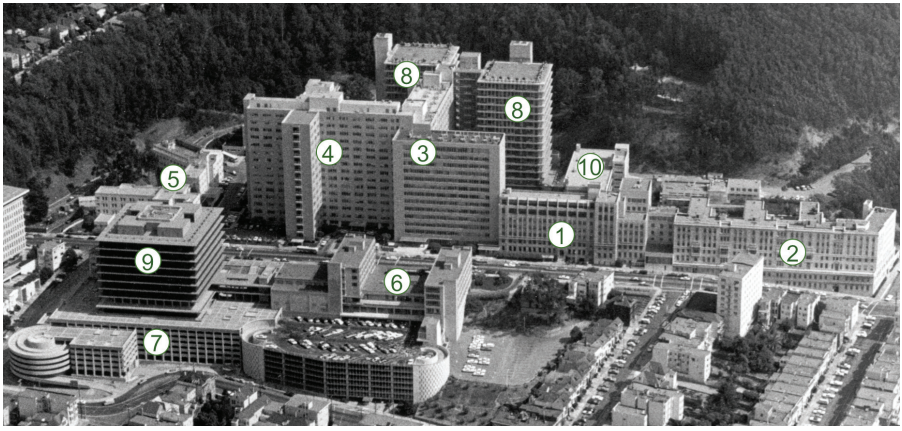
One of these pressures came from Ronald Reagan, whose 1966 campaign for Governor of California featured his intention to "clean up the mess at Berkeley" by getting rid of Kerr. Kerr's sin was his perceived excessive lenience toward the Berkeley Free Speech Movement of 1964, in which radical students protested against UC policies and railed about an increasingly corporate America, racial discrimination, and the widening conflict in Vietnam. Once elected, Reagan and others used false evidence provided by the Federal Bureau of Investigation to attack Kerr, and, in 1967, persuaded the University Regents to terminate his presidency. On the way out, Kerr said he was leaving the presidency just as he had entered it—"fired with enthusiasm." In the meantime, as we shall see, one of Kerr's bravest decisions determined UCSF's future.

During the early years of his presidency, Kerr's most important influence on UCSF reflected his determination to decentralize the University of California. According to Kerr's memoir, the Regents appointed him as President in part because they thought he would do just that.[9] His predecessor, Gordon Sproul, had administered the entire, ever-expanding University almost as if it were confined to a single campus. Sproul's detailed control over decisions and appointments at UCLA, the growing Los Angeles campus, led to resentment and opposition from faculty and,

more important, from Regents based in southern California, who sought to empower UCLA (and, later, other UC campuses). For his part, Kerr felt that proper growth and development of each campus required a strong degree of independence—a point of view that helped to overcome the southern Regents' objections to his liberal politics. (In 1949, he had signed an anti-communist "loyalty oath" required of UC faculty, but argued courageously that not all faculty should be required to sign it.) As president Kerr gave chancellors of individual campuses much greater autonomy than they had enjoyed under Sproul.

As applied to the newly consolidated medical school in San Francisco, decentralization meant rendering it clearly distinct from its erstwhile parent, UC Berkeley. It was no longer the UC Medical School, because UCLA was developing its own medical school, as would other UC campuses a few years later. Moreover, while the Parnassus campus lacked an undergraduate college, it housed four schools—Medicine, Pharmacy, Dentistry, and Nursing—and needed to take over its own governance.

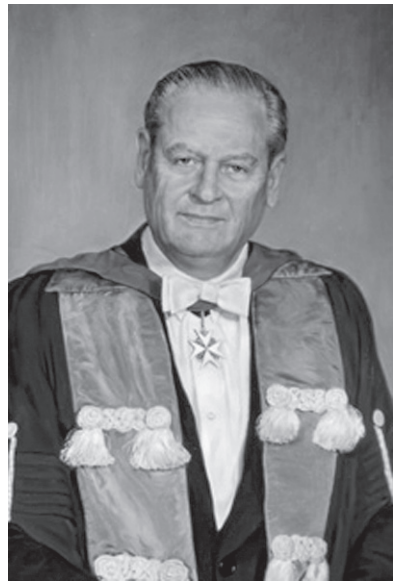
Recognizing that the Medical Sciences Building would not be adequate for all four schools, in the late 1950s Kerr set in motion plans for building new fifteen-story towers for research and teaching, later called the east and west Health Science towers, or HSE and HSW. This decision, and the towers themselves, which were completed in 1966, were to become the material foundation for UCSF's expanding research effort over the next thirty years.




Parnassus campus in the 1970s, aerial view. Important buildings (date of construction in parentheses) include: (1) Clinics Building (1934); (2) UC Hospital (1917); (3) Medical Sciences Building (1951); (4) Herbert C. Moffitt Hospital (1955); (5) Langley Porter Psychiatric Hospital (1943); (6) Millberry Union (offices, student services, 1958); (7) parking facility; (8) East and West Health Science towers (left and right, respectively, 1964); (9) Robert H Credé Ambulatory Care Center (1972).

Table 1: UCSF Leaders, 1950s-1970s

| Name | Position | Dates |
|---|---|---|
| Julius Comroe | Director, CVRI | 1957-1973 |
| Stuart Cullen | Chair, Dept. of Anesthesia | 1958-1966 |
| | Dean, School of Medicine | 1966-1970 |
| | Chair, Dept. of Anesthesia | 1970-1973 |
| Engelbert Dunphy | Chair, Dept. of Surgery | 1964-1975 |
| | Acting Dean, School of Medicine | 1965-1966 |
| Isidore Edelman | Faculty, Dept. of Medicine | 1952-1978 |
| | Acting Chair, Dept. of Biochemistry | 1967-1968 |
| Richard J. Havel | Faculty, Dept. of Medicine and CVRI | 1956-now |
| | Director, CVRI | 1973-1992 |
| Clark Kerr | President, University of California | 1958-1967 |
| Julius R. Krevans | Dean, School of Medicine | 1971-1982 |
| | Chancellor, UCSF | 1982-1993 |
| Frances Larragueta | Staff Chief for the Dean of Medicine | 1956-1982 |
| John B. deC. M. Saunders | Faculty, Dept. of Anatomy | 1931-1971 |
| | Chair, Dept of Anatomy | 1938-1956 |
| | Dean, School of Medicine | 1956-1963 |
| | Provost at Parnassus campus | 1958-1964 |
| | Chancellor, UCSF | 1964-1965 |
| | Regents Chair of Medical History | 1966-1971 |
| William O. Reinhardt | Dean, School of Medicine | 1963-1965 |
| | Associate Dean, School of Medicine | 1966-1982 |
| William J. Rutter | Chair, Dept. of Biochemistry | 1969-1982 |
| | Director, Hormone Research Laboratory | 1982-1991 |
| Lloyd H.("Holly") Smith, Jr. | Chair, Dept. of Medicine | 1964-1985 |
| Gordon M. Tomkins | Professor, Dept. of Biochemistry | 1970-1975 |
| Harry Wellman | Vice-President, University of California | 1958-1967 |



John B. DeC. M. Saunders, UCSF Chancellor, 1964-65. Detail of a portrait that hangs—with paintings of other UCSF chancellors—in the first floor hallway of the Medical Sciences Building.

In 1956, when he was still chancellor at Berkeley, Kerr had appointed a Professor of Anatomy as dean of the School of Medicine in San Francisco. This was John Bertrand de Cusance Morant Saunders, who was to play a singular role in the evolution of UCSF. While he may not have enjoyed every aspect of that role, it was perfectly fitted to his remarkable background and personality. (For a list of UCSF's leaders in the 1960s and 1970s, see the accompanying Table.)

Born in South Africa, Saunders was tutored as a child in Latin and Greek by his father, a surgeon who loved the classics. After attending Rhodes University College in South Africa, he earned medical and surgery degrees in Edinburgh, Scotland, where he captained the golf team and was an ace tennis player. Following a residency in surgery, he moved to the US and joined the UC Medical School's Department of Anatomy in 1931, at age twenty-eight. At UC he rose rapidly. At age thirty-four, his abiding interest in medical history led him to take the chair of the Department of the History of Health Sciences. He became chair of the Department of Anatomy as well during the next year, and Librarian of the Parnassus campus five years later. His research produced more than 100 publications, including eight books, with titles like *Illustrations from the Works of Andreas Vesalius of Brussels*, *Ancient Egyptian and Cnidian Medicine*, and *Manchu Anatomy and its Historical Origin*. A connoisseur of wine and cigars, Saunders was an erudite, eloquent speaker and a skilled raconteur. Once he started talking, however, no one found it easy to make the stories stop—as others told me, and as I observed myself on one occasion. Aside from medical history,

Saunders's research focused on the functional anatomy of human bones, muscles, and joints. Studying the body "as a machine," he wrote monographs on the shoulder and knee, some in collaboration with Verne Inman, chair of orthopedic surgery. As campus Librarian and a genuine bibliophile, Saunders took great pleasure in reading and handling the library's rare books, which he often carried home with him—many such books were returned to the library after he died.[11]

As dean, Saunders enjoyed great popularity among practicing clinicians with offices located at the San Francisco campus, because he valued their clinical ability and their teaching of medical students. These so-called "geographic full-time" faculty practiced medicine in University offices but earned their incomes primarily from direct payments by patients rather than in salaries paid by the University. In 1958 Kerr gave Saunders a dual title—he would serve both as dean of Medicine and as provost of the Parnassus campus. In 1963, Kerr elevated Saunders to a new title—he became UCSF's first chancellor. The medical school's new dean was William Reinhardt, another member of the Anatomy faculty who had moved over to San Francisco with Saunders.

Despite appointments to these responsible positions, Saunders was not blessed with administrative skills. He had a reputation for delaying decisions unnecessarily, and that getting an appointment to speak with him was often difficult (see Chapter Two).

## Isidore Edelman and Richard Havel

In the late 1950s and early 1960s, scientific research was a marginal pursuit at the newly consolidated medical school on Parnassus. Stanford and a number of other medical schools, especially those on the East Coast, were quicker to leap at growing opportunities to attract research grants from the NIH. As late as 1964, UCSF received 174 separate grants from the NIH, many fewer than leading schools like Baltimore's Johns Hopkins (255) and New York's Columbia (289).[12]

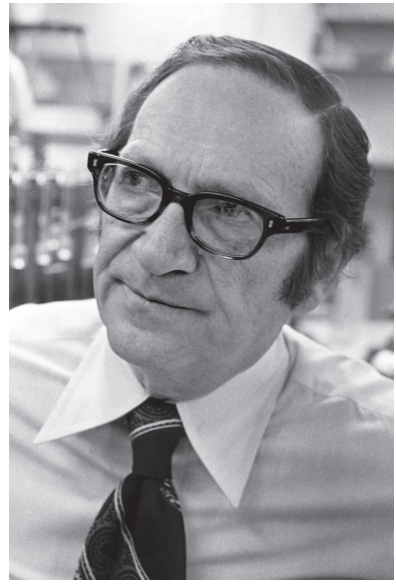
In this period at UCSF, however, four especially daring and persistent individuals—Izzy Edelman, Dick Havel, Julius H. Comroe, and Lloyd H. Smith—began to pave new avenues for experimental research. Despite their varied backgrounds, personal styles, and roles at UCSF, their reasons for coming to UCSF and committing themselves to its future reveal motivations and goals that put the institution on the road to becoming a national powerhouse in biomedical research.

Izzy Edelman came to UCSF in 1952, when he was thirty-two. Son of the owner of a small business, he grew up in the (then) lower middle-class Bedford-Stuyvesant

section of Brooklyn, where he ranked at or near the top of every class at PS 54 and Boys' High School. While attending Brooklyn College he decided to become a doctor. Knowing he could not be admitted to a medical school from Brooklyn College, Edelman transferred to Indiana University in Bloomington, from which he graduated fifth in a class of 1,000 students, with strong recommendations for medical school. Still, of the twenty-two schools to which Edelman applied (including "some of the lousiest in the country"), only Indiana's medical school admitted him.[13]

In 1941, just before World War II, the most likely reason for those turndowns, antisemitism, was common at every level of academia. He excelled in medical school as well, but "the only internship I could get" was at a small city hospital in Brooklyn. Nine months later, the intern was inducted into the US army, just as the war was ending. After six weeks of classes, and after atomic bombs caused Japan to surrender, the army shipped him to Panama, to serve as a "specialist" in psychiatry.

In Panama Edelman thought hard about his future, deciding to combine an academic career in medicine with research—although he knew almost nothing about such a career and had no obvious role model to follow. In 1946, about to be discharged from the army and unable to find a residency position, he used a friend's contact with a prominent doctor to land an unpaid "externship" at Montefiore Hospital in New York, in a unit that treated cancer. Four months later, he was offered, and accepted, a chief residency in neoplastic diseases, along with a real salary. In his



Isidore Edelman, physician-scientist. He came to UCSF in 1952, and worked in fields ranging from blood electrolytes to molecular biology. Photo from the early 1970s.

residency, Edelman began research on the body's handling of salts and water in congestive heart failure and kidney diseases. In a year he published three papers. "That convinced me. I never would go into practice. I wanted a full-time academic career."[13]

Then Edelman was awarded a "Postdoctoral Research Fellowship in Medical Application of Isotopes" from the Atomic Energy Commission. Deeming him a security risk, the AEC would not let him take the fellowship to the "birthplace of nuclear medicine," the Donner Lab in Berkeley. (Senator McCarthy's crusade against suspected communists in American universities and government was in full swing. In medical school, Edelman had attended two communist party meetings and bought a subscription to the Sunday issue of The Daily Worker.) The AEC did allow him to go to Harvard, which didn't require a security clearance. There he worked in the Surgical Laboratories headed by Francis D. Moore, a legendary Harvard surgeon and scientist who was using deuterium (an isotope of hydrogen) and other isotopes to determine the amount of water and salts in the body.

"Fran Moore was terrific," Edelman felt.[13] In their first interview, after they discussed Edelman's research at Montefiore and the future of isotopes, Moore asked him "'Well, that's great, but why do you want to do this?' So I said, 'Well, I want to become a professor in a medical school and do research and clinical work at the same time.' He said, 'You mean full-time, on a salary?' I said, 'Yes.' He said, 'Well, do you have private means?' And I thought to myself, Yeah, I got about $150 in the bank." Moore replied, "You should think about that," and went on to explain that most of the professors at Harvard could not afford to do research if they weren't also independently wealthy. In 1948, research in medical schools was still a luxury. "I didn't tell him what I thought at the time," Edelman said, "that I don't need much."[13]

Edelman's research in Moore's lab was successful, and his publications garnered a good deal of attention. In the meantime, he was called before two congressional committees as a possible security risk, and Harvard asked him to give up the AEC fellowship—which Moore arranged to replace with a fellowship from the American Heart Association. "He rescued my career."

In 1952, Edelman began to look for a job. He was interviewed at several schools, but "the only one that came through with an offer, including money, was UCSF." Why other schools turned him down is not clear. I suspect anti-semitism played a part, although Edelman denied it. In addition, as Moore had warned him, he was applying very early for a kind of job that didn't exist in many medical schools. At UCSF, he would teach and see patients at San Francisco General Hospital and was promised a lab of 800 square feet. The University paid only $400 of his $5,200 yearly salary, because he had an "Established Investigatorship" from the American Heart Associa-

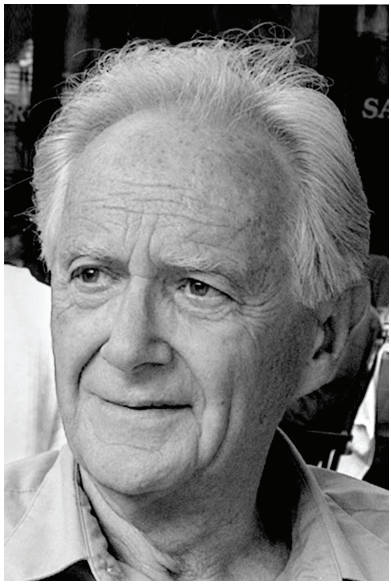
tion (AHA), for $4,800 a year. (The chair of Medicine, Ted Althausen, ungraciously announced that the $5,200 was a mistake, too high by $400, but honored his commitment to pay the $400.[13])

The promised lab proved to be a small dark locker room in the basement of an old SFGH building, without lab benches, running water, or even a working light switch. "I thought, Oh my god, first the Red-baiters were going to kill me, and now these guys are going to kill me. It's all hopeless."[13] But good luck intervened. An endocrinologist at the Parnassus campus, Gil Gordan, told Edelman, "Wait a minute. I know Mrs. Fleischmann. From Fleischmann's Yeast. She has a little foundation, and they give money for medical research." Mrs. Fleischmann's check for $12,000, along with $4,200 cadged from colleagues and the local heart association, sufficed to convert the basement room into a real lab. (In today's dollars, the $16,200 would amount to approximately $130,000.) Supported by NIH grants and the AHA, Edelman set to work using isotopes and other developing technologies to analyze transfer of salts and water among body "compartments" (cells, blood, individual organs, etc.).

The medical school insulted Edelman, a brilliant researcher and future academic leader, by claiming that he was paid too much and by offering him a nearly nonexistent lab. This niggardly treatment opens a window into the bleak circumstances of researchers in most medical schools before NIH funding began to increase, later in the 1950s. Nowadays a bright research prospect like Edelman is wined, dined, and offered close to a million dollars in start-up funds. When Edelman came to UC, however, the medical school received only twenty individual NIH grants—$223,000 in total, distributed between Berkeley and San Francisco, and a leading school like Columbia had only 64 NIH grants.[12] By 2008, UCSF had 889 NIH research grants, totaling more than $448 million.

Edelman's distinguished career as a biomedical scientist contrasted sharply with the ungenerous welcome he received when he came to UCSF. His quantitative studies of the body's handling of salt and water set the stage for fundamental understanding of important aspects of kidney function and actions of hormones on the kidney and the cardiovascular system. As he grew older, his work began to focus on molecular mechanisms and heredity. Later in his career, Edelman assumed the chair of Biochemistry at Columbia University's medical school in New York, where he went on to become director of the Columbia Genome center.

In the 1970s at UCSF, I knew Edelman as a highly respected senior faculty member. Packed with a prominent brow, nose, cheekbones, and chin, his rugged face radiated intelligence and verve, but his residual Brooklyn accent was flavored with



Richard Havel, physician-scientist. An expert on lipid transport in the blood, he came to UCSF in 1956 and succeeded Julius Comroe as CVRI director in 1973, serving until 1992. Recent photo.

warmth and a bubbling sense of fun. Superficially he contrasted with Richard Havel, who had learned from Edelman that he had "nowhere to go but up!" Unlike Edelman, Havel appeared ascetic, thin, reserved, measured and precise. He took great care to choose exactly the right words and to get each fact precisely straight. Although usually friendly and never dour, Havel appears unusually serious, not given to frequent or easy laughter. The two men were equally intense, however, and shared an unwavering commitment to the rigor and fascination of experimental biology.

Havel was raised in Seattle, Washington, the son of a credit manager for a newspaper and a former actress, both moderately leftist, neither a college graduate. In high school he gravitated toward quantitative sciences, especially chemistry. He attended Reed College, in Oregon, partly because he thought he'd get better teaching there than at the University of Washington, but also to move away from home. At Reed he majored in chemistry, and loved it. He also met the woman he would marry. When Havel turned nineteen, in 1944, defective vision in one eye protected him from the draft, and after a bit more than two years in college he moved to medical school at the University of Oregon in Portland, the "provincial medical school" he remembered later in San Francisco. In the last year of medical school he earned an MS degree doing research on metabolites in the blood of patients undergoing cardiac catheterization, then a relatively new procedure.

In 1949 Havel became the only member of his medical graduating class to do his residency (in internal medicine) east of the Mississippi. He and his wife Virginia

("Ginny") drove an old clunker across the country to Cornell's medical school and hospital, in New York City—where he soon found that, despite his earlier deferment, he was likely to be drafted to serve in the Korean War. But Havel, who favorably impressed his mentors at Cornell, was quickly rescued from this predicament. In 1950, the NIH was building a huge new Clinical Center in Bethesda, Maryland. James Shannon, then head of the National Heart Institute and later head of the entire NIH, called buddies at leading medical schools in the eastern US and asked them to recommend young, research-oriented individuals to take care of Heart Institute patients in the new center. Soon Havel was invited for an interview and inducted into the Public Health Service, which protected him from the draft. Because it took three more years to finish construction of the Clinical Center, Havel continued his residency at Cornell.

At the NIH, as one of the first eight young doctors to supervise care of patients in the Heart Institute, Havel joined a bright and very select group. Almost all were chosen from Harvard, Yale, Cornell, and Columbia. Two of the eight, Donald Fredrickson and James Wyngaarden, later became directors of the NIH; another, Robert Gordon, became Clinical Director of the National Institute of Arthritis and Metabolic Diseases; still another, Roy Vagelos, went on to a brilliant career as a biochemist at Washington University and subsequently as president of Merck.

During his three years at the NIH, in addition to his clinical duties, Havel chose to work in a new group formed by Chris Anfinsen, who would later receive a Nobel Prize for his work on protein folding. The group was charged with understanding the normal and pathologic functions of lipoproteins, blood proteins that bind and transport fatty molecules like cholesterol and triglycerides through the blood. In atherosclerosis, however, some of these lipoproteins deposit fat molecules (aka "lipids") in blood vessel walls, where they lead to coronary thrombosis, vascular occlusion, and stroke.

At the NIH, Havel adapted a recently published new method for separating and characterizing different classes of lipoproteins in blood. His revised method made it easier to identify a host of lipoprotein disorders, and allowed him to discover a new human disease in which abnormal lipid patterns result from genetic deficiency of lipoprotein lipase, an enzyme discovered by another NIH scientist.

After his stint at the NIH, Havel decided to look for an academic job. He and Ginny inclined toward the West Coast, in order to rejoin their families, but his friends at NIH worried "that I'd go out there and nothing would happen," because the West Coast was so far from the real "action" concentrated in the East. He had to decide whether "to stay at the NIH and really have a research career there, or head out on my own."[1] His trip out West was not reassuring. Chicago was not especially

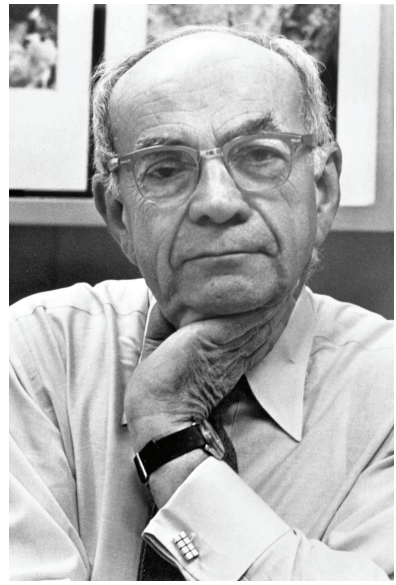
attractive. The University of Washington's medical school in Seattle seemed the best prospect for real scholarship, but the chair of Medicine, noting that lipoprotein disorders cause lipids to accumulate under the skin, suggested Havel learn dermatology first, then head a dermatology division and do research on the side. Portland, with its "provincial medical school," seemed pedestrian.

In San Francisco, the future seemed hopeful but uncertain. Taking solace from the prospect of joining the Cardiovascular Research Institute, Havel still worried that teaching and patient care responsibilities could make it hard for him to run a lab, especially on a campus where NIH-supported labs were rare. In the future, three basic science departments would migrate from Berkeley to San Francisco, but no one knew which Berkeley scientists would actually move.

San Francisco was a risk, but the Havels had relatives in the Bay Area, and in the end he decided to take the job. Fortunately, after a couple of years he was awarded an AHA "Established Investigatorship" like the one Edelman received earlier. It paid all but $1,000 of his total $7,500 salary. For the University, this constituted a real bargain—for much less salary than it had promised to pay, it got a new assistant professor to teach Laboratory Diagnosis. That he was likely to prove an excellent scientist carried weight with people like Edelman, although it wasn't clear that department leaders felt the same way.

For Havel, however, the AHA Investigatorship was a very special bargain, because it formally required the University to allow him to devote a substantial portion of his time to research. "That protected time was key. If that hadn't happened, things could have developed for me very differently. There were times in those first years when I said—because of all the teaching, running the lab, doing ward rounds—[that] my colleagues who had stayed behind [at eastern schools or the NIH] were full-time in the lab, practically. So I did ask myself, Am I going to be able to keep up?"[1]

Havel did find a way to "keep up," with a great deal of help from the NIH and the American Heart Association. He went on to a distinguished career, as director of UCSF's Cardiovascular Research Institute, and in the laboratory, where his focus on lipoproteins and their travels through the blood stream laid key elements of the foundation for modern understanding of inherited and diet-induced disorders of fat metabolism and for atherosclerosis and heart disease. In addition, he became director of the Cardiovasclar Research Institute at UCSF. Like Izzy Edelman, Havel later profited from and contributed importantly to profound changes in the research orientation of medical schools like UCSF in the latter half of the twentieth century. We shall return later to other causes and consequences of these changes. For now it is enough to recognize that, contrary to Francis Moore's message to Izzy Edelman in



Julius H. Comroe, physician-scientist. A specialist on cardiopulmonary regulation, he came to UCSF in 1957, as the first director of UCSF's Cardiovascular Research Institute. Photo in 1975.

1948, medical scientists would soon be able to work in laboratories and make a living at the same time.

## Julius Comroe and Holly Smith

Throughout the 1960s and 1970s, both Edelman and Havel contributed to the flowering of scientific investigation at UCSF, both as scientists and as administrators. The contributions of Julius H. Comroe and Lloyd Hollingsworth ("Holly") Smith were even greater. UCSF would have been a very different place—in the 1970s and ever since—if either Comroe or Smith had not come to California when they did.

Before we meet Comroe, I'll describe the genesis of the Cardiovascular Research Institute (CVRI), destined to occupy the empty shell Havel saw on the thirteenth floor of newly built Moffitt Hospital. An earlier chair of the Department of Medicine (William Kerr, no kin to Clark Kerr), had worked to get the University to build Moffitt Hospital (as well as the Medical Sciences Building). Realizing that the new structure's thirteenth floor might be unpopular with prospective patients, and knowing scientists would be embarrassed to admit to superstition, he assigned the thirteenth floor to research. Responsibility for planning how to use that space fell to "the Cardiovascular Board," a small group of clinical faculty interested in heart disease. They envisioned an interdisciplinary unit in which scientists from multiple departments would focus on the cardiovascular system.

Born in 1911, Julius H. Comroe, Jr, was the youngest son of a prominent doctor

in York, Pennsylvania.[14] Graduated from college and medical school at the University of Pennsylvania, he began a two-year internship in surgery. During an operation, one of his eyes accidentally became infected, but the infection was not effectively treated, and eventually the eye had to be removed. At the age of 25, Comroe turned to medical science.

As an instructor in the University of Pennsylvania's Department of Pharmacology, Comroe's logical powers, determination, and knack for devising decisive experiments led to rapid success. In 1937 and 1938, he and a colleague identified the precise locations of the body's chemosensors for oxygen and carbon dioxide in circulating blood, one on the carotid artery in the neck, the other in the chest. In 1943, they showed that specific brain chemosensors for blood carbon dioxide are the primary regulators of normal respiration, while the neck and chest sensors act primarily when oxygen is severely depleted.

During and after World War II, Comroe's academic career at the University of Pennsylvania involved chairing a Department of Physiology and Pharmacology, continuing his meticulous studies of pulmonary gas exchange and mechanical properties of the lung, and devising a new approach to teaching basic science to physicians, structured around organ systems rather than departments. Then highly original, this idea was later adopted by other medical schools, including UCSF.

By 1956 Comroe was forty-five, and a combination of slow promotion and opposition to his administrative efforts in Philadelphia prompted him to search for new opportunities.[15] One of these was in San Francisco, where he was interviewed for the recently vacated chair of the Department of Pharmacology on Parnassus. He concluded, however, that the Pharmacology faculty were too entrenched in their old-fashioned attitudes. Then, on the way to the airport after his visit, UCSF's Ellen Brown (who was a member of both the Pharmacology search committee and the Cardiovascular Board) asked Comroe to suggest potential candidates to serve as the the nascent CVRI's first director. His response was characteristically direct, and immediate: "Me," he replied.[14] The School offered him the job, and he accepted.

Upon arrival in San Francisco, in August 1957, Comroe promptly requested a key change in the organization of the CVRI and his status in the School of Medicine. In the Board's plan for the CVRI, its director was to coordinate research activities of faculty from various existing departments in the School, and to be responsible to a committee composed of chairs of those departments—an arrangement, Comroe saw, that would severely hamper his ability to hire and retain good scientists in the CVRI. To direct an independent institute, he needed to be responsible, like department chairs themselves, to the dean of the School. According to Comroe, "it was

hard for [the Board] to believe that a director of a scientific institute could have an interest, let alone concern, for the School as a whole."[14] He persuaded them otherwise, and got his wish.

Comroe's first opportunity to show his concern for the School came within two weeks of his arrival on Parnassus. He learned that UC had a serious problem with the specialty of anesthesia, then a division of the Department of Surgery, chaired by Leon Goldman. Anesthesia at UCSF had lost its accreditation for residency training and its director had left. A year's search for a replacement was unsuccessful, largely because Goldman was unwilling to make Anesthesia a department on its own, independent of surgery. Comroe's approach to the problem reveals the essence of his administrative style—decisive, direct, timely, persuasive, and usually successful.

As Comroe told the story, at a national meeting early in September 1957 he met an old acquaintance, Stuart Cullen, the highly respected chair of Anesthesia at the University of Iowa. Cullen wanted to know "what the hell was going on in San Francisco, anyway." UCSF was considering one of his own faculty members, Cullen said, for chief of Anesthesia, but was uncommunicative and slow to make decisions. Comroe replied that UCSF had interviewed ten candidates, and all had turned down the job because Anesthesia was not an independent department. Then, in Comroe's words:[14]

> I told Cullen that if I were Dean, I would erase the slate and start over again. I would select the number one man in the country and tell him we would be delighted to have him come as a Chairman of a separate Department of Anesthesia. I asked Cullen, "Would you accept the position in San Francisco if it were a separate department?" I think he thought about 20 seconds and said, "Yes, I think I would."

I got to a telephone and called Goldman in San Francisco and told him that I had just talked with Cullen and that I was sure that Cullen would accept if the school would agree to create a separate Department of Anesthesia. I then asked Goldman whether he would agree to this. There was a long, long pause (much longer than Cullen's); I could hear him gulping several times. He then said, "Yes, I will recommend a separate department."[14]

The deed was done. Cullen became a distinguished chair of the new Department of Anesthesia. Later, at a crucial time in the School's history, he was to serve as dean of the School. From this experience, Comroe drew a critical inference:

> The Cullen recruitment convinced me that this School of Medicine could and should be the best in the country. If we could recruit a #1 Chairman of Anesthesia in 20 seconds, we should be able to recruit the #1 man for every

> open position. The reason for this optimism was obvious. Between 1954 and 1960, everything had suddenly come together in San Francisco. For the first time in 50 years, there was a structurally complete medical school with basic scientists and clinical faculty, a magnificent connected group of high-rise buildings, many not occupied. And all this in American's favorite city that had major league opera, symphony, theater, baseball, football, a spectacular shoreline, and nearby mountains and lakes. It was becoming more and more difficult for [our] faculty to be satisfied with being [just] "best in the West"![14]

I came to UCSF well after Comroe made his most dramatic and important contributions. In my memory he was a stern senior figure, revered and even feared by some of my own seniors. To everyone, he gave the impression of immense determination. In addition, his contemporaries found him funny, perceptive, forceful, and forthright but genuinely supportive of anyone he found capable and energetic. When they first met, Edelman said, he told Comroe that he "hoped he would find fertile soil. [Comroe] said, 'Well, I think it is, because it's my intention to make this the greatest cardiovascular research institute in the world.' Before he had recruited anybody and the space wasn't even finished, that was the driving ambition."[13]

Working with Comroe, Edelman went on, was "an interesting experience . . . because there were gears going on in his head, and you never knew what exactly was going on there until he laid it out. But he was absolutely focused on the target, that this was going to be the greatest teaching, training, research center in the world, and he sure as hell made a great enterprise out of it."[13]

William Reinhardt, who had replaced Saunders as dean of the medical school in 1963, reviewed the performance of several UCSF department chairs and managed to persuade them to step down and be replaced. Of the clinical departments in a medical school, the Department of Medicine usually has the biggest teaching load and the largest faculty, and cares the most about research. It is fitting, then, that the person Reinhardt recruited to chair this department on the Parnassus campus played such a major role in shaping the future of the school and the UCSF campus.

Lloyd H. Smith, Jr, was called "Hollingsworth" by his family, but his friends later shortened this to "Holly." He grew up in Easley, South Carolina, a town of 6,000 located where the northwestern corner of the state wedges tightly between North Carolina and Georgia. Modestly better off than most of their neighbors, his family had to move into his grandmother's house when Smith's father's bank failed. Although Easley schools were not sophisticated, he was bright and enjoyed learning. In 1941, just as World War II began, high marks on a state exam earned him

LH (Holly) Smith, Jr., chair of the Department of Medicine. Taking the chair in 1964, he profoundly influenced UCSF's history. Photo from about 1964.

a scholarship to attend Washington and Lee University, in Lexington, Virginia. In keeping with the tendency of men in his family to pursue a profession, he enrolled in a pre-medical course.[16]

In 1944 Smith entered Harvard Medical School, which he found academically challenging and more engaging than college. By the end of medical school, in 1947-1948, he was assigned to a clinical research project focused on constructing an artificial kidney machine for patients with acute renal failure. Invented by a Dutch physician, Willem Kolff, this machine bathed blood, circulating through tubes connected to the patient, in a special bath of salts and water. Connections between tubes were made from condoms, which Smith bought at a drugstore near the Peter Bent Brigham Hospital. Weekly purchases of a gross of condoms "earned me considerable respect in the drugstore," Smith says, although he says the respect was largely unearned.[17] The artificial kidney looked clumsy and unsophisticated, but worked rather well.

After internship and residency at the Massachusetts General Hospital (MGH), Smith was a very busy man. Among other things, by 1956 he had begun his scientific training in earnest by learning to do organic chemistry at Harvard; served in the army during the Korean War, where he set up and operated a Kolff machine under battle conditions to treat acute renal failure in wounded soldiers; undergone further chemistry training in New York; married the sister of a medical school classmate; studied enzymology and metabolism for a year in Stockholm; and returned to the MGH for a year as chief resident in Medicine. Then Walter Bauer, the chair of Medicine at the

MGH, invited Smith to join the faculty and—because Bauer was "given to breathtaking extrapolations"—asked him to direct a subspecialty division in endocrinology.[16]

"I'm going to give you five years," Bauer told him. "I'm going to support you to the very hilt. I think you can make it in academic medicine. But if you don't, I'm going to kick your ass out of here." Smith found this "a very sporting proposition, because I knew he meant it precisely. He liked me and wanted me to succeed, but he was brutally honest."[16]

In fact, Smith did an excellent job, both academically (in developing the endocrine division) and as a physician-scientist (he discovered the precise biochemical basis of several inherited metabolic diseases). In 1963, after Bauer's death, Smith himself became a leading candidate for MGH's chair of Medicine. Instead, Harvard chose Robert Ebert, an eminent physician and scholar who was twelve years older than Smith, then just thirty-nine. Once the decision was announced, Smith was free to take a year of sabbatical leave at Oxford, where he worked in the lab of Hans Krebs, a Nobel laureate. Passed over for a very prestigious position, the young man knew his own worth. As the year at Oxford wound down, both UCSF and the nascent UC medical school in San Diego invited him for a visit. Now he was ready to pay attention to such invitations, although for years he had imagined himself staying at Harvard.

Reinhardt's search for a chair of Medicine had focused on nationally prominent physician scholars, older than Smith but with strong scientific credentials. These included people like Arno Motulsky, who studied the genetics of human diseases in Seattle, and Robert Berliner, an immensely talented renal physiologist. Reinhardt would have seen in Smith a young man skilled in biochemical investigation of disease and—according to grapevine information from the MGH—well-regarded as a physician and potential leader at the premier academic hospital in the country. In person he would have met a tall, patrician figure who spoke in pungent, well-rounded sentences tinged with cadences of South Carolina. His interviewers would also have found him immensely perceptive, a man on whom nothing is lost. Unfailingly gracious and affable, Smith maintains the reserved but receptive persona of a leader skilled at keeping his own counsel while others urgently exhibit their own assets and needs.

In a three-day visit to San Francisco, Smith met several science-oriented faculty, including Edelman and Comroe. The latter was impressive "in that little office of his," although "trying to figure out which eye was watching you" was disconcerting.[16] Saunders was not in evidence, and Reinhardt gave a clear message: "There's going to be a lot of change. I knew Reinhardt had the guts. He was the key—quiet, self-effacing, saturnine, said very little. In his shrewd way, rather like a very quiet

Harry Truman, not flashy. And he had the guts to fire a lot of chairs." [17] (The outgoing chair of Medicine, Henry Brainerd, had been chair for ten years.)

On the way to the airport, Reinhardt "offered me the job, and I accepted immediately."[17] Reinhardt mentioned the salary ($30,000 per year) and said Smith would be given three faculty positions to fill. Smith asked for nothing else, because—he wrote later—"in the long run the leadership of the campus would want me to succeed in leadership of this largest clinical department. In that prediction I was never disappointed."[18] Even in 1964, endless haggling over details—money, space, positions, bizarre perquisites like membership in a golf club—often bedeviled academic recruitments at UC and elsewhere. Smith's refusal to initiate the usual ritual became almost a legend at UCSF. In doing so, he signaled his own confidence and shrewdly let the School's leadership know he trusted them to do their part.

Later Smith listed many reasons for deciding to come to UCSF. He could see plenty of "mediocrity, missed opportunities, weaknesses, [and] incompetent people in high places." On the other hand, this medical school was "the oldest in the West; connected to Berkeley, one of the great universities, [and in] the country's most visceral and attractive city;" jet travel now made it possible to travel in a little more than four hours to the NIH and scientific meetings on the East Coast; Stanford had decamped from the medical scene in San Francisco, opening great clinical opportunities; UC's brilliant president, Clark Kerr, had just written *The Uses of the University*, heralding the rise of research universities; California's economy was strong, with Governor Pat Brown investing heavily in higher education, including two research towers under construction on Parnassus; and the NIH was rapidly increasing its support of biomedical research.[19]

"But the main thing was, it hit [at a time when] I was susceptible," Smith says. "My old chief at the Mass General had died, I'd just been passed over as chief, I was in transit and my house had been rented. So I said, 'Jesus! Let's do it!' It was like a perfect storm, a perfect opportunity. When you are young, life stretches out endlessly ahead of you. That's the time to go for it. All those things came together. Change was in the air, anything was possible."[16]

Smith was not the only future leader attracted to UCSF because he perceived opportunities for change, in contrast to medical schools back East. This was true of all four leaders we have discussed—Smith, Edelman, Havel, and Comroe—to say nothing of many other important individuals who came to San Francisco in the 1950s and 1960s (see Table 1). For Izzy Edelman, anti-semitism, red-baiting politicians, and the economic and social entry barriers surrounding mainstream medical science made it hard to become a researcher. UCSF may have offered him a nearly fictional

lab, in a basement locker room, but it was also the only medical school that offered him a paying faculty job. To Dick Havel, determined to return to the West Coast after flourishing in supportive research environments at Cornell and the NIH, one risky faculty position appeared marginally better than his other choices. Comroe and Smith had prospered on the east coast, but also felt constrained by the traditional status quo at its most prestigious medical schools. Most important, all four had been trained and developed their early careers at academically superior institutions, and were acutely aware of UCSF's limitations, but also sensed great opportunity and exciting challenges—"Nowhere to go but up!" Thus begun, their stories traced patterns that were eventually to transform UCSF.

Chapter Two

# Deciding a Future

## The Wild Man's Victory

Barely forty and barely a month into his new job, Holly Smith was still trying to learn who was who and what tasks to tackle first. In September 1964, he had taken the chair of UCSF's Department of Medicine. Now, in mid-October, his secretary announced that Julius Comroe, Director of the CVRI, would soon pay him a visit. Smith had already learned to respect Comroe—"a remarkable person, very smart, very determined to get his way, and generally right."[1] But why this sudden visit?

Comroe was on time, as always, and dressed in his usual gray suit and tie. He came right to the point. Smith doesn't remember the precise words, but the message was straightforward: It is time for UCSF to get rid of John Saunders, our Chancellor, and you, Holly, are the man to lead the charge. "So, much to my astonishment," Smith recalls, "I found myself a member of a cabal, and being pushed to the front of this cabal."[1]

Smith had made an appointment to meet the chancellor a week earlier. Saunders had talked for the entire fifty minutes without asking a question. "He talked about human ecology, gave me a lecture on it"—although what human ecology really was, Smith couldn't figure out. But others assured him that Saunders was "an impediment to progress."[2]

Smith, who likes to masquerade as "a gentle Presbyterian boy from the south," had been summoned to lead a bold, risky flanking attack.[2] The long-standing battle harked back to the medical school's long geographical separation into two parts, clinical medicine in San Francisco and medical "science" in Berkeley. Science-oriented faculty at Berkeley, exemplified by Herbert Evans, the chair of anatomy, had considered "pure science" superior to mere clinical practice. In substantive conflicts, however, the more numerous clinicians usually prevailed. Several had patients who were members of the University's Board of Regents—relationships that partly accounted for UCSF's reconsolidation in San Francisco, rather than Berkeley. In addition, the medical school's chair of surgery, neurosurgeon Howard Naffziger, was himself a Regent.

The battle may have begun before Saunders was appointed dean of the School of Medicine or Comroe came to Parnassus, but now the stakes were higher and many of the combatants were new. In the late 1950s and early 1960s, scientists were asking exciting medical and biological questions, in experiments supported by generous federal grants. Not yet numerous or powerful, this new generation of medical scientists nonetheless began to sense that the future could belong to them. Once a petty skirmish between scientists and clinicians, the conflict but now looked more like an inexorable future striving to overcome a fading past. If the flanking attack failed, the losers would pursue their futures elsewhere, leaving UCSF to return to its comfortable status as a good regional medical school. But if Comroe's battle plan proved as decisive as he hoped, the winners could build a new future, making UCSF a nationally recognized leader in biomedical research.

## The Comroe-Saunders Battle Begins

As Izzy Edelman remembered it, the battle's first major skirmish dated back as far as 1956.[3] Soon after Saunders became dean, and before Comroe appeared, UC's Regents announced a contract in which Franklin Hospital, a private facility, would be located on University land, directly across Parnassus Avenue from UCSF. Edelman worried that the faculty had not been consulted about committing University resources to a program that would contribute nothing to research or teaching, and to create a facility over which UCSF would have no control. When he persuaded UCSF's Academic Senate to ask for more information, Saunders replied that the new facility, in essence, was none of their business. Clark Kerr later surmised that the Franklin Hospital plan originated with Naffziger, the UCSF neurosurgeon, who pressured Saunders to go along. Once Naffziger retired as a regent, other regents lost enthusiasm for the plan and, in 1962, pulled out of the deal altogether. To do so, the University had to pay Franklin Hospital's trustees more than $200,000 (then a very great sum), to defray planning and other costs. As a result, Kerr thought, Saunders lost popularity with some Regents.[4]

Saunders's high-handed style and his habit of interminably delaying meetings also rubbed many faculty and department chairs the wrong way. None of these faculty was as determined as Julius Comroe, or less susceptible to Saunders's attempts to deflect him from his chosen course. As CVRI director, Comroe began by renovating the thirteenth floor of Moffitt Hospital and filling its new labs with first-rate researchers. He asked Havel and Edelman to serve as associate directors, and hired from outside UCSF an array of outstanding cardiac and pulmonary physiologists, biophysicists and biochemists. (One of these was John Clements, whose research on

pulmonary surfactant was to transform both understanding and treatment of respiratory distress in newborn babies, saving many lives.)

Spending most of his time and effort on the CVRI, rather than his own research, Comroe created a first-rate training program for young scientists. Supported by an NIH training grant, the program paid stipends to postdoctoral fellows and grew from $70,000 to $191,000 per year between 1957 and 1966. Research at first was supported by grants awarded to individual CVRI scientists by the National Heart Institute (NHI). In 1962, the NIH director offered to merge all NHI grants to the CVRI into a single "center" grant, which had to be renewed every seven years. In its first year this center grant, with Comroe as the "Principal Investigator," or PI, amounted to about $875,000 per year.[5] This was nearly 30% of the total $3.045 million in NIH funds awarded to UCSF in 1962—a proportion that reflected both the research prowess of the CVRI's faculty and the relative weakness of UCSF's other researchers.[6] Comroe's center grant continued to grow, and by the early 1970s was bringing nearly $2 million to the CVRI.

By consolidating its NHI funds into a single grant, the CVRI managed to avoid the bureaucratic drudgery of submitting many separate applications to the NIH, increased the long-term stability of its funding (individual grants were awarded for shorter periods of four to five years), and enjoyed the flexibility of "rearrang[ing] budgets within the institute [in order] to support exciting new discoveries immediately or to obtain special equipment or services . . . that might be deemed excessively expensive for a single laboratory."[5] In the 1960s grants of this size were rare—indeed, unknown—at UCSF, and consequently threatening to some faculty, who worried that Comroe and his colleagues were going to take over the medical center. Frances Larragueta, who supervised the dean's office staff from early in Saunders's time until the 1980s, recalls that administrative approval for submitting Comroe's grant fell to Robert Credé, Saunders's deputy in the dean's office. Credé "really didn't want to approve that one," she says. "His fear was that here was Comroe, the leader on the campus, and if he got this $1 million grant and went back to Pennsylvania with it, here would be UCSF down at rock bottom again."[7] Credé did approve the grant, however, and Comroe did not return to Pennsylvania.

To solve several problems, Comroe appealed directly to UC President Kerr for help. For instance, soon after his arrival he discovered that grant applications from UC schools did not reach the NIH until eighty days after they were submitted to the UC bureaucracy. At all other universities, the average delay was ten days. The long wait at UC was caused by requirements for administrative approvals at many levels, including the school or campus, the president's office, and the Regents. Similarly, it

was proving almost impossible for a school or campus to offer a job to a new faculty recruit without incurring a year's delay, as the request for administrative approval moved through echelons of administrators at higher and higher levels. Kerr solved both problems by implementing reforms based on his policy of decentralizing UC's administration.[5]

In addition, the CVRI needed to hire researchers whose salary support would be derived primarily from grant funds, rather than from University coffers. University rules made this very difficult, because they forbade such faculty not only to earn tenure, but also to be appointed as professors, to become members of the Academic Senate, or to apply for their own grant support through University channels. Such policies dated back to the old days, when research support from foundations and charitable donors was awarded to faculty whose salaries were already fully supported by the University. Comroe was bitterly opposed by some regular faculty and many administrators, who felt that researchers' salaries should be paid mainly, or perhaps exclusively, by the University. But Comroe did his homework, and his survey of ninety-four university medical schools in the US and Canada showed that UC's were the only ones that provided neither academic titles nor privileges to grant-supported scientists. He communicated this finding to Kerr in December 1961. In July, 1962, the Regents granted many faculty privileges to "the previous second-class citizens, in return for about four hours teaching per week . . . (which in most cases was much less than they already did)."[5] UC was belatedly joining other schools in a practice that helped transform all biomedical research institutions. Nowadays, in clinical departments of most such institutions, including UCSF, scientists on the faculty draw big chunks of their salaries from research grants, and the institution uses indirect costs from those grants to fund buildings. (To help compensate for institutional expenditures that support research, the NIH pays "indirect costs" to host institutions, in proportion to each research grant awarded to an investigator.) Such arrangements converted researchers from drains on the institutions' budgets into sources of revenue for expansion. Contrast this with a senior academic's warning to young Izzy Edelman, just one decade earlier, that medical school faculty can afford to do research only if they are independently wealthy (see Chapter One).

Other difficulties were less inherent in the UC system and more directly attributable to Saunders himself. Indeed, Comroe wrote later, in 1962 he "told Clark Kerr that resignation[s] of certain professors, including mine, were imminent unless he appointed a new dean."[5] As we noted earlier, Kerr did appoint William Reinhardt as dean of the School of Medicine in 1963, keeping Saunders on as provost; in 1964, he made Saunders chancellor of the UCSF campus. Presumably, Kerr—and perhaps

Comroe, as well—hoped that promoting Saunders to a higher office would reduce his meddling with the School of Medicine. Comroe had asked for appointment of a new dean, but his request may not have been as decisive as he believed. Kerr later denied that Comroe played a large role in his decisions about UCSF.[4] Like his rescinding the requirement for central administrative approval of grant applications and new faculty appointments, appointment of a new dean and retaining Saunders as provost and chancellor conformed to Kerr's policy of decentralizing UC.

Two major conflicts between Comroe and Saunders involved the usual bones of contention in a medical school—money and laboratory space. The first began with a large bequest from a cardiac patient, Samuel Neider, whose will specified that half his fortune be devoted to research on cardiovascular disease at UCSF. After Neider's death, his physician stated that his patient had intended the money to go to the CVRI, but Chancellor Saunders opposed this idea. In a separate conflict whose outcome was equally critical for the CVRI's future, Comroe requested additional space for institute laboratories in the Health Science research towers, scheduled to open in 1966. Saunders was opposed, and stubbornly delayed planning for use of the new space.

Most egregiously, Saunders allegedly delayed or prevented academic promotions of faculty who opposed him. Comroe said Saunders kept dossiers on several such faculty members. Larragueta confirms that as provost or chancellor he could hold promotion papers in his desk drawer indefinitely, if he chose to do so.[7] The faculty whom Saunders considered his opponents included Edelman and Havel in the CVRI, plus faculty in other departments. In several cases, Comroe wrote, "Kerr's Vice President, Harry Wellman, came to our rescue and overruled" Saunders.[5]

Opposition to Saunders was not confined to Comroe or the CVRI. One case involved Gil Gordan, the endocrinologist who had advised Izzy Edelman to ask "Mrs. Fleischmann" for renovation money. One day Gordan found himself discussing the Saunders problem with Leon Goldman, Naffziger's successor as chair of the Department of Surgery. In response to Goldman's doubts about how many people opposed Saunders, Gordan offered to bring a group to talk with Goldman that very afternoon. Six individuals (I have not been able to find their names) told Goldman what they thought of Saunders, and Goldman promptly told Saunders what people were saying about him. Saunders asked for names, Goldman gave them, and Saunders "blacklisted" Gordan and all the rest.[8] Although some condemned Goldman for naming names, it seems likely that he eventually agreed with Gordan and his allies—as I infer from Saunders's unsuccessful attempt, soon thereafter, to fire Goldman from the chair of surgery. It was widely rumored that Saunders telephoned Goldman at home, and told Goldman's daughter Diane—now Diane Feinstein, California's senior US Sena-

tor—that he was calling to ask her father to resign. The daughter—according to the rumor—replied that Saunders would have to deliver that message himself. He did so later, but Goldman's furious response, it was said, dissuaded him.[9]

## Comroe Mounts a Flanking Attack

Comroe's papers, stored in cardboard boxes at UCSF, include a maroon loose-leaf notebook, two inches thick, with "Saunders" printed on its cover in big, bold letters and black ink.[10] Meticulously kept, with three holes punched into almost every sheet to keep the papers in order, the notebook is stuffed with correspondence, typed or handwritten documents, newspaper clippings, and voluminous notes in Comroe's beautifully crafted handwriting.

A hand-written letter to Clark Kerr, dated December 18, 1961, begins with "I find it increasingly difficult to function as Director of the CVRI under the present circumstances," and goes on to request an interview with Kerr soon, because only UC's President "can solve several important problems and enable our campus to achieve true excellence." At least a dozen subsequent letters to "Dear Clark," along with a few handwritten replies, deal mostly with the CVRI's problems, including increased needs for research space and the Neider affair, described above. Comroe repeatedly provides Kerr with documentation of his complaints, often listing other faculty or non-UC officials and experts able to corroborate his story.[10] I do not know whether other UC faculty members have inundated the President with so many letters, but very few can have matched Comroe's tenacity and attention to detail. Kerr's briefer, always thoughtful responses are similarly impressive—in addition to the results he produced, of course, if Comroe's later account is correct.[5]

The Saunders file also includes numerous notes from Comroe to himself.[10] Many are short and cryptic, and few are dated. The most remarkable are four comprehensive lists of specific grievances against Saunders and possible plans for dealing with them. One early list begins with the need for "new men" (including women) in campus leadership, with chairs that need replacing and possible candidates. Next he lists Saunders's "advisors," commenting that the list does not include the best academic faculty and that the advisors are biased in favor of hospital beds rather than research. He then notes instances of "complete admin breakdown" in which Saunders's actions (or failure to act) hurt CVRI faculty, as well as his failures to answer multiple letters on many topics and to work with the Academic Senate (on the Franklin Hospital affair and appointing a Dean of Graduate Studies). To these Comroe adds, "(Dossier on me) re my 'irresponsible' statements." In the upper right corner Comroe wrote, "Min Requirements—New Dean, Flexibility with Associate Prof level, and Deptl

status [for the CVRI, presumably]." This was probably written rather early, before Comroe asked Kerr to replace the dean and when he was working to make sure that he, like a department chair, would be directly responsible to the dean. (What "Flexibility with Assoc Prof level" refers to is not clear.)

By late 1963, the numbered list of grievances against Saunders, fifteen in all, had expanded to fill four typewritten pages.[10] The first grievance, "Incompetent as an administrator," refers to Saunders's office as "an administrative shambles" and states that he is unwilling to delegate authority, unavailable to faculty, and given to inexcusable delay. Other grievances, each replete with multiple examples, indicate that Saunders is "unable or unwilling to admit errors of fact, judgment or policy;" fails to seek expert advice and surrounds himself with "yes men;" keeps faculty ignorant of new space allocations, campus development plans, and recommended appointments, promotions, and budgets; fails in recruiting chairs (including four failures to fill the chair of Biochemistry); and keeps "dossiers" on faculty who oppose him. Finally, Comroe refers to a "Tremendous waste of faculty's time (monologues, historical accounts, avoidance of major issues)."

In addition to its repetitive and persistent laser-like focus on detail, the Saunders file reveals a degree of emotional intensity not usually expected in research administrators. Comroe appears an implacable avenger, bound and determined to prevail. Still, letters and notes about Saunders occupy a tiny fraction of the correspondence in Comroe's files. During this conflict, the CVRI director was busy hiring faculty, teaching students and postdocs how to do research, training other faculty to supervise postdocs, writing grant applications, running a small lab, and keeping up with CVRI research as well as discoveries and developments elsewhere, in the general fields of cardiopulmonary physiology and disease. He also dealt constantly with the questions of finance, personnel, and conflicting egos that arise daily in any growing, successful organization. These tasks kept Comroe very busy, and he was proving a competent and effective leader, who got things done.

Comroe may have masked his emotional intensity in interactions with his colleagues, who do not mention it explicitly, but he probably needed it to fuel his unquenchable energy and effectiveness as a catalyst for change. Clark Kerr, a perceptive and experienced administrator himself, was troubled by the CVRI director's apparent capacity for implacable aggression. "I had the impression," he said years later, "that he was a very able guy, but something of a wild man, maybe a very aggressive, perhaps even impossible person. I'd still say that [of him] as an administrator, but I developed a great respect for him as a scientist. [I] discounted him early on, but not in the end, because I became convinced that he was something of a genius."[4]

Whether or not Comroe was a "wild man," he was also immensely perceptive about others. My guess—based on hints in his Saunders file and his management of the final flanking attack on the chancellor—is that Comroe began to realize, sometime in 1964, that Kerr considered him too intensely focused on his own and the CVRI's interests, so that it would be a real advantage to enlist allies outside the CVRI. One such ally would be William Reinhardt, Dean of the School of Medicine. Earlier comments in the Saunders file indicate that Comroe considered Reinhardt somehow in thrall to his former anatomy department chair, Saunders, and lacking in courage and leadership. In a later letter (dated October 14, 1964), however, we find Comroe telling "Dear Clark" that "Dean Reinhardt and I have now come to the conclusion that, with Dr. Saunders as Chancellor, the matter [of assigning space in new research towers to the CVRI] cannot be settled except by the University-wide administration." He goes on to ask Kerr (or Harry Wellman, his assistant) to see him and Reinhardt in the near future. A note from Reinhardt at the bottom of this letter states his agreement with Comroe's analysis.

As the drama unfolded in the early 1960s, Reinhardt's own role and attitudes remained shrouded in mystery. A man of few words, and by no means an ebullient enthusiast—"saturnine," Holly Smith called him—he appears to have maneuvered very carefully. Earlier, Reinhardt worked with Saunders in the Department of Anatomy, and was widely thought to be Saunders's ally. Nonetheless, his regime ushered in changes that proved crucial. In 1963 and 1964, with the new dean's approval, five chairs of clinical departments were reviewed, and four were not recommended for re-appointment. A fifth chair retired because of age. Consequently, between 1963 and 1966 the new dean and his immediate successor appointed five new chairs from outside UCSF. As we have seen, one of these was Holly Smith in the Department of Medicine. The other new chairs—all, like Smith, recruited from leading institutions outside UCSF—were in Radiology, Surgery, Pediatrics, and Neurology.[11] How and why Reinhardt accomplished this feat without meeting direct opposition from Saunders is not clear. Given the dean's delicate position, adopting a Comroe-like attack mode would have proved disastrous. Instead, and probably deliberately, Reinhardt relied mainly on his own distinctive personal style, with its quiet temperament, tenacity, and capacity for keeping his own counsel. Such a person, and perhaps only such a person, could have maneuvered adroitly below Saunders's often-inattentive radar, maintaining the Chancellor's trust while secretly improving the School of Medicine. Smith characterizes Reinhardt as a brave unsung hero.[1] We shall always want to know more about this extraordinary man, who was so silent, crafty, cunning, and effective.

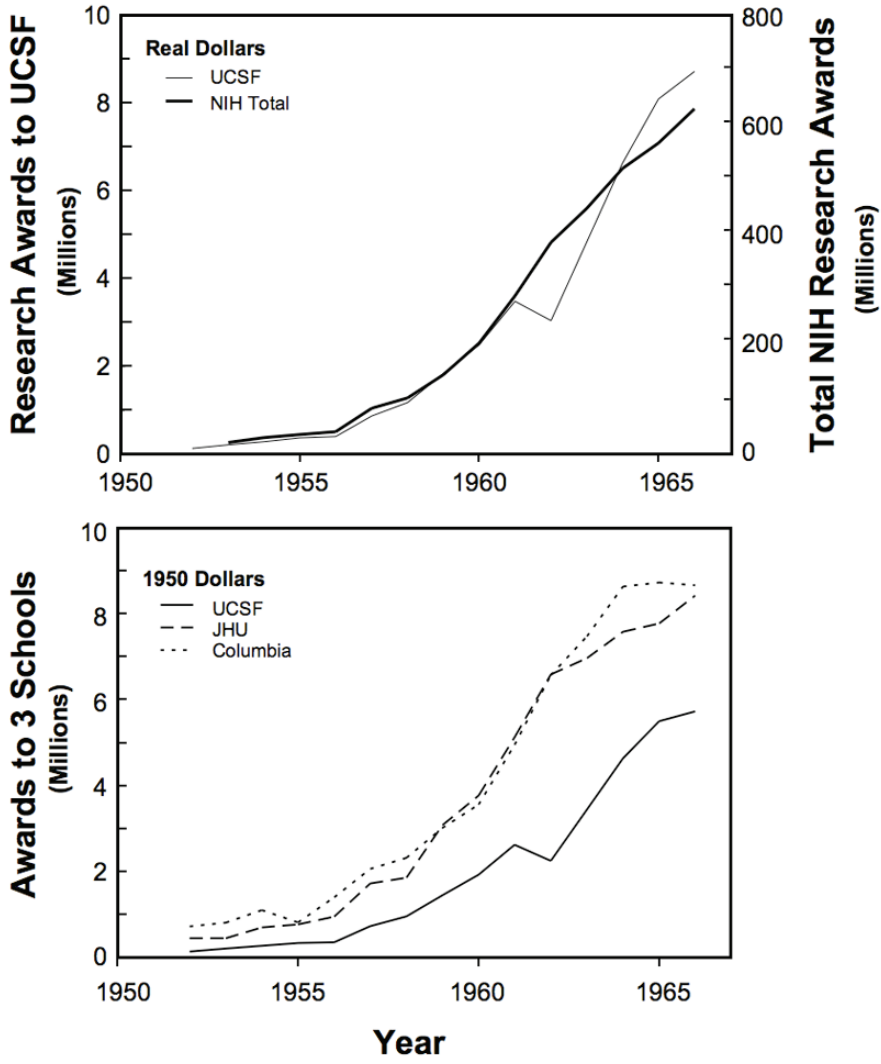
In the autumn of 1964, the Neider bequest and CVRI lab space in the new towers were both in abeyance, with the chancellor aligned against Comroe. Decisive action was necessary. Comroe, Smith, and the other leaders recruited by Comroe set his daring plan in motion by sending Clark Kerr a letter.[12] Signed by ten senior UCSF faculty and dated November 20, 1964, the letter stated simply:

> In the immediate future decisions will have to be made that will determine much of the future course of this campus. We, the undersigned, urgently request an appointment to see you at your earliest convenience for a high-level decision of utmost importance to this campus.

The message's weight was evident mostly in its signatures, which included those of Comroe, Smith, J. Engelbert (Bert) Dunphy, the recently appointed chair of Surgery, and William Reinhardt, the Dean of Medicine. Other signers included Maurice Sokolow, a prominent cardiologist, Izzy Edelman, and the chairs of Anesthesia, Pharmacology, Psychiatry, and Radiology.[12] Politically, the group's great strength was its representation from chairs of five important clinical departments, as well as the dean. Of the signers, only Comroe and Edelman were well-known researchers directly associated with the CVRI, although Sokolow, highly respected as a doctor and UCSF leader, had served on the old Cardiovascular Board responsible for planning the institute.

At that November 27 meeting in the President's office, Smith remembered,[1] he and Dunphy, as chairs of prominent clinical departments, were pushed to sit on the front row, with 10 additional UCSF representatives beside or behind them.[12] They directly faced the President's representative, Harry Wellman, sent to the meeting by Kerr, the consummately careful administrator, to avoid creating the impression that he agreed with the UCSF cabal. No one took careful notes, but the message was unmistakable. Comroe remained rather quiet, and Smith and Dunphy did most of the talking. The medical school on Parnassus, they said, qualified as a good regional school, but not a great one. Long-standing problems with Saunders, they added, made it clear that UCSF could never become a leading biomedical center without first getting a new chancellor. The Parnassus leaders said they were all committed to the task of making the campus the best clinical and research institution in the US—and that if Saunders remained they would be forced to leave.

Like Canute fighting the raging surf, Saunders was waging a battle he could not win. He feared that rapid increases in NIH grant funds would lure UCSF's faculty into the laboratory, at the expense of teaching medical students and caring for patients. Powerful forces arrayed against him included Clark Kerr's vision of the research university, the burgeoning of biomedical research in medical schools across the United




Graph 1: NIH research grants, 1952-1966

The top panel shows total NIH research grant awards (thin line; right-hand scale, in millions of dollars) and research grant awards to UCSF (thick line; left-hand scale, in millions of dollars) for the same time period;[6] amounts shown are in actual dollars for each year. The bottom panel compares NIH research grant awards to UCSF (solid line), Johns Hopkins University (JHU, dashed line), and Columbia University (small dashes) for the same years.[6] Here the left-hand scale is in "constant" 1950 dollars, calculated using the Biological Research and Development Price Index, or BRDPI.[13]

States, and UCSF's new cadre of recently recruited scientists and science-oriented clinicians from other leading medical schools—people like Edelman, Havel, Smith, Comroe, and many others. The upper panel of the accompanying graph [Graph 1 here] shows what Saunders was up against. From 1952 to 1966, total annual NIH research grant awards increased forty-fold, from $15.6 to $628 million dollars, while NIH research grants to Saunders's own medical school in that period increased sixty-two-fold, from $141 thousand to $8.7 million dollars per year.[6] The lower panel shows that the big increase in UCSF's NIH grant awards lagged four or five years behind those of two leading eastern schools, Johns Hopkins University in Baltimore and Columbia University in New York City. Beginning around 1958, when it consolidated in San Francisco, UCSF began to increase its awards at a rate comparable to those of the other two schools—increasing more than six-fold, in constant 1950 dollars, from $946 thousand in 1958 to $5.72 million in 1966.

## Saunders Falls, as a Separate War Rages in Berkeley

After the November 27 meeting, Kerr saw he would have to make a decision, which would spell conclusive victory or defeat for the "cabal"—Comroe, Dunphy, Smith, and their allies—or for Saunders and his supporters at Parnassus, led by private practitioners who worked and taught at UCSF. Both sides felt sure that deciding the chancellor's fate would irrevocably set the medical center's future course.

For Kerr personally, the Saunders affair must have appeared almost trivial in comparison to a much greater problem, the worst he faced as UC's president. This was the so-called "Free Speech Movement," or FSM, which claimed his primary attention during the fall of 1964 and early 1965. According to Kerr's memoir, *The Gold and the Blue*, the FSM arose largely because Edward Strong, Kerr's successor as chancellor of the Berkeley campus, made a bad decision.[14] That campus had an agreement with student groups, approved by the University and Berkeley police, that students could not mount political protest demonstrations on the campus proper, but had the right to do so within a small strip of land (twenty-six feet wide, forty feet long) located near Sather Gate, a much-traveled entrance to the campus. On September 14, 1964, without consulting Kerr, Strong revoked permission for protests in this tiny strip. On October 1, after escalating conflicts with police, radical students attacked and turned over a police car. Kerr called off the police, and people began to calm down. Indeed, on November 20—the same day the "cabal" mailed its "decisions will have to be made" letter to Kerr, and a week before the UCSF delegation met with Wellman—the UC Regents met to discuss the fracas. They decided to discipline two students by putting them on probation, and let the others off. The FSM smoldered

quietly on, ready to break out again anytime.

In the meantime, Kerr learned from his deputy, Wellman, that the Parnassus delegation had offered persuasive arguments for removing Saunders from the chancellorship. As a first-rate administrator, Kerr devoted several months to gathering and carefully marshalling facts. He began by asking Robert Brode, a Physics faculty member who assisted him in the President's office, to look into UCSF's standing among national medical schools. Brode's assessment, based on opinions of other medical school deans and previous published rankings, placed UCSF somewhere close to twentieth in the nation, trailing UCLA's School of Medicine, which ranked fourteenth. Kerr was famous for requiring that each of Berkeley's departments be ranked within the top six in the US. Now Berkeley's own former "medical college" appeared to rank barely twentieth.

Kerr had already read the 1963 accreditation report by the American Association of Medical Colleges (AAMC), which did not please him. The AAMC accredited UCSF's medical school, but its judgment of the school's quality was lukewarm at best. The report stated that the "overall impression is . . . a degree of disappointment, [which] began with . . . failure to find the stimulating, progressive, exciting forward looking thinking expected in a school located in a community otherwise full of the vitality of surging growth." The accreditors perceived "a seeming lack of a vigorous attempt to create an educational environment of excellence . . . [based on] the implications for medical education and practice of the scientific and social revolution." Worse, they noted that the "relatively abundant resources possessed by this medical school . . . out-strip the quality of the faculty and the program . . . . This program is not of the quality or the distinction that characterizes other fields in the University of California."[15]

In addition, Kerr sought the advice of a blue-ribbon committee of advisers, mostly deans of respected medical schools outside the University of California.[16] Unanimously, these advisers told him that Smith, Dunphy, Comroe and their allies were right, and Saunders should go. Investigating the specific allegations against Saunders, Kerr and his staff interviewed UCSF faculty not associated with the cabal and found that Comroe was not exaggerating. Finally, Kerr consulted his own personal physician, Morton Meyer, who was a highly respected member of the San Francisco medical community and at the same time a friend and admirer of Julius Comroe and colleagues on the UCSF faculty. Meyer could tell Kerr how the practicing physicians felt and who among the clinical and science faculty at UCSF sided with Saunders or Comroe.[9]

The fact-finding process was punctuated by explosive events, only one of which

related to the problems at Parnassus. First, student protesters staged a sit-in in Sproul Hall on December 2. Kerr tried to keep the police out, but California Governor Pat Brown bowed to political pressure and asked them to arrest the demonstrators. Berkeley's Academic Senate voted for "free speech" and against disciplining the students, but then moderated its stand. On December 17 and 18, the Regents met and agreed with Kerr's handling of the situation. For almost three months, Berkeley calmed down.

In January 1965, however, a group of San Francisco doctors circulated petitions in pharmacies asking patients to protest against removing Saunders, who in the past 50 years had done more than anyone "in establishing a proper rapport between the Medical Center and the practicing physicians of the state of California." On January 19, newspaper stories took up the story, describing rumors that Saunders was about to be deposed as Chancellor, and that Comroe or Reinhardt would replace him.[17] Holly Smith, who had imagined a quietly "donnish affair," was surprised that community clinicians sought publicity for their pro-Saunders cause.[2] In February, Kerr and Saunders talked for an hour at UCSF. A newspaper reported that Saunders "insisted that much of the agitation against him stems from researchers on the faculty who demand the lion's share of prestige, power, and budget," while "the leading researchers contend the conflict is between 'mediocrity and excellence' in academic medicine."[18]

In March, Kerr had to weather yet another storm. By using the F-word in public, a protesting student brought the "Filthy speech movement" to Berkeley, triggering more frantic regential behavior than did the previous political protests. At one point Kerr had to threaten to resign if the Regents tried to take over the chancellor's office at Berkeley.

Fact-finding about Saunders continued, however, and by June or July 1965 Kerr made up his mind. He judged that UCSF's academically excellent faculty sided with the cabal, but the rest—amounting, he thought, to approximately 90% of all faculty—supported Saunders. Treading lightly, he made no overt decision until the UC Regents themselves began to feel Saunders had to go. Then Kerr told them "we've got to make a change," meaning "not just in leadership, but I looked upon this thing as we were making a decision about the future. And to make this change to a new kind of medical school you had to have a new type of leadership."[9] At a Regents meeting in Los Angeles that summer, Kerr and Ed Carter, chairman of the Board of Regents, called Saunders in and gave him the verdict.

Kerr made the transition easy for Saunders, who took a sabbatical leave in July, 1965, and returned in 1966 as Regents Chair of Medical History. Bert Dunphy re-

placed him as acting chancellor. Saunders's allies required that if he went, Dean Reinhardt must not be perceived to triumph over his former mentor, and so must resign from the dean's office. (Quiet, steadfast, and competent as ever, he soon returned to the office to assist other deans.) His replacement as Dean of Medicine was Stuart Cullen, the University of Iowa professor Comroe had talked into taking the Anesthesia chair back in 1958.

Almost to the very last, Kerr had trouble understanding Reinhardt's role in the whole affair. The crafty dean appears to have fooled Kerr into considering him a Saunders ally. Kerr didn't remember that Reinhardt had signed the cabal's letter and joined in the visit to Wellman in November, 1964. Years later, when Reinhardt received the UCSF Medal for service to UCSF, he wrote Kerr to say that he owed the award to Kerr—an unexpected sentiment from a man Kerr mistakenly thought was in the enemy camp.[9] At some time thereafter, before he wrote his memoir, Kerr had learned that Reinhardt, as he wrote, was "the major hero of the historic change" that included Saunders's ouster.[19]

## The Battle Won, Changes Loom

The fall of Chancellor Saunders from power, in 1965, produced immediate consequences. Julius Comroe and his CVRI got the extra laboratory space they sought in the new Health Science towers, and the Neider bequest for cardiovascular research at UCSF was finally assigned unequivocally to the CVRI. Primarily comprising a large shopping center in nearby Marin county, this bequest was to provide the CVRI with a large and extraordinarily useful income for many years, underwriting its continuing position of leadership in research at UCSF. Over the subsequent decades the Neider income, blessedly distinct from research grants and awards given for specific purposes from the NIH and other sources, would be used for construction, laboratory renovation, startup funds for new faculty, and supporting new research initiatives.

In his last years Saunders had managed to delay replacements for two of the five clinical department chairs that became vacant during Reinhardt's time in the dean's office. In 1966, those departments got their new chairmen, both hailing from Columbia in New York City—Melvin Grumbach in Pediatrics, and Robert Fishman in Neurology.[11] And, as described in the next chapter, Stuart Cullen, the new Dean, continued Reinhardt's search for a new chair of Biochemistry, eventually succeeding in 1969.

In addition to the exit of Saunders from the chancellor's office, the year 1965 brought to UCSF—and to all other US medical schools—a much more momentous driver of profound change, with an economic impact even greater than that gener-

ated by the rise in NIH funding. This was the beginning of Medicare and Medicaid support for care of aged and indigent patients. In his history of American medical education in the twentieth century, *Time to Heal*, Kenneth Ludmerer details the profound effects of these new medical care funds on medical schools.[20] In the 1920s and through the 1950s, medical teaching had been conducted largely on indigent "ward" patients, who were unable to pay hospital bills but provided invaluable "clinical material" for learning by medical students and residents. In contrast, well-to-do patients with insurance were cared for by physicians on a medical school faculty, but rarely seen by students in training. Rather suddenly, beginning in 1965, Medicare and Medicaid provided medical schools with new sources of ready income, as indigent patients became paying patients. Now, in the twenty-first century, medical schools correctly complain that such funds often pay only a fraction of the cost of medical care. In the 1960s and 1970s, however, this "new" money was a valuable windfall for medical schools.

As Ludmerer shows, the new sources of money drove rapid increases in income of clinical faculty members and spelled the end of the old "geographic full-time" arrangement, in which the medical school provided facilities for care of private patients who paid their faculty physicians directly. With new sources of funding, medical schools could more easily pay the entire salaries of clinical faculty, which of course gave them greater control over the relative time and effort their faculty devoted to research, administration, teaching, and patient care. The resulting salary increases were not confined to clinical faculty, but also spread to researchers on the basic science faculty, who never saw patients. This spread occurred in part because researchers demanded salaries more comparable to those of their clinical colleagues, but also because medical schools found they could apply some of the new money from patient care—in addition to the increasing funds from NIH and other federal sources—to pay higher salaries to researchers, and even in some cases to defray costs of the research itself.

The dramatic ouster of Saunders furnishes a useful symbol for changes that now appear to have been inevitable. These changes were first set in motion at the end of World War II, when soldiers committed themselves to education and economic opportunity. In California, perhaps more than any other state, political leaders like Pat Brown (state governor from 1958 to 1967) and Jesse Unruh (Speaker, California State Assembly, from 1961 to 1969) used tax money to expand and improve education as an essential underpinning of technical innovation and future prosperity. As an integral part of this effort, the state and the federal government (with the NIH and Medicare) sought to advance biomedical knowledge and improve treatment of

disease. In California, Clark Kerr anticipated and augmented these investments by skillfully guiding the birth and development of immensely powerful new research universities, including UCSF.

In San Francisco, a small group of pioneering scientists and science-oriented physicians from other schools joined together to convert a good provincial school into the best biomedical research institution they could imagine. Their mutual determination set the stage for the remarkable explosion of imagination and scientific creativity in labs at UCSF labs in the 1970s, as we shall see in the next eight chapters.

Chapter Three

# New Brooms

## And a New Template for Research

I used to think that UCSF's history began in 1969, the year I arrived in San Francisco. This solipsism seemed to explain a stark difference between UCSF and other medical schools I knew. In UCSF's halls and elevators, professors with white hair were hard to find. Hopkins and Columbia, in contrast, were filled with white-haired professors in their fifties and sixties. The oldest UCSF professor I met, Holly Smith, had a few gray hairs, but he was only forty-five. Where, I wondered, had UCSF stashed its wise, grizzled leaders?

Scarce white hair signaled recent changes at UCSF and served as a harbinger of even greater changes in the 1970s. In clinical departments, new brooms had begun to sweep the old order away. Smith in Medicine and new chairs in Radiology, Surgery, Pediatrics, and Neurology were replacing grizzled heads in their departments with young people excited by the prospect of applying new technology to understanding and treating disease. By 1969, four years had passed since Clark Kerr removed John Saunders's heavy foot off the brake pedal at UCSF. Nourished by steadily increasing NIH grant support, research in the CVRI and clinical departments flourished. Scientists had nearly filled the new Health Science towers, HSE and HSW. Foreseeing an exciting future, the whole institution gathered momentum, beginning its headlong charge into the future.

At the beginning of this book, I outlined the prevailing narrative of UCSF's scientific success, seen from one point of view as the successive accomplishments of heroic "Great Men," but from another standpoint as the product of discoveries by working scientists. I called them face cards and wild cards, respectively. This chapter completes the heroic story line, with appointments of three key individuals to pivotal positions at UCSF. Subsequent chapters will turn to the wilder world of the laboratory.

In 1969, a young biochemist from the University of Washington, William J. Rutter, took the Biochemistry chair. As his first act, he hired from the NIH a brilliant, charismatic guru of molecular genetics, Gordon Tomkins. Together, Rutter and Tom-

kins jump-started a new research era for the entire campus.

In 1971, Julius R. Krevans, a hematologist from Johns Hopkins, became Dean of the School of Medicine. During his term in the dean's office, UCSF became a leader in biomedical research in the US and the world.

## Recruiting Rutter and Tomkins

Among basic science departments in many medical schools, Biochemistry is often considered "first among equals," a view biochemists readily agree with. In the mid-1960s, however, the Biochemistry department at UCSF was considered undistinguished, both in comparison to Comroe's rapidly growing CVRI and in the view of the new clinical chairmen who were seeking to raise the campus's scientific aspirations. Accordingly, Dean Reinhardt appointed a committee to find a new chair for Biochemistry. Led by Holly Smith and Izzy Edelman, the committee interviewed a number of distinguished candidates, but by 1965 no one had been hired.

In July that year, a small group of dissident biochemists and their friends met to discuss the search for a Biochemistry chair—a search already two years old. The occasion was a dinner at Jack's Restaurant in downtown San Francisco, in a private room, which had served, in a more raffish past, as a boudoir in a notorious bordello. Manuel Morales, a biophysicist and muscle biochemist brought to UCSF by Julius Comroe, had invited the guest of honor, John Saunders, who—Morales thought—might be able to push the dean and the search committee in the right direction. The attendee who told me about this dinner, Leon Levintow, had joined UCSF's Department of Microbiology only two weeks earlier, after sixteen years' service as a virologist at the NIH.[1]

During the discussion, Morales spoke forcefully in favor of his friend Alton Meister, a distinguished forty-three-year-old biochemist at Tufts. Other distinguished candidates included Sol Spiegelman, who studied nucleic acids at the University of Illinois, Earl Stadtman, a first-rate enzymologist from the NIH, and Gordon Tomkins, a brilliant and charismatic biochemist at the NIH. Tomkins was known to be Holly Smith's leading candidate, but almost everyone at the dinner, Levintow remembers, considered Tomkins "a scientific lightweight, insufficiently grounded in basic physical and chemical principles." Tomkins had visited UCSF but turned down the job, fearing that administration would get in the way of his real passion, laboratory research.[1]

Levintow soon discerned the dinner's governing subtext, which was Morales's disdain for a new variety of upstart "replicators," guilty of an improper interest in the replication of DNA (deoxyribonucleic acid)—a group that would have included Spiegelman, Tomkins, and a host of recent and future Nobel Prize winners.

In contrast to these misguided souls, old-line biochemists like Meister and Stadtman were thought to be "concerned with serious issues." Whether or not he recognized Levintow, a virologist, as the "closet replicator" Levintow knew himself to be, Morales—like his allies—didn't believe that DNA and RNA offered keys to understanding fundamental life processes. Although the dinner laid bare the poverty of Biochemistry's vision in 1965, its effect on the Biochemistry search was effectively nil, because Morales was unaware of Saunders's recent fall from power. Levintow found Saunders distracted and bored at the dinner—hardly surprising for a man the University's President, Clark Kerr, had just forced to take a sabbatical leave and then return to UCSF, but no longer as chancellor.[1]

In the course of the search several candidates, including Meister and Stadtman, turned UCSF down, just as Tomkins had. According to Holly Smith, UCSF was probably lucky not to attract the traditional biochemists. "The good lord loves UCSF. He allows us to stumble around, but he looks after us."[2] The key step, however, was not a lucky stumble, but rather a painstakingly deliberate campaign aimed at landing a candidate who had already turned UCSF down without even visiting San Francisco.

This was William Rutter, whom the committee first contacted in 1965, soon after he took a professorship in Biochemistry at the University of Washington. Two years later, in 1967, Smith and William Reinhardt, at this point associate dean of the School of Medicine, flew up to Seattle, hoping to persuade Rutter to visit San Francisco. According to Smith, "We took him out to dinner and talked to him. 'Come here, you get in on the ground floor' [We] got him stirred up."[2]

The man they met in Seattle was confident, energetic, bright, and determined, with piercing blue eyes and a hawk-like beak of a nose.[3] Rutter had turned down earlier invitations to San Francisco because "UCSF at that time was unpopular and considered a mediocre institution, " and also because it was hard to do "the best science" in a medical school, owing to lack of adequate technology for addressing important medical problems from a molecular point of view. In Seattle, for instance, he felt that good science in the Biochemistry and Genetics departments was not integrated between the departments or with clinical medicine.[4] The visit from Smith and Reinhardt conveyed a very different impression. "Here are these two medical guys saying there should be an integrated program between basic and clinical science."[5] Well stirred, Rutter decided to visit San Francisco after all.

Rutter's biography hints at other reasons for thinking hard about a chairmanship in San Francisco, including an inveterate thirst for new challenges. Born in 1928, in Malad, Idaho, Rutter left high school at age fifteen, attended Brigham Young University for a year, and lied about his age to get into the US Navy during World War

William J. Rutter, chair of Biochemistry, 1969-1982. As chair, Rutter crafted a template for basic research that influenced many other departments at UCSF. Photo from 1978, with insulin's amino acid sequence as background.

II. After the war and a BA in biochemistry at Harvard, he turned down Harvard Medical School because attending a few medical classes at the University of Utah showed him that medicine didn't offer the scientific challenges he sought. Instead, he entered graduate school at the University of Illinois, earning a PhD in biochemistry in 1952. In postdoctoral work in Wisconsin and Sweden, he did pioneering work in enzymology. Back at the University of Illinois as a faculty member, Rutter studied enzyme mechanisms for seven very productive years (1955-1962)—his longest stay in one place since the first fifteen years in Malad.

Suspecting that technical obstacles would soon limit further advances in enzymology, Rutter turned to a more biological problem—formation, from the gut of an embryo, of the primordial bud that becomes the pancreas. During a sabbatical year at Stanford in 1962-63, working with an undergraduate student, Ed Penhoet, he showed that tissue overlying the pancreatic bud produces a substance that can also induce bud formation in other parts of the gut. Rutter wondered how this "induction" might regulate specific genes to induce cells to form a pancreas, rather than another stretch of gut wall. By the time he returned to the University of Illinois, Rutter was determined to analyze gene expression biochemically, by isolating and characterizing the enzymes (DNA-dependent RNA polymerases) that transcribe DNA sequences into RNA sequences.

Penhoet, who became Rutter's graduate student when he returned to Illinois and followed him to the University of Washington in 1965, thinks "itchy feet" and

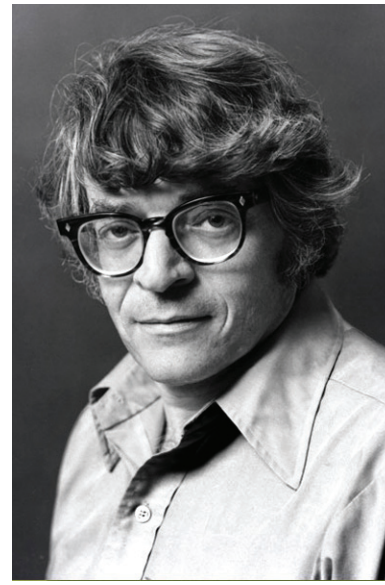
a penchant for new challenges were not his mentor's only reasons for thinking seriously about the chairmanship in San Francisco.[6] In addition, by 1967 Rutter was thirty-nine, and ready to become his own boss. He chafed under a dominant chair at Illinois, and similarly (Penhoet suspects) under "another domineering chairman," Hans Neurath, in Seattle. (For instance, Neurath imposed a dress code: no shorts in the lab, except on weekends.)

The first stage of UCSF's active courtship of Rutter began in June 1967 and lasted for about four months. The participants' memories and their correspondence indicate that Rutter immediately saw the UCSF offer as an attractive opportunity, not only because it combined a large number of empty faculty positions with appropriate space, but also because the School appeared genuinely committed to making Biochemistry a great department.[7] Unimpressed by incumbent Biochemistry faculty, he liked the virologists in Microbiology, including Levintow and Michael Bishop. He found Izzy Edelman impressive for his intellect, acute perceptions about science and people, and practical grasp of administrative realities. (Beginning, in 1952, with a dank basement lab, lacking lights or water, Edelman had now become the acting chair of Biochemistry.)

A recommendation from Arthur Kornberg, chair of Biochemistry at Stanford, persuaded Edelman to propose Rutter as a candidate to the search committee, and Edelman pivotally influenced Rutter's recruitment by introducing him to Gordon Tomkins, the NIH scientist who had turned down the job earlier. "I knew Gordon reasonably well," Edelman said.[8] "I'd only met Rutter once, on his first visit. I decided my best bet was to let them recruit each other.

"So I arranged to have them come together to my house, . . . in my living room, because I wanted them to be able to free-associate with each other, rather than my trying to sell them. We plied them with coffee and cookies or cake, and the three of us started talking. Pretty soon, they started talking to each other, and within two hours they sold each other on the idea of coming together." Just as Tomkins was immensely intrigued by San Francisco, but didn't want to be chair, Rutter was ready to take a chair but needed a first-rate scientist and extraordinary person to help make the project work—and judged Tomkins exactly right for the task.

Rutter found Tomkins persuasive and attractive for the same reasons that made Tomkins such an important player in the subsequent history of research at UCSF. First, Tomkins was immensely bright and talented, in many realms. Son of an endocrinologist father and a mother who was a musician, he graduated from UCLA at nineteen, as a philosophy major and an accomplished musician—classical and jazz, clarinet and saxophone. After two years of medical school at UCSF, he transferred to



Gordon M. Tomkins, biochemist. He joined Rutter in the Biochemistry department in 1970 and died in 1975, but his enthusiasm and ideas influenced research careers of many biomedical scientists. Photo from the early 1970s.

Harvard, graduated with an MD in 1949, and took a year of internship. In 1950-53 he earned a PhD at Berkeley, working on the biosynthesis of cholesterol in the lab of I.L. Chaikoff, one of the prominent scientists who would later refuse to move to San Francisco with the rest of the medical school. Thereafter he worked at the National Institutes of Health (1953-1969), studying biochemical transformation of adrenal steroids, effects of these steroids on expression and activities of enzymes, and regulation of a set of bacterial genes, called the lac operon, which will reappear several times in our narrative.

This bare recitation does not begin to describe the effect of Gordon Tomkins on almost everyone who knew him. Tomkins had an extraordinary sense of humor, an unquenchable curiosity, a photographic memory, broad knowledge of biology and many other subjects, and a boundless capacity for imagining possible connections between disparate facts. Many of the connections he imagined turned out to be correct, although he freely admitted that at least 90% were wrong. Tomkins also had a genuine talent for friendship. Never intimidating, his knowledge and erudition stimulated friends to think they were smarter and more perceptive than they really were. His greatest gift was a remarkable generosity of spirit, a quality as rare among scientists as it is in any walk of life. Tomkins's generosity helped myriad scientists and friends to weather life's adversities and bolstered their daring to tackle new questions and pursue previously unimaginable answers.[9]

Edelman had known that "Gordon was the kind of guy that, if you got him

on the right note, you'd get a positive resonance and [he and Rutter] would start to resonate cooperatively. They'd each convince each other that it's a great idea."[7] The plan eventually worked, but the process took longer than Edelman had anticipated.

After Rutter's first visit to UCSF, the courtship ritual proceeded through four months of telephone conversations and letters. Rutter expressed great enthusiasm, but wanted more faculty positions, more contiguous lab space, and extra support for non-faculty personnel, renovation, and equipment. He also asked that he be made a member of the School's Executive Board, a group of faculty appointed to advise the dean. Holly Smith and Stuart Cullen, Reinhardt's successor in the dean's office, sweetened the package, but could not come up with everything Rutter asked for. After a couple of weeks of silence, Rutter wrote, in early November, that "the romance of the San Francisco opportunity kept tugging at my heart," and sought a few more concessions.[10]

Smith wrote back that some of Rutter's requests would have to wait. UCSF, he said, needs someone with the "vision to create an outstanding department and a basic faith in the future of this school and campus to make him realize that the ingredients are here to build upon. All of us here now came from top flight departments around the country into situations which were less than ideal but gave good opportunities for growth. We have not been disappointed. This is what we need in biochemistry."[11]

Smith's eloquence didn't overcome Rutter's reservations, and he turned down the offer, with genuine regret. Smith's reply to this disappointing turndown expressed his own regret, capped with a gentle barb. Writing that he was sorry the school couldn't offer Rutter all he needed, he added, "Drop by to visit us again sometime. I hope it will be as a guest of a first-rate, nationally recognized Department of Biochemistry. I am sorry that it has to be someone else who will build it."[12]

In the spring of 1968, Rutter changed his mind and re-ignited the flame. "I [had] turned it down several times. When I did call Holly up and say, 'Okay, I'm ready to come,' I got a categorical offer—you've got to accept under no conditions. No negotiation on salary, no whatever, period. I had to do that. So I accepted it."[5] (That is, Rutter did not give up the terms he had previously negotiated, but could not ask for more.) By July 1968, the deed was done. Rutter's first task was to make sure that Gordon Tomkins would join him in San Francisco. He traveled to Washington, went canoeing with Tomkins and his wife, Millicent, and signed him up. Rutter would chair a department with approximately twenty full-time faculty positions, abundant lab space, and promises of more positions and space to come. In 1968 Rutter was forty, Tomkins forty-four.

## Transforming Biochemistry—1969-1975

A young faculty member recruited to UCSF in the twenty-first century would find it hard to imagine the world as it looked to new faculty who entered the Department of Biochemistry in 1969 and the early 1970s. Now Biochemistry is just one bright star in a tightly-packed constellation of basic science departments, most of whose faculty interact closely with one another in both research and teaching. The new department organized by Rutter and Tomkins evoked a feeling more urgent and compelling. Former Biochemistry faculty recall the 1970s as a special time, mingling hard work, intense thinking, friendly (and not so friendly) rivalries, and a stimulating, constant give-and-take of ideas, plus ample opportunities for fun. In a department that saw itself as the only game in town, many were barely aware of faculty in other departments. In fact, they and their leaders were busily weaving a complex new model for scientific interaction. Generations of UCSF scientists in many departments would try to emulate that model, which in the 1970s began to influence the "wild card" scientists we shall meet in later chapters.

In his first six years as chair, Rutter was the center of a whirlwind of activity and ideas. After working part-time in San Francisco during the latter part of 1968, he formally took the chair on a full-time basis in 1969, the year Tomkins arrived. From the outset Rutter revealed an immense capacity for adroit multi-tasking and plain hard work—renovating labs, moving his own lab people from Seattle, judging and recruiting prospective faculty candidates, and plotting out the Department's future. "Most people have no idea how hard people worked at UCSF in the 1970s," says Penhoet,[6] Rutter's former student, who watched the department develop from his own vantage point at UC Berkeley, across the Bay. Rutter's extraordinary intensity, according to Penhoet, was not only necessary to get things done—something Rutter was very good at—but also served to generate intensity in people around him, accelerating the pace of their lives.

Within the first two years—that is, by 1971—Rutter and Tomkins had signed up seven new faculty members, and by 1973 they hired three more. As a former department chair myself, I can't imagine evaluating and offering jobs to ten scientists—eleven, counting Tomkins—in such a short time, let alone housing each of them in new labs, with the myriad administrative tasks each new arrival must entail. The quality and variety of the first seven scientists Rutter and Tomkins recruited were impressive (see Table 2). Five were rising young research stars in fields that included genetics of a bacteriophage (a virus that infects bacteria), DNA chemistry, enzymology, muscle biochemistry, and neuroscience. The other two included a senior UCSF faculty member we have already met (Izzy Edelman) and an African-American biochemist (John

## Table 2: Biochemistry Faculty Recruits, 1970-1973

| Year | Name | Initial Field | Future Direction |
|---|---|---|---|
| **1970** | Gordon Tomkins | Molecular genetics, hormone action | Imaginative, charismatic intellectual leader. Died, 1975 |
| | Harvey Eisen | Bacteriophage lambda | Moved to Switzerland, 1972 |
| | Isidore Edelman | Regulation of salt and water | From Dept. of Medicine; moved to Columbia, 1978 |
| | Howard Goodman | DNA chemistry and sequencing | Moved to Harvard, 1980 |
| | Daniel Santi | Enzymology, chemistry | Prof., Biochemistry and Pharmaceutical Chemistry, UCSF |
| | James A. Spudich | Muscle | Cytoskeleton; moved to Stanford, 1977 |
| | John A. Watson | Cholesterol, lipids | Associate Dean of Medicine |
| **1971** | Regis Kelly | Synaptic transmission | UCSF faculty; Director, California Institute of Quantitative Biosciences |
| **1972** | Roger Cooke | Biophysics of skeletal muscle | In residence position (salary paid from grants, rather than by the University) |
| | Brian J. McCarthy | Evolution of DNA and RNA | Moved to UC Irvine, 1980 |
| **1973** | Christine Guthrie | Bacteriophage tRNA | Gene splicing, expression |

Watson) who also served as an associate dean in the School of Medicine. Soon thereafter (see Table) department added a biophysicist who worked on skeletal muscle (1972), a senior biochemist studying evolution and inter-species variation of DNA and RNA (1972), and the Department's first woman faculty member, a young biochemist working on transfer RNA (tRNA) in a bacteriophage (1973).

As Rutter describes it,[5] he and Tomkins chose to develop a new kind of biochemistry department, focused primarily on mammalian biology and consequently

relevant to human (and medical) biology. Presciently, they also hired scientists (Eisen, Goodman, McCarthy, Guthrie, and Tomkins himself; see Table 2) skilled in molecular genetics and the nascent technology that would soon give birth to powerful new tools for studying biology. This vision differed substantially from prevalent models for constructing such a department. At Harvard and Illinois, and in Seattle, Rutter had learned to distrust the "cover the waterfront" model, which tried to attract experts in most subfields of biochemistry. "Is that necessary to teach? Does that provide any insight into overall science? Is it productivity-driven? No way." Instead, he considered it a device for segregating faculty from one another and from the rest of biology. For similar reasons, he would not try to mimic the "tremendous focus" of Arthur Kornberg's department at Stanford, which "didn't really [try to] integrate biology." Finally, the CVRI, UCSF's successful model, was not as open to individual initiative as he would like.[5]

Science was changing, Rutter points out. "This was all happening in the early days of DNA discovery. It was obvious how the direction of science was going. You could just see methodologies developing [so that soon] collaborations would be more important than individual programs."[5] So, he and Tomkins sought to hire scientists whose expertise focused on fundamentally important functions of animal cells, and to combine them with geneticists and molecular biologists, along with experts on the biochemistry and function of nucleic acids. One of his new hires, Jim Spudich, also emphasizes that he saw coming to Biochemistry as a real adventure. "UCSF in those days," he adds, "selected for a particular personality [that liked] really unusual adventures."[13]

Most importantly for Rutter, these disparate, independent, adventurers would have to learn to communicate openly, work together, and collaborate on problems of mutual interest. "You find new things at the interstices between disciplines. Young people provide innovation, and if you limit insights and directions, you temper enthusiasm." Openness was essential, "just inherently part of our mission."[5]

That was the plan, but the practice wasn't always easy. Rutter had to deal with existing faculty, relics of an older way of looking at the world. "Cleaning up was a big problem, and there were rough edges in that, but I had to get it done."[5] A few older faculty chose to focus on teaching medical students and graduate students, while several others faded quietly from the scene. One faculty member, Manuel Morales—the rigorous biophysicist who had strenuously opposed Rutter's appointment—kept on "try[ing] to intimidate us," referring to Tomkins as "your lieutenant."[5] Morales didn't leave until 1978.

Rutter's legendary ability to exploit any and every opportunity impressed his col-

leagues. He still likes to tell, for example, how he used an apparently discarded set of platinum "weighing boats" (small cups for weighing potentially corrosive chemicals) he found in the first lab he moved into at UCSF. Not needed for his experiments, platinum was immensely valuable, so he promptly sold it to finance faculty recruitment.

The young people Rutter and Tomkins hired came to UCSF with excellent credentials. For instance, Reg Kelly and Jim Spudich both earned their PhDs in Arthur Kornberg's lab at Stanford, and did postdoctoral work at prestigious places—Kelly in the Harvard Neurobiology program and Spudich in the Medical Research Council Laboratory in Cambridge, England. Nowadays a young faculty recruit with such credentials finds herself ensconced in a modern, luxurious lab with a spacious office, surrounded by an established department full of prominent scientists and located in a large and diverse biomedical research center, where it is easy to find experts on almost any subject in biology. Instead, new Biochemistry faculty at UCSF in the early 1970s were housed in small labs with tiny offices, in a medical center not renowned for the breadth and quality of its research. Rutter and Tomkins were the only potential mentors available. While individuals ready to join such a department would have to be fearless risk-takers, some must have wondered what they got themselves into.

Still, after four decades of hindsight, those I talked to remember Biochemistry as an exciting place with great expectations. Now senior scientists in their own right, they see Rutter and Tomkins as different and complementary leaders. For Rutter they tend to emphasize his uncompromising focus on excellent research and his uncanny ability to identify fields and individuals likely to make critical scientific contributions in the future.

In the early years of Rutter's chairmanship, however, some young faculty saw things rather differently. For instance, Harvey Eisen, the bacteriophage lambda geneticist, took a negative view of his chairman. Brilliant, feisty, and mordantly funny, he liked Tomkins but disliked Rutter's style and distrusted his goals. He worked hard, albeit unsuccessfully, to persuade his colleagues to rebel against the chair, and within two years he left to take a faculty position in Switzerland.

Other young faculty in the department in those years found Rutter variously "a real builder, [with] a personality that made you want to be involved" (Jim Spudich), "an aggressive man" who knew exactly what he wanted and usually got it (Roger Cooke), a ruthless man who occasionally went too far because he "wasn't good at drawing the line" (Christine Guthrie), and a somewhat distant figure, often out of town, who acted effectively at higher levels but wasn't terribly sympathetic to the work or lives of his young faculty (Reg Kelly).[14] At one point, Rutter got wind of the fact that Kelly was feeling discouraged about his neuroscience research, and told him,

"Someone said you're not sure you're going to be making it here. Now, let me tell you our very tight promotion policies." The lesson, Kelly felt, was simple: "I learned that even [with] your best friend, you never tell them if you're feeling insecurities about yourself." [15] Kelly carefully distinguishes his opinion in those days from his present view of Rutter, whom he now values as a brilliant, wise leader whose advice he still seeks and often follows.

In their early years at UCSF, Kelly and Guthrie both considered Gordon Tomkins their real leader. Tomkins, who had traveled to Harvard to recruit Kelly to UCSF, was, Kelly says, "always so smart and always asked those very perceptive questions, and was so incredibly funny. The fact that someone could be so smart and so amusing at the same time was a joy."[15] He was also the person who asked the best questions about Kelly's research, and seemed to care most about his progress. Kelly also thought Tomkins's postdoctoral scholars (postdocs) were of higher quality than Rutter's, and were doing better research. Guthrie thinks of Tomkins as "really the only mentor I ever had."[16]

Tomkins's most important contribution, Kelly and Guthrie felt, was to foster growth of a community in which people loved to share experiments and ideas. Tomkins played a key role in fostering cooperative, open interactions among the faculty. Keith Yamamoto, a postdoc in the Tomkins lab and later a Biochemistry faculty member, vividly recalls the constant laughter he overheard from the tiny room—located near his own lab bench—in which the faculty met every week for beer, conversation, and talking science. The laughter, he knew, was fueled by Tomkins's irrepressible sense of humor, which smoothed egos and made communication fun.[17] Tomkins tried to "set a tone for how scientists should live," Kelly says, stressing that "we've got to see ourselves as . . . a new community of scientists that interact with each other in a friendly and a supportive way. We were going to create a new type of scientific ambience. That's resonated strongly with me."[15] Guthrie says, "It was that atmosphere. It wasn't that he was saying, 'Let's sit down and talk about your science.' It was, 'Wow, wouldn't it be neat if . . . .' He used to hold forth [at the] coffee machine . . . every morning, . . . spouting his latest ideas . . . . Gordon was fabulous. Gordon was really the glue. Bill had a lot of political strengths, but Gordon really kept the social contract going."[16]

On the other hand, Kelly says, he was enormously impressed at Rutter's reponse to the challenge posed by Harvey Eisen, especially when his rebellion progressed to entertaining an offer from the University of Geneva. Instead of being delighted at the prospect of getting rid of an irritating young adversary, Rutter "bent over backwards to try and keep him." On a different occasion, when Kelly went to Rutter to com-

plain about what he saw as weak graduate education in the department, he learned a lesson: Rutter said, "You've detected an important problem. Fix it." "I had been expecting him to deal with it himself, and he told me it was my job to solve it. That was empowering. A very important lesson."[15]

In fact, Rutter made no bones about his view that research was the principal task of department faculty, far more important than teaching. Kelly remembers Rutter stressing that "our first focus is to be first-class researchers. Yes, we'll worry about all those teaching things in graduate programs, but let's get our research programs going first."[15] Indeed, in 1971, the year Kelly was hired, Biochemistry was a top-heavy department dominated by Rutter's and Tomkins's laboratories. Rutter's had made a big research splash just before coming to UCSF, when Robert Roeder in his lab identified and characterized the functions of several DNA-dependent RNA polymerases. At UCSF Rutter began to phase out work on these polymerases, and a few years later turned his attention to the exciting and difficult task of cloning the insulin gene—work described in Chapter Six. Tomkins's lab in these early years focused on regulation of mammalian gene expression, with a special emphasis on effects of adrenal steroid hormones and an intracellular "second messenger" for other hormones, called cyclic AMP. In 1971 the two labs together employed 71% (thirty-five of forty-nine) of the department's postdocs. By 1975, as the research of younger faculty prospered, the two labs together accounted for a much smaller percentage of the Department's postdocs—39%, or twenty-four of sixty-two.

Then, in 1975, cataclysm struck. In mid-July, following a brain operation, Gordon Tomkins died. Dizziness had led to diagnosis of an acoustic neuroma. He underwent surgery in New York to remove the tumor, but failed to wake up afterward because of post-operative intracranial bleeding. His death was a devastating loss for Rutter, for Biochemistry faculty, and for UCSF. Although I was only a young scientific collaborator of Tomkins, located in a separate department (Medicine) and ignorant about the Biochemistry department and his role in it, this death was a watershed event in my own life. I was not alone. Indeed, almost every person who knew Tomkins felt his death as a profound change in their own lives. Many also saw it as a dire threat to the direction and style of basic science research at UCSF. No one could know what the future would have been like if Tomkins had lived, but all felt that the world would somehow have developed very differently.

For Rutter, the loss was wrenching. He had depended on Tomkins, not only as a wise and generous friend and a superb scientist, but also for his help in shaping administrative strategy. Tomkins, he says, was "not dogmatic at all. He was quite sensitive—he was not interested in [becoming a chairman], but when it came to un-

derstanding what needed to be done, in two minutes, no problem, he'd find something, some way to go ahead."[5] Losing Tomkins forced Rutter to think hard about the department's future, and his own. He had seven more years to serve as chair of Biochemistry—a period that unfolded very differently from the first six years, in ways closely intertwined with important events in our story, as we shall see in later chapters.

Young scientists like Kelly, Spudich, and Guthrie lost a friend and valued mentor. Kelly remembers a memorial service for Tomkins in which UCSF's scientific community pledged to continue working to "create an institution which did reflect his vision of a new way of doing science."[15] Still, these young scientists and their colleagues in Biochemistry wondered how the collegial, cooperative atmosphere of their nascent research community could survive and prosper without Tomkins's sympathy, humor, and commitment to open communication about every aspect of science.

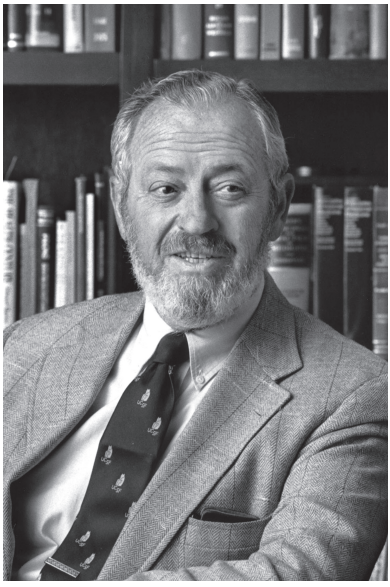
Later chapters will recount subsequent events in Rutter's Biochemistry department, as they intertwined with scientific discoveries and changes at UCSF. At this point, however, we turn to a different "new broom," the medical school's new dean.

## Julius Krevans, Dean of Medicine

Stuart Cullen, who had replaced Reinhardt as Dean of the School of Medicine in 1966, returned to his position in Anesthesia in 1970. Philip Lee, the chancellor, chose a new dean, a hematologist from Johns Hopkins. Julius R. Krevans served as dean for eleven years, and UCSF's chancellor for eleven additional years (1982-1993).

The new dean's parents had immigrated from a village in the Ukraine, where his mother was a nurse-midwife-general-practitioner and his father an actor. In New York, his father supported the family during the Depression as a retailer of women's clothing accessories. Graduating from New York University in 1944, and from medical school at the same university in 1946, Krevans took a medical internship in Queens. He served in the US Army, in the Philippines and at Walter Reed Hospital (1948-1950). Like Izzy Edelman, he found that antisemitism made it difficult for Jews to get into medical schools or find jobs in academic medicine in the 1940s, but saw the situation greatly improve in the 1950s.[18] Fortunately, a hematologist at Johns Hopkins, Lockhard Conley, saw promise in the young man. Krevans completed a hematology fellowship, finished his medical residency, and took a position on the Hopkins faculty in 1953.[19]

Krevans's intelligence, ability, and hard work brought him success at Hopkins. His flair for teaching and administration led to his appointment as chair of Medicine at Baltimore City Hospital, a Hopkins affiliate, where he transformed weak resi-



Julius R. Krevans, Dean of the School of Medicine 1971-1982 and UCSF Chancellor 1982-1993. For 22 years, he presided over major expansions of UCSF's clinical programs and research efforts. Photo from the late 1970s.

dency, teaching, and patient care programs into efforts better suited to the parent institution's high standards. In 1969, at forty-five, Krevans moved back to the medical school as Associate Dean of Academic Affairs—an appointment that marked him as a man to watch.

When UCSF began to show interest in Krevans as a possible new Dean, he quickly compared NIH research awards to UCSF with those of other medical schools. To his surprise, UCSF's ability to garner NIH research funds ($11.4 million in 1969) ranked close to that of leading medical schools like Hopkins and Columbia ($12.3 and $11.8 million, respectively, in the same year).[20] Before visiting UCSF, Krevans received a note from Julius Comroe and a personal visit from Izzy Edelman, both urging him to consider UCSF. During his visit in San Francisco, he was impressed by Comroe, Rutter, and several clinical chairs (Holly Smith was out of town), but underwent a discouraging session with a committee of UCSF's Academic Senate. "It looked [to me] like a bunch of jailhouse lawyers."[21] Perceiving discouragement, Frances Larragueta, who was in charge of Krevans's schedule, asked if he had time for one more visit. "I said, 'Yes.' In came Gordon Tomkins, after which I wanted to know, 'Where do I sign?'"[18] Enthusiastic about UCSF's future, Tomkins worked his magic on Krevans, as he did on many others.

Many years after his job interviews at UCSF, Krevans wryly summed up his impression. "What I saw out here was a place that undervalued itself. Nobody was talking about themselves as being part of a great research enterprise." Grinning, he

continued, "I figured if I could get out there in time, I could get credit for it . . . . This place was great when I came, and it had the enormous advantage of not being full of itself. They still felt we've got to do more."[19]

Krevans became dean in January 1971. During his twenty-two years at UCSF, he faced many challenges that lie outside the scope of this book. These included the AIDS epidemic, which struck San Francisco very hard, compelling UCSF to become an international leader in treating the disease; substantial expansion of clinical care facilities, including renovation of Moffitt Hospital and construction of the fifteen-story Long Hospital at Parnassus; changes in medical insurance and modes of paying for health care; and introduction of a vast panoply of effective and expensive technologies, including some pioneered by UCSF clinician-scientists, such as magnetic resonance imaging.

Discussing basic research at UCSF, Krevans frequently returns to two critically important themes. The first, he says, came as a welcome surprise. Not only did UCSF's clinical departments work to strengthen basic research, as Smith and his colleagues did during the Saunders episode, but they also proved willing to contribute financial support, via the dean's office, to help the basic sciences grow. Krevans says such an arrangement was unusual among US medical schools, including Hopkins and Harvard. The money came in the form of a "dean's tax on clinical income." Chairs of several departments—surgery, vascular surgery, and neurosurgery—felt "the school has to put its discretionary resources into basic science."[19] Emphatically, he added, "Not only was there no objection, there was enthusiastic endorsement. J. Engelbert Dunphy [the Surgery chair] took the lead."[21]

Krevans may be exaggerating how unusual it really was for UCSF to contribute clinical dollars to research. Ludmerer's history, *Time to Heal*, indicates that it was common practice for many medical schools, taking advantage of the new income from Medicare and Medicaid, to use part of the dean's tax on clinical earnings to improve research.[23] It is impossible to know whether UCSF's clinical faculty in these years parted with their income more readily than was the case at other schools.

The amounts of money—a few hundred thousand dollars, perhaps more in some years, Krevans says—were substantial but not immense. (Bear in mind that $100,000 in laboratory expenditures in 1975 would be more than $530,000 in 2010.[22]) Mostly used in basic science departments, he says, the money was spent to renovate labs, help new faculty start their lab research, and jump-start new ventures. One of these was the Neuroscience Graduate Program, founded in the late 1970s at the instigation of Robert Fishman, chair of Neurology, and Francis Ganong, chair of Physiology. The two chairs told Krevans, "Juli, you've got to do something about this place. It's

a heart/lung/kidney preparation, and it has no brain."[19] Other chairs agreed with their rationale for a new neuroscience program, he says, and an outstanding young neuroscientist from Harvard, Zach Hall, came to San Francisco to lead UCSF's first interdepartmental graduate program, which was to play an important role in changing the future of graduate education at UCSF.

I have not been able to determine the precise dollar amounts of dean's tax money used to support research in basic science departments, because memories are vague and because the dean's office at UCSF—now, as in the past—is not enthusiastic about documenting its discretionary income or how that income was spent, even when the money passed through the office more than thirty years ago. Although Rutter recollects negotiating with the dean about salaries for his department's faculty, he remembers no direct contributions from the dean's office to the Biochemistry department, aside from funds and faculty positions promised by Dean Cullen during his negotiations about taking the chairmanship.[5] It is possible, of course, that Krevans used part of the dean's tax money to fulfill previous agreements between Rutter and Cullen.

When he talks about clinical departments cooperating with basic science departments in the 1970s, Krevans stresses the importance of regular monthly meetings of what he calls "Jack's Club." Every month a subset of Department chairs used to meet for a convivial dinner in an upstairs room of Jack's Restaurant (in yet another boudoir of the former bordello). This "club" was an informal old boys' club, of a kind that has more or less disappeared at UCSF and elsewhere in the past forty years. Charles B. Wilson, chair of Neurosurgery in the 1970s and 1980s, says that the dean was not a member of the group, strictly speaking, although he was often invited to the dinner.[24] Sometimes the dinner served only as an enjoyable venue for meeting friends, but often the chairs and the dean discussed substantive issues with implications for UCSF's future. For instance, Krevans says he brought up the idea of funding a genetics program, under the aegis of Biochemistry, and that club members supported use of discretionary funds from the dean's office to recruit faculty to strengthen genetics research and teaching.

Interdepartmental cooperation went both ways, Krevans says. As an example, he cites an urgent need, near the end of his deanship, to purchase steel for constructing Long Hospital and renovating Moffitt Hospital. "Frank Sooy was chancellor, I was dean. The campus didn't have the money to go ahead. They needed money to buy the steel. I called an emergency meeting of my department heads, and we met on a Saturday morning and laid out the problem. Bill Rutter got up and said, 'We can't have a great med school unless we have great clinical work. How much do they need?' And I said '$500,000, to buy the steel.' He said, 'Where can you get it?' I said, 'Well, I have

that much in the comp[ensation] plan that was going to go to basic science.' He said, 'Buy the steel.' They voted on it. The med school bought the steel to build [the new hospital]."[19] Later, the Chancellor's office paid the money back to his office, Krevans says, with state funds. Krevans adds: "That's what we had that was so precious. Every major player, with a couple of exceptions, really believed in the vision for the whole—not just what was good for medicine but what was good for the school and the center and the hospital."[19]

Krevans also sounds a more threatening theme, which bedevils deans of many medical schools. This was the inadequacy of space for patient care, teaching and research. Success in a biomedical enterprise always creates a space crunch. During Krevans's tenure UCSF built or bought new hospitals, but gradually tightening space constraints made it increasingly difficult to retain research stars or hire new ones. The recently built research towers at Parnassus filled rapidly, as Bill Rutter and other successful scientists continued to clamor for bigger labs. One stopgap measure, ridiculously inadequate, reflected Parnassus's earlier history as a campus for (almost exclusively) male students: men's toilet facilities were converted into laboratories, on almost every floor.[21] But the space problem at Parnassus was never really solved. In the late 1970s the campus agreed with neighborhood activists to abide by a "space ceiling" that would not be exceeded. Finally, early in the twenty-first century, UCSF constructed a new campus in a San Francisco district called Mission Bay, several miles from Parnassus.

One effect of the space crunch was that the revitalized Department of Biochemistry increasingly dominated research in the basic sciences at UCSF. With few exceptions (e.g., the Neuroscience program in Physiology), other basic science departments stood still while Biochemistry strode ahead. Their backwater history after moving from Berkeley to Parnassus played a part, but in any case they would have found it difficult to recruit new faculty—or a new chair—into inadequate research space.

I asked Krevans whether he consciously decided to treat other basic science departments with benign neglect. "No," he answered. "The decision was created in recruitment. I wouldn't release the FTE ["Full-Time Equivalent" position, available for recruiting] unless the [Department of Pharmacology] had somebody from Biochemistry on the recruiting team." That is, in essence, Krevans trusted the judgment of Rutter and his faculty more than he trusted the chair of Pharmacology. Rutter, Krevans says, was blessed with a sommelier's taste in judging scientific promise. When he made this clear to Eddie Way, Pharmacology chair at the time, Way "went berserk on me" and said, "That's an insult."[19] The upshot was that Pharmacology couldn't replace departing faculty. Krevans says he applied the same criterion to the Department

of Anatomy, but not to Physiology or Microbiology—whose chairs, presumably, he trusted more fully. As a result of this policy, by the time I took the Pharmacology chair, in 1984, the department had accumulated six unfilled faculty positions. With respect to Pharmacology, at least, I think he made the right decision.

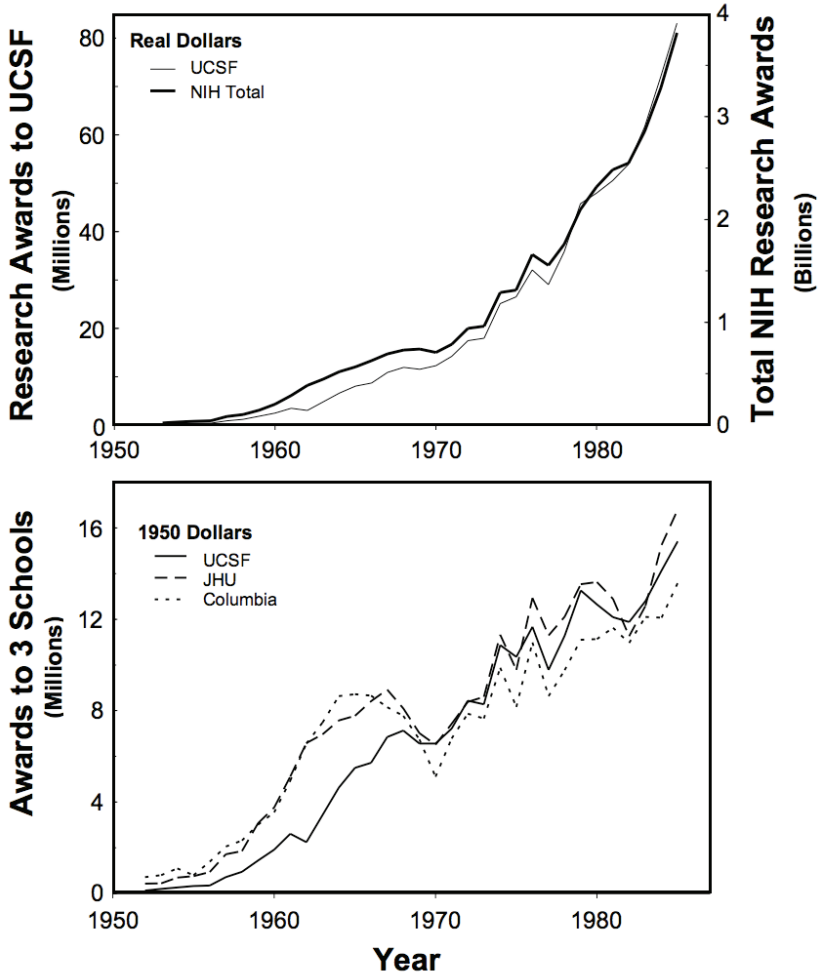
Overall, Krevans proved an excellent steward, allowing UCSF to grow smoothly and rapidly throughout the 1970s. He inherited a medical center that was already prospering and expanding mightily. In the 1970s and 1980s, the national reputation of UCSF's basic science research began to match and then to surpass that of the CVRI and clinical departments. In this regard, Krevans's guidance was less hands-on and far-reaching than Rutter's. Nonetheless, at several key points he made contributions that proved essential.

## A Template for Future Basic Research

By about 1970, as we saw in the previous chapter—and as Krevans found, just before coming to San Francisco—UCSF's share of NIH research grants had pretty much caught up with those of leading biomedical research institutions in the eastern US. As shown in Graph 2, NIH largesse continued during Krevans's tenure in the dean's office, and beyond.[25] After a dip in 1970, both total NIH funds and UCSF's NIH research grants continued to climb. Grants at UCSF continued to match grant awards to schools like Johns Hopkins and Columbia. (From 1982 to 2008 UCSF's share of NIH research grants would remain nearly constant.[26])

The steadily increasing flow of federal funds for research furnished a stable environment that allowed a quiet but profound change in the goals and organization of research at UCSF. Simply stated, the school gradually began to emphasize research focused on fundamental questions relevant to human biology and medicine, as well as the relatively more "applied" investigation of diseases and organ functions in patients, which was already flourishing on the campus. The change began when Rutter and Tomkins took over Biochemistry and eventually came to encompass much of the research conducted in UCSF's basic science departments. Clinical departments continued to conduct excellent research, but in the 1970s UCSF's basic science efforts also began to bring it considerable renown, nationally and internationally.

Before Rutter came, Comroe's CVRI represented the very best of a dominant mode of research on the San Francisco campus. In this institute PhDs and MDs studied the functions and physiological regulation of the heart and lungs in animal models and in patients, usually with an eye to understanding mechanisms relevant to pathogenesis and treatment of common diseases. In the CVRI and in most labs, whether in clinical or basic science departments, the dominant science was physi-




Graph 2: NIH research grants, 1952-1985

The top panel shows total NIH research grant awards (thick line; right-hand scale, in billions of dollars) and research grant awards to UCSF (thin line; left-hand scale, in millions of dollars) for the same time period.[20] Amounts shown are in current dollars. The bottom panel compares NIH research grant awards to UCSF (solid line), Johns Hopkins University (JHU, dashed line), and Columbia University (small dashes) for the same years.20 Here the left-hand scale is in "constant" 1950 dollars, calculated using the Biological Research and Development Price Index, or BRDPI.[23] Note that values shown for UCSF include research grants to the Schools of Dentistry, Nursing, and Pharmacy, as well as the School of Medicine; Medicine accounts for approximately 85% of the total. Values for Johns Hopkins and Columbia represent NIH grants to the corresponding medical schools and to their respective parent University campuses.

ology, with its fundamental underpinnings in physics and chemistry. The research itself, especially in the CVRI, used advanced technology and was first-rate in quality, producing important advances. Investigation at its best was original and based on individual initiative of superb scientists, but it was usually organized in laboratories directed by a chief who occupied a "stand-alone" position in his department's hierarchy, sometimes collaborating with others, but often not. Research funds for CVRI labs, and to a lesser extent for those in many clinical departments, came for the most part in the form of NIH grants awarded for large research programs. With a brilliant, hard-driving, perceptive, and benevolent leader like Comroe, hierarchy and support from a big central grant worked quite well.

Goals and organization of the Biochemistry department were quite different. Rutter and Tomkins brought together a cadre of investigators broadly focused on molecular genetics and the chemistry and function of DNA and RNA. In addition, they envisioned Biochemistry as a group of highly independent but intensely interactive scientists, whose combined ideas, discoveries, and collaborations would make each investigator smarter and more effective than he could hope otherwise to become.

In the event, the model they constructed proved remarkably successful. It would survive the cataclysm of Tomkins's death, and grow despite personality clashes and other serious problems during the next seven years of Rutter's chairmanship, which ended in 1982. As a template for organizing and fostering research, Biochemistry's example set the standard for the later flowering of research in other basic science departments at UCSF.

In the meantime, as we learn in the chapters that follow, UCSF departments outside Biochemistry recruited four scientists whose discoveries changed the fundamental underlying ideas and experimental basis of biological research. While their initial discoveries were largely independent of Rutter and his department, the template he laid down provided key elements of the fertile research environment that nourished their later progress and made UCSF into a powerful magnet for attracting smart researchers and financial support in the 1980s, 1990s, and the first decade of the twenty-first century.

Chapter Four

# Bizarre Little Binary Point

## An Unlikely Revolutionist

Four years after he came to San Francisco, Herb Boyer's research was moving slowly, and he wasn't happy about it.

In 1966, his dream as a new faculty member had focused on exciting new evidence that bacteria use specific enzymes to protect themselves from harboring DNA from viruses or other kinds of bacteria. Because the defensive enzymes of each kind of bacteria were thought to cut exquisitely specific sequences of foreign DNA, Boyer had imagined they might help him understand how proteins recognize a DNA sequence. But repeated disappointment had stymied the project at its very first step—finding and purifying the right enzyme. He worked out a way to measure the enzyme, but found no enzyme activity in bacterial strains, even when genetics showed they had to be present. After a competing lab revised the conditions for measuring the enzyme activity, Boyer could finally show that the enzyme he was working with did cleave DNA, but then he discovered, to his dismay, that it didn't cut at a specific site. In 1970 he was thirty-four, and another lab had beaten him to the punch, by reporting a DNA-cutting enzyme that specifically cleaves a well-defined DNA sequence.

This litany of failure required a quick decision, and it had to be the right decision. Should Boyer persist, or switch to another project? The project, he felt, was still a good one. So, because different bacteria must have fashioned a vast variety of defensive enzymes to cut different sequences of DNA, he would have to survey a rich and various source to find the kind of enzyme he needed. Bacteria, he knew, used small circular pieces of DNA, called plasmids, to transmit resistance to many different antibiotics from one to another, and occasionally such plasmids had been found to transmit genes for DNA-cutting defensive enzymes also. So he asked a graduate student to bring from UCSF's clinical laboratory every single strain of *Escherichia coli*—aka *E. coli*, a gut bacterium that often causes urinary tract infections—that was resistant to more than one antibiotic. In more than 200 *E. coli* strains, the student found more than thirty different plasmids that transmitted antibiotic resistance. Six

of these also transmitted genes for DNA-cutting defensive enzymes. Five of the six carried an enzyme unsuitable for Boyer's purpose, but the sixth carried the enzyme Boyer was seeking. This enzyme, which he dubbed EcoRI, was the jumper-cable he used to start the Great DNA Revolution, which would transform experimental biology and the entire biomedical enterprise.

Today Boyer cites isolating EcoRI as one of many "bizarre little binary points in life" that change everything.[1] It was a rare stroke of very good luck indeed. In the subsequent forty years or so, Boyer says, only one additional published paper has reported, anywhere in the natural world, a restriction nuclease activity that cuts the same DNA sequence EcoRI does.[2] In the early 1970s, as we shall learn, many other brilliant scientists also hoped to plumb DNA's genetic secrets. If Boyer had not found EcoRI, one or more of them would certainly have done so first. But without EcoRI, Boyer would not have played such a central discovering role, and the early history of recombinant DNA and the birth of DNA-based biotechnology would probably have been very different.

Herbert W. Boyer, the primary focus of this chapter, was the first of our wild card discoverers to arrive at UCSF. He remains the straightforward, friendly, low-key person his colleagues remember from four decades ago, with no hint of self-importance or the more subtle elder-statesman persona sometimes affected by eminent older scientists. Long retired, Boyer sometimes consults for biotech companies, but spends much of his time traveling, fishing for trout, playing golf, and having fun with his family. Multiple interviews[3] haven't dulled his keen memory for experimental detail or his delight in explaining what they mean. Obviously smart, but straightforwardly un-intellectual, he is bored by unnecessarily complicated explanations, and tries hard not to exaggerate, dramatize, or otherwise shade his accounts of critical events and remarkable individuals.

No one ever expected Herb Boyer to start a revolution.

## Leaving Derry, Beckoned by Bacterial Genetics

Boyer grew up in Derry, Pennsylvania, a town of 3,000 people, in which most families depended on wages earned in the local Westinghouse factory or on the Pennsylvania Railroad. His father, a freight train brakeman, rode the rails in regular two- or three-day stints, each beginning with a 100-yard walk from his home to the dispatch station. While away, his father lived in the train's caboose—a detail his son thought "rather romantic."[1]

High school for Boyer was "football, basketball, baseball, girls, hunting, and fishing," plus a variety of odd jobs—digging ditches, painting houses, mowing lawns,

and delivering newspapers.[1] His family was not in the least academic—his mother finished high school, his father the eighth grade. One dynamic teacher in the small high school (thirty-two students in the graduating class) made a real difference. He coached Boyer on the football team and taught math, chemistry, physics, and biology—courses Boyer liked, because they were "orderly [and] logical. They made sense."[4] No one in the family encouraged (or discouraged) pursuing education beyond high school, but Boyer chose to go on to college. "I had to get out of Derry, and that was the only way I knew how to do it!"[1]

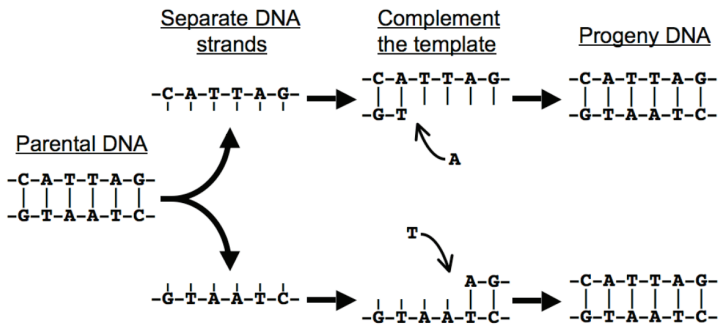
The first step out of Derry, a short one, took Boyer to St. Vincent College. This small Catholic school, run by the Benedictine order, was close enough to home that he was able to live with his family until graduation. Still, St. Vincent's furnished a key turning point in his life, resulting from an assignment in Father Joel's physiology class. Each student was asked to present a seminar on a different textbook chapter. "Which one did I get? 'The Structure of DNA.' I was really taken with the Watson-Crick structure of DNA," he says.[1] The structure, which had just begun to appear in textbooks, fascinated Boyer because it offered a simple, elegant explanation of genetic inheritance—that is, how fruit flies, bacteria, and humans transmit physical and functional characteristics to their progeny. Boyer's own work would make the Watson-Crick structure's implications accessible to experiments by thousands of scientists.

One simple feature of the new DNA structure initiated profound changes in the ways scientists thought about biology. The double helix is composed of two intertwined helical strands, which stick together because each of the linked chemical building blocks in one strand binds specifically and uniquely to a "complementary" building block in the opposite strand. A DNA strand can contain millions of linked building blocks, but the individual blocks come in only four different shapes, conferred by a particular chemical compound found in each. These similar but distinct blocks, termed "bases," have different names—adenosine, thymosine, cytosine, and guanosine, which are abbreviated A, T, C, and G. To understand how one DNA strand complements another, consider a strand that contains a particular sequence of bases, such as CATTAG. If so, because A always binds T, and C always binds G, in a DNA double helix the opposite strand will show the exact complementary sequence, GTAATC. Complementary "base-pairing" aligns the complementary sequences like this:

```
-C-A-T-T-A-G-
 | | | | | |
-G-T-A-A-T-C-
```

Why was Boyer, along with thousands of biologists around the world, excited to learn that base sequences in DNA helices complement one another? After all, it was 1957 when Father Joel assigned him that chapter—a time when neither Boyer nor anyone else knew the base sequence of any DNA strand in the natural world. But they did know that DNA is the so-called "genetic material," the substance that transmits hereditary characteristics in every organism from parents to their progeny, and the new DNA structure produced the pairing rule, that A binds T, and G binds C. If so, how did the pairing rule explain this transmission? Together, the DNA structure and the pairing rule explain—at least "in principle," as practitioners of molecular genetics like to say—how double-stranded DNA can transmit inherited traits from parents to progeny. More precisely, p*airwise complementation of bases allows a cell to replicate exactly the base sequence of each helical strand of its chromosomal DNA*.

To visualize this notion concretely, imagine that the parental cell first separates the two complementary strands of its DNA, and then uses each strand as a template for creating a complementary strand, using the A-to-T/G-to-C base-pairing rule. The replication process produces two identical helices, each composed of two precisely complementary strands. Each helix can then be packed into a separate chromosome and assigned to one or the other progeny of a dividing cell. In this way, base-pairing guarantees that the DNA sequence of each progeny cell will be identical to that of its parent—like this:




Back in 1957 the idea that DNA is the genetic material raised a second question, even more mysterious: no matter how faithfully the parental cell manages to replicate a DNA sequence, exactly how does that sequence determine the physical and functional characteristics of that cell's progeny? Rather vaguely, scientists imagined that DNA sequences somehow determine the structures of a cell's proteins, which in turn

specify the physical shapes and functions of cells and organisms. Protein molecules also were made by folding together long chains of building blocks, each of which is an amino acid, chemically very different from the bases of DNA. Many biologists believed that a particular DNA sequence (a gene) constituted a coded version of the amino acid sequence of a particular protein. In 1957 no one knew how this code worked or even that it was linear—that is, no one was absolutely certain that the sequence of DNA in a gene can be aligned to match the sequence of amino acids in the corresponding protein.

Before Herb Boyer could immerse himself in the intricacies of genes and DNA sequences, he faced a more pressing question. In 1958, at twenty-two, he would graduate from St. Vincent's, and must decide what to do with his life. He had earned good grades in math, logic, and pre-medical science courses, but failed to shine in a course on Chaucer and got a D in metaphysics. Eventually, Boyer applied to the medical school at the University of Pittsburgh, where the interviewer, a "tough old biochemist," asked him how he planned to pay for medical school. "I looked at him and said, 'You mean I have to pay for it?'"[1] When the medical school turned him down, it wasn't clear whether metaphysics or economic innocence did him in.

Boyer then applied, more successfully, to graduate school in biology, also at the University of Pittsburgh. That was still close to Derry—"A small town boy doesn't stray too far from home"[5]—but too far away to commute, so he moved to Pittsburgh. After a year of grad school, he married. His new bride, Mary Grace, also grew up in Derry.

In an interview more than thirty years ago, Boyer characterized his switch from medicine to biology as an unequivocal decision: "I got very interested in [the DNA structure]. I said, 'To hell with medicine. Who needs all these sick people to take care of? I want to do something that's interesting.'"[5] Recently, he described the switch more subtly. "Someone suggested going to grad school for a couple of years, improving my grades and reapplying to medical school."[1] Probably, both accounts are correct. Like many young people faced with such a decision, he may have temporized for a while, taking one path while reminding himself he could still choose to follow the other.

Soon after starting graduate school, the decision became clear. Boyer "latched on to" a young professor who had just come to Pittsburgh, a bacterial geneticist named Ellis Englesberg. "That was my awakening . . . . My whole interest in modern science was awakened when I went to graduate school."[5]

Boyer was assigned an exciting project, based on a recent discovery by Roger Weinberg, a collaborator and departmental colleague of Englesberg. Weinberg had

found a bacterial genetic defect, or mutation, which made it easy to obtain mutant bacteria carrying additional rare but specific mutations affecting multiple genes in a stretch of DNA called the "arabinose operon." (The genes of an operon lie close to one another in the bacterial genome, and are often expressed at the same time in the cell.) His mentors had devised a complex strategy (much too complex to detail here, especially since it soon proved thoroughly impractical[6]) for Boyer to grope his way into the mysterious sepulcher containing the Holy Grail of molecular genetics—deciphering the DNA sequences of normal and mutant genes, and eventually determining how DNA sequence dictates amino acid sequence in proteins. Precise mapping of this operon's mutations was only the first step, and even that little step posed real problems.

Boyer was glad to learn that bacterial genetics, like the subjects he preferred in high school, was orderly and logical, and made sense. But gradually he came to realize that the route his mentors had planned would not find the Holy Grail of molecular genetics any time soon. Mapping genes required him to "recombine" mutations from one genome into another, with genetic tricks designed to incorporate a small part of one bacterium's DNA into the genome of another bacterium. But the process was cumbersome, localizing mutations so slowly and so imprecisely that centuries might not suffice to map even a short stretch of operon sequence. "I was getting worried, 'Am I ever going to get out of here?'" [1] The project "had me chasing windmills."[4]

Three years after Boyer began his PhD work, a lab at the NIH began to attain the Grail Boyer was seeking. Beginning in 1961 and continuing over the next four years, Marshall Nirenberg and his NIH colleagues used a much more direct and efficient biochemical approach to show that each of the twenty amino acids found in proteins is encoded in DNA by one or more "triplet codons"—called triplets because an individual codon comprises three consecutive bases in a DNA sequence.

DNA's four bases can be arranged into sixty-four different three-base sequences. This large number allows almost every amino acid (eighteen of the twenty, in fact) to be specified by two or more different triplet codons, which serve as redundant synonyms encoding the same amino acid. A few non-coding triplets provide punctuation marks in the DNA sequence, indicating the end of a stretch of codons that corresponds to a protein. I shall defer discussion of a second kind of nucleic acid polymer, RNA (ribonucleic acid), because it will reappear in later chapters.[7]

In the meantime, like many a scientific beginner before and after him, Boyer decided he needed new tools to do the job. This beginner, who made it his business to read everything he could find on bacterial recombination, found papers describing a more efficient way to recombine pieces of recombinant genomes. To do so, he

would increase the rate and frequency of genome recombination by speeding up the bacterium's sex life.

As a first step, Boyer asked Ed Adelberg, a scientist at Yale, to send him a "high-mating" strain of male bacteria, able to mate at a high frequency because each carries an "Hfr" (for high mating frequency, or high fertility) plasmid. A plasmid—a tiny circular piece of DNA, separate from the bacterium's own chromosomal DNA—can replicate itself inside a bacterium. In addition to replication genes, an Hfr plasmid's DNA contains genes for part of the tiny mating bridge that transports the plasmid, or a portion of its DNA, into another bacterium, in a kinky variety of sexual congress called "conjugation."

Now that the Grail was in good hands, Boyer became intrigued by the unexpected but striking results of his first experiments with Adelberg's over-sexed bacteria. He was working with two different strains of the same bacterial species, *E. coli*—the K12 strain from Adelberg and the B/r strain from Englesberg. To recombine genes in the arabinose operon, Boyer first had to make sure the B/r and K12 strains could mate and produce hybrid (recombinant) genes in their progeny. They did mate and recombine, but the data looked strange. As compared to K12 x K12 crosses, in B/r x K12 crosses, recombination inserted DNA fragments into the other bacterium's genome less frequently, and the recombined genes were less consistently linked. Further genetic crosses showed that these peculiarities were caused by genes, quite separate from the arabinose operon, that differ in K12 *vs*. B/r. Without knowing it, Boyer had just taken his first faltering step on a long trail that would lead to his most exciting discoveries.

Despite the frustration of chasing windmills, Boyer had accumulated enough genetic results to obtain a PhD. He didn't need to publish the funny results with K12 x B/r crosses, and had no idea what they meant, but they did kindle his interest in plasmids, conjugation, and bacterial sexuality. So he applied for a postdoctoral fellowship to work in the lab of Ed Adelberg, who had authored many papers and a book on these subjects. In 1963, a newly minted PhD, he joined Adelberg's lab at Yale.

At Yale Boyer worked on several projects, but became more intrigued by the strange crosses in his PhD lab notebook, which he explained to a Yale graduate student, Noel Bouck. One day, during a conversation about recently published papers[8] from the lab of Werner Arber in Geneva, Switzerland, Bouck made an intriguing suggestion: perhaps Arber's papers could explain the results of his funny crosses. Boyer is adept at grasping a new insight and running with it. When he realized that Bouck was right, new questions began to unfold before his mind's eye, questions that might reveal key connections between DNA sequence and function. At the time, few other

scientists were asking such questions, so the promising new prospect still remained sufficiently obscure for a beginning scientist to make a significant contribution.

Arber and his colleagues had uncovered key weapons that bacteria use primarily to defend against the relentless attacks of viruses (called bacteriophages, or "bacteria-eaters"). The viruses keep trying to insert viral DNA into bacteria, DNA that can take over the bacteria's metabolic machinery and kill them. To defend itself, each strain or species of bacteria makes one or more enzymes that slice up the invading viral DNA before the foreign genetic material gets a chance to destroy the host. These host enzymes are called "restriction endonucleases," because they "restrict" foreign DNA from surviving inside the host bacterium. A restriction endonuclease first recognizes and binds to a specific short DNA sequence, four or more bases long, and then cleaves the DNA into pieces. To protect itself from damage by its own defensive measures, the host has to modify endonuclease-susceptible sequences in its own genome. To do so it makes a separate "modification" enzyme, which recognizes and chemically disguises the same short sequences in the host's own DNA that would otherwise be cut by the nuclease. K12 and B/r strains of *E. coli*, it turned out, make different pairs of restriction and modification enzymes, which recognize different DNA sequences. As a result, K12 can't recombine its genes efficiently into B/r, or *vice versa*. Inadvertently, Boyer's failed attempts to recombine genes had tripped over the incompatible anti-viral defense systems of the two bacterial strains.

By 1965, his last postdoctoral year at Yale, Boyer had decided that restriction and modification offered exciting opportunities for tackling a biologically critical question: how do proteins recognize and interact with specific DNA sequences? He would purify a restriction endonuclease from a particular bacterial strain and then mix the pure enzyme with pure DNA to identify the DNA sequence the enzyme recognizes and cleaves. Boyer sensed a glimmer of more exciting possibilities as well. Learning how restriction enzymes recognize specific DNA sequences might point the way to understanding how recombination works in bacteria. Mixing pure enzymes with the right DNA might even allow him to make recombination work outside the bacterium, in a test tube. To complement his expertise in bacterial genetics by becoming a biochemist as well, he spent his last months at Yale learning how to purify enzymes.

Boyer was eager to start his own project, although he knew that wouldn't be easy. To do so, he needed an academic position and a lab. Yale showed tentative interest in giving him an assistant professorship, but his postdoctoral adviser, Ed Adelberg, suggested he think also about UCSF, which was getting ready to upgrade its basic science departments. (In 1965, as we know, Julius Comroe, Holly Smith, and their allies had

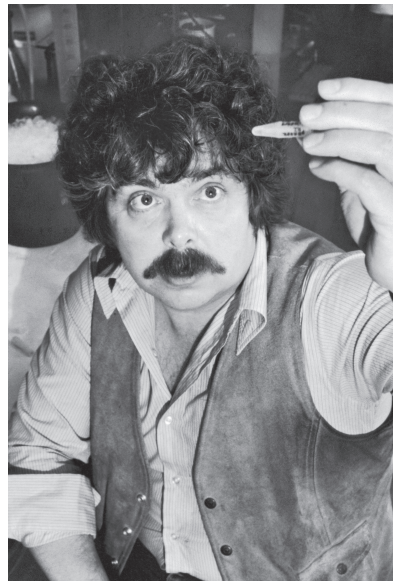
just managed to oust UCSF's Chancellor, John Saunders.) Adelberg mentioned Boyer to his friend, Ernest Jawetz, who had co-authored a microbiology text with Adelberg and was chair of Microbiology at UCSF. An old-school bacteriologist, Jawetz had little interest in molecules, but he needed a faculty member to teach bacterial genetics. In a brief visit to San Francisco, Boyer met Jawetz and other department members, and gave a seminar to a very small audience. Everyone was cordial, but no one knew anything or cared in the least about restriction and modification in bacteria.[4]

Boyer thought Yale was "terrific," but chose UCSF instead, despite its lack of interest in his work and his judgment (correct at the time) that San Francisco was a scientific backwater. A big reason for his choice was simply that UCSF was in California. "I had always wanted to come to California. I had read about California and watched all the movies about California. It was pretty exciting. San Francisco was so different from the east coast. So we decided to come here."[9] In addition to the idea that California would satisfy his need for adventure, Boyer got the impression that the position in Microbiology would allow him to get down to serious work on his own projects, without much in the way of teaching obligations and without direction by anyone else.[4] After seven years of graduate and postdoctoral training, he was more than ready to become his own boss.

So the Boyers crossed the US, ready to start new lives in San Francisco.

## Seeking the Right Restriction Enzyme

San Francisco was indeed a good place to live, and it was exciting to work on his own projects, but Boyer's new job bristled with trials and disappointments. He found he wasn't very good at teaching medical students, but fortunately his class schedule was light. Much worse, the Department reneged on an explicit promise to give him lab space on the fourth floor of Health Sciences East (HSE-4), one of the recently built research towers. This was because Leon Levintow, a virologist in Microbiology space on HSE-4, feared that Boyer's bacteria might contaminate his cultures of virus-infected mammalian cells.[4] In retrospect, Levintow's fear was not justified. For many decades now, biology labs have propagated bacteria and mammalian cells in adjacent rooms, and find that ordinary bacteriological techniques prevent contamination. In other ways, Levintow—who joined Microbiology the year before Boyer, and whom we met in Chapter Two—was a friendly and supportive senior colleague. Still, his fear was a symptom of departmental xenophobias that afflicted scientists in an era when bacteriologists, virologists, biochemists, and physiologists could afford to go about their business without mixing with one another. The DNA revolution swept away such xenophobias by showing how much all these specialists could learn from each other.



Herbert W. Boyer, pioneer of recombinant DNA. Boyer came to UCSF in 1966, collaborated with Stanley Cohen to create a new technology for cloning DNA, and later with Robert Swanson to found Genentech. Photo from 1981.

Denied departmental lab space on HSE-4, Boyer was assigned to several small laboratory rooms in a separate building, Medical Sciences. Although all the rooms were located on one corridor, they needed renovation and were not all connected to one another. Worse, the alternative lab space provided limited access to facilities Boyer badly needed to do biochemistry, including a refrigerated "cold room" for purifying proteins at low temperatures; large centrifuges, called "ultracentrifuges," indispensable workhorses in several biochemical procedures; a machine for making ice, which was necessary to keep enzymes and other reagents cold. He and his lab people could walk to HSE-4 to use ultracentrifuges and a cold room, and to another building to use an ice machine, but the extra steps slowed experiments, and ultracentrifuges were accessible only at night.

For his first four years at UCSF, Boyer wondered whether he had come to the wrong place. Adelberg had told him that UCSF would furnish him an opportunity to "get in on the ground floor" of a rapidly growing scientific community, but "I didn't know it was the sub-basement."[4] In those early years, Boyer felt scientifically isolated. He enjoyed scientific conversations in the cold room with Mike Bishop, a bright, hard-working departmental faculty member who came to UCSF in 1968. Jawetz, chair of his department, seemed resolutely uninterested in anything Boyer was doing in the lab. So was just about everyone else. "I thought, 'Are they trying to tell me something? Maybe I should get the hell out of here.'"[4] Because he and Grace were

starting a family, Boyer didn't actively look for jobs elsewhere, a step he considered both drastic and risky.

The real difficulty, though, was that he had chosen a tough problem and experiments didn't pan out. First, he worked hard to devise a biochemical assay for restriction enzymes, based on assessing the ability of a bacterial extract to cut radioactively labeled DNA from a bacterial virus, lambda bacteriophage. To detect sliced DNA, he looked for radioactive fragments smaller than untreated lambda DNA. He would put the radioactive samples into different centrifuge tubes, each containing sucrose at concentrations that increased gradually from the top to the bottom of the tube. Several hours' spinning the tubes caused each DNA fragment to concentrate in the tube at a level dependent on its size—bigger fragments at the tube's bottom, smaller ones nearer the top. "What a mess! [After] a six-hour run[, you'd] take the little centrifuge tubes, punch them at the bottom, collect the contents drop by drop on little squares of filter paper hung on a pin on a piece of styrofoam, dry the papers, and [count their radioactivity in a] scintillation counter."[1] Depending on the availability of centrifuges, he and his lab co-workers could do up to three runs per day, assessing a total of only twenty-four DNA samples.

For two years, to Boyer's great disappointment, this assay showed no restriction enzyme activity whatever in extracts of K12 and B/r strains. Then, in 1968, another lab discovered that restriction enzymes from K-strain bacteria did show brisk activity, but only if measured under special conditions.[10] Using those conditions, Boyer could readily measure and purify restriction enzyme activity from B-strain *E. coli*. Now the enzyme delivered a second great disappointment: it did cut DNA, but not at a specific site.[11] This unexpected result was especially disappointing because Boyer hoped to study a restriction enzyme's interaction with a single specific DNA sequence, and had wrongly assumed that the interaction site would always be where the enzyme cut the DNA. But restriction enzymes come in dozens of flavors, and he had inadvertently chosen a flavor that cuts DNA at sites separate from the sequence the enzyme recognizes in order to bind to the DNA—making it quite worthless for the purpose he had in mind.

Despite the debacle with B- and K-strain restriction enzymes, Boyer continued to look for a restriction enzyme that *did* cut DNA at a single specific site. Luckily, he managed to maintain his lab's grant support, by publishing a painstaking genetic analysis of *E. coli* B-strain restriction and modification enzymes.[12] But then came a third disappointment, which must have been especially bitter. In 1970, a lab at Johns Hopkins showed that one such restriction enzyme, now known as HindII, does indeed cut DNA at a one site. They even determined the six-base sequence, which is

cut right in the middle, between the third and fourth base of each complementary DNA strand.[13] This disappointment taught a useful lesson, with a double edge. Yes, it was bad luck to pick bacterial strains with endonucleases unsuitable for Boyer's purposes, but nature had created many additional restriction enzymes with different specificities, and his luck might improve—especially if he hunted in the right part of the woods.

So Boyer kept on looking. Japanese scientists had reported that plasmids that transfer genes for antibiotic resistance from one bacterium to another sometimes also carry genes for restriction and modification enzymes. So Boyer asked his graduate student, Robert Yoshimori, to gather samples of different antibiotic-resistant *E. coli* isolated from patients in UCSF's clinical bacteriology lab, and determine which bacteria contained plasmids with genes for interesting restriction enzymes.[1] Testing 214 different *E. coli* samples, Yoshimori found thirty-three plasmids carrying antibiotic resistance. Of these, six also carried genes for restriction and modification activities. Five of the six seemed to encode restriction enzymes similar to those already reported from Japan, of a type (now called EcoRII) that would not be useful for Boyer's purposes. The sixth plasmid, isolated from an *E. coli* infecting the urinary tract of a woman treated at UCSF, was different.[14]

Yoshimori went on to purify the restriction nuclease activity, now called EcoRI, transmitted by the sixth plasmid. He then asked whether the pure enzyme produced DNA fragments different from those produced by the EcoRII activity purified from bacteria infected with a different plasmid. "We were so thrilled with the first centrifugation experiments," says Boyer.[1] After exposure to either enzyme, lambda DNA was cut into neatly separated fragments, but the patterns produced by the two enzymes clearly differed (See Figure 1).

After 1970 Boyer began to feel happier at UCSF. His lab remained a poorly equipped and inconvenient congeries of separate small rooms, but the advent of Bill Rutter and Gordon Tomkins in Biochemistry began to make him feel part of a larger community. The new emphasis on molecular genetics, and two new Biochemistry faculty members in particular, offered welcome promises of a new and exciting environment, even if it was centered in a different department. Boyer quickly made friends with Howard Goodman, a young biochemist already well versed in determining the base sequences of small pieces of RNA, who planned to extend his efforts to DNA as well. Goodman's expertise in the chemistry of nucleic acids meshed nicely with Boyer's interest in cutting and recombining DNA fragments. The two young men joined forces in writing grant applications that proposed to identify DNA sequences recognized by restriction and modification enzymes, and soon began to conduct joint lab meetings.

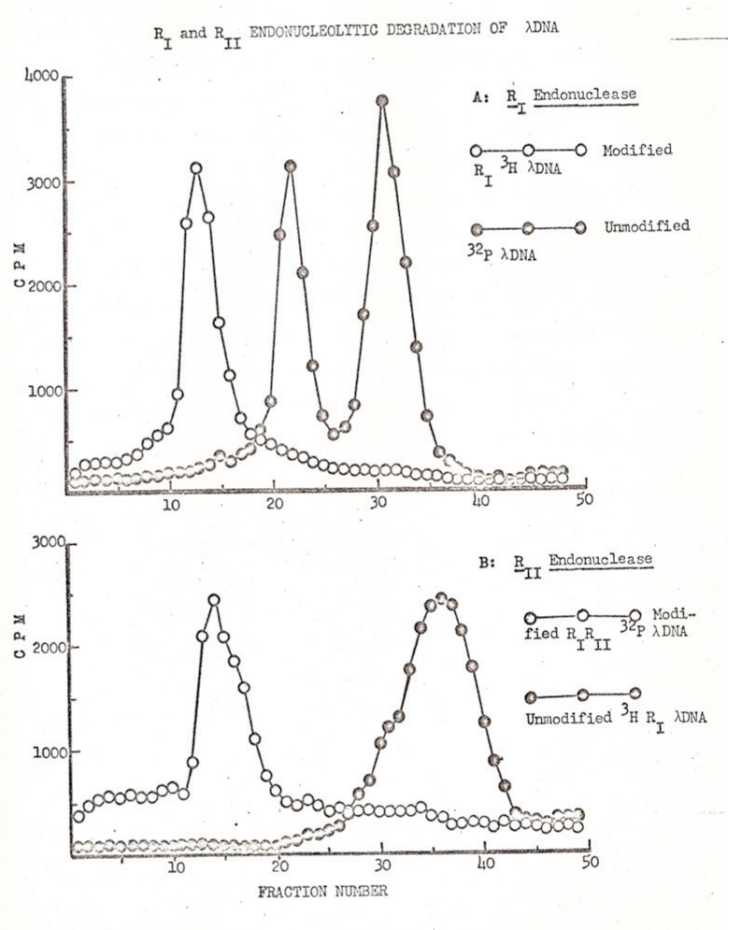



Figure 1: RI and RII endonuclease activities

Yoshimori's comparison of EcoRI and EcoRII endonuclease activities (top and bottom panels, respectively). Each panel depicts the radioactivity (counts per minute, CPM) of lambda DNA in drops taken sequentially from the bottom of a centrifuge tube. DNA fragments in individual drops are larger on the left-hand side of the panels, smaller on the right, owing to increasing densities of the sugar solution at lower levels in the centrifuge tubes. In each panel, open circles represent radioactivity of uncut DNA, which had been modified to resist clipping by the enzyme tested. The filled circles represent radioactivity of clipped DNA. The top panel shows that EcoRI produced two sharply demarcated fragments, at positions equivalent to sizes of thirteen or 3.8 million daltons (one dalton is the weight of a single hydrogen atom). In contrast, EcoRII (bottom panel) produced smaller fragments, of approximately two million daltons. These two graphs first appeared as Figure 7, on page 47 of Robert Yoshimori's PhD thesis.[14]

In addition, like many young scientists at UCSF, Boyer soon got to know Gordon Tomkins. Finally he found a senior faculty member who was genuinely curious about all of biology—even restriction and modification genes in bacteria!—and who enthusiastically encouraged his own research. Boyer vividly remembers an interchange following one of his presentations to a weekly meeting of Biochemistry faculty, where he talked about restriction enzymes and ended by mentioning that one of their uses might be to cut and recombine pieces of DNA in the test tube. Tomkins liked the idea, and suggested that it would be much easier to recombine DNA fragments if the restriction enzyme, EcoRI, were to make staggered breaks in the DNA, cutting the two strands at sites near but not directly across from one another and therefore leaving short strands of overhanging sequence at each end of the severed double helix. Such "sticky ends" would complement the sticky ends of any DNA cut with the same enzyme. This would make it much easier to recombine any two DNA fragments in the test tube, if both had been cut by the same enzyme.[4] A biological precedent for this use of sticky ends was already known: lambda DNA, linear in the bacteriophage, can form a circle once it enters the bacterial cell because base-pairing between complementary sequences on overhanging strands at each end of the DNA allows them to recombine and form a circle.

Before we describe further experiments, let's take a moment to understand why recombining DNA in a test tube seemed so exciting in the first place. We begin with an analogy, in which individual bases are said to be equivalent to letters on a page, three-base codons (in either DNA or RNA) are equivalent to words, and a gene is equivalent to an extended sentence—which is later translated into the corresponding protein "sentence" in a language composed of amino acid "words." By the late 1960s, molecular geneticists realized that to understand how genes make an organism they would have to *read and understand individual sentences in the DNA text*.[15] But they were faced with an almost insuperable problem—the many thousand genetic sentences in every organism's DNA appeared to be jumbled together in ways nobody had figured out. Before reading, it was first necessary to separate sentences from one another and then to xerox many copies of each sentence of interest. Multiple copies were essential, because no lab could read or edit a single DNA molecule. Instead, accurate biochemical analysis required analysis of (literally) millions of identical copies of an individual sentence in a single test tube.

Well before the twenty-first century, biologists would learn how to do each of these tasks. In the 1960s they learned the alphabet of bases and the genetic code. In the early 1970s, Boyer and molecular biologists in other laboratories learned to cut, paste together, and xerox DNA sequences—that is, to slice them at specific sites and

recombine them in test tubes, and then to make many copies of any DNA sequence by inserting recombined DNA into replicating bacteria. In this way, as we shall see, recombinant DNA was born. In the mid-1970s, 1980s, and 1990s, scientists would learn how to read DNA sequences easily and rapidly, how to make recombinant proteins in bacteria and other organisms, and how to record complete genomic DNA sequences of many organisms.[16]

## Glomming onto Sticky Ends

Boyer's was not the only lab intrigued by the idea of using sticky ends to recombine DNA fragments in the test tube. By 1971 the same idea was hot currency in at least three other laboratories, including two located thirty-five miles south of UCSF, in the Biochemistry department of Stanford's medical school.[17] Founded more than a decade earlier by Arthur Kornberg, this department was widely considered the most distinguished in the US, and probably the world. Kornberg assembled an extraordinarily gifted faculty, many of whom were former students. Everyone in the department focused on DNA and RNA or on viruses—lambda bacteriophage in Dale Kaiser's lab, and Simian Virus 40 (SV40), a mammalian DNA virus that can induce cancer, in Paul Berg's lab.

The combination of razor-sharp minds and strongly focused effort made Stanford Biochemistry an exciting place to study molecular biology and molecular genetics. Its freezers contained a cornucopia of purified enzymes that proved essential tools for the DNA revolution—enzymes for cutting DNA, for tying together (technically, "ligating") separate small DNA fragments, and for trimming (or filling in) short complementary stretches of DNA near breaks in the helix. In Kornberg's department the atmosphere was cerebral, intense, and intellectually integrated—a far cry from UCSF's catch-as-catch-can gang of beginners, including Boyer in Microbiology and the diverse set of risk-takers who were joining Rutter and Tomkins in Biochemistry.

At Stanford, the Berg lab and a graduate student in the Kaiser lab, Peter Lobban, came up with the same idea independently. They would link two DNA fragments to one another in the test tube by adding complementary bases to one end of each, using an enzyme (from the department's freezer) to add multiple copies of a single base to one end of a strand in the double helix. Attaching a string of A's to one strand and a string of T's to the other would allow base-pairing to bring them together, and other enzymes would "fill in" missing bases and tie the two DNAs tightly together.[18] Lobban planned to work with fragments of one kind of bacteriophage, while the Berg lab would try to link a fragment of a different phage, lambda, to the SV40 genome. Because SV40 was known to infect and then express its genes in mammalian cells,

they imagined the virus could serve as a vector for introducing a chosen piece of non-viral DNA into cultured mammalian cells.

The Berg lab's effort, which progressed further than Lobban's,[17] quickly revealed that EcoRI (supplied by the Boyer lab, and "enough enzyme to last a lifetime," Boyer says[1]) would cut SV40 at a single site, and did the same with the lambda fragment they had chosen. By the spring of 1971, the Berg lab had successfully applied the approach they planned earlier. Using Boyer's EcoRI, plus five enzymes from Stanford's freezers, they had performed the first successful test-tube recombination of DNA.[19] In July, however, the effort struck an unexpected snag. Taking a course on animal cells and viruses at the Cold Spring Harbor Laboratory, Janet Mertz, Berg's graduate student, described the work she and others were doing with SV40 and the lambda fragment, along with their plan to introduce the recombined DNA into *E. coli*. Robert Pollack, the course instructor, suggested that their plan to introduce the recombined DNA into mammalian cells might be dangerous. SV40 DNA was known to induce tumors in animals, and *E. coli* lives in the human intestinal tract, so he worried that bacteria containing this foreign DNA might cause cancer. Although disappointed and aware that these fears were probably exaggerated, Berg and his colleagues decided to defer introducing the DNA chimera into bacteria until safety could be judged more accurately.[17] In the next chapter we shall return to the safety question, which affected the subsequent course of the whole DNA revolution.

Discoveries in the spring of 1972 suddenly increased the value of Boyer's EcoRI as a tool for engineering DNA recombinants. Knowing that EcoRI cut SV40 at a single site, converting its normally circular DNA into a linear piece of the same length, Mertz was surprised to find that EcoRI-cut SV40 could infect mammalian cells, albeit only 10% as efficiently as the circular version. More interesting, the progeny virus produced by the infected cells contained circular DNA, with an intact EcoRI site at the same location as in the original virus. This indicated that bacteria could convert the linear DNA into circles by re-attaching the ends. Perhaps, she imagined, EcoRI's cuts leave sticky ends. (This would be unlike the cleavage observed with HindII, which cuts both strands of the double helix at the same site, producing "blunt ends," which are not sticky at all.)

To find out, she enlisted the help of Ron Davis, then an Assistant Professor in Stanford's Biochemistry department, who knew how to make images of pure DNA fragments with the electron microscope (E/M). E/M pictures of normal SV40 DNA always showed circles. EcoRI-cleaved SV40 DNA could also form circles, as well as "concatenated circles, " in which two or more linear DNA molecules joined their ends to one another. The cleaved DNA formed circles, concatenated or otherwise,

much more efficiently at low temperature (at or below 15° Centigrade), and re-warming the cooled samples caused the DNA to resume its linear form.

The conclusion was straightforward—DNA cut by EcoRI has sticky ends, with sequences that complement one another. Circles form but are reversible because the overhanging DNA strands are short, so that paired bases bind less tightly to one another at higher temperature. The substantial ability of a bacterium infected with EcoRI-cleaved SV40 DNA to produce stably circular SV40 progeny probably meant the bacterium contains DNA-ligating enzymes that tie loosely-tethered DNA into tightly linked circles. Thus "any two molecules with [Eco]RI sites can be recombined at their restriction sites by the sequential action of RI endonuclease and DNA ligase to generate hybrid DNA molecules."[20] At virtually the same time, a third Stanford investigator, Vittorio Sgaramella, performed less elegant but nonetheless persuasive experiments to support the same inference—that DNA cleavage by EcoRI generates cohesive or "sticky" ends.[20]

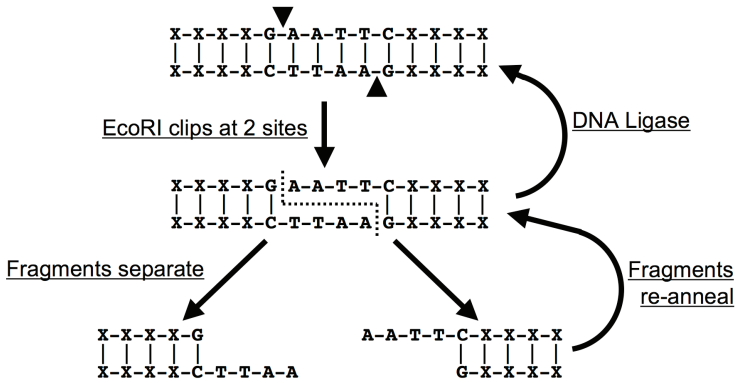
Boyer's lab was also trying to recombine EcoRI-cut fragments with one another in the test tube, but without success. For them the problem was harder, because they lacked an electron microscope and measuring recombination was tough. Using a technique and equipment borrowed from Mike Bishop's lab in an adjacent building, they tried to separate cleaved and recombined fragments in "tube gels." Propelled by an electrical field, DNA fragments would migrate toward the bottom of the "gel," at speeds that depended on their size. "We would put the gel in a small metal tube and . . . mechanically push it into a guillotine-like device, and slice small fragments into scintillation vials. It would go chop, chop, chop, and invariably pieces would fly across the room and we'd be down on the floor looking for slices . . . like looking for a fallen contact lens."[1] The process was not just laborious—it was also too unreliable to detect recombination.[21]

When Boyer heard the Mertz-Davis result from Berg, his lab was trying to determine the sequence of the site cleaved by EcoRI, and had already identified the base at one side of the cleavage. Now that the wild dream he had discussed with Tomkins proved a reality, Boyer quickly called Mike Bishop to borrow an enzyme (reverse transcriptase, which we'll meet again in Chapter Seven), which could "fill in" the sequence of the shorter strand at the cut site with nucleotides that base-paired with the overhanging sequence. By the next morning, judicious use of radioactive nucleotides had revealed the sequence cleaved at the EcoRI site. Boyer reported the sequence in the November 1972 issue of *Proceedings of the National Academy of Sciences* (usually referred to by its abbreviation, *PNAS*).[22] In the same issue, Mertz and Davis and (separately) Sgaramella reported evidence for sticky ("cohesive") ends of DNA

cleaved by EcoRI.[20]

To recapitulate, EcoRI produces sticky ends by cleaving a six-base sequence (GAATTC) at different sites (arrowheads in the drawing below) in the two strands of a double helix. The separate fragments may transiently anneal to one another at low temperature, but eventually separate unless DNA ligase has tied them stably together. The scheme looks like this:

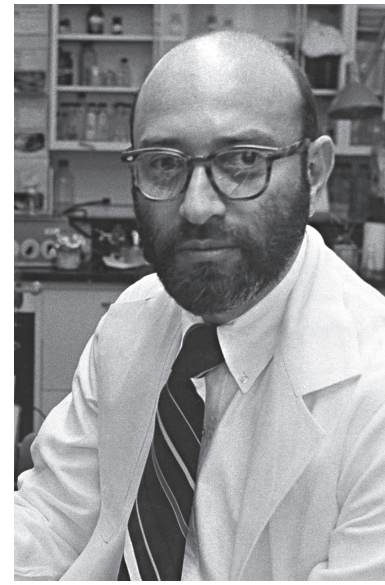
Note that the complementary sequence of the second strand, CTTAAG, if read




from right to left instead of left to right, is identical to the GAATTC sequence in the first strand. "Nature" can read the two strands in opposite directions because the strands of the actual helix point in opposite directions (left to right for the one we write on top, right to left for the one at the bottom). Because of this peculiar symmetry, the EcoRI enzyme sees precisely the same sequence at the symmetrical cleavage site, regardless of which strand it nuzzles up to first.[23]

In October 1972, Boyer was invited by a Stanford scientist, Stanley N. Cohen, to speak at a joint US-Japan conference on bacterial plasmids,[24] scheduled to begin in Hawaii in three weeks. The invitation was late because Cohen had just heard from a colleague that Boyer would soon publish the sequence cleaved by a restriction enzyme, and that the gene for the restriction enzyme had been transferred by a plasmid from one *E. coli* bacterium to another. Although the two scientists didn't know each other, their research interests would soon coalesce in experiments destined to transform the practice of molecular biology.

Stan Cohen had come to Stanford four years earlier, as an Assistant Professor of Medicine. Growing up in Perth Amboy, New Jersey, where his father ran an electrical



Stanley Cohen, physician-scientist at Stanford. Collaborating with Boyer to develop basic recombinant DNA technology, he contributed a sharp mind and invaluable expert knowledge of plasmids responsible for resistance of bacteria to antibiotics, which provided essential tools. The photo was probably taken in 1975.

appliances business, he already planned to go to medical school when he was still in high school. Premed courses at Rutgers didn't prevent him from joining the school debating team or from co-writing a popular song good enough to make the nation's Hit Parade.[25] Cohen went on to earn his MD degree from the University of Pennsylvania, and got further training in internal medicine in New York, Michigan, and North Carolina. To avoid the Vietnam draft, he spent two years at the NIH, working on the interaction of an anti-malarial drug, chloroquine, with DNA. This project, plus meeting an exciting cadre of NIH scientists, including Gordon Tomkins, greatly expanded Cohen's research horizons, leading him to augment his clinical training with postdoctoral research in the New York lab of Jerry Hurwitz, a former student of Arthur Kornberg. His work in the Hurwitz lab, focused on the ability of a bacterial enzyme to transcribe DNA of bacteriophage lambda into RNA, further revealed the beauties of lambda genetics and the nitty-gritty power of purifying enzymes.

At Stanford, Cohen's lab research asked basic scientific questions about the mechanisms underlying a critically important clinical problem, the increasing incidence of infections by bacteria resistant to multiple antibiotics. Scientists in Japan and elsewhere had begun to show that this phenomenon depended on plasmids that carry "Resistance factors" (aka "R factors"), which can transfer from one bacterium to another the ability to resist killing by an antibiotic—or, in some cases, more than one antibiotic. Cohen very much wanted to figure out what the antibiotic resistance genes were, and how bacteria could become resistant quickly to multiple antibiotics.

His invitation to Boyer was based on a coincidence—the fact that EcoRI was identified by taking advantage of the occasional chance inclusion of genes for restriction enzymes in R-carrying plasmids.

Hearing Boyer's talk at the meeting, Cohen began to suspect that EcoRI could point a way to understanding R-factor genes. Because the six-base sequence recognized by EcoRI should arise by chance about once in every 5,000 bases of a random sequence, he calculated, the enzyme should cut a plasmid 100,000 bases long into about twenty fragments, which could then rejoin one another in different combinations. "If cleavage left the replication functions of the plasmid intact, the replication region might join to different antibiotic resistance genes in the mix and form DNA circles containing different fragment combinations . . . . Maybe we could isolate . . . plasmids containing different combinations of antibiotic resistance genes."[24] Cohen's hopes were bolstered by the fact that he had just learned a new way to "transform" bacteria with naked plasmid DNA—that is, how to introduce plasmid DNA into bacteria without requiring one bacterium to create a bridge for transferring the DNA to a separate bacterium.

The evening after Boyer's talk, Cohen and Boyer, along with several other attendees at the meeting, took a long walk down the street parallel to Waikiki beach, discussing possible ways to isolate DNA encoding specific antibiotic resistance genes. In Cohen's memory, Boyer at first was not very interested in plasmid genes, and offered to give Cohen some EcoRI to do the experiments on his own. Boyer's interest kindled more strongly, Cohen recalls,[26] when he realized that plasmids offered a unique avenue to isolating and propagating individual genes, because their very small genomes can be introduced into a bacterium at will and then replicate independently of the bacterial chromosome. This must have come as a welcome revelation to the young man whose PhD research had chased windmills until he realized that the bacterial genome was too big and complex for studying natural recombination after bacterial mating. Moreover, Cohen also reminded him that "Your lab has spent a lot of time isolating the enzyme and we should really do this as a collaboration."[24] The walk ended with a late-night stop at an undistinguished delicatessen, where the two laid out detailed plans for experiments.

Cohen and Boyer agreed that, as Cohen put it, "there was no assurance that any of this experimentation would work."[24] Sure, the basic components were available. They could transform bacteria with naked DNA, cut DNA at appropriate intervals with an enzyme that creates sticky ends, separate fragments from one another according to size in the test tube, and recombine them via their sticky ends. In addition, antibiotic resistance would provide a significant advantage, by allowing them to iden-

tify and isolate bacteria that had become selectively resistant to a specific combination of antibiotics. For instance, a DNA fragment from plasmid 1 might confer resistance to tetracycline, while DNA from plasmid 2 conferred resistance to ampicillin. If EcoRI cleavage sites were located in the right places, they might be able to recombine the key resistance genes of plasmids 1 and 2, plus the necessary replication functions, into a novel plasmid 3, and use plasmid 3 to transform antibiotic-sensitive bacteria, creating bacteria resistant to *both* tetracycline and ampicillin.

But the crucial question was "whether biochemically linked DNA fragments could be propagated [and would function] in living *E. coli*," as Cohen pointed out, adding, "[T]he answer was not known."[24] Joining fragments at *Eco*RI sites would bring DNA sequences together artificially, rather than via the normal biological processes—most of them unknown or poorly understood—used by naturally evolved organisms. Stanley Falkow, a Stanford microbiologist who witnessed the now famous delicatessen conversation, was skeptical. "If it works," Cohen quotes him as saying, "let me know."[27]

## Perspective

In his first six years at UCSF, Boyer produced a modest number of solid papers, but they sufficed—in 1971 he was promoted to tenure, as an Associate Professor of Microbiology. Still, he remained well below the radar of high-flying talent-spotters like Bill Rutter. His efforts to identify and purify a useful restriction enzyme had met with repeated failure. A cumbersome enzyme assay found no restriction enzyme activity, and when he got it to work, the first restriction enzymes he tested proved to cut DNA at random sites. Finally, another lab apparently beat him to the goal, by finding, purifying, and determining the DNA sequence cleaved by the first well-characterized restriction enzyme.

Boyer trusted his betting strategy—find the horse with a restriction endonuclease—but unaccountably kept picking horses that didn't run. Finally he took a long shot, screening hundreds of bacteria isolated from UCSF patients. Persistence led to EcoRI, a "bizarre little binary point in life" resulting from a very lucky, very clever bet. As he had imagined, the pure enzyme proved a genuine boon. He generously provided supplies of EcoRI to many labs, and rival labs showed that when it cleaves DNA it produces sticky ends. While Boyer couldn't claim to "own" the new finding all by himself, determining the sequence at the site where the enzyme cuts DNA allowed him a share of the credit, and would later prove invaluable in experiments. Its other critical contribution (see Chapter Five) was to attract an invitation from Stanley Cohen to give a talk in Honolulu.

In these early years, UCSF's support for Boyer's effort was at best rather modest. Microbiology gave him a crowded, poorly designed laboratory far from equipment he needed every day, colleagues and a chair who showed little interest in his work, and an opportunity to write requests for grants from the NIH. Its most useful gift to Boyer was to leave him alone and let him follow his own nose, wherever it might lead. His teaching responsibilities were small, and though the department provided little encouragement for his efforts, no one told him what to do, or how, or why.

Boyer brought his own considerable gifts to UCSF. In addition to his knack for detecting and profiting from insights of others, he recognized the value of his own insight, which told him to persist in pursuit of the "right" restriction enzyme. His patient, persistent refusal to give up was essential. Without his openness to new ideas, ability to recognize good ones, and persistence in the face of adversity, his work would have gone nowhere.

Now that Biochemistry's rejuvenation began to attract colleagues like Tomkins and Goodman, Boyer's situation at UCSF was looking up. Still, he could not have predicted the remarkable events of the ensuing decade, described in Chapter Five—the birth of the DNA revolution, followed by his move into the rapidly growing Biochemistry department. These events, in turn, set the stage for an exciting race to clone the first human gene and for Boyer's second major discovery, both described in Chapter Six.

Chapter Five

# The DNA Revolution is Born

## As a New Department Prospers

Soon after the Honolulu meeting, Herb Boyer chanced to find the third and last essential element required to recombine DNA and clone genes. The first was Eco-RI, which made it possible to cut and re-attach manageably small pieces of DNA. The second was Stanley Cohen, who suggested a key idea—to recombine plasmid DNA encoding genes for antibiotic resistance—and knew how to make it work. The third element, allowing Boyer to cobble together an extraordinary trifecta, came in January 1973, when—Boyer recalled—"Manna from heaven dropped out of the sky."[1]

Boyer was visiting the Cold Spring Harbor Laboratories on Long Island, to give a talk. Joseph Sambrook and Philip Sharp, two scientists then at Cold Spring Harbor, picked him up at the airport, took him straight to a darkroom near their lab, showed him an agarose gel, and exposed it to ultraviolet light. Suddenly he saw a series of starkly bright fluorescent bands, each representing a single cleaved DNA fragment, separated according to its size from other fragments in a biochemical mixture. The DNA fragments were stained with ethidium bromide, a chemical no one had previously used for that purpose. "It was one of the most exciting things I could have looked at. I said, 'Thank you, lord!'"[2] The reason for his excitement was simple: now, instead of tediously spinning DNA samples in sugar solutions and testing individual drops from the bottom of centrifuge tubes, Boyer could not only determine whether DNA was cut, but also assess the precise size of each separate fragment. "You could see the results from your experiments in a couple of hours, and you could essentially do endless experiments."[2]

The Revolution was about to begin.

### Recombining and Cloning Genes

As soon as he returned to San Francisco, Boyer asked Robert Helling in his lab to standardize details of the Sambrook-Sharp procedure. (Helling, once a graduate student with Boyer in Pittsburgh, was now a professor at the University of Michigan

and spending a sabbatical year in Boyer's lab.) Staining DNA with ethidium bromide, Boyer estimates, accelerated his lab's work more than ten-fold. This new wrinkle shifted the collaboration with Cohen's lab into high gear. Soon it was progressing almost as fast as the scientists could design experiments.

Collaboration between the labs, thirty-five miles apart, was made easier by the fact that Annie Chang, a technician in the Cohen lab, lived in San Francisco and commuted to work at Stanford, in Palo Alto. After plasmids were isolated and purified in Palo Alto, in the evening Chang would take the DNA to San Francisco, where Boyer and Helling would cleave it with EcoRI and link the fragments stably to one another with DNA ligases. Before her morning commute, Chang would pick up the (putatively) recombined fragments for transport back to Palo Alto. Cohen's lab used the cut, recombined fragments to transform bacteria and assessed resistance or sensitivity of the resulting bacteria to appropriate antibiotics. In San Francisco, Helling assessed sizes of cleaved or recombined DNA fragments on gels, in samples analyzed either after they had been cut and ligated in the test tube, or, alternatively, after the Cohen lab had recovered the plasmid DNA from transformed bacteria.[3]

The key result was wondrously simple. One of Cohen's plasmids, designated pSC101, conferred resistance to killing by tetracycline, and had only one EcoRI cleavage site. A second plasmid, pSC102, had more EcoRI cleavage sites, and conferred resistance to a different antibiotic, kanamycin. Helling used EcoRI to cleave DNA from the two plasmids, mixed the resulting DNA fragments together, and then stably ligated whichever fragments had stuck to one another by virtue of their sticky ends. Then Chang used the resulting DNA mixture to transform antibiotic-sensitive bacteria, and tested bacteria from separate colonies (each derived from a single parental cell in the transformed population) for resistance to tetracycline and/or kanamycin. A combination of electron microscopy and ethidium bromide-stained bands told the tale. *Bacteria from several colonies had been transformed by a single plasmid each, but were resistant to both kanamycin and tetracycline. The plasmids from these doubly-resistant bacteria were larger than pSC101, because an EcoRI-cleaved DNA fragment derived from pSC102 was inserted at the single EcoRI cleavage site of pSC101.* From the size of the inserted fragment, Cohen, Boyer, and their colleagues could identify which EcoRI-produced fragment of pSC102 DNA contained the gene for kanamycin-resistance.[4]

Boyer remembers joining Helling in the darkroom to look at the crucial set of plasmid DNA fragments, separated on gels and stained with ethidium bromide. "[T]here it was. It actually brought tears to my eyes, it was so exciting. . . . [W]hen I looked at those gels, I knew we'd be able to isolate any piece of DNA that was cut

with EcoRI, regardless of where it came from."[5] Boyer was excited because now it seemed likely that recombinant DNA from many sources could eventually be manipulated, analyzed, and "amplified," pretty much at will. ("Amplified," like "xeroxed," is a technical term denoting production of a very large number of copies.)

Cohen and Boyer had used scissors (EcoRI) and paste (DNA ligase) to produce a text composed of a novel combination of sentences, and converted replicating plasmids and bacteria into an extraordinarily efficient xerox machine, able to "print" (that is, propagate) unlimited numbers of perfect copies of the new text. Cohen drew the same exciting inferences, but makes no bones about his less ambitious original motive for doing the experiments. "In my case, the technology was developed out of necessity, so that we could study antibiotic resistance plasmids."[3]

In their November 1973 paper reporting these results, despite their elation, Cohen and Boyer resisted the temptation to crow. The paper's summary contains little more than this deadpan sentence: "The general procedure described here is potentially useful for insertion of specific sequences from prokaryotic or eukaryotic chromosomes or extrachromosomal DNA into independently replicating bacterial plasmids."[4]

To test this notion, Cohen and Boyer sought to extend the principle to other organisms. Now that they had introduced DNA obtained from one population of *E. coli* into other *E. coli* bacteria, what about transferring DNA between different bacterial species? Cohen soon performed the species-crossing trick in his own lab, by inserting into an *E. coli* plasmid a gene (for resistance to yet a third antibiotic, ampicillin) that was obtained from a plasmid in a quite different bacterial species, *Staphylococcus aureus*.[6]

But what about the vast array of bigger and more complex species biologists categorize as eukaryotes? Rather than store their DNA in a chromosome that floats about with the other cellular machinery, as prokaryotic bacteria do, eukaryotes have evolved a distinctive subcellular structure called the nucleus. Larger than bacteria, eukaryotic organisms range from single-celled yeast species to multi-celled plants and animals, including birds, reptiles, humans, and other mammals.

Crossing the potential barrier between bacteria and eukaryotes would significantly raise the ante. For one thing, it was easy to imagine that eukaryotes had evolved very different ways of handling their genes, so that their DNA might not replicate normally in a bacterium. Cohen and Boyer also faced a serious practical problem—it could prove very difficult to *know* whether a piece of inserted foreign DNA came from a eukaryote. By contrast, DNA transfer from one bacterium to another had been easy to detect, because judiciously chosen plasmids carrying resis-

tance to specific antibiotics allow experimenters to select bacterial colonies that grow in the presence of an antibiotic, revealing the presence of a gene that could only have come from a specific plasmid. Because eukaryotic DNA genes don't confer antibiotic resistance, they would have to screen huge numbers of individual bacterial colonies for a distinctive feature of the foreign DNA. What distinctive feature(s) would they seek? Worse, eukaryotic DNA, whether from yeast or rats, represents an organism's entire genome, containing a vast array of unknown sequences. In 1973 it was not easy to find a homogeneous supply of eukaryotic DNA with conveniently distinctive features.

The unexpected answer came from a conversation between Herb Boyer and a graduate student, which took place at a June 1973 meeting, the annual Gordon Conference on Nucleic Acids. (Gordon Conferences on different research topics meet each year at small preparatory schools in New England, for scientists to share their latest work with others.) Although he and Cohen had agreed not to talk in public about their DNA recombination experiments before the paper was published, Boyer found himself unable to resist the temptation to tell such an exciting story. After the conference session where he gave this talk, he began discussing possible future directions with a Stanford graduate student, John Morrow. They knew each other because Morrow, finishing his PhD work in Paul Berg's lab, had found that Boyer's EcoRI cleaves SV40 DNA at a single site. Boyer remembers telling Morrow that "The next thing to do is to ask if we can put eukaryotic DNA into *E. coli* and get it to replicate . . . . I said, the problem is, we have to find the right DNA. And [Morrow] says, well I've got some of the right DNA."[7]

The right DNA, Morrow suggested, was a special variety of frog DNA, termed ribosomal DNA because it is transcribed into the RNA used for making ribosomes, which serve as the protein-making machines of the cell. A procreative quirk of the amphibian life-style makes it easy to separate homogenous ribosomal DNA from the rest of the animal's DNA, and several chemical characteristics of ribosomal DNA nicely distinguish it from other frog DNA.[7] Morrow had already found that EcoRI cuts frog ribosomal DNA, which he had obtained from Donald D. Brown, director of the lab where he planned to pursue postdoctoral training.

Upon returning home, and after confessing to Cohen that he had revealed their findings in public, Boyer told him that Morrow had what might be the right eukaryotic DNA to insert into bacteria. Cohen agreed that they should collaborate, but at first Morrow demurred. He wouldn't be an author on the paper, but would just give them the ribosomal DNA. He then changed his mind, deciding he would be an author. Curiously, however, he said nothing about the experiments to Berg, his thesis

supervisor. Later, when Berg discovered he had been left out of the loop, he was furious.[8]

After all the talking, the experiments worked. This time, Cohen, Boyer, and their colleagues used EcoRI to pluck "clauses" (ribosomal DNA fragments) out of a frog's DNA. They obtained these copies by inserting the ribosomal DNA clauses into a plasmid that also encoded tetracycline resistance. Then they xeroxed many, many copies of the clauses by putting the plasmid in bacteria, and allowing the bacteria to divide and multiply, in the presence of tetracycline, to form colonies in a culture dish (each bacterium in such colonies would contain plasmid DNA, in addition to its chromosomal DNA).[9]

How did they screen the tetracycline-resistant colonies to determine which of them contained plasmids that also encoded ribosomal DNA? Stanley Falkow at Stanford, a witness at the delicatessen foray in Waikiki, later remembered asking Boyer how he found those colonies. "He just said he kissed every colony on the plate, until one turned into a prince."[10] In actual fact, Boyer would have had to kiss only about five frogs to get a prince! The initial screen identified colonies containing plasmid fragments, in addition to pSC101, identical in size to those produced by treating "native" ribosomal DNA (obtained directly from frog cells) with EcoRI. Approximately one fifth of the colonies did contain such fragments. The collaborators also applied several more exacting criteria to confirm that these fragments were derived from frog ribosomal DNA.[11]

## Early Responses to Recombining DNA

Rather than talking about copying sentences, scientists adopted a more telegraphic term. Boyer and Cohen had "cloned" stretches of DNA in bacteria by persuading colonies (aka "clones") of rapidly-growing bacteria to replicate multiple copies of a single DNA sequence. Their 1973 paper reported cloning an *E. coli* gene for resistance to kanamycin,[4] and their second paper (May 1974) reported cloning frog ribosomal DNA.[9]

Scientists responded to the new findings in different ways. For instance, Keith Yamamoto—then a postdoc in Gordon Tomkins's lab, and now a scientist and administrator at UCSF—remembers that Boyer gave an informal talk to the Tomkins lab sometime in 1973 or early 1974. After the talk, several members of the audience appeared less than impressed by the significance of what they had heard, but Tomkins quickly saw that Boyer's work would transform the way every scientist studies biology.[12] Almost forty years later, Boyer recalls Tomkins's response to the frog DNA result. One day Tomkins "came around the corner, and I said, 'Gordon, we cloned

Xenopus DNA!' He gave me a kiss on the cheek."[1] In contrast, Cohen remembers that he gave a seminar in 1974, after which a scientist asked, "Well, these experiments are kind of cute, but why in the world would anyone want to put DNA from a frog into bacteria?"[3]

Still other scientists worried that recombining DNA might prove extremely dangerous. Although their worries would later kindle conflagrations in the public arena, as we shall see later in this chapter, the first public recognition of the new recombinant DNA technology was quite positive. Victor McElheny, a science reporter, heard about the Boyer-Cohen experiments from a scientist friend, David Baltimore, and interviewed Cohen. McElheny's *New York Times* story, in May 1974, emphasized possible uses of the technology in agriculture and medicine.[13]

The *Times* story exerted a crucial effect at Stanford. The University's news director sent it to a remarkable Stanford administrator named Niels Reimers. Six years earlier, in 1968, Stanford had hired Reimers, a former engineer and business executive in the electronics and aerospace industries, to manage research grants and contracts for the University.[14] Early on, Reimers discovered that Stanford was not doing a good job of exploiting new technology discovered by its faculty, despite the school's crucial role in fostering explosive growth of the semiconductor and computer industries in nearby communities, collectively nicknamed "Silicon Valley."[15] The history of Silicon Valley probably did help Reimers to persuade skeptical University administrators to set up an Office of Technology Licensing (OTL), a thing almost unheard of in universities in 1968. By 1974 the OTL, directed by Reimers, was already funneling significant income to Stanford from companies willing to pay for permission to use new technology invented by Stanford faculty. Intrigued by the McElheny article, Reimers picked up the telephone to call Cohen. If he had not made that call, the complex relations between academic biologists and the unborn biotech industry would have played out very differently.

As the conversation progressed, Reimers told Cohen it would be a good idea for him and Stanford to apply for a US patent on the new DNA technology. Cohen replied that he didn't want to apply for a patent, because the discovery depended on enzymes found and purified by other labs and because its practical usefulness would be realized so far into the future that a patent would not be financially worthwhile.[3] Reimers finally persuaded him that the patent application was worth a try, and Cohen pointed out that Boyer was a co-inventor. Reimers agreed to contact UCSF, and Cohen said he would call Boyer. Not pursuing patents efficiently, Reimers found, UC would not assume any risk. (UC would join the patent only if Stanford paid for the patenting process and if UC was guaranteed not to lose a dime in the event the patent produced no income.[16])

Boyer says his first response to Cohen's call was firmly negative: "Stan, you can't patent that, we're funded by NIH." Cohen responded that Reimers had convinced him the work was patentable and permitted by agreements between universities and NIH.[3] "It was certainly not Stanley's idea or my idea to do it." Indeed, Boyer says, "Very few molecular biologists knew anything about patents" in the 1970s.[2] Applied to me and many other experimental biologists in 1974, the statement is accurate. Many of us imagined that the NIH forbade patenting results of research in our labs, and some considered such patents little more than greedy attempts to profit from knowledge that rightfully belonged to the world. Most of us had never heard of the idea that a patent can be essential for a business to exploit a new idea. Such ignorance came within an ace of killing the patent on the method Boyer and Cohen developed, because US rules allowed only a one-year delay between publishing the experiments and submitting the patent. Reimers only learned about the published work when it was almost too late to prepare a submission.

"We were just ignorant," Boyer said later. "But the rest of the scientific community was all over us, because they were ignorant also."[2] Especially at UCSF and Stanford, the Boyer-Cohen patent application triggered considerable consternation and controversy, based on ignorance plus a roiling mixture of genuine concerns about possible consequences of mixing business and academic pursuits, and perhaps more than a tincture of plain envy. Because Cohen was not a Biochemistry faculty member at Stanford, Biochemistry would receive none of the patent money awarded to Stanford, although the research had used enzymes from its communal freezers and Cohen had received advice from some of its faculty. Over more than three decades anger has cooled, but its residue can still be sensed today. Describing the patent, for instance, Paul Berg and Janet Mertz recently wrote that its "claims to commercial ownership of the techniques for cloning all possible DNAs, in all possible vectors, joined in all possible ways, in all possible organisms were dubious, presumptuous, and hubristic."[17]

The controversy over patenting and commercial exploitation of recombinant DNA was to prove especially disturbing at UCSF. At the same time, the Boyer story became closely entwined with that of the UCSF Biochemistry department, partly because several Biochemistry labs entered the race to devise medically effective uses of recombinant DNA, but also because Boyer approached Bill Rutter in 1975, asking to be appointed as a Biochemistry faculty member. "I always had a certain resentment about the fact that I didn't get the space I was promised when I went to UC," Boyer says. "I never felt I had support from Microbiology. . . . From my perspective, I got into some new lab space."[2] Boyer moved from cramped, inconvenient quarters in Mi-

crobiology to a larger, newly renovated facility in one of the Health Science towers.

## Safety Fears Arise

For Boyer, the collaboration with Cohen ushered in a tumultuous period of joyful excitement, punctuated by unwelcome jolts of discord and confrontation. We begin with the controversy about safety of recombinant DNA—a controversy Boyer ignited, quite unwittingly, with his unscheduled talk about recombining antibiotic resistance genes at the Nucleic Acids Gordon Conference in June, 1973. In addition to prompting the conversation with John Morrow that led to cloning frog ribosomal DNA, unveiling the new findings also raised qualms among some conferees about possible dangers of future recombinant DNA experiments. Following a vote at the conference's business meeting, its two chairs, Maxine Singer and Dieter Söll, wrote a public letter to the US National Academy of Sciences (hereafter, the NAS) to express concern that scientists had too little solid information to predict the actual dangers, and to suggest developing explicit guidelines for future experiments.[18] The Singer-Söll letter prompted the NAS to form a committee, chaired by Paul Berg, to "examine the scientific prospects and potential risks of what came to be known as recombinant DNA."[17]

Later in 1973, in a draft of the Discussion section of their paper[19] on frog ribosomal DNA, Cohen and Boyer touted the implications of "a general approach for the cloning of DNA molecules from various sources," but added this cautionary sentence:

> However, the implications and potential biohazards of experiments employing this approach should be carefully considered, since the biological role of molecular chimeras containing both prokaryotic and eukaryotic genes is unknown.[20]

Calling this "ridiculous, . . . a vague ominous warning," a reviewer of the manuscript pointed out that the authors had no information to offer about potential dangers, and urged removing the sentence.[3] The authors did so, but others would have more to say.

Seven members of Berg's committee met at MIT in April, 1974,[21] after news of the paper on frog ribosomal DNA had begun to circulate through the scientific community. (It would be published in May.) Cohen and Boyer were not members of the committee, but learned of its deliberations when, by chance, Cohen gave a talk at MIT. There a committee member, David Baltimore, told him the committee was considering proscribing experiments that involved either DNA containing viral tumor genes or "the use of any antibiotic resistance genes or plasmids in any DNA cloning

experiments."[20] Pointing out that the committee lacked members who worked with antibiotic resistance or plasmids, Cohen objected to forbidding experiments that used antibiotic resistance genes. Instead, he told Baltimore, he sent plasmids to others with a more limited request—insert no novel combinations of antibiotic resistance genes into a bacterium.[22]

Upon returning to California, Cohen telephoned Boyer to tell him the committee was planning to proscribe experiments like the first one they had reported. He then began to draft a statement, to be signed by him and Boyer and published, "indicating our feelings about the importance of continuing the study of antibiotic resistance genes and plasmids and their use in DNA cloning," and repeating the suggestion he had made to Baltimore.[3] Berg heard about the draft statement and suggested that Cohen and Boyer compromise with his committee and co-sign its letter. Cohen and Boyer got a statement on plasmids they could live with, and co-signed what came to be called the "Berg letter."[23] Published in July 1974, the letter proposed that scientists voluntarily honor a moratorium on recombinant DNA experiments involving tumor viruses or novel combinations of antibiotic resistance genes; that the NIH assess risks of recombinant DNA and establish guidelines for experiments; and that an international conference be convened so that involved scientists could discuss the problem.

Up to this point, the safety question had remained firmly in the hands of scientists, with little or no involvement of anyone else. Now, however, the NAS decided—with Berg's concurrence—to hold a press conference, at which the committee's recommendations would be made public. Cohen and Boyer disliked the idea of calling a press conference, feeling that the public arena was the wrong place to debate scientific issues as complex as the safety of recombinant DNA. While the putative dangers were not supported by scientific evidence, they thought scientists should proceed cautiously with recombinant DNA. The subsequent international meeting, held in February 1975 in Asilomar, California, distressed them even more. "[T]he Asilomar conference was a nightmare," Boyer said. "There were a lot of accusations and shouting from the floor. [It was] an absolutely disgusting, . . . exhausting week. I was so upset . . . I couldn't sleep. . . . [T]he whole thing was counterproductive."[2] Cohen's response to the conference revealed a similar disgust. "What I did not expect was the almost religious fervor. . . . [It was] not so much a meeting called . . . to address an issue . . . , but rather . . . an emotionally uplifting event. The mood among some of the organizers was self-congratulatory. [A]n issue . . . raised because of scientific concerns [was] taken out of the hands of scientists and . . . turned into a sort of witch hunt. [P]eople who had contrary positions were afraid to say so. It was the first time I had encountered a situation where scientists were fearful of speaking their mind about

scientific issues. [A] steam roller had taken over."[3]

Although the organizers tried to argue that the conference reached a consensus, the "acrimonious discussion" Cohen observed in the final session argued to the contrary. As the meeting ended, the organizers asked for a vote approving a document they would write after the meeting. "I . . . voted against giving [them] . . . authority to prepare post hoc a statement that would be presented as the 'consensus' of the group at Asilomar."[3] Voting against senior figures like Paul Berg and David Baltimore was a brave gesture, joined by few other attendees. Indeed, Cohen saw the uplifted hands of only two other scientists voting with him—James Watson and Joshua Lederberg,[3] each of whom had been awarded a Nobel Prize.

The organizers' recommendations, once published, proved relatively mild.[24] They proposed that the informal moratorium on recombinant DNA experiments should cease as soon as an appropriate national "body" was able to formulate clear guidelines matching levels of caution to levels of risk, and suggested rules for scientists to follow in the interim. In October 1974, the NIH established the Recombinant DNA Molecule Program Advisory Committee—RAC, for short—to set up the actual guidelines.

Now we know that the danger of recombinant DNA research, in and of itself, was (and is) close to zero. As Berg and Mertz wrote in 2010, "In the over three decades since adoption of these various regulations for conducting recombinant DNA research, many millions of experiments have been performed without reported incident [or] documented hazard to public health."[17] The RAC guidelines themselves have been revised to reduce the degree of precaution required for most kinds of experiments. In hindsight, the safety controversy can appear moot or even comical, and the resulting biohazard committees little more than, as Boyer put it, "an incredible waste of time and money."[2]

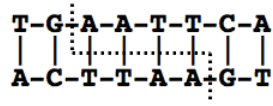
Disagreement persisted nonetheless, because the issue was always as much a public, political problem as a scientific one. One side saw Boyer, Cohen, and their allies as arrogant, narrow experimentalists, loath to delay the gratification of immediate experiments and scientific success, while the others pictured Berg and his allies as posturing pseudo-saints, cloaking themselves in sanctimony. My view, as a non-expert, is that a public and judiciously cautious course was the better alternative. Yes, from the point of view of "pure science," the controversy was simply a waste of money and time. Science, however, is rarely pure and never simple.[25] It was prudent for scientists to take charge of the issues beforehand, rather than to risk possible politician-formulated rules that could hobble scientific investigation for decades to come.

## The Power of Synthetic DNA

Herb Boyer cared less about safety politics than about what was happening in the lab. He could always recognize good ideas, and proved a master at latching onto them and finding exciting ways to make them work. Between 1975 and 1980, he would encounter many new ideas. Of these, the first two—one good, one truly marvelous—introduced him to synthetic DNA. Together, they worked superbly.

The good idea came after Boyer's lab determined the sequence of the EcoRI cleavage site, in a conversation at a Cold Spring Harbor meeting with Hamilton Smith, a Johns Hopkins scientist who had purified the first restriction enzyme, HindII, and identified the sequence it cleaves.[26] (Unlike EcoRI, HindII cuts straight across a different sequence in complementary DNA strands, leaving blunt ends rather than sticky ones.) Smith mentioned that he was about to ask someone to synthesize a short stretch of DNA matching the cleavage site for HindII. Immediately after returning to San Francisco, Boyer told his colleague, Howard Goodman, "We need to get some chemically synthesized DNA"—specifically, a short stretch of chemically synthesized DNA containing the EcoRI cleavage site, GAATTC.[27] Goodman persuaded a chemist at Roche Pharmaceuticals in New Jersey to make an octanucleotide (TGAATTCA) containing the sequence Boyer asked for—a task he performed "on the sly," because Roche frowned on the notion of its employees making chemicals for their friends. The bases pair with one another, following the A-with-T/G-with-C rule, so that the octanucleotide complements itself to form a short piece of double-stranded helical DNA. A sequence may sometimes complement itself because, as described in Chapter Four, base sequences of complementary strands run in opposite directions.

(Dotted lines indicate the sites at which EcoRI cleaves the two strands.)




Test tube experiments with the octanucleotide—"We had a ton of it!" Boyer said—confirmed its susceptibility to cleavage by EcoRI, but were not especially exciting.[27] Then came the truly marvelous idea, born in a pivotal conversation with Arthur Riggs, a molecular biologist with a boundless capacity for generating fertile

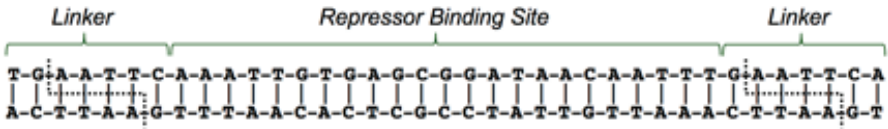
ideas.[28] Boyer had just given a seminar at The City of Hope, a research institute in southern California, where Riggs was his host. They had known each other for years, and Riggs had spent several months in Boyer's lab to learn new recombinant DNA techniques.

Riggs was known for his pioneering studies of the best-understood example of gene regulation in biology, the euphoniously named "lac operon." For much of the past decade, Riggs had been fascinated by the ability of *E. coli* to orchestrate its preferences for nourishment by different sugars. If an *E. coli* bacterium, happily making its living by consuming glucose, is suddenly faced with an environment that supplies a different sugar, lactose (an abundant sugar in cow milk), it adapts by increasing its ability to use lactose. In the absence of lactose, a protein called the "lac repressor" is bound to a short DNA sequence, thereby preventing a gene nearby from producing a lactose-using enzyme, β-galactosidase (aka β-gal). Replacing glucose with lactose causes the repressor to dissociate from its binding site, unleashing production of the lactose-using enzyme by the β-gal gene.[29]

By 1973, science had progressed to the point that Riggs could entertain a quite specific version of the misty dream Boyer had brought to California eight years earlier—that someday he would understand how proteins bind specific DNA sequences. Specifically, Riggs proposed to solve the 3D structure of a protein-DNA complex, that of the lac repressor protein bound to its DNA binding site. A serious snag remained, however. Riggs and his collaborators would have to crystallize the complex, which would require large amounts of pure repressor and repressor binding site. Riggs's lab had purified the protein, but not the DNA, so he was thrilled when his department chair showed him an application for a faculty position from a brilliant young chemist, Keiichi Itakura. Riggs remembers that Itakura's application mentioned, almost as an after-thought, that he knew how to synthesize the repressor binding site chemically.[30] The department offered Itakura a position and, after a short delay,[31] he came to California.

Their collaborative effort stalled, however, because Itakura's chemically synthesized DNA, twenty-one nucleotides long, wasn't quite pure and abundant enough to make crystals in association with the repressor protein. Thinking they might make enough pure operator by cloning it in bacteria, Riggs invited his cloning buddy, Herb Boyer, to give a seminar at City of Hope.[30] After the seminar Riggs unfolded his plan for solving a co-crystal of the repressor, together with the repressor binding site—which Boyer recognized as "everything I had originally been interested in doing with protein-DNA interactions."[27] As they discussed how to clone the lac DNA, Boyer remembers, "I said, 'Make the sequence [with the EcoRI] cohesive ends on it and

we'll clone it.'" Boyer's seminar had just described the octanucleotide that included the EcoRI cleavage site, so Riggs suggested using a DNA ligase to connect the octanucleotide's blunt ends to those of the 21-nucleotide binding site DNA. Then they would treat the resulting 37-nucleotide DNA (8 + 21 + 8 = 37) with EcoRI, insert it into a plasmid's EcoRI cleavage site, and clone it. Boyer objected that no known ligase could attach blunt ends of two DNA sequences to one another. Despite claims to the contrary, Riggs replied, a viral enzyme called T4 DNA ligase would do the job nicely—as he had just learned from a brilliant molecular biologist at Harvard, Wally Gilbert.[32] "So we tried it," Boyer said, "and it worked."[27] The resulting construct looked like this, with the repressor binding site in the middle and a "linker" at each end containing the EcoRI cleavage site (dotted lines):




Treating this synthetic nucleotide with EcoRI produced a repressor binding site flanked by cleaved EcoRI sites and their sticky ends, ready for insertion into an EcoRI-cleaved site in any plasmid. Boyer and Heyneker, a young Dutch biochemist in his lab, inserted it into a plasmid that would make thirty copies of itself (with the repressor binding site) inside the bacterial cell, and devised a clever stratagem—which they hoped would work—to detect *E. coli* colonies containing the repressor binding sites. If the normal bacteria incorporated thirty repressor binding sites, they would turn blue.[33]

The moment of truth came in February 1976. As Boyer later described it, "We were in the lab late one night, around nine or ten o'clock, and Herb Heyneker had done the experiment that morning. He brought [bacterial culture] plates in to look at. We knew there were clones if the colonies turned blue. Herb said, 'It didn't work.' I said, 'Let's look a little bit more closely.' If you looked *real* closely, it was very obvious that they were turning blue. They were very faint, but there were quite a few blue colonies on the plate."[34] Heyneker had expected a more dramatic blue, but says "the project changed my life."[35]

The experiment's principal value, as Heyneker, Boyer, and Riggs would probably agree, lay in its use of synthetic DNA. In Heyneker's words, "it was the first example

[in which] DNA . . . chemically synthesized from off-the-shelf chemicals could be stitched together. . . [and] you create a biologically active entity [from] a very small beginning."[35] In this way, a short 21-nucleotide DNA sequence altered a behavior of an entire micro-organism. In a more narrowly practical sense, Riggs, Boyer, and their colleagues had invented an immensely versatile and useful strategy for stitching previously unattached stretches of DNA to one another. The octanucleotide Boyer had stashed in his freezer became the first of many linkers. Any biologist who uses recombinant DNA technology—most biologists, that is—regularly uses similar linkers, each based on a specific DNA sequence recognized by a different restriction enzyme. Each enzyme, one of dozens in nature, recognizes a unique DNA sequence. As we shall see in the next chapter, man-made DNA was to play a pivotal role in the birth of biotechnology.

Boyer's delight in linkers and synthetic DNA furnished a welcome counterpoint to churning emotions elicited by the 1975 Asilomar conference on dangers of recombinant DNA research. In the same years, he also faced the gradual, uncomfortable unraveling of his long-term collaboration with Howard Goodman. Working together since 1971, the two had become good friends.[2] They wrote grant applications together, their labs held joint lab meetings, and Goodman's technical expertise in DNA chemistry and sequencing had made his friend glad to list him as an author of almost every paper from the Boyer lab. Goodman was not a co-author on Boyer's 1973 paper with Cohen, however, to which he had not contributed. To Goodman's protest at being omitted from the list of authors, Boyer replied, "Howard, we've got another one coming. We'll put your name on it."[1] And in fact, despite Cohen's disagreement, he did.[2]

Later, Goodman became upset that he was not invited to a joint meeting of the Academies of Science of the US and the USSR, held in Russia, which Boyer and two other UCSF faculty members attended. (The other two were Christine Guthrie and Michael Bishop, the first a member of the Biochemistry department, the second—whom we shall meet in Chapter Seven—from UCSF's Microbiology department.) By 1976, about the time he was starting Genentech, Boyer "just didn't want to fight about authorship anymore," so he and Goodman "decided that it was time to go our own ways."[1] At UCSF, Goodman's lab was to play a pivotal role in the race to clone the insulin gene, described in Chapter Six. Later, in 1980, he took a position at Harvard.

## Despite "Fracture," Biochemistry Hires and Thrives

In 1975, as Boyer was juggling exciting experiments, a nascent safety controversy,

and troubles with a colleague, Bill Rutter faced the daunting challenge of guiding a Biochemistry department that reeled from grief at the loss of Gordon Tomkins. Looking back on the subsequent seven years of Biochemistry's history, Rutter sees both a dynamically growing, productive scientific enterprise and a "real fracture in the department"[36] caused by the unexpected opportunities and risks of recombinant DNA. Deferring the impacts of recombinant DNA on Boyer, Rutter, and their colleagues to Chapter Six, here I shall describe the growth of the Department of Biochemistry as a scientific enterprise.

## Table 3: Biochemistry faculty recruits, 1975-1982

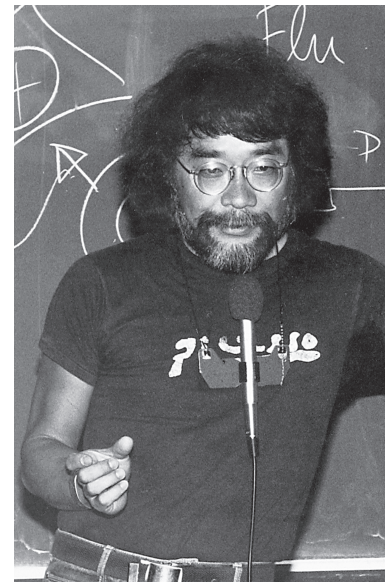
| Year | Name | Initial Field | Future Direction |
|---|---|---|---|
| **1975** | Herbert Boyer | Restriction endonucleases DNA cloning | Transferred from Dept. of Microbiology (hired 1966); retired 1988 |
| **1976** | Keith Yamamoto | Action of adrenal steroid hormones | Pharmacology Chair (1994-2003); Vice Dean, Medicine 2002-present |
| | Bruce Alberts | Chromosome replication | Dept. chair, 1985-1990; NAS Pres,, 1993-2005 now Editor, *Science* |
| **1977** | John Sedat | Chromosome structure | Organization of the cell nucleus; light microscopy |
| | Robert Stroud | Protein structure | Protein structure |
| **1978** | Robert Fletterick | Protein structure | Protein structure |
| | Marc Kirschner | Cytoskeleton | Cell and developmental biology; moved to Harvard, 1993 |
| | Thomas Kornberg | Fruit fly development | Developmental biology |
| | Patrick O'Farrell | Fruit fly development | Developmental biology |
| **1981** | Ira Herskowitz | Yeast genetics | Chair 1990-1995; died 2003 |
| **1982** | David Agard | Protein structure | Chromosome and protein structure; light microscopy |
| | Peter Walter | Protein traffic *via* cell organelles | Protein traffic; chair 2001-2008 |

In his energetic first four years (1969-73), Rutter brought eleven faculty members into Biochemistry (Chapter Three). After a short hiatus, in the seven subsequent years, from Tomkins's death in 1975 until his own retirement from the chair in 1982, Rutter hired twelve additional faculty, beginning with Herb Boyer (see Table 3). In at least one way, the two cohorts differed. Of the eleven Biochemistry faculty hired before 1973, six were gone by 1980—five moved to positions elsewhere, and Tomkins died. In contrast, of the twelve Rutter hired between 1975 and 1982, nine remain productive faculty members at UCSF in 2010—thirty-one years, on average, after joining the department. Of the others, one moved to Harvard after fifteen years, one retired to private life and one died (each of the latter two after twenty-two years at UCSF; see Table 3, above).

The difference may indicate that Rutter learned better how to judge the staying power of faculty candidates, or that several early hires were more willing than most to risk joining an unproved department, a risk-taking tendency that may also have accelerated their later departures. My conversations with faculty who stayed on suggest a third explanation—that sharing the heady joys of the DNA revolution, despite its conflicts and troubles, made the department a very exciting and satisfying place to work after 1974, and the faculty became bound to one another and to UCSF by pride in their joint experience and accomplishments, as well as the new culture they created.

Three decades later, recalling what he was trying to do with Biochemistry back in the 1970s, Rutter said he was trying to develop "human medicine." Because he "didn't exactly know where the breakthrough was coming, [he had] . . . to keep [his] tentacles more or less unprogrammed and receive all signals, and then [had to be able to] refocus as sharply . . . and as quickly as possible." Moreover, he noted, young scientists he hired should be smart and work hard, but they should also be free to tackle problems without heavy administrative supervision and open to collaborating and exchanging ideas. Finally, he admitted, sometimes he had to be "kind of ruthless about people who were not productive or . . . not contributing."[36] My interviews suggest that most of his faculty would judge Rutter's description accurate.

In choosing a new faculty member, Rutter's practice was to invite suggestions from all everyone, make sure the candidate was thoroughly vetted and discussed, and try to develop a department consensus—all without a yea-or-nay vote. Once the right choice had been made, he would apply his formidable charm to get the individual on board. A few months after Gordon Tomkins's death, such a process led to Rutter's initiating a conversation with a brilliant young scientist named Keith Yamamoto. As a postdoc in the Tomkins lab, Yamamoto had pioneered genetic approaches to under-




Keith Yamamoto, a postdoc in the Tomkins lab, joined Biochemistry in 1976. He is now executive vice dean in the School of Medicine and vice chancellor for research. Photo taken in 1982 at the Cold Spring Harbor Laboratories in New York.

standing how steroid hormones regulate gene expression in mammalian cells. Rutter faced an uphill battle because Yamamoto, after considering multiple job offers from leading research universities, had just accepted a faculty position at MIT. Yamamoto remembers thinking that "it wasn't that good a deal to have Bill saying 'Stay.'" In fact, he adds, "I really didn't know what was going to happen. Everybody felt very nervous about [Biochemistry. It] was a small department—Bill and Gordon and a bunch of other people they were bringing in. And suddenly [Tomkins,] the charisma guy who was really able to get anybody to come that he wanted to, was gone. So what was going to happen to the place?" Rutter's argument began with praising MIT, but then pointing out that at MIT "I would be the 44th rung in the best 44-rung biology ladder in the country, whereas if I stayed here, then I would be involved in every single decision that was made in the department, from that day, that . . . no decision would [be] made without my being able to say what I thought."[37]

Then Rutter posed a crucial for-instance question: "Who do you think should be recruited next?" Quickly, Yamamoto suggested Bruce Alberts, who had been his PhD thesis adviser at Princeton. "Bruce would be the person that would most put my mind at ease," he told Rutter, who "immediately seemed to resonate to that. [I]t made me think, well, if that could happen, then I could see trying this. That was very exciting."[37]

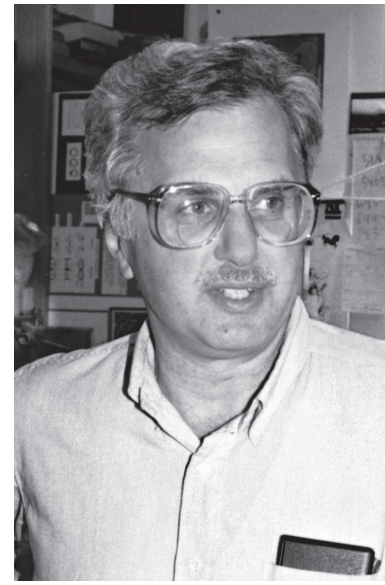
Yamamoto does not know whether Rutter was already thinking about hiring Alberts, who would be a superb choice as a scientist because of his outstanding work

on how a bacteriophage orchestrates replication of its DNA. Rutter had additional reasons for hiring Alberts, who was widely respected for his collegial style and devotion to teaching and scholarship.[37] First, recombinant DNA was creating a widening "fracture" in the department, in which one faction had what Rutter calls "a real mean attitude about anybody who wanted to do anything that was not purely academic." At this stage "anybody" meant mainly Herb Boyer, who was being attacked for using his UCSF lab to do experiments for Genentech (see Chapter Six). But Rutter—who was already planning to clone the medically important insulin gene—could readily imagine that he and Boyer might soon be tarred with the same brush. Certainly, at some future time "things were going to change dramatically, and I should be prepared to . . . move on. . . . I wanted to get on with my own stuff." If he were to leave, Alberts would make a superb academic leader. So, in 1975 Rutter began working to hire Alberts, as well as Yamamoto. As he describes his strategy, "I wanted Bruce to be recruited by the faculty, so quite deliberately I was in the background. The factions which existed at that time, I did not want to persist. And thank God that happened!"[36]

In January 1976 Yamamoto chose UCSF Biochemistry, rather than MIT. (He still feels bad about letting down the chair who hired him at that institution, who was justifiably furious.) A few months later, Alberts joined the department as well. Yamamoto, who also worried about a fracturing department, as well as its relative lack of interest in graduate education (which was not one of Rutter's priorities), pushed for hiring Alberts because he would bring the department "the same focus on education and departmental coherence I had seen at Princeton."[37]

In hindsight, Yamamoto sees an even more compelling argument for hiring Alberts in 1976—Alberts, the right kind of strong leader for Biochemistry and for UCSF, arrived at precisely "the right moment." Together, Rutter and Tomkins made a superb combination—with Rutter as "the operator, a real tactician, with very good taste in people, . . . scheming and plotting ways to pull resources together, which he was fantastic at," and Tomkins as the charismatic intellectual leader, who "made everyone feel like they were fantastic. . . . All of us." Then, soon after Tomkins's death, "Bruce [stepped] in at exactly the right time for his kind of leadership. [He is] one of the few scientists where everyone would agree he's completely above reproach. He doesn't do anything for himself, . . . a very, very, very unusual person. So when he would say, 'We should do things together and work together,' . . . if Bruce said it, that's what he meant." In contrast, "If Bill said that, he was scheming to get full power, right?"[37]

Some of his colleagues share a similar image of Rutter, whom they see as an



Bruce Alberts, biochemist and cell biologist. He joined the Biochemistry department in 1976, became its chair in 1985, and in the 1990s became a national scientific leader. The photo was taken in his lab in the late 1970s.

unusually skilful, ruthless, and manipulative schemer. One faculty member told me, without qualification (but also not for attribution), that he joined Biochemistry despite the fact that he did not respect or trust Rutter, and that he still doesn't. Nonetheless, many of Rutter's critics qualify the schemer image, arguing that it is correct but incomplete. His clever schemes, they contend, focus on getting good things done, and he inevitably promotes high-quality science, never acting from spiteful or malevolent motives. Rutter knows and understands the criticisms, I suspect, and may even agree with them. Recognizing his own limitations may have played a key role in his crucial decisions to hire both Tomkins and Alberts. Uncannily adept at judging unique qualities of others, he is smart enough to have seen how they could complement his own deficiencies and help to make Biochemistry the extraordinary enterprise it did become.

According to Yamamoto, Alberts "became a leader the day he set foot in the place, . . . [with] an immediate impact in bringing an education perspective."[37] In fact, Alberts was recognized as a leader not only in Biochemistry, but elsewhere as well, at UCSF and nationally. He formally took the chair of Biochemistry in 1985, after Rutter retired from it three years earlier. In 1993, Alberts moved to Washington DC to become president of the NAS, and returned to a faculty position in Biochemistry 12 years later, in 2005. Since 2007, he has also served as the editor-in-chief of *Science* magazine. In choosing new faculty, Rutter sought "people who were moving towards molecular biology, focusing on systems which were human-oriented but also building

in technological diversification."[36] The nine additional individuals he hired between 1977 and 1982 (see Table) included an expert on structure of chromosomes; two skilled at analyzing the 3D structures of proteins; three geneticists (two studied genes of the fruit fly and one worked on genes of budding yeast); two cell biologists (who studied the cell's internal "skeleton" or transport of proteins into, out of, and within cells, via internal vesicles); and a structural biologist who also developed innovative light microscopy.

More important than the fields they represented, the new faculty all met Rutter's primary criterion by producing excellent science. Rutter's superb taste in choosing scientists brought to UCSF individuals who excelled by a quantitative criterion—the proportion of faculty members elected to membership in the prestigious NAS. Thirteen of the twenty-three faculty Rutter hired from 1970 to 1982, including ten of the twelve he hired after 1975, were elected to NAS membership. Moreover, four of Rutter's hires shared with him and with Alberts a remarkable capacity for leadership—a rare quality, quite distinct from scientific ability. In the 1980s, each of these four inspired and led their departmental colleagues, but also played critical roles in extending the aura of scientific excellence beyond Biochemistry to include other basic science departments at UCSF. Two, Ira Herskowitz and Peter Walter, served as Biochemistry chairs after Alberts moved to the NAS. A third, Keith Yamamoto, took the chair of the Department of Pharmacology at UCSF in the early 1990s, and subsequently assumed a vital leadership position at UCSF, where he serves as both vice chancellor for research and executive vice dean in the School of Medicine. The fourth, Marc Kirschner, a brilliant young cell biologist attracted to UCSF by Bruce Alberts, his faculty colleague at Princeton, would spearhead formation of a cell biology graduate program at UCSF. In 1993, he moved to Harvard Medical School, where he has founded not one, but *two* outstanding academic departments.

The growing size and excellence of Biochemistry's faculty in the 1970s made it all too obvious that UCSF's other basic science departments—Anatomy, Microbiology, Pharmacology, and Physiology—were failing to thrive. The old-fashioned style and attitudes of their faculty, and especially of their chairs, proved no match for Rutter's energy, capacity for innovation, passion for excellence, or knack for capturing any lab space that was not optimally used. A general space crunch, afflicting the entire campus, made it hard for Dean Krevans to hire new chairs for these departments, and similarly limited incumbent chairs' ability to attract first-rate faculty. Still, the space crunch was probably not the main obstacle to rejuvenating the other basic science departments. Indeed a new dean, with help from new Biochemistry leadership, hired new chairs and fostered much-needed change in *all* these departments in the latter

half of the 1980s, without providing new space for laboratories. Instead of the space crunch, it seems more likely that Biochemistry's extraordinary success during Rutter's reign abetted persistent inertia elsewhere, both by consuming limited resources and by furnishing the dean and the School of Medicine a source of justifiable pride, which effectively drew attention away from torpor and inanition in other departments.

Rutter sometimes exerted a salutary impact on another department by offering a joint membership in Biochemistry to selected members of its faculty. In the latter 1970s, for instance, he offered joint membership to two Microbiology faculty members, Michael Bishop and Harold Varmus, who were famous for discovering the first bona fide cancer gene a few years earlier (see Chapters Seven and Eight). Biochemistry's growing reputation for excellence also made joint membership a coveted perquisite, valuable in recruiting new faculty. In 1976 Rutter also offered joint membership in Biochemistry to the bright young neurobiologist, Zach Hall, who was being recruited by Physiology to head an interdepartmental Neuroscience Graduate Program, one of the first of its kind in the US.

Hall's subsequent attempt to attract and retain one of his own first recruits gives the flavor of Rutter's hard-nosed persistence and flair for beating the odds. Based on their credentials and interviews, two candidates for Hall's Neuroscience position appeared impressive, and Rutter told Hall he would be glad to offer either A or B a joint appointment. After due deliberation, Hall offered the position to A, who said yes. Another school offered a financially more attractive startup package, however, persuading A to turn down UCSF's offer and commit himself to the position elsewhere. Disappointed, Hall decided to offer the position to candidate B. He told Rutter, who replied that he would not offer B a joint appointment, despite Hall's reminder that he had been willing, earlier, to welcome either candidate. Rutter suggested that Hall augment the offer to candidate A instead, but Hall replied that Physiology didn't have the money. In that case, Rutter advised, get the extra money from the dean—an idea Hall had not considered, because it would never have worked at Harvard, the school he came from. But he asked, and the dean did help. As a result, A changed his mind again and came to Neuroscience, jointly appointed in Biochemistry.[38] By dangling a joint appointment, urging persistence in the face of apparent defeat, and knowing where the money was, Rutter—unfazed by minor scruples about baldly reversing an earlier promise—pushed another department's academic decision in what he deemed a better direction.

## Perspective

Among the influences that led to kindling the DNA revolution, one of the most important was simply that the time was right. Many bright, ambitious scientists were asking the same set of questions and considered them ripe for plucking, if only someone could devise a simple, generally applicable approach to isolating specific DNA fragments and propagating them to make multiple copies—exactly the approach Boyer and Cohen came up with, despite formidable competition from colleagues who might have appeared more likely winners—and who occasionally provided critical help, as when Mertz and Davis discovered that cleavage by EcoRI leaves sticky ends. We have seen how the initial lure of a very basic biological question—how do proteins recognize specific DNA sequences?—led eventually to Boyer's trifecta. EcoRI, a key to the trifecta, was very much his own contribution, but the others came from colleagues—Stan Cohen, the brilliant collaborator who knew exactly the right kind of plasmid to use for isolating recombinant DNA sequences and how to characterize recombinants with the electron microscope, plus the team of Sharp and Sambrook at Cold Spring Harbor, who uncovered the magic of ethidium bromide.

Boyer's local environment at UCSF was a thoroughly mixed bag. Early on, the institution gave Boyer little in the way of material resources or a stimulating and encouraging intellectual environment. His local colleagues were not helpful or even faintly interested in anything he did. Certainly he didn't get the treatment accorded budding experimental biologists in the twenty-first century, for whom a first-class research university furnishes state-of-the-art research facilities, generous financial support, superb colleagues, brilliant students, rich seminar programs, and opportunities to travel to frequent meetings. But for Boyer, at least from 1966 until about 1973, minimal interest and low departmental standards, combined with an NIH funding environment more generous than the one US biologists currently enjoy, may have furnished just the right combination—low-pressure environment, freedom to do whatever he wanted, and little interference from others—for a young man struggling with experimental approaches that stubbornly failed to work. During the last two of these seven years, Boyer's jumbled local environment changed for the better, and at just the right time. New colleagues—interested, encouraging, and competent—appeared as he was in the process of creating a key technology for recombinant DNA, based on the trifecta that began with EcoRI.

Boyer came to his new job with a good idea, although nobody at UCSF seemed to think so. The fact that restriction enzymes could provide insight into how these proteins recognize specific DNA sequences—insight that would not be achieved until years later—may have been premature in the 1960s, but it pushed Boyer to keep on

seeking an enzyme that cleaves DNA at a definable site.[39] With his unflappable, low-key style, Boyer was both persistent and unusually receptive to good ideas, wherever they originate. The list of other people's ideas Boyer embraced and exploited effectively is long, beginning with Noel Bouck's suggested explanation of the funny recombination patterns in K x B/r crosses and extending to and beyond the founding of Genentech, described in the next chapter.[40] Scientists sometimes argue that using someone else's ideas reflects deficient imagination, but Boyer's knack for connecting with other scientists and their ideas amounts to real genius. Once he grasped Cohen's arguments in favor of working with plasmids, Boyer leaped at the opportunity. The two were intrigued, not daunted, by differences between a bright but laid-back Catholic kid from the other side of the tracks, fascinated by racing cars, and a brilliant, meticulous, resolutely assertive, and intellectually demanding Jew.

It may be instructive to contrast the effectiveness of the Boyer-Cohen collaboration with the unproductive relation between Cohen and Berg, both at Stanford. Cohen, who worked just down the hall, was allowed to borrow enzymes from freezers in Biochemistry, Berg's department, but the department denied him a joint appointment because his clinical training and affiliation were thought to make him unfit for work in a basic science department.[3,41] Berg passionately wanted to recombine DNA fragments in ways that would allow him to understand genes, so it should have been an easy leap to combine the Berg lab's idea for creating sticky ends with Cohen's idea for taking advantage of the unique properties of plasmids. Why, I asked Cohen, did he and Berg never consider working together, although each had ample opportunity to learn what the other was up to? Among other reasons, he said, referring to Stanford's Department of Biochemistry, "I don't think they would have wanted to do that. These were world-famous scientists in the leading department of biochemistry in the world. I was a physician studying a quiet area of infectious disease, antibiotic resistance. When I first came to Stanford and talked with Arthur Kornberg, Arthur said, 'Why are you studying plasmids?' It was an elitist group of people, so it didn't create a culture for active collaboration."[42] Cohen, like Boyer, actively sought collaborations and new ideas, but appears to feel that he could not scale barriers raised by Stanford's Biochemistry department.

Bill Rutter, of course, was trying to make a Biochemistry department in which collaborations could flourish, but he had lost a charismatic catalyst for exchanging ideas, Gordon Tomkins, and his department would be nearly fractured by faction and fierce competition, as the DNA revolution inspired several of its members to engage in a race to clone an important human hormone, insulin. We'll hear that story in Chapter Six.

Chapter Six

# A Transforming Harvest

## The Revolution's First Fruits

From 1976 to the early 1980s, dramatic episodes in the early history of the DNA revolution unfolded at (or near) UCSF. Awash in excitement, glowing promise, and a certain amount of *Sturm und Drang*, Herb Boyer, Bill Rutter, and their colleagues in the Biochemistry department competed in a major race to clone human genes. In addition, Boyer co-founded Genentech, the first truly successful biotech company, which served as a model for most of modern biotechnology—a still-growing industry that now contributes billions of dollars to the US's gross national product.

### Founding Genentech

As early as 1974, Herb Boyer was thinking about practical applications of the new recombinant technology he and Cohen had devised.[1] In addition to basic biological questions, he imagined it could be applied to commercial production of antibodies and hormones like insulin. By August 1975, Boyer came up with a more definite proposal, for "a general synthesis procedure" to make hormones like angiotensin, a blood pressure regulator.[2] The idea was to apply recombinant techniques to man-made DNA fashioned by chemical synthesis, rather than to genes isolated from animal or human DNA.[3]

Boyer's ideas had not progressed much further in mid-January, 1976, when he received a telephone call from Robert Swanson, who wanted to talk about possible commercial applications of recombinant DNA. Boyer said he would see Swanson for ten minutes, in his UCSF office on the next Friday, which was January 17.

Raised in Florida, where his father was an electrical maintenance crew leader for Eastern Airlines, Swanson had a bachelor's degree in chemistry and a master's degree in business management, both from MIT. He began his career in 1970 as a venture capitalist, investing money in small businesses for a New York bank. After moving to the bank's California office in 1973, he moved again in early 1975, to a job as junior

partner at Kleiner and Perkins (K&P), then a small venture capital outfit south of San Francisco. Late in the year his bosses at K&P told him that in 1976 he would be on his own, although they'd let him use a desk and telephone "until you find what you're going to do." Later, Swanson saw this as a lucky break—"Talk about environmental factors that give you motivation!"[4]

At the age of twenty-nine—a year younger than Boyer was when he took a position at UCSF in 1966—Swanson suddenly found himself interviewing for a new job, and hoping to find an opportunity that involved promising new technology. In one of his last assignments for K&P, he heard about recombinant DNA from someone at Cetus, a recently formed microbiology-based company. The new technology, he was told, was "coming along and is going to be wonderful stuff. You're going to be able to make insulin and other hormones." After some thought, Swanson talked to Cetus for a job on his own. Recombinant technology looked "really exciting," he said, "and I'd like you to hire me to do it." Developing this new approach would take a long time, Cetus replied, so at that point it wouldn't be fair to ask him to tackle it.[4] After reading more about DNA, Swanson talked to the business development group at another company, Syntex, who agreed with Cetus's pessimistic judgment of how long the project would take. But the idea still sounded good to Swanson, so he started making cold telephone calls to prominent scientists in the field. Remembering that Swanson said he listed the scientists alphabetically, Boyer later inferred that Paul Berg's name must have come up before his own.[5] Berg, however, says Swanson did not contact him.[6] Swanson himself wasn't sure exactly who he called, but said most people told him that practical applications of DNA technology were too far in the future. Early in the process, Swanson called Boyer, and the need for further cold calls disappeared.[4]

Both Boyer and Swanson remembered that Swanson began their first conversation by raising the question of whether recombinant DNA could be commercially useful, and that Boyer answered something like, "Sure, why not?"[7] Hours of further conversation unfolded their complementary expertise and compatible views. It is hard to exaggerate the astonishing luck that brought this unlikely pair together. Searching without success for someone interested in commercial application of a new, untried approach, which he felt sure would work, Boyer found a bright young man with enormous drive and untapped ability to build a new company. Swanson sniffed the new technology's potential promise, but his meager knowledge of genetics and biology made it hard for him to counter the deep-rooted reservations of molecular biologists he talked to. In Boyer, by contrast, he found a scientist, of a kind very rare at the time, who combined deep knowledge of the new field with confidence that the


Genentech's founders celebrate Halloween. At a Genentech party in 1984, Herbert Boyer (left) and Robert Swanson danced as jokers from *Alice in Wonderland*. In reality, the hard-driving entrepreneur and the deceptively laid-back scientist made an incongruous but highly-effective team.

right experiments could overcome technical obstacles—and who also saw that the right time to try was now.

Together Boyer and Swanson weighed the relative advantages of different commercial targets for the new technology, concentrating on small protein hormones. They chose insulin as their first target, for obvious reasons—that is, the human hormone's structure (the sequence of its amino acids) was known, and insulin from pig or cow pancreas was already a proven useful treatment for diabetes, a disease whose patients comprised a large, easily defined market. Within a few months the two formed a partnership and crafted a business plan. In mid-April 1976, they put up $500 each to form a new company, named Genentech—a hybrid term that announced its guiding strategy, *Gene*tic *En*gineering *Tech*nology.

Swanson was modestly well-versed in chemistry, extremely bright, and a quick study who asked good questions, but the first scientific decisions were up to Boyer. From the outset he stressed that the company would begin by relying mainly on synthetic DNA, rather than on cloning DNA sequences from natural sources. Boyer based this crucial decision—which he later estimated gave Genentech a five-year head

start over its rivals—on two considerations.[8] First, the successful collaboration with Riggs and Itakura had taught him that synthetic DNA makes it much easier to "edit" DNA sequences to order, as they did to construct the synthetic EcoRI-linker-plus-repressor-binding sequence (see Chapter Five). This kind of editing would be necessary to define starts and ends of the separate A and B chains of insulin and to persuade bacteria to make them. Second, limited experience in his own lab and arduous but so far not very successful attempts in other labs led him to suspect (correctly, it turned out) that a year or more would pass before scientists would learn how to accomplish rapid and facile cloning of natural DNA sequences. In contrast, the synthetic route, though labor-intensive, clearly worked already. A third potential advantage of the synthetic route was not a prime motivation, according to Boyer.[5] This was the fact that public fears about safety of recombinant DNA experiments focused on natural DNA from humans, not synthetic DNA made from off-the-shelf chemicals. This subtle and scientifically indefensible distinction would later provide Genentech a significant advantage over its main rivals.[9]

Relying on synthetic DNA meant inviting Arthur Riggs and Keiichi Itakura to join the effort. Riggs's enthusiastic response came with a disturbing new suggestion. For their first effort, he proposed that they make a 14-amino acid hormone called somatostatin, because the synthetic DNA sequence required would be shorter than that for either of insulin's two chains (twenty-one amino acids for the A chain and thirty for the B chain).[10] In fact, Riggs had already applied for an NIH grant to make somatostatin. The application would soon be turned down, because NIH reviewers thought the project would take too long, and objected that it "seems like just an intellectual exercise."[11] But Boyer immediately saw it as a useful "proof-of-principle" experiment, which would teach the experimenters how to make a protein and also put the new company on the map. Then, as Riggs later recounted the story, "It was Boyer's job to convince Robert Swanson we should do somatostatin, and that was not easy."[11] Concerned about precarious funding for a fledgling enterprise, Swanson worried that making somatostatin, with no obvious medical use, would delay making insulin—a medicine that most certainly *would* produce value for the company. With considerable difficulty, Boyer persuaded him that somatostatin would establish feasibility of the new technology, giving the company much-needed visibility and credibility.

The project proceeded rapidly, with Riggs and Itakura making the DNA in southern California and Heyneker recombining it into appropriate plasmids in San Francisco. Several months later the bacteria had incorporated the right plasmid, and they were ready to measure whatever somatostatin the bacteria might make with an

antibody-based procedure good enough to detect as few as six hormone molecules per *E. coli*. Swanson and the experimenters gathered at City of Hope to celebrate, but to everyone's dismay the antibody detected no somatostatin whatever. Devastated, Swanson looked "deathly white," said Riggs, and felt sick enough to check briefly into a hospital. The bacteria did make hormone, it turned out, but in a form that allowed them to degrade it immediately. Three months later, the team had devised a way to produce hormone in a more stable form, so the bacteria could accumulate plenty of somatostatin.[12] With his company firmly back on track, Swanson's face could regain its normal healthy color.

As scientific strategy, Riggs's idea to tackle somatostatin before insulin gave the new company indispensable opportunities to learn, by asking it first to crawl before running the more complex gauntlet of obstacles posed by the bigger hormone. "If we had skipped somatostatin," Riggs said later, "we would not have won the race."[11] As a business strategy and as a nudge for shifting political winds from fear to anticipation of a nationally profitable new industry, production of somatostatin in bacteria was welcome triumph, and a necessary one.

## Discord in Biochemistry

Closer to home, however, Genentech's experiments triggered argument and disarray in UCSF's Department of Biochemistry. The local controversy began in the summer of 1976, at about the same time the City Council of Cambridge, Massachusetts was debating the moratorium it imposed on recombinant DNA experiments. A Harvard Nobelist argued for extending Cambridge's moratorium, complaining that, "We're asked to turn over the products of three billion years of evolution to a group of scientists eager to play around with them."[13] Instead of threats to evolution, argument in Biochemistry corridors at UCSF focused on Genentech experiments being performed in Boyer's lab, which some considered a threat to the moral integrity of academic science. Boyer had the blessing of Bill Rutter, who told him, "You should do it. Good luck. I've always wanted to start a company myself."[5] Opponents, however, were unswayed by the fact Boyer and Swanson had obtained the necessary University approvals for these experiments and for Genentech to pay salaries of two postdocs, plus a stipend to Boyer himself.

The fiercest and most articulate criticism of Genentech's experiments in a Biochemistry lab came from the department's least senior faculty member. In faculty meetings, written articles, and a television interview, Keith Yamamoto accepted the need for industry to exploit new discoveries, but argued that the University was terribly mistaken in allowing academics to do company experiments in its own facilities

and to profit from the resulting discoveries. He cited several dangers: the prospect of monetary gain could shift the faculty's focus from teaching and seeking new knowledge to competition for profit; working on company projects could lure graduate students away from research focused on fundamental questions; free communication of ideas in academia could be compromised by the need to protect valuable intellectual property.

More than three decades later, Yamamoto realizes his arguments were naïve and wrong, not just because he over-estimated the dangers. For instance, he points out, graduate students chose not to join labs of biotech entrepreneurs who were not committed to graduate education, flocking instead to labs of faculty who cared more about teaching them. In fact, academia proved able to control this and other dangers and still work—as Boyer did in forming Genentech—to transform academic ideas and discoveries into socially useful commercial products. This transformation process now occupies much of Yamamoto's energy in his present role at UCSF as a vice chancellor for research and an executive vice dean. In the 1970s, however, his youthful daring spurred him to take risks and to indulge, at least once, in a carelessly cruel jab. He vividly recalls the television interview in which someone asked, "Are you saying, Dr. Yamamoto, that Dr. Boyer is going to start a company, right here . . . on the premises of the University of California, a state institution?" Quickly, Yamamoto responded, "Well, he didn't hang a sign out." At this point, he learned later, a producer of the program whispered to another listener at the interview, "Does [Yamamoto] have tenure?" (He didn't.)[14]

In the Biochemistry faculty meeting where he first voiced his concern about Boyer's Genentech experiments on campus, Yamamoto was surprised that only one faculty member, Christine Guthrie, overtly joined him. Others strongly disagreed (like Bill Rutter), quietly tried to persuade Boyer to give up his involvement with Genentech (Bruce Alberts), or shifted uncomfortably back and forth on the fence.[5] Despite his power and position, Rutter characteristically made little effort to make life difficult for Yamamoto, and all parties carefully maintained civility, at least on the surface.

His colleagues' negative response hurt Boyer deeply, however. Lacking Cohen's zest for confronting opposition, he found the local controversy "very difficult. I had a lot of anxieties and bouts of depression," he said later, adding, with a laugh, "Here I thought I was doing something valuable to society, something that would make a contribution, and then to have the accusations and criticisms, it was extremely difficult."[5]

Now we know that the anxiety and anger were vastly out of proportion to the danger. While it is tempting to dismiss the controversy as a tempest in a fragile glass

of academic sherry, we can't help wondering what it was that really bothered Boyer's opponents so much. Although we can't know for sure, the unmistakable whiff of academic snobbery in their attitudes, which Boyer calls "intellectual elitism," suggests that the real threat was that of impending change, which was about to engulf biomedical academia.[15] In the pungent words of one UCSF official, "In the 1970s, for an academic to make money from a research project, that was like a priest pissing in the chalice."[16]

If we try to frame the local controversy in the context of social class, it may seem fitting that the putative desecrator of the holy-of-holies was the son of a railroad switchman. As a teenager, Boyer had declared his ambition "to become a successful businessman."[17] Yamamoto, Boyer's nemesis in the Biochemistry fracas, was not cast for his role quite so perfectly—he was a Princeton graduate, to be sure, but one born to parents just released, after World War II, from one of the infamous internment camps in which the US incarcerated its citizens of Japanese origin.[14] In any case, the railroad switchman's son saw nothing whatever wrong with applying his knowledge and expertise for a purpose that promised genuine benefit for society and might prove commercially valuable as well. As Boyer points out, academic scientists in chemistry, agriculture, and engineering were already quite used to interactions with industry and the commercial marketplace at the time.[15] In contrast, earlier basic biological discoveries had produced little of real practical (or monetary) value, so it was easy for biologists to distance themselves from crass commercial pursuits.

Herb Heyneker—Boyer's postdoc, and then Genentech's first chief of molecular biology—emphasizes that biology's estrangement from practical applications had important consequences.[18] "The molecular biology community was not ready for biotechnology or practical applications. . . . [M]ost people were practicing science for science"—that is, in their bones scientists would rather concentrate on understanding how nature works than on developing innovative methods for studying natural phenomena. Even in scientific terms, he adds, "Herb Boyer had a very difficult time to convince the pure academics. . . . [S]ynthetic DNA caught on slowly [because] people were not eager to copy Genentech. There was skepticism that it would work." That skepticism, of course, furnished Boyer and Genentech a real advantage as well. "Only after we came out with a paper on somatostatin would people realize, wait a second, we can make foreign proteins in *E. coli*. [T]hat might have been the start signal for other groups to look at more applied molecular biology. And they did. But by that time, Genentech was off and running."

Similarly, but more broadly, Berg and Mertz recently argued that scientific breakthroughs often depend crucially on "new tools that make possible new approaches

to formerly intractable problems."[19] Indeed, recombinant DNA technology changed the ways that biologists pose questions and find solutions. Early on, it loomed as a threat to many biologists because it was new. When Genentech made somatostatin in *E. coli*, the threat began instead to beckon as an opportunity.

## The Insulin Race: UCSF Wins Round 1

By the time Genentech had expressed somatostatin and began to tackle insulin, two academic teams had already entered the race. All three sets of competitors knew the task would be more challenging than somatostatin, because insulin is bigger and more complex. But the prize should also be more lucrative, providing that human insulin proved superior to cow or pork insulin, as the racers expected.[20] Genentech focused unwaveringly on developing human insulin as a commercially useful drug, while the academic teams—one led by a brilliant molecular biologist at Harvard, Walter Gilbert, the other in UCSF's Biochemistry department—sought to isolate and study the insulin gene itself, hoping that nitty-gritty details of the encoding and genetic regulation of a biologically important protein would furnish clues to puzzles posed by myriad regulatory circuits in complex organisms.

The academic "race" for insulin at UCSF began in late 1975, when the Rutter and Goodman labs were astonished to find that they were competing. A UCSF endocrinologist introduced the visiting seminar speaker, an insulin expert, to a young German postdoc in the Goodman lab, saying, "This is Axel Ullrich. He is . . . attempting to clone the rat insulin cDNA." Overhearing, Bill Rutter promptly stepped up with his own postdoc to announce, "Well, here's John Chirgwin. He's doing the same thing!"[21] Afterwards, Rutter told Ullrich they needed to talk. Then, Ullrich says, "Howard [Goodman] and I were essentially ordered into Bill's office and told, 'If anybody in this department clones insulin, it's either in my lab or in collaboration with me.'" Ullrich adds, "I don't know how Bill operated otherwise, but he clearly knew what was important.'"[22]

Thus began an awkward, uncomfortable collaboration, which was not improved by the uneasy relationship between the chair and the faculty member. Goodman's "uneven temperament," Rutter says, sometimes made him hard to work with, but his contributions were valuable.[23] The two labs brought very different expertise to the project. Although Rutter had worked on embryonic development of the pancreas, he and his lab were by no means expert at molecular biology. At this point, Ullrich wasn't either, but he had been attracted to San Francisco by Goodman's reputation for expertise in DNA technology. In a field so new, however, single-minded determination tended to trump expertise. Indeed, despite repeated clashes the mutual deter-

mination of Rutter and Ullrich combined to overcome daunting obstacles—including the fact that nobody in 1975 had cloned an animal gene, with the single exception of DNA encoding frog ribosomal RNA, incorporated into a bacterial plasmid by Cohen, Boyer, and their colleagues.[24]

While Boyer, Riggs, Swanson, and their colleagues would bet on synthetic DNA, the UCSF and Harvard teams opted for an uncharted course, which they hoped would connect two elusive landmarks. One landmark, called "messenger ribonucleic acid" (aka mRNA) would have to lead to the second, called "cDNA" (complementary DNA), in which the sequence of bases complements that of the corresponding mRNA molecule. mRNAs carry genetic messages from the cell's nucleus to its cytoplasm, where proteins are made. Each of a cell's thousands of different mRNAs is copied ("transcribed") from the sequence of a nuclear gene and transported to molecular machines in the cytoplasm, called ribosomes. Then the ribosomes make the corresponding protein by "translating" the mRNA's triplet codons into the new protein's amino acid sequence. While their structures differ subtly, RNA and DNA are close relatives, so that the sequence of an mRNA can predict the (complementary) sequence of its corresponding DNA, just as complementation allows one strand of DNA sequence to specify (or predict) the sequence of the strand it associates with in a double helix.[25]

To navigate from mRNA to cDNA, the UCSF team would first have to isolate insulin mRNA from the pancreas, in which a few hundred clumps of cells, called islets, make virtually all the body's insulin. They would proceed to the second landmark by converting the insulin mRNA sequence into a cDNA, with a viral enzyme fittingly called "reverse transcriptase." The Boyer-Cohen technology would then persuade a bacterium to make multiple copies of the cDNA. A third step, making insulin itself, remained murky. With luck, the bacterium might make insulin, but the hormone is composed of two separate amino acid chains, A and B, encoded by a single gene. Making and separating the two chains might prove a problem.[26] At the outset, the main task seemed to divide itself neatly into two parts—isolating the insulin mRNA and using it to make and replicate the corresponding cDNA. At UCSF, these chores would be tackled primarily by two postdocs, Chirgwin in the Rutter lab and Ullrich in the Goodman lab.

In May 1976, Eli Lilly, a pharmaceutical giant and the world's principal purveyor of cow and pork insulin, hosted an insulin conference in Indianapolis. There the UCSF researchers heard a Boston scientist, William Chick, describe a rat islet cell tumor that made lots of insulin. Realizing that insulin mRNA should be abundant in the tumor, the UCSF team approached Chick after the talk. He said he would gladly

collaborate with them, but soon thereafter Walter Gilbert and one of his colleagues from Harvard also asked for the tumor, and Chick chose to go with Gilbert. Asked if Gilbert's entry into the fray would be a problem, Rutter replied, "No. There's nothing better than a good battle."[27] Still, in losing an apparently convenient source of insulin mRNA, UCSF had acquired a formidable new rival.

The two academic efforts would have been well matched, but fortune soon handed UCSF a big advantage—for months, the Harvard effort was stymied by the Cambridge City Council's moratorium on lab experiments with recombinant DNA. In San Francisco, isolating mRNA from pancreas proceeded slowly, partly because there was so little of it, but also because the pancreas is rich in enzymes (RNAases) that chew up RNAs in seconds after cells are broken. In March 1976, after months of testing possible RNAase inhibitors, Chirgwin found a chemical, guanidinium thiocyanate, that stops RNAases in their tracks. Now the Rutter lab faced the nasty prospect of killing and dissecting a horde of rats to isolate islets, and then mRNA, from their pancreases. Screwing up their courage, the UCSF team decided to kill the rats.

In the fall of 1976, scientists elsewhere suddenly discovered that they couldn't obtain collagenase from commercial distributors. Rutter is said to have bought the nation's entire supply of this digestive enzyme,[28] which breaks down the connective tissue that holds an organ's cells together, and was necessary to separate insulin-making islet cells from surrounding pancreatic tissues. A week's work by half a dozen people harvested 200 pancreases, yielding a tiny white pellet of islet cells at the bottom of a tube. Then Ullrich broke the islet cells in the presence of guanidinium thiocyanate and isolated about thirty millionths of a gram of mRNA—too little, even, to determine how well-preserved the mRNA was, because the test would use up the whole sample.

What fraction of those mRNA molecules encoded insulin, rather than some other islet cell protein? No one knew, but insulin's amino acid sequence was known. The only way to know whether insulin mRNA was in the mRNA fraction isolated from islets was to produce cDNAs from that mRNA, clone them in plasmids, and determine whether the sequence of at least one cDNA matched insulin's amino acid sequence.

By January 1977, Ullrich had made cDNAs from his sample of islet mRNA, and was ready to clone them into a plasmid. He could choose either of two plasmids, both constructed in the Boyer lab. RAC (the NIH's Recombinant DNA Molecule Program Advisory Committee) had approved pMB9 earlier, while pBR322, constructed in August the year before, had just been approved. He chose pBR322, which suited his purpose slightly better.[29] In the latter part of January, Ullrich put EcoRI linkers at

each end of the cDNAs he had made from islet mRNA, inserted those cDNAs into the single EcoRI cleavage site of pBR322 DNA, and put the plasmid DNA into bacteria. Using the plasmid's convenient antibiotic resistance markers, he grew up colonies likely to contain a plasmid with a cDNA insert, isolated DNA from several plasmids, and asked John Shine, a sequencing expert in the Goodman lab, to determine the base sequences of their inserts.[29] In those days DNA sequencing took a considerable time, but Shine eventually found cDNAs that coded for an amino acid sequence identical to that of insulin!

As Ullrich and his colleagues felt victory in their grasp, disaster threatened. In February 1977, it became clear that choosing pBR322 had been a mistake because the plasmid, although "approved" by RAC, had not been formally "certified" by the Director of NIH. pMB9 was certified somewhat earlier. Knowing on scientific grounds that pBR322 and pMB9 were equally safe, the team dithered. Rutter discussed the problem with a highly placed NIH official, but otherwise he, his colleagues, and UCSF chose to stay mum, as institutions usually do in such cases. The team finally made a painful decision, and on March 19 Ullrich poured hydrochloric acid on the pBR322-containing bacteria he had isolated. Starting with extra islet-derived cDNA (transcribed earlier from islet mRNA), he and his colleagues re-did the cloning experiment with pMB9. Three weeks later, Ullrich had a complete rat insulin cDNA, and its sequence confirmed that it really coded for rat insulin. This time the plasmid was legitimately certified. Published in June, the resulting paper described the pMB9 experiments but did not mention pBR322.[30]

## Round 2: Genentech Wins

Cloning the rat insulin cDNA was a real triumph. At first it was marred, at least for Ullrich, by only one fact—Rutter and Goodman received almost all the credit, in preference to him and others, who had done almost all the work. The disparity, which first appeared when UCSF announced the feat at a press conference in April, continued to rankle.[22] Then, in September 1977, the pBR322 affair came back to haunt UCSF's Biochemistry department, in the form of a news article in *Science* magazine, entitled "Recombinant DNA: NIH rules broken in insulin gene project."[31]According to the article, the incident had not exposed anyone to danger, but it also showed that Rutter, Ullrich, and their colleagues had not only misinterpreted the difference between "approved" and "certified," but had also shrouded the entire story in secrecy. The article mentioned department rumors that the pMB9 experiment may not have been performed as it was published, based on the fact that it was completed only 3 weeks after the plasmid was certified. One un-named Biochemistry department

member was quoted as saying, "It is conceivably possible to do such an experiment in three weeks if everything works perfectly the first time, but you know as well as I do that science never works as well as you hope."[31] To this day some at UCSF remain convinced that the rumors were true, but Ullrich and Rutter vigorously denied the charges, which were never substantiated.

Universities and their laboratory scientists found this negative publicity especially dangerous, because it threatened to sway the ongoing national debate in state legislatures and the US Congress about the possible dangers of recombinant DNA and whether it should be regulated by law—a possibility academics wanted very much to avoid. In November 1977, Senator Adlai Stevenson III, a longstanding critic of the NIH, called a Senate Subcommittee hearing to investigate the question of recombinant DNA. Invited to speak at the hearing, Rutter and Boyer planned to describe the promise of recombinant DNA technology and to explain why regulating it by law would not be appropriate. Instead, they quickly found themselves on the defensive in responding to barbed questions about the pBR322 incident. Their part of the hearing was not UCSF's finest hour. Rutter was miffed by the hearing's inquisitional tone and Boyer, who had little to do with the story, except that his lab made the plasmids—was quite dismayed: "We were . . . at the cutting edge of technology," he said, "and doing beneficial things, and here were these guys trying to nail us to the cross."[32]

At a national level, fortunately, the grilling of Rutter and Boyer made little difference. Indeed, public and political views of the controversy were rapidly changing, and soon Congress would find itself celebrating recombinant DNA as a much-needed source of innovation and commercial opportunity rather than condemning it as a danger to public safety. The change came about partly because the vaunted dangers of recombinant DNA failed to appear, but mainly because it became clear that recombinant DNA was more than a promise of pie in the sky. At the same hearings that dismayed Boyer, Paul Berg and Philip Handler, president of the NAS, took a key step in this direction by leaking the fact that Genentech would soon announce it had persuaded bacteria to make a protein hormone, somatostatin. The fact that bacteria were able not only to replicate a DNA, but also to construct a hormone, made it more likely that recombinant DNA would make a difference in the real world.[33]

Now let us return to the race. Delayed by the moratorium, Gilbert's lab cloned a rat insulin cDNA in the summer of 1978, a year after the San Francisco group published its rat insulin cDNA.[34] Both groups were seriously delayed by US regulations for experiments with recombinant *human* DNA, which specified safety conditions almost impossible to find outside military laboratories, and required both labs to move their experiments to Europe. Ullrich took the UCSF effort to France, while the

Gilbert group, which had progressed a bit farther, adopted a preposterously inauspicious venue—a "safe" but extraordinarily inconvenient lab in England, designed to prevent escape of dangerous microorganisms. Worse, the Harvard experiment fell victim to a chance intrusion of an unwanted cDNA, probably derived from a contaminating mRNA (or cDNA).[35] That contaminant must have been derived from rat insulin mRNA, because the Gilbert group re-cloned a *rat* insulin cDNA, instead of the human cDNA they sought. SS Hall's book, *Invisible Frontiers*, vividly recounts the whole tragicomic story.[28]

The winning horse in the race for human insulin, Genentech, was also the last to start. Genentech's success with insulin depended on several key elements, including Boyer's prescient insistence on using synthetic DNA made by Itakura and Riggs, the invaluable experience gained earlier from making and expressing somatostatin, and the appearance of new players who drove the project faster than anyone earlier would have imagined possible. The plan was for the City of Hope team to make DNA encoding separate A and B chains of insulin. The San Francisco group would then clone these DNAs separately in bacteria, by attaching the front end of the DNA encoding each chain to the hind end of DNA encoding β-galactosidase (β-gal) )—that is, precisely the scenario that worked with somatostatin. After cleaving each chain from β-gal, City of Hope would use published procedures—which they practiced with A and B chains from real insulin—for re-folding and connecting the chains to one another as an intact insulin molecule.

Although Boyer later described his role in cloning insulin as "avuncular,"[28] his contributions were essential. First, his choice of the synthetic route for making separate DNAs encoding insulin chains made it easy to tailor each encoded protein to fit neatly into β-gal, and offered the valuable bonus of avoiding the ultra-stringent safeguards that drove the Harvard and UCSF groups to Europe. In addition, Boyer worked hard to make sure that Genentech researchers got appropriate credit for their work. From the outset, against Swanson's strong objections, he insisted that important results in Genentech laboratories be published as soon as the appropriate patent had been submitted. In part to avoid hogging the limelight, moreover, he elected not to put his name on published reports of the insulin work or on later Genentech discoveries. And he worked with Swanson to make sure that each laboratory scientist was offered a stake in the company, in the form of low-cost stock or stock options. Over the long term, these policies set a standard that would be followed by almost the entire biotech industry.

In the short term, these policies allowed Genentech to attract first-class young scientists and to retain them on its payroll. In January 1978, the company hired two

scientists, Dennis Kleid and David Goeddel, from a research institute south of San Francisco. Both made major contributions to the insulin project, initially at City of Hope and then, after June 1978, in Genentech's new lab facility, located in a renovated warehouse in South San Francisco. Goeddel, a rock and mountain climber in his non-lab life, showed a special genius for getting experiments done faster than anyone else. (Later, his shirts would be emblazoned with an imperative—"Clone or die!"[28]) Working harder, longer, and more effectively than most, he shifted the cloning into high gear and then helped spearhead assembly of the insulin protein from separate chains isolated from bacteria. Despite a month's setback caused by a mistake in the B chain DNA sequence, by late August 1978 Genentech had made insulin protein, detectable by an antibody that would only recognize intact insulin. To announce their success, the company held a press conference at City of Hope on September 6, 1978—less than a year after the effort began.

The three-way race for insulin produced two big winners. Bacterial expression of the first human insulin primed Genentech to lead a new industry, and the UCSF group earned bragging rights for one of the first cDNAs encoding a mammalian protein. Circumstances constrained both victories. Genentech's assembly procedure for insulin had to be scaled up to make gram quantities, but eventually the Eli Lilly company purchased the new product, providing Genentech ample capital for future expansion. In 1982 the Food and Drug Administration approved Humulin for clinical use.

The UCSF group's victory, although slightly tarnished by the apparent advantage furnished by the Cambridge moratorium and by the much-debated pBR322 affair, nonetheless set the stage for submitting a patent, focused on insulin and a different protein, human growth hormone. Peter Seeburg, a second German postdoc in the Goodman lab, had cloned growth hormone cDNAs from mRNA in pituitary tumors that secrete excess growth hormone. He worked with John Baxter, an entrepreneurial UCSF endocrinologist who brought him the tumors fresh from the operating room. Submitted in 1978, the patent listed three inventors—Rutter, Goodman, and Baxter.

Boyer and Swanson recognized that both Ullrich and Seeburg could make valuable contributions to Genentech's future, and offered them jobs. They demurred at first, but then Seeburg decided to accept the offer. For Ullrich the patent was the last straw, because its list of inventors excluded the scientists who, in his opinion, did most of the work. Furious, at Biochemistry's annual retreat in September 1978 he told Goodman he would move to Genentech.[28] Seeburg, by this time already preparing to move, was becoming increasingly at odds with Goodman over the growth hormone project. Indeed, according to Hall's dramatic account,[28] Goodman abruptly terminated Seeburg's work at UCSF by transferring all his clones to a locked freezer.

The departure of these bright, ambitious young men to Genentech would have far-reaching consequences.

## Perspective

As fomenters of a revolution that transformed experimental biology in academia and created a new biotechnology industry, Herb Boyer and Bill Rutter make an odd couple, with remarkably different styles and personalities. Rutter comes across as intense, competitive, sharply focused on the task at hand, and quick to take charge when a project moves slowly. In contrast, Boyer masks competitiveness and keen insight with an apparently laid-back persona and a quietly laissez-faire style in the lab.

Rutter almost seemed to thrive on opposition, tolerating (or even encouraging) colleagues who criticize, disagree, or compete against him, providing he judged them smart and competent—colleagues like Harvey Eisen, an early hire in Biochemistry, Keith Yamamoto, who fought commercial commitments by Biochemistry faculty members, or Wally Gilbert, a fierce competitor in the race for insulin. His tactical skills and insights into people allowed him to overcome or bypass multiple "fractures" in the department—conflicts about faculty involvements with businesses, competition for recognition, academic promotions, the list of inventors in a patent, and rumors about pBR322. Similarly, his energy and determination pushed the insulin project in precisely the direction he chose, despite behind-the-scenes disagreements with Howard Goodman and the head-strong self-direction of a brilliant postdoc like Axel Ullrich.[36]

Unlike his department chair, Boyer was deeply distressed by colleagues' jibes about Genentech's filthy lucre and hated the confrontations and posturing he perceived at the Asilomar meeting on the purported dangers of recombinant DNA. Not adept at swashbuckling adroitly over and through fractures, Boyer achieved his triumphs in cordial, remarkably cooperative joint ventures with brilliant colleagues like Stan Cohen, Art Riggs, and Bob Swanson. Still, his laid-back style didn't stop him from prevailing against Swanson, a brilliant but hard-nosed businessman, in the decision to make somatostatin before insulin, and also when his empathy for young scientists at Genentech persuaded him that exciting findings of company scientists should be published promptly, as soon as the patent was submitted.

Despite the differences, both Rutter and Boyer were smart, competitive, and ambitious—qualities that account for their success, but only in part. They also share a rarer, more crucial qualification—unremitting receptivity to new ideas and to anyone who knows or imagines something they don't yet understand. They found this receptivity especially attractive in Gordon Tomkins, and it marked both men before

they came to UCSF. Rutter stressed its importance in picking new recruits and in grasping new ideas to build a department, and Boyer proved exquisitely open to good new ideas throughout his career, especially in his collaborations with Cohen and Swanson.

Along with a few dozen other pioneers in scattered laboratories, these two scientists created recombinant DNA as a tool for understanding and manipulating nature. It is eerily fitting that their creation vastly increased the value of receptivity and "unprogrammed tentacles" for every other experimental biologist. I exaggerate only a little in characterizing biology before recombinant DNA as a congeries of small labs, each focused on an isolated question, cell, organ, or species in relation to a single narrowly defined function or property. The object focused on in one lab might or might not relate to objects studied in other labs, but we would not know for sure anytime soon.

After recombinant DNA, our world changed. Genomes, DNA sequences, and the evolutionary process that produced them indissolubly link all life forms to one another, so that virtually any question tackled in one phylum, species, organ, or cell has its counterpart—leading to a related or occasionally quite different answer—in myriad other phyla, species, organs, and cells. Consequently, every biologist must constantly be poised to leap nimbly from one question, answer, or technology to another, regularly enlisting aid from other scientists who know facts, systems, and techniques they have not mastered. Before the mid-1970s, many biologists worked for decades on a single enzyme or function, fully expected to stick with it many more years, and interacted with a tiny cohort of like-minded specialists who got together once a year to compare notes. Ten years later, it was becoming clear that any young biologist might hope to specialize, but had to recognize that she/he would inevitably be forced to don the generalist's hat when the problem transforms itself into a different one. Problems change so frequently because related DNA sequences have irrevocably unified biology, so that a new finding or question of a scientist somewhere often bears precisely on the very question one is pursuing. Scary and sometimes hard to manage, this new world offers endless new opportunities—all brought to us by Boyer, Rutter, Berg, Gilbert, and other pioneers of recombinant DNA.

More locally, at UCSF, the recombinant DNA revolution gradually wrought a sea change in the organization and goals of experimental biology and clinical medicine. Later, the Biochemistry department figured critically in spreading the DNA gospel to other departments. During Rutter's chairmanship, rather than offering direct help to other basic science departments, it set an inspiring and rather daunting example.

In the meantime, Rutter showed increasing interest in the possibility of play-

ing an entrepreneurial role himself, outside the University. He and Goodman briefly considered the possibility of consulting for Genentech,[5] and Rutter conducted a short-lived flirtation with Amgen, another early biotech company, about organizing an affiliate, "Amgen North," in the Bay Area.[37] Early on, during the insulin race, Rutter also began talking about starting his own company, a step opposed by most Biochemistry faculty. It was one thing for a regular faculty member like Boyer to start a company, but such a commitment, they felt, would be far too demanding for a department chair.[38] Rutter did start Chiron, a new biotech company, and also gave up the Biochemistry chair to become head of UCSF's Hormone Research Laboratory, in 1982. His gift for organizing complex ventures and his receptivity to new ideas—along with the requisite amount of good luck— combined to make Chiron very successful. After leaving UCSF in 1991, Rutter's entrepreneurial spirit reveled in founding additional new biotech companies.

After the cloning of insulin, Genentech prospered mightily. With Goodman and other colleagues at UCSF, Peter Seeburg cloned rat growth hormone in 1978, and in 1979 co-authored Genentech's report of cloning human growth hormone by combining synthetic DNA and a cDNA.[39] In October 1980, Genentech became a publicly owned company, selling one million stocks at $35 each. Since then it has become one of the leading biotech companies in the world, having created and marketed many profitable drugs, mostly made with recombinant DNA, and having accumulated thousands of patent approvals. In 2009 Roche Pharmaceuticals bought Genentech outright, paying more than $46 billion to Genentech stockholders, other than Roche itself. A small blot on Genentech's record came in UCSF's suit claiming infringement on the University's growth hormone patent. In the 1999 settlement, Genentech paid UCSF $200 million, including a $50 million donation for a building on UCSF's new Mission Bay campus in San Francisco—the building, called Genentech Hall, in which I am writing this book.[40]

In October 1980, at almost the same time as Genentech's first stock offering, came the announcement that the Nobel Prize in Chemistry would be awarded to Paul Berg, Walter Gilbert, and Frederick Sanger.[41] The award sparked speculation that Boyer was passed over because he started Genentech, or that Berg received the prize in part because of his perceived role in defusing public fears of recombinant DNA by organizing the 1975 Asilomar meeting. Boyer says that "winning the Nobel Prize . . . should not be the recognition you strive for. What are you going to do if you don't get it?" Admitting to some disappointment, he emphasizes that criticism from UCSF colleagues was worse. "Gee! I thought what I was doing was a pretty good thing! And you'd think I was a criminal! That I found to be much more difficult than not

getting a Nobel Prize."[42]

Like Boyer's former postdoc, Herb Heyneker, I consider Boyer's amazing twofold accomplishment far more important than many discoveries that merit a Nobel. First, he and Stan Cohen created what Heyneker terms a genuinely "dislocating" technology. "Only with this technology," Heyneker says, "was it possible to [produce] human insulin in bacteria. That's dislocating, a completely different way of thinking. [A] biologist in the pre-recombinant DNA era would not have comprehended it." It was so dislocating, in fact, that some scientists, and many businessmen, had trouble seeing its potential value. Second, Boyer and Robert Swanson unequivocally demonstrated that this dislocating technology works in the real world. Heyneker calls "Genentech . . . the best thing [Boyer] ever did, and he knows it. . . . [K]nowing what the technology was capable of doing, . . . and extrapolating into the future, I give Herb a lot of credit."[18]

Let us reflect a moment about how UCSF may have influenced the work of Boyer and Rutter. In most ways, UCSF treated them quite differently, virtually ignoring one for his first six or seven years at UCSF, while allowing the other to establish the kind of department he envisioned and rewarding him with substantial resources and high regard. One received meager resources, toiled long in the wilderness before finding collaborators, and had to weather fierce criticism from colleagues when he founded Genentech. In contrast, the other entered the insulin race with a large laboratory and the invaluable help of first-rate molecular biologists in his own department.

The two did share one crucial contribution from UCSF—when it really mattered, the institution allowed each to do just what he wanted to do. At the outset, lack of interest from supervisors allowed Boyer to pursue restriction endonucleases without interference. Once he and Cohen discovered the technology, the campus administration permitted him to found Genentech and to conduct its early experiments in a University laboratory—permissions rare in medical schools at the time. Rutter enjoyed unfettered opportunity to organize Biochemistry as he saw fit, and resources and power from the University that helped him win the first round in the race for insulin.

The next chapter, focused on two very different scientists, will unfold some of the same issues, but in a rather different light.

Chapter Seven

# Lone Wolf and Literary Maven

## Parallel Tracks Converge

In October 1989, Michael Bishop and Harold Varmus learned they would receive a Nobel Prize for discovering the genes that cause cancer, now known as oncogenes. Baseball's world series was about to commence in San Francisco, so the new Nobelists were scheduled to throw out the first ball at the beginning of Game Five. But the home team lost the first four games, so Bishop and Varmus had to settle for throwing the first pitch at a Giants-Dodgers game during the next season. As Bishop wrote later,

> I had been living in hope of (but not practicing for) that moment since the age of 10. In front of 38,000 people, I unloaded a one-hopper to the catcher, Terry Kennedy, who then ran out from behind the plate to shout: "You should let go of the ball earlier, Doc." True to form, Harold threw a perfect strike. I later learned that he had practiced on a regulation pitching mound. . . . I arrived back at my seat in the stands, to be greeted by the good-natured razzing of the fans who now knew me for what I was.[1]

Like his passion for baseball, the deft irony, self-deprecation, and what he likes to call "enlarged expectations" are Bishop trademarks.[1] Beneath the melody, however, we pick up an insistent beat. His colleague may have thrown a perfect strike, but—as Bishop carefully informs us—Varmus practiced beforehand. The same leitmotif runs through the lives of both men—relentlessly competitive, they strive for excellence with an intensity that is rare, even among first-rate scientists.

Success may depend on a competitive yen for excellence, but yen alone rarely suffices. The success of Varmus and Bishop is more complicated. Remarkably similar external influences and internal proclivities shaped them. Both grew up in small towns, with fathers in service professions. Caring deeply about words and books, each became an unusually articulate adult, speaking in complex sentences and crisp paragraphs. Medically trained, both did research at the NIH in lieu of service in the

Vietnam War, although in other ways they followed different paths into experimental biology.

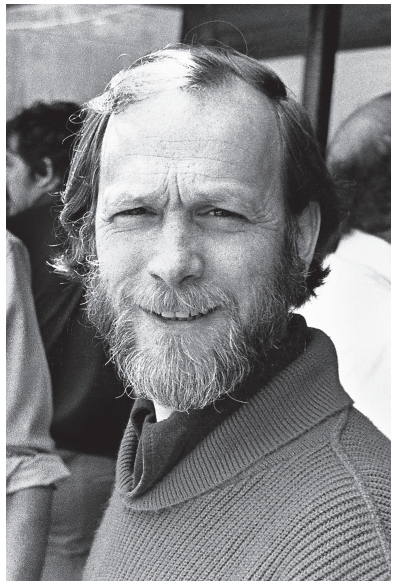
## PK Into Lone Wolf

Born in 1936, the oldest child of a Lutheran minister, Michael Bishop grew up in a small Pennsylvania town near the Susquehanna River. Determined not to behave like a PK (preacher's kid), the youngster threw himself into sports. Still, his formidable academic prowess and passion for music and books fitted the PK stereotype, as did as his short stature and modest athletic ability. Even as a student at Gettysburg College, he ruefully observes, he was "just this side of a nerd, respected because I did a hell of a job putting together the fraternity float every year." In college Bishop also met Kathryn Putnam, another PK, who would become his wife. ("All good Lutheran children in central Pennsylvania went to Gettysburg, particularly PKs.")[2]

In college, Bishop followed a family doctor's advice and took pre-medical courses. While he didn't want to be a country doctor, he did like to figure out how things work and "I wanted to do something that challenged my intellect." Finding literature and history more fascinating, he took only the bare minimum of four courses in his chemistry major—a major he chose to enhance his chances for medical school. In 1957, two medical schools offered the *summa cum laude* graduate a place. Learning that Bishop saw medicine as a gateway into a life of the intellect, the wise admissions officer at one of them, the University of Pennsylvania, advised him to go to the other, Harvard.[2]

At Harvard, Bishop found the medical school's first-year courses almost uniformly "drab," and had to learn that DNA forms a double-stranded helix from a *Scientific American* article in the medical school library, rather than in a class.[2] Still, Harvard proved a revelation, owing to a few science-oriented classmates who introduced him to the idea of research as a possible career.[3] By the end of Bishop's second year, a microbiology class touched on the mysterious topic of molecular biology. "I thought, what the hell is that? Near the end of the second year it was becoming painfully clear that I really wanted to do science. [But] I had no clue how to go about it."[2]

Then Bishop kicked his powerful self-starting mechanism into high gear, and Harvard Medical School, much to its credit, let him navigate his own course. The first step was to take a post-sophomore year (1959-1960) off from medical school, just after he and Kathryn were married. Working that year in the Pathology Department at the Massachusetts General Hospital (MGH), Bishop performed 50 autopsies. "It was an incredible year. I was totally my own boss," he says, and "pathologists are nine-to-fivers, so I had a lot of free time."[2]



J. Michael Bishop, co-discoverer of the first oncogene. A mutant version of a normal chicken gene transmitted by Rous Sarcoma Virus, this oncogene caused sarcoma in chickens. Photo taken at a 1978 RNA virus meeting, which he co-chaired with Varmus.

Devoting that time to wide reading and to occasional lectures—including one by a famous visitor, Francis Crick, co-discoverer of the double helix—Bishop soon "became infatuated with protein synthesis and molecular biology."[2] Crick had lectured on the ribosome, the small intracellular machine we met in Chapters Five and Six. The ribosome translates one language, "read" in the base triplet code of messenger RNA (mRNA), into a second language, the sequence of amino acids that makes a protein. A special set of "adaptor molecules" (Crick's term), now called "transfer RNA" (tRNA), assists the ribosome by "reading" both the mRNA *and* specific amino acids, making it possible to add the right amino acid to the growing protein chain at the right time.[4] In 1960 the actual triplet code was yet to be deciphered (see Chapter Four), but Paul Zamecnik and Mahlon Hoagland had discovered tRNA the year before at the MGH, where Bishop was spending his post-sophomore year.[4]

Toward the end of this year, Bishop told his Pathology sponsor, "I'm so into science that I'd like to quit medical school and go to graduate school." His sponsor sent him to talk with Hoagland, who advised him to delay becoming a researcher until he completed medical school—advice he followed, but in his own way. Bishop arranged to work part-time in the one-room laboratory of Elmer Pfefferkorn, who taught him in a virology course. There he was to isolate so-called "conditional mutants" of Sindbis virus. (Conditional mutants can perform a particular function—in this case the mutant virus he sought would be able to replicate itself under one condition, but not another. For instance, at a slightly cool temperature a mutant replication protein

might work fine, but a higher temperature would render the mutant protein inactive.) Bishop never found a conditional Sindbis mutant, however, because the replication procedure "stone-cold stopped working." He worked on the problem for six long months, but failed.[2]

Undiscouraged, Bishop boldly changed direction. He could still grow the virus in cells, and others had shown that its genome was RNA rather than DNA. Accordingly, he set out to purify the virus and ask whether its genomic RNA could be "translated" into an amino acid sequence in a test tube. Pfefferkorn had never performed such an experiment, but with his blessing Bishop got advice from Hoagland's technician, synthesized a radioactive amino acid[5] necessary for the experiment, purified the virus, and prepared the ribosomes and cell extracts he would need.

By the time this effort was well launched, it became time to return to senior year of medical school, with its mandatory clinical clerkships in surgery, medicine, obstetrics and gynecology, etc. But Bishop had "decided this is what I wanted to do for a living, and why not spend the whole fourth year of medical school doing it?"[2] The dean of Students warned that he would "be committing professional suicide," but Bishop persisted and the dean caved in. A single clinical clerkship would still be required, however, and the headstrong student would have to convince chairs of the other clinical departments that he need not rotate through their subspecialty. Most students would have quailed at the thought of facing those department chairs, but Bishop talked to all of them, and prevailed. His single senior clerkship, in internal medicine at the MGH, was a success, and that department accepted him for an internship the next year.

Bishop's lab work that year was not so successful. Ribosomes translated normal (control) mRNA, but made no protein whatever when he added Sindvis virus RNA instead. Later, others found that Sindbis genomic RNA cannot be translated into protein because it is not the coding strand. Instead, the genomic RNA is complementary to the mRNA that encodes Sindbis virus proteins, which is transcribed in the infected cell.[6] Despite the failures, Bishop treasures the time in Pfefferkorn's lab as a gift of inestimable worth, because he was allowed to "develop a sense of self-sufficiency. Nobody was telling me what to read, nobody was telling me what to do. I went and set these techniques up. It didn't matter when I failed, because I always had another chance to do it again, and nobody was keeping score."[2]

After medical school, Bishop spent two years as an intern and resident at the MGH—an experience he was delighted to finish in 1964. Ready to tackle research full-time, he was awarded a research associate position at the National Institutes of Health as a "yellow beret"—so-called because the Vietnam War was heating up, and

two years at the NIH would allow him to avoid the military draft for physicians.[7]

At the NIH Bishop worked for three years in a small lab, focused on polio virus. His mentor, a seasoned virologist called Leon Levintow, assigned him a starter project, but after a few months Bishop concluded that it wouldn't work. Instead, he "wanted to do molecular biology on the virus—and it was about that vague."[2] So he proposed an audacious experiment aimed at determining how the polio virus replicates its genome in infected cells. Developing approaches new to the Levintow lab, Bishop soon found that infected cells contained three distinctive species of RNA derived from the virus. With Gebhard Koch, a visiting scientist in the lab, he tried to work out relations among these three species, at the NIH and during a subsequent year (1967-1968) in Koch's lab in Hamburg, Germany. They made progress, but the replication story didn't become clear until after Bishop moved to a permanent faculty position in the US.[8]

In guiding him to that position, Bishop's first NIH mentor played a key role. When Levintow left the NIH one year after Bishop arrived, to take a position as Professor of Microbiology at UCSF, he had already become a trusted "gadfly" for his younger colleague, thoughtful and critical but never dictatorial—the right balance for a young advisee determined to be his own boss. During that first NIH year, "Leon sat at his desk, he read, and he would discuss my day with me. I was semi-independent, because I was doing stuff he had never done. Leon was definitely an alter ego, a sage advisor and a pretty good critic."[2] As Bishop's NIH stint came to an end, he talked with two east-coast universities about a possible faculty position. Hopkins made him an attractive offer, but in the end Harvard did not. Levintow, now at UCSF, talked with the Microbiology chair, Ernest Jawetz, and they invited Bishop to pay a visit to San Francisco.

That visit was "a very 'Leon' recruitment. I was quite taken by the city. We ate at the late lamented Doro's [a traditional San Francisco seafood restaurant]—my first abalone. I'd never heard of abalone. Then Leon took me down the street to see Carol Doda [America's first topless dancer], and then we went to City Lights Bookstore [famous for selling books of the Beat Generation], and I was ready to sign the contract! I would be intellectually comfortable because Leon was here. It would be just like NIH, essentially." In comparison to Hopkins and Harvard, "the prospects at UCSF were much more welcoming—lots of lab space, nothing resembling an academic pyramid, a patron in Leon. It looked like endless opportunity. If you look at the history, I was a lone wolf from the get-go, doing molecular biology in Leon's lab."[2]

In 1968, after his year in Koch's lab in Hamburg, the lone wolf joined the Microbiology faculty at UCSF. As Bishop worked to complete the polio virus work he

started at the NIH, Levintow introduced him to Warren Levinson, a third virologist in the Department. Levinson had completed his postdoctoral work at UC Berkeley, across the San Francisco bay, in the laboratory of a prominent animal virologist named Harry Rubin. There he had learned to work with the first known cancer-causing virus, an RNA virus, called Rous Sarcoma Virus (RSV) because Peyton Rous discovered in 1911 that it transmits a sarcoma (connective tissue cancer) between chickens. Levinson—like Rubin and Rous before him—had little knowledge of molecular biology, but Bishop thought the way he put RSV through its paces was "absolutely arresting. [Warren] would infect cells with this virus, and depending on how he did the experiment, within twenty-four to thirty-six hours, there were cancer cells. It was stunning."[2] Howard Temin, trained (like Levinson) in Rubin's lab, had already worked out an elegant, quantitative method for assessing the virus's ability to transform cells into cancer. In a lawn of infected non-cancerous cells ("fibroblasts" from connective tissue of a chicken embryo), the experimenter counted small aggregations (foci) of rapidly growing cancer cells. The lone wolf had found a new hunting ground, full of promise.

## Literary Maven Into Scientist

As the twentieth century began, Harold Varmus's grandparents, poor Jews from Poland and Austria, landed at Ellis Island. Capable and hard-working, both families prospered in the rich new country. By 1932, in the midst of the Great Depression, Varmus's father had graduated from Harvard and Tufts Medical School and his mother was headed for the New York School of Social Work, after graduating from Wellesley College. Meeting in New York, they were married in 1936 and moved to Freeport, New York, a small town outside New York City. Harold, their first child, was born in 1939. Later, he marveled at his family's transition "from persecution and poverty in one part of the world to a contented existence in the professional class in another"—a transition he took, as a child, "more or less for granted, growing up with a sense of entitlement and a confidence about the future that were probably unwarranted, however useful they proved to be."[9]

In Freeport, for the "bright and earnest son of a general practitioner . . . a career in medicine seemed preordained."[9] While Varmus did not actively resist that destiny, he found science courses in high school rather unexciting. Instead, a powerful attraction to English literature enticed him to read (and carefully annotate) James Joyce's *Ulysses*, and to write short stories. For college, Varmus chose Amherst over Harvard, feeling that the smaller school would give him more opportunity to know members of the faculty.

At Amherst, Varmus took the required pre-med courses, and considered majoring in philosophy, physics, or English literature. He eventually chose the latter, which allowed him to revel in close reading of poems and to write a senior thesis on the novels of Charles Dickens. The aspiring literary maven was a generally good student, but poor grades in organic chemistry, a pre-med course, forced him to move from a boisterous fraternity to a solitary dorm room in order to earn a "gentlemanly" C in the course.[9] A year as editor-in-chief of Amherst's college newspaper was Varmus's "headiest experience." Controversial editorials (like supporting Kennedy *vs*. Nixon in the 1960 presidential race, criticizing the influence of philanthropic foundations on educational policy, or championing intramural rather than intercollegiate sports) led to a student attempt "to recall some of us with a referendum, which we managed to survive. A few of the controversies made me uncomfortable, but I also found them exhilarating."[9] As a senior Varmus entertained a wide variety of careers and applied for admission to multiple medical schools, graduate programs in English, and travel fellowships in other countries, plus a couple of jobs in journalism. Responses to his applications were mixed, but in the end he chose to enter the PhD program in English literature at Harvard.

That choice was not a final decision. Graduate courses in English literature seemed a bit too much like college, and other stimuli reactivated his interest in medicine. These included visits to occasional conferences at nearby Harvard medical school, as well as growing awareness that English literature did not have to be a full-time occupation—indeed, he noted that leading authors of his favorite literary period in England, the 17th century, were also deeply "involved in the practical world . . . [while they] wrote on the side."[9] So, Varmus re-applied to medical school. Harvard Medical School had turned him down flat a year earlier, but granted an interview this time. Now the interviewer "found me too inconsistent and immature in judgment to be admitted to his school," and suggested that Varmus might profit from a maturing stint in the military.[9] Fortunately, Columbia University's medical school greeted him more warmly. In the fall of 1962, Varmus became a medical student in New York.

By 1968, Varmus had enjoyed four years of medical school, followed by two years of medical house staff training (also at Columbia). Lectures about molecular discoveries may have planted seeds for Varmus's future, but at the time he found more satisfaction in diagnosing and treating disease. He thought he was headed for a career as an academic internist, focusing on medical practice, teaching, and perhaps clinical investigation—but not laboratory science. Unfortunately, by this time the US had committed vast resources and many young men's lives to the Vietnam War. Opposed to this War on principle but unwilling to avoid the draft by emigrating to Swe-

den or Canada, Varmus—like Bishop, four years earlier[7]—applied to the NIH for a yellow-beret position, requesting assignment to a lab doing disease-related research.

The competition was tough, especially for a young man with no experience in research, but this one was lucky. During the interviews, in Bethesda, Maryland, a senior scientist "took pity on" Varmus, suggesting that he meet Ira Pastan, a young scientist who worked on the thyroid. "Ira rescued me. I knew what the thyroid was."[10] Pastan decided to take Varmus into his lab because he stood out, among a set of extraordinarily smart and attractive candidates, as one who also cared about literature.[11] After he returned to his residency following the interviews, a "shocking" telephone call from Pastan interrupted his pleasant anticipation of the coming stint at the NIH. Pastan announced what he said was a startling discovery—"cyclic AMP reverses catabolite repression of the lac operon of *E. coli*." Varmus had "no idea what that meant," but was so busy tending patients that he had to break off the conversation without finding out.[9]

Later, in the hospital library, Varmus did his homework. Published papers on the lac operon told him a story we met earlier, in Chapter Five.[12] Adding lactose (a sugar usually found in milk) to a culture of *E. coli* increases the bacterium's ability to break down lactose and use it for energy. Lactose does this by binding to a "repressor" protein and preventing it from turning off production of a lactose-using enzyme, β-galactosidase (β-gal). In the absence of lactose, the repressor does this by binding to a regulatory DNA sequence near the β-gal gene. The lac operon, a much-studied region of the bacterial genome, contains both the β-gal gene and the repressor binding site.

Varmus's homework also dispelled the mystery of "catabolite repression," the process in which a second sugar, glucose, *inhibits* β-gal production, even when lactose is present. In other words, glucose prevents production of β-gal. How glucose exerted this effect was not known, but Pastan had discovered an exciting clue. He and a colleague, Robert Perlman, found that the chemical he mentioned on the telephone, "cyclic AMP" reverses glucose's inhibitory effect.[13] Pastan and Varmus both knew that certain hormones act at the outer membrane of animal cells to increase the amount of cyclic AMP inside. In turn cyclic AMP serves for these hormones as an intracellular "messenger," triggering their biochemical effects on the cells. These effects include, for instance, increases in heart rate or glucose release from the liver (in response to adrenaline), as well as augmented thyroid hormone secretion (in response to thyroid stimulating hormone). Later work would reveal that in bacteria glucose inhibits β-gal production by reducing cyclic AMP accumulation.

Instead of working on the thyroid at the NIH, Varmus suddenly realized, he would work on a problem he knew almost nothing about. At that early stage Pastan

also had a lot to learn about the genetics and molecular biology of bacteria, so that he and his research fellow would have to learn together. In fact, Varmus says, entering Pastan's lab in 1968 was "incredibly good timing. Ira had just cracked a big problem, and there were so many things to do."[10] For a bright young fellow thoroughly ignorant about science, these two years in the Pastan lab proved an extremely lucky break, introducing him to key ideas and technology at a rapidly advancing frontier of experimental biology.

Once at the NIH, Varmus focused on a key question—how did cyclic AMP stimulate production of β-gal, reversing the effect of glucose? Did it reduce the rate at which β-gal protein breaks down or prolong the lifetime of mRNA encoding β-gal in the bacterium? Alternatively, did cyclic AMP increase β-gal production by increasing the rate at which β-gal mRNA is copied ("transcribed," in technical language) from its gene in the lac operon?

To distinguish these possibilities, he would have to measure the amount of β-gal mRNA in *E. coli* exposed to glucose, plus or minus cyclic AMP. Such measurements required Varmus to learn how to use two kinds of tools, both of which would play crucial roles in his work after leaving the NIH. One tool, called molecular hybridization, was a technique for detecting and measuring the amount of a specific DNA or RNA sequence in solution. It exploits a key chemical interaction we met in Chapter Four—that is, specific association of one nucleic acid sequence with another, dependent on base-pairing between complementary sequences (C with G, A with T or, in the case of RNA, U; U stands for a base called uridine, which is similar to thymidine).[14]

To measure mRNA transcribed from the lac operon, Varmus would need to obtain a second tool—that is, a specific DNA "probe" representing the β-gal gene. He would then assess the probe's "molecular hybridization" with complementary mRNA sequences, which carried a radioactive tag and had been extracted from appropriately treated *E. coli* cells. The amount of radioactive mRNA associated with the probe would directly reflect the amount of β-gal mRNA present in the bacteria while they were alive.[15]

For his immediate purpose, this second tool was simply a convenient probe for a particular set of molecular hybridization experiments. But as the new tool was forged, it became one example of a powerful, more general idea—that a virus's genetic simplicity can open ways to replace vague speculations with critical experiments and answer concrete questions—a notion that later would help to find the first cancer gene.

For now, Varmus and Pastan took advantage of a bacterial virus, called a bacte-

riophage. Chapter Four showed how the relatively short DNA sequence of a similar bacteriophage allowed Herb Boyer to assess the ability of EcoRI to cleave DNA at specific sites. Pastan and Varmus profited from a different property of bacteriophages, their propensity for incorporating small parts of the bacterial genome into their own viral genomes. Others had already found a particular bacteriophage that had captured part of a bacterium's lac operon, so that its purified genome could furnish the proper DNA probe to complement and hybridize specifically with radioactively labeled β-gal mRNA.

For a fledgling physician who had never worked in a lab, making the idea work was not simple. He remembers that his ignorance of a simple laboratory reagent prompted Pastan's "half-joking" comment—"Now remind me why I took you into the lab."[9] Still, the ignorant fledgling successfully made the β-gal probe and adapted a convenient procedure, with the probe attached to pieces of filter paper, for measuring β-gal mRNA by molecular hybridization.[15] He found that cyclic AMP reverses inhibition of β-gal expression by making transcription of the β-gal gene into mRNA faster and more efficient—so there was no need to invoke a prolonged lifetime of either the mRNA itself or its protein product. Others in the Pastan lab began to work out the mechanism by which cyclic AMP stimulates production of this mRNA. The simple fact that cyclic AMP *did* stimulate its production was in itself important, because until that point the only known mode of regulating expression of a gene was "active" repression by a repressor protein binding near the gene (as first shown for the lac repressor). Now the Pastan lab was finding that the very same gene was also controlled by the positive effect of an *activator* of expression, cyclic AMP. Now, with abundant hindsight, we know that many animal genes are subject to both positive and negative regulation.

Varmus recognizes that his introduction to science was quite unusual. A young beginner, even someone bright and ambitious, is rarely plunged into a situation where experiments are so feasible and revealing. "Experiments occurred," he says, "at a pace I found disconcertingly fast. We could do two experiments a day."[10] From just two years in the Pastan lab, he became first author or co-author of nine reports of new findings. In contrast, most of his present postdoctoral fellows—faced with mice, human cancers, and animal viruses vastly more complex than the bacterium's lac operon he and Pastan had to deal with—"don't publish *anything* their first two years." From his NIH experience, he says, he got "a sense that this was stuff I can do. Once I got the hang of it, I felt powerful. I could do things. I saw a question and I could answer it. Things moved incredibly quickly, and I just didn't have any failures. It was weird."[10]

Although Varmus was one of Pastan's first postdocs, he can now judge him in comparison to a host of others who have passed through his lab. "It took him almost no time to figure out what the important thing was to do and to get it done," he says. "He can . . . figure out what the core [of a problem] is and how to deal with it very efficiently. He doesn't get sidetracked. He's not chatty. He identifies it and gets it done. I don't think I've worked with anyone ever who has the skills that Harold has."[11]

Mike Bishop had come to laboratory experiments by a very different route. A self-professed "lone wolf," he set up methods new to his mentors and tackled hard questions with experiments that worked, but produced nothing publishable in medical school. His NIH mentor, Leon Levintow, knew little about the molecular biology that fascinated his postdoctoral fellow, and departed after a year. Resourceful and determined, Bishop forged ahead by himself, eventually forcing poliovirus to reveal secrets of its replication.

Despite their different initiations into science, by the time Bishop and Varmus met each had decided on his own that viruses would open the door to understanding the molecular basis of cancer.

## Converging on Cancer

As Varmus was learning to do experiments with Pastan, events outside the lab brought other life-altering changes. During the summer he came to the NIH, in 1968, he fell in love with Connie Casey, the woman who would become his wife. He also paid a vacation visit to old friends in San Francisco, where he was entranced by the sunny weather, beaches for swimming and fishing, and convenient access to the Sierra mountains. Before the summer was out, "enamored with San Francisco," Varmus had "resolved that I would come to California."[9] Sadly, the same summer also brought a devastating blow—his mother found she had breast cancer.

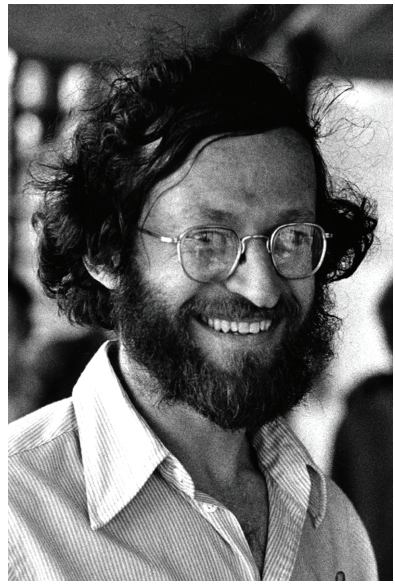
By the time his mother died three years later, Varmus would already be engaged in trying to unravel the molecular riddle of cancer, with the hope that scientific understanding would benefit other patients.[9] The hope had begun at the NIH, when Varmus took several courses taught by senior scientists. A course on cancer biology taught the central idea that would underlie much of his life's work—that most cancers are caused by genetic mutations (changes in DNA, inferred but at that time not yet specifically identified). Even more intriguing, a virology course suggested that the molecular simplicity of tumor viruses might reveal exactly how mutant genes cause cancer. In that course Varmus read controversial papers by Howard Temin, in which he proposed that the life cycles of RSV or other RNA tumor viruses involve production of a "provirus" with a DNA genome.[16] In other words, when an RNA

tumor virus infects a cell, its RNA is copied into DNA. That DNA, once integrated into the host cell's genome, is transcribed into RNA to generate genomes of progeny viruses. (The postulated RNA-to-DNA copying enzyme is now called "reverse transcriptase," to emphasize that it copies nucleotide polymers in the opposite direction from the usual DNA-to-mRNA mode.) Although the evidence was far from conclusive, Varmus found Temin's provirus hypothesis impressive.[16] Moreover, it was easy to imagine experiments, logically similar to those he was doing with Pastan, which would use a DNA probe, wrested from the virus itself, to identify the provirus by molecular hybridization.

In the summer of 1969, it was time for Varmus to look for a job. He applied only two criteria—he would find a tumor virus lab and it would be located in California. Although a famous Italian tumor virologist in southern California didn't grant him an interview, Varmus did arrange to visit Bill Robinson at Stanford and Harry Rubin at UC Berkeley. Both interviews were disappointing—Robinson was "not inviting," while Rubin spent much of the time bashing the provirus notion proposed by Temin, his former student, and revealed "a surly attitude toward molecular explanations of cell behavior."[10] Clearly, Varmus would have to look elsewhere. Fortunately, however, Rubin mentioned that a former student, Warren Levinson, worked in a virus group at UCSF, across the Bay—a group that also included a former NIH virologist Varmus had heard of, Levintow, plus "someone named Mike Bishop, about whom I knew nothing."[10] (Bishop summarizes: "Harold had not chosen me nor I him. He had been deflected to me by a senior figure . . . who apparently thought that neither Harold nor I deserved any better."[1])

A few days later, without mailing an application or even placing a telephone call, Varmus crossed the Bay and walked into the Bishop lab, on the fourth floor of Health Science East (HSE-4). Informed that the faculty were having lunch, he sat down to wait. That afternoon Varmus was interviewed by Bishop, Levintow, and Levinson. For Varmus, the "life changing . . . conversation was with Mike." Bishop was already pursuing "the kind of reductionist, rational approach I hoped would become possible with the tumor viruses. So, in the course of about an hour, we both recognized we had very similar aspirations for turning the molecular revolution into a bonanza for the study of cancer, using viruses."[10] For his part, Bishop was delighted that Varmus came from the same NIH virology background he did, found his credentials and accomplishments "spectacular," and thought him "exceedingly bright, almost preternaturally so."[2] Offered a position as a postdoctoral fellow, Varmus said yes.

Months before this interview, Bishop saw Warren Levinson's RSV transform normal cultured cells into tiny cancer foci and decided to switch his lab's focus. Pre-



Harold E. Varmus, co-discoverer of the first oncogene. The collaboration between Varmus and Bishop triumphed over strong competition from other labs. Photo from the 1978 RNA virus meeting, which he co-chaired with Bishop.

paring to wind down the polio work, he had assigned a graduate student to launch the RSV campaign. Her task was to isolate a conditional RSV mutant that could infect cells and replicate its genome to make new infectious virus but would, under certain conditions, *not* be able to induce cancer in infected cells.[17] Cells infected by the mutant virus she was to seek would grow quite normally at a higher "restrictive" temperature, but would form distinct cancerous foci at a lower "permissive" temperature. The virus would propagate briskly at either temperature. (A Siamese cat gets its distinctive pigmentation from a similarly conditional mutation, albeit in a different gene. The cat mutation causes a mutant enzyme responsible for producing black hairs to be inactive in the skin at body temperature—the restrictive temperature—but permits it to work normally in the cooler skin of ears and feet.)

Unfortunately, Bishop's graduate student lost interest in a conditionally mutant cancer gene, and dropped the project. Then Steve Martin, a bright postdoc in Harry Rubin's lab at UC Berkeley, decided to isolate the conditionally defective RSV mutant himself. Flouting Rubin's firm opposition to genetic analysis, Martin obtained from Bishop and Levinson a "cloned" population of RSV (in which all the viral genomes would be identical).[18] Then he treated the cloned RSV with a chemical known to induce mutations, and went on to isolate the conditional mutant Bishop had sought. At the restrictive temperature, 41°C, the mutant virus infected cells efficiently and the virus propagated itself just as rapidly as it did at the permissive temperature, 36°C, but the infection produced no cancerous foci. At 36°C, however, virus-infected cells

rapidly turned cancerous. The converse switch, from lower to higher temperature, caused cancer cells to revert to normal growth. Thus the ability to induce cancer was completely dispensable for RSV's efficient infection and replication. The simplest and most exciting interpretation of this result was that normal *RSV carries a gene responsible for inducing cancerous growth* of infected cells—rather than causing cancer, for instance, by activating cancer genes already lurking in the host cell's genome.[19]

Bishop was delighted to hear about the new mutant, if disappointed that his lab had not isolated it. A greater disappointment lay ahead.

In early 1969, while preparing lectures on the molecular biology of RNA tumor viruses for a graduate course, Bishop had carefully reread the papers by Howard Temin that Varmus also read for a virology course at the NIH. "The whole thing just made sense," Bishop thought, despite the fact that many virologists and molecular biologists cast doubt on Temin's hypothesis, and despite the weakness of Temin's evidence.[2] Accordingly, because virions (infectious particles, composed of a protein coat enclosing a genome) of certain other viruses contain polymerases, he decided to ask whether RSV contains an enzyme with a biochemical activity that must exist, if the Temin provirus hypothesis was correct, but which had never been identified—that is, the "reverse transcriptase" able to use the virus' genomic RNA to make a complementary *DNA* copy, the hypothesized proviral DNA. Pressed at the time to finish the polio virus work, Bishop tried "three or four times" to measure activity of a reverse transcriptase in RSV particles, without success.[2] He thought he knew what his mistake was, but Levintow and others reminded him that his NIH grant didn't pertain to RSV and the idea was in any case far-fetched, so he should return to polio virus.[20] This first part of the reverse transcriptase story, including Bishop's decision to shelve his search for the enzyme, took place around the time that Varmus paid his impromptu visit to the lab and was offered a postdoctoral position.

Several months later, early in 1970, Bishop received a rude surprise. His fellow faculty member, Gordon Tomkins, telephoned to tell him that two labs had separately identified a reverse transcriptase in RNA tumor viruses! At a conference, Tomkins had just heard presentations by David Baltimore and Temin. Tomkins didn't say how they succeeded but, Bishop says, "literally, I went in the lab and ran [the experiment] and in 30 minutes I knew exactly what I'd done wrong."[2] His mistake, distressing but simple, had been to follow the advice of a visiting guru rather than his own inclination with respect to the concentration of a single chemical component of the enzyme assay.[20] Now the same experiment, but substituting the concentration he had originally planned to use, gave him exactly the result Temin and Baltimore would soon publish.

"The discovery of reverse transcriptase was a devastating blow," Bishop later wrote. "I grieved for months; I still grieve in weaker moments."[1] He berated himself for letting a "momentous secret of nature" escape his grasp, simply because he didn't trust his own imagination. Postdocs later regaled Bishop with accounts of his unusual behavior in the aftermath—working away at the lab bench, he would suddenly mumble profane obscenities. Known for his meticulous verbal precision, this PK has not been heard using such language, before or since. He was "exorcising my anger at myself for just not carrying through. I just didn't have the strength of my convictions. It was a terrible lesson."[2]

Soon thereafter, Bishop presented his data at a Gordon Conference on animal cells and viruses, held in Tilton, NH. At the same conference, Temin and Baltimore gave talks about discovering reverse transcriptase and Steve Martin presented his conditional RSV mutant as clear evidence that RSV's genome contains a cancer gene. Varmus also attended the conference, in the course of his drive to San Francisco with his wife, Connie. "Mike's group," he says, "was one of about five desperately trying to catch up, doing competent biochemistry, . . . the incremental experiments that follow any huge discovery."[10] Bishop recalls Varmus "sort of blithely saying, 'Well, good luck with reverse transcriptase,' and then [he] took off."[2]

That memory of an off-hand remark forty years ago made me wonder. Back in 1970, what outcome would a thoughtful observer have predicted from the convergence of these two young men in San Francisco? Varmus was thirty, Bishop thirty-four. To a small virology lab, each brought a nimble mind, an MD degree, research training at the NIH, and limitless ambition and competitive drive. They also shared the very specific dream of using RSV to find a cancer gene. Their most recent laboratory experiences differed. Bishop, a savvy academic busy building a lab able to run against fast competitors, was still playing catch-up ball after the reverse transcriptase debacle. In contrast, Varmus was a naïve, optimistic recruit whose NIH success may have made him a bit too sure that everything would turn out right, even though hindsight tells him now how crucial it was to join the right NIH lab at just the right moment. In 1970, he says, "I did think I could do anything." In addition, he was moving to San Francisco, a city he loved, where two of his closest friends already lived. "I had, in a sense, come home. I felt like this was where I wanted to be."[10]

Although I didn't know either man in 1970, I think I would have predicted that their potentially volatile mix of similarities and differences would eventually generate a fruitless battle of egos and personalities. As we shall see in the next chapter, the actual story evolved in a very different direction, and for a reason that points to a condition necessary for real bursts of discovery.

Chapter Eight

# A Rare Partnership

## Fishing Out the First Cancer Gene

UCSF's VIROLOGY EFFORT at UCSF in 1970 was a small, self-contained operation. Harold Varmus was joining a loose confederation—Michael Bishop, Warren Levinson, and Leon Levintow, plus a few postdocs—all located in contiguous small laboratories on the fourth floor of Health Sciences East (HSE-4). Herb Boyer was also a member of the Microbiology department, but his lab was in a separate building, and they saw him less often. Within the local world of HSE-4, Bishop's experience was, he says,[1] "a latter-day version of Renaissance patronage. . . . Number one, I had a patron." This was Levintow, who served as his conduit to the powers that be at UCSF. "Number two, there were no administrative demands, I didn't feel any pressure." One example of patronage took place before Varmus arrived. Bishop welcomed a new postdoc, who didn't yet have a fellowship to support his salary. "I was running out of money, so I wrote a letter to the [acting] dean of Medicine,[2] whom I had never met. [The letter said,] 'I'm in desperate straits. I have two postdocs, and I don't have any money to pay them.' And I got a note saying, 'We'll take care of it as long as necessary.'" Bishop suspected that the acting dean checked with Levintow first, but this quiet and effective support gave Bishop a welcome sense of security. "I understood that I was expected to be accountable, but I wasn't facing an academic pyramid. I had people who clearly thought I belonged here and wanted to keep me here, and that's about all that mattered."[1]

Scientifically, Bishop says, his local environment in 1970 was confined to a small number of colleagues. "Herb [Boyer], and Harold [Varmus] and Leon [Levintow] and Warren [Levinson] with the viruses, that was my intellectual milieu." In the days before Rutter had attracted a cadre of young biochemists to San Francisco, Bishop says, most of the virus group's intellectual input came from "the external community, [which] quickly became my home in science."[1] David Baltimore and Howard Temin, in particular, were accessible and very supportive.

For Varmus, the first year in San Francisco was scientifically exciting but person-

ally a little disturbing. In contrast to Bishop, who appeared to understand how to build a laboratory and a career, he sees himself then as remarkably innocent about practical issues, including his own professional path. Immersing himself in the new lab and enjoying it, Varmus says he "was basically kind of lazy about career thinking, and I just didn't even want to think about trying to go somewhere else or apply for a job."[3] Almost immediately after leaving the Pastan lab, he had attended the summer Gordon Conference where he watched Bishop and others trying to catch up with Baltimore and Temin. "I could see in a minute when I arrived in the tumor virus world that it was very, very different[, and] big enough to feel pretty competitive. Everybody was going to be doing the same thing." Consequently, he wondered whether "it would make more sense to stay in San Francisco if I felt there was some kind of clinically oriented support option." These doubts prompted him to talk with Holly Smith, chair of the Department of Medicine, and to attend a genetics clinic in Pediatrics. Many who know the ultra-confident Harold Varmus will find it difficult to imagine his experiencing what he refers to as "this little trough of concern after arriving." In fact, hard work and satisfying results in the lab soon dispelled his doubts.

Reminiscing about Varmus and Bishop, co-workers often emphasize their focused approach to a scientific problem. In the lab, they always sought to identify the first task to be completed, and tackled it forthwith. Soon after Varmus arrived, he and Bishop agreed that his first task should be to demonstrate the presence of the DNA provirus in RSV-infected cells. The presence of reverse transcriptase in the virus predicted that the virus's RNA genome must first be transcribed into a DNA sequence in an infected cell and subsequently, as Howard Temin had proposed, incorporated as a provirus into the cell's genomic DNA.[4] Because no experiments so far had tested and confirmed these predictions, the cancer riddle would have to wait. For now the essential task was to establish firmly the basic life cycle of RSV in infected cells.

At the NIH Varmus had vaguely imagined experiments using molecular hybridization to fish out proviral DNA from RSV-infected cells. He would cast a lure, an RSV probe, in much the same way that he and Pastan used bacteriophage lambda DNA to fish out lambda mRNA in the Pastan lab. But the fishing expedition in San Francisco posed a greater challenge, because an animal's genome is so much bigger and more complex than that of *E. coli*. In fact the specific mRNA he had sought (and measured) in Bethesda was much more abundant, relative to other mRNAs, than RSV proviral DNA could ever be, relative to the vast DNA genome of an animal cell. Consequently, a fisherman could not land the provirus without devising a more sensitive method for detecting hybridization—that is, a more effective procedure than the "filter hybridization," described in the last chapter, which Varmus had used in

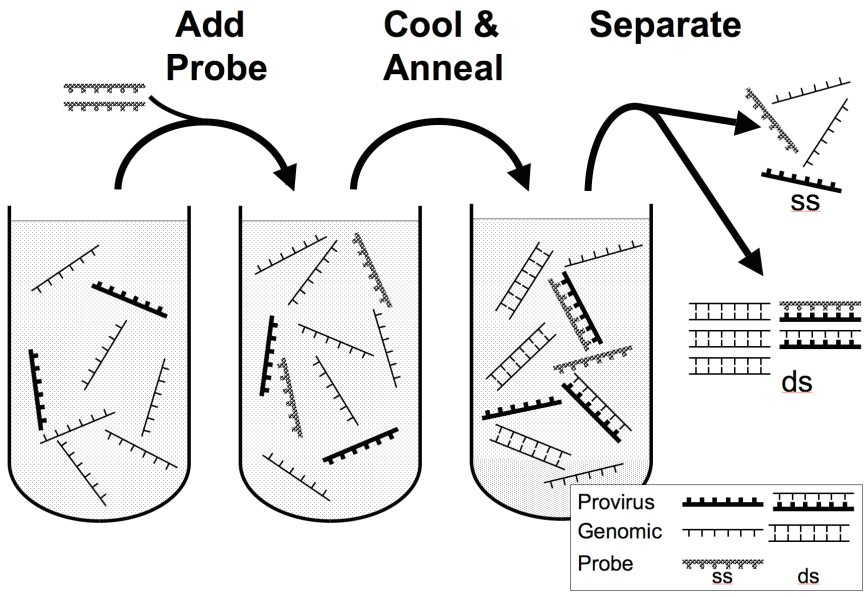



Figure 2: Fishing for RSV Provirus DNA

As shown in the test tube at the left, double-stranded cellular DNA, sheared into pieces about 400 nucleotides long, contains “right” and “wrong” fish—that is, respectively, a relatively small number of proviral sequences (thick studded bars, solid black) amid a host of uninteresting normal “anonymous” genomic DNA (thin studded bars). These DNA pieces have been heated to a temperature that separates one strand from its complementary counterpart. Then (middle test tube) the experimenter adds a small amount of radioactive probe (thick gray bars), which is single-stranded (ss) DNA copied by reverse transcriptase from the virus’s RNA genome. After the probe is added, the mixture is cooled to a temperature at which pieces of single-stranded DNA can re-anneal with their complementary counterparts to form double-stranded (ds) DNA. After a period at the cooler temperature, some pieces will have reannealed, while others will not. In the final step, reannealed (ds) and unannealed (ss) DNA are separated by virtue of their different affinities for associating with hydroxylapatite. The relative amount of radioactive probe in the ds population—that is, annealed to complementary proviral DNA—depends directly on the number of proviral genomes available for annealing in the mixture. The illustration greatly exaggerates the number of DNA fragments representing proviral sequences or radioactive probe (thick black or gray bars), relative to the number of DNA fragments representing anonymous DNA (thin bars). In reality, the latter would be many thousand-fold more numerous than proviral or probe sequences.

Bethesda.[5] Today's molecular biologists can dismiss this problem as trivial, because they enjoy the luxury of using many different restriction endonucleases, including Herb Boyer's EcoRI, to identify unique pieces of DNA.[6] But in 1970, as Boyer discovered, restriction enzymes were hard to find.

At this point Bishop recalled a strategy recently developed in other labs, in which molecular hybridization took place in solution rather than on filter paper. Applied in clever variations, this "solution hybridization" approach was to play indispensable roles in pivotal discoveries he and Varmus reported over the next decade. In both the new approach and the earlier procedure, the fishing lure finds its target sequence by virtue of the complementary base-pairing we met in Chapter Four. In the procedure Varmus used in the Pastan lab, he caught the fish he wanted with lures attached to a piece of filter paper, like hooks attached to a boat's hull.[5] Now he sought to count (but not exactly to catch) individual members of a particular "right" kind of fish species by trawling a special net through a "pond" (water, in a test tube) teeming with a huge number of "wrong" (uninteresting) fish, which he hoped to ignore. Casting distinctive lures (radioactive, in fact) would allow him to net the right fish, at a rate that would depend directly on the number of right fish in the pond. (The rate might be slightly slowed, of course, by constant nibbling from hordes of wrong fish unable to bite the hook.)

Luckily, the Bishop lab already had both the lure and the specialized net required for this experiment, depicted schematically in Figure 2. The lure was a radioactively tagged single-stranded DNA probe copied (by reverse transcriptase, in a test tube) from the virus's RNA genome. In a test tube pond, this DNA probe would "hook" the right fish, single-stranded proviral DNA from the cell, because the two sequences would specifically complement and "reanneal" to one another, forming double-stranded DNA. The fishing net—that is, the procedure for separating reannealed, double-stranded DNA from un-annealed, single-stranded DNA—would distinguish radioactive lures that had snagged the right fish from the bare hooks of unbitten lures. (The net similarly separated wrong fish, hybridized to complementary genomic DNA, from single-stranded wrong fish in the pond. But this kind of wrong fish was not radioactive, and so would not be counted.)

The straightforward procedure could literally count the number of proviral (right) sequences in genomic DNA prepared from cells or animal organs. In the real world of the lab, optimizing every detail of the procedure[7] was not easy, and it proved (like real fishing, at least for non-aficionados) tedious to perform. But, Bishop says, "It was a kick the first time [the procedure] worked. We were the first people to use it for DNA/RNA assays, and it was magic, just fantastic. That technique hooked

Harold. I mean, he said, 'Okay, this looks so much better [than the procedure he used in Bethesda].'"[1]

While Bishop suggested switching to the solution hybridization strategy, Varmus brought to the lab his own special impetus, according to Leon Levintow, a close observer of the collaboration. Rapidly mastering complex techniques and analytic procedures, the new recruit devised novel ways to apply them, and molecular hybridization became a powerful tool for discovery. "Without Harold," Levintow says, it's impossible to imagine the picture unfolding in any way similar to what actually happened."[8]

After setting up the new procedure and testing its reliability, Varmus, Bishop, and their colleagues used the RSV DNA probe to count the number of hybridizing sequences in chicken cell genomes. As expected, genomes of RSV-infected cancerous cells, like RSV-induced tumors, contained sequences (more than a dozen or so per genome) that hybridized with the probe. But the resulting 1972 publication, Varmus's first major paper from his new lab, did *not* demonstrate an actual RSV provirus in RSV-infected cells.[9] Instead, the probe showed that genomes of non-cancerous chicken cells, not previously exposed to RSV, *also* contained similar numbers of hybridizing sequences. These were proviruses inserted into the genome when ancestors of modern chickens were infected by non-tumorigenic RNA viruses related to RSV.[9]

Soon Varmus and Bishop found that RSV infection really does induce production of proviral DNA.[10] Twelve hours after infection of duck or mouse cells, whose genomes normally lack the high background of RSV-like sequences seen in chickens, most newly formed DNA that hybridized with the RSV probe had not yet been integrated into the cell genome. If the mouse cells were cultured for days or weeks after RSV infection, however, sequences that hybridized with the RSV probe became incorporated into genomic DNA as proviruses. In 1974 Varmus, Bishop, and their colleagues reported that viral DNA is reverse-transcribed from RSV's RNA genome in the cytoplasm (the intracellular fluid surrounding the nucleus) rather than in the nucleus.[11]

Later work by Bishop, Varmus, and investigators in many other labs would reveal more detail of the life cycles of RSV and other "retroviruses"—the name for a family of viruses whose RNA genomes are converted by reverse transcriptases into DNA proviral sequences, and then integrated into the host cell's genome. Some retroviruses directly induce cancer, but others, including Human Immunodeficiency Virus (HIV), the cause of Acquired Immunodeficiency Syndrome (AIDS), do not.

The tumor virus field had been competitive enough in 1970, and would become even more so. Within a few years, nonetheless, scientists in labs across the US found

the "Bishop-Varmus team" able, productive, and hard to beat. Like UCSF's virology community, competitors recognized Varmus as Bishop's coequal, and he progressed rapidly up the academic ladder, with Levintow as Renaissance patron. Like all truly effective patronage, it worked mainly in the background, nearly invisible. "It didn't feel like there was any simple hierarchy [and] I never really felt like Mike was my mentor," Varmus says. Instead, "This [was] a free-floating organization, and we'd do what we wanted. We were all learning it together."[3] In his first year as a postdoc, he became a Lecturer in Microbiology, in his second year he advanced to the rank of Assistant Professor in Residence, and by 1974 he was an Associate Professor with tenure. "I was just saying to myself, how is the experiment going? . . . I hadn't thought about jobs. I bought a house after I'd been there a year. [T]his was craziness, in a way." Varmus's work was going very well, and his relation with Bishop was scientifically exciting and satisfying. They didn't talk about career issues. "I can't remember sitting down and discussing how we were going to work together. We never did that," Varmus says, suggesting that such talk may have felt awkward for Bishop, and "Frankly, I wasn't that good at it either."[3]

It appears that one-on-one discussions about science were also relatively uncommon for Bishop and Varmus. "The conversations we had, just the two of us, were much, much fewer than conversations that involved other people." For the most part, science was discussed in the presence of a student or a small group of postdocs. "These meetings were almost always in the company of other people. . . . The conversations were terrific, free-ranging and high-spirited. We treated our trainees, for the most part, like equals and what we had to say to each other was freely expressed in their presence." [3]

Useful as these sessions must have been, I find the absence of one-on-one conversations between these two partners surprising. Virtually every other bright, articulate scientist I know finds it impossible *not* to seek out the brightest scientist in the vicinity for conversation about questions they both know and care about. It is possible, of course, that Varmus and Bishop explicitly opted for efficiency, judging that key experiments would be tackled sooner if they included a student or postdoc in their meetings. I favor a subtler explanation, perhaps even more crucial to the success of their fruitful collaboration. We shall return to this issue later.

## Fishing for v-src and c-src

When he arrived in San Francisco, Varmus dreamed of using molecular hybridization not only to explore RSV's life cycle in cells, but also to identify RSV's presumed cancer-causing gene, subsequently called v-src. (Pronounced "vee-sark," the name

was coined to indicate the gene's presence in a *v*irus and its propensity for inducing *sarc*omas, a distinctive kind of cancer.[12]) Formally, Bishop and Varmus did not "discover" v-src, the gene whose existence was first revealed by Steve Martin's conditional RSV mutant and confirmed in key experiments by others. Instead, v-src gave them an essential tool for fishing out an even more surprising animal gene—the gene for cellular src, aka c-src ("see-sark"), a very similar protein, present in most cells of the body, that does not cause cancer. In fact, discovering c-src made it certain that v-src was a genuine cancer gene.

By showing that RSV's v-src gene is neither required nor even helpful for the virus's replication, Martin's conditional mutant signaled a great boon.[13] In contrast, replication of many DNA tumor viruses does depend on a gene or genes that induce infected cells to form tumors. If v-src had been necessary for RSV's replication, Bishop, Varmus, or anyone else would have had a much tougher time finding it, as we shall see.

But Martin's conditional mutation also posed a stark question—if v-src is fully dispensable in the virus, what is it doing there? Did it perhaps originate as a normal gene, accidentally captured by RSV—just as lambda bacteriophage occasionally carries (more technically, "transduces") host DNA sequences from one bacterial cell to another? One answer, proposed by George Todaro and Robert Huebner in 1969, was a somewhat fantastic hypothesis, in which all cancers result from activation of cancer-causing "virogenes" introduced into animal genomes eons earlier, by viral infection. This latter notion might imply either that RSV itself carries such a virogene or that (like X-rays or chemical carcinogens) RSV somehow activates a hitherto quiescent c-src gene already present in cells before infection. Strictly speaking, Martin's conditional mutant didn't rule out the second alternative, but neither Bishop nor Varmus took this more complex hypothesis very seriously, although it helped to spur their search for v-src and c-src.[14]

In addition to the boon of working on a tumor virus whose cancer-producing gene was not necessary for its replication, Varmus and Bishop had acquired another practical advantage—that is, they understood how to use solution hybridization of nucleic acid sequences to identify a single copy of a particular gene in an animal's genome. Unfortunately, however, the search for v-src and c-src had been thoroughly stymied until now by an apparently insuperable obstacle—lack of a radioactive probe able to recognize the src sequence, and only the src sequence. Both the viral RNA genome and its reverse transcriptase-produced DNA version contained v-src sequence, to be sure, but also contained other genes (e.g., for the virus's envelope and for reverse transcriptase). It must have seemed a frustrating paradox that Varmus

and Bishop needed to have radioactive src already in hand in order to look for the src sequence in cells!

The solution came in the form of another set of RSV mutants, studied by Peter Vogt, an RNA tumor virologist at the University of Washington who moved, in 1972, to the University of Southern California in Los Angeles. Vogt told Bishop and Varmus about a fascinating set of RSV mutants he and Peter Duesberg had described. These mutants, lacking a small stretch nucleotides at one end of their RNA genomes, could still infect cells and replicate briskly (faster, in fact, than normal RSV), but—unlike Martin's conditional mutants—did not induce infected cells to form foci of cancer cells at any temperature.[15] They were termed "transformation-defective" or *td* mutants, to indicate their inability to "transform" infected host cells into cancer cells. If v-src were necessary for RSV to replicate, Vogt would not have found *td* mutants, and no one would have been able to use a *td* mutant to make the desperately needed probe for v-src.

Varmus came up with an ingenious plan for isolating that probe,[16] based on the assumption, shared with Bishop and Vogt, that the RNA lost from the *td* mutant's genome originally encoded v-src. Early work by Ramareddy Guntaka, a postdoc, showed the approach could work, but he returned to a separate project and Dominique Stehelin, a talented French postdoc, stepped in.[1] Stehelin isolated enough pure radioactive probe to use in real experiments, and found, as expected, that the DNA probe associated nicely with the RNA genome of normal RSV, but not with genomes of *td* mutants or several other RNA viruses.[16] Now Stehelin was ready to look for src in cells.

Before the probe had been used for even more exciting experiments,[17] Bishop recalls mentioning, in a "chat" with Varmus, that he had to give a lecture somewhere. As Bishop remembers it, the conversation continued: "I said [to Varmus], 'I'm not sure what I'm going to talk about.' He says, 'Well, why don't you talk about this probe stuff?' And I said, 'Well, Harold, that was really your idea.' And he said, 'Well, you know, we're in this together. Of course you can talk about it.' I felt a little sheepish about that, but he was right."[1] That chat marks explicit recognition by both men that theirs really was the Bishop-Varmus laboratory.

With the src probe as a lure, Stehelin went on to search for src-like genes in genomic DNA from a variety of birds,[18] using the fishing tackle (solution hybridization; see Figure) that worked earlier to identify proviral DNA. The search proved more difficult than a schematic summary can easily convey, but the src lure eventually detected src-like sequences in genomes of chicken cells that had not been infected with RSV, and in the genomes of four other avian species. Parallel experiments, using a

radioactive lure made from RSV *td* mutants, did detect hybridizing DNA in uninfected chicken cells—where it presumably represents previous infections, eons earlier, by relatives of RSV that did not induce tumors—but detected *no* hybridizing DNA in other vertebrate genomes. The src probe's ability to find complementary DNA sequences in genomes of every bird species tested, contrasting with the *td* probe's ability to hybridize only with chicken DNA, strongly suggested that bird genomes must contain a "cellular" src gene, inherited in evolution because it was useful. Thus it seemed likely that c-src, as it was later called, did not derive from a virus at all. Instead, it appeared likely that RSV probably obtained src from a chicken, rather than the other way around.

At this point, however, the evidence for a c-src gene in normal animal genomes was by no means unequivocal. Nowadays a molecular biologist could quickly furnish straightforward evidence, simply by showing that the v-src nucleotide sequence and those of c-src in various bird species are similar. In 1976, however, available technology could not reliably decipher these sequences. To convince scientists that the c-src gene predated RSV would require stronger corroborating evidence.

Fortunately, in addition to their capacity for focusing on the task at hand, the Bishop-Varmus team was exceptionally good at finding and adapting techniques and ideas that originated outside their immediate field and were not already popular in the tumor virus community. Adapting solution hybridization to identify proviral sequences was one good example. Now Varmus and Bishop exploited the simple notion that nucleotide sequences of individual genes, just like the shapes of limbs and organs, must diverge in the course of evolution. If, as they surmised, c-src originated in the bird lineage before chickens appeared (and well before RSV filched it from a chicken, converted it into a cancer-causing gene, and "transduced" it by infecting other chickens), then c-src sequences should have diverged significantly in genomes of bird species distant from chickens. No one seems to remember exactly how the idea came up, but Varmus invited an evolutionary biologist at UC Berkeley, Allan Wilson, to cross the Bay and give a talk to the lab.[19] Wilson suggested they extend their search to the distantly related ratite family, which includes ostriches and emus. The Sacramento zoo was persuaded to contribute an emu egg, which hatched in Varmus's office.[3] There his "delightful little bird" enjoyed twenty-two days of life before it was sacrificed and its DNA examined.[20]

Stehelin needed to compare the relative similarities of c-src sequences in various bird species to those of chicken c-src and the provirus itself (that is, v-src, integrated into the genome of rat cells infected with RSV). To do so, he measured the "fidelity" of base-pairing—a touching term for the cold, impersonal association of one single

DNA strand with another—between the v-src probe and the c-src of each species.[21] Higher base-pairing fidelity makes a duplex DNA more resistant to "melting"—that is, a higher temperature is required to separate it into two single strands. In descending order from chicken to quail to turkey to duck to emu, bird c-src sequences showed progressively reduced base-pairing fidelity with v-src. This order clearly reflected the estimated phylogenetic distances between the corresponding genomes—distances that indicate how many million years ago the respective lineages separated from one another in the course of evolution.[18] "It was beautiful," Bishop says. "Considering the technologies we were using, the way those melting curves tracked with the phylogenetic distances was uncanny."[1]

The evolutionary divergence of c-src genes in different bird lineages neatly confirmed the notion that c-src is much older than RSV, which must have captured it by mistake well after chickens split off from other bird species. Even more important, however, the c-src gene is still recognizable in species that separated many millions of years ago—unequivocal testimony that the corresponding c-src protein is useful for these animals to survive.[18]

By this time the Bishop-Varmus lab had developed its distinctive style. Acting as coequals, both leaders received applications for postdoctoral positions. Once arrived, a postdoc would meet regularly with one of the two leaders, and sometimes with both. At a weekly "Rous Lunch," named for RSV's discoverer, postdoctoral or graduate student speakers would present their work. At these sessions, according to Art Levinson—a postdoc from 1977 to 1980 who later became chairman and chief executive officer at Genentech—"Everybody would listen and critique and tell you that it was good or you were full of shit. [It was] an aggressive, a macho type of environment that most people liked." At Rous Lunch, Bishop's criticisms "were less detail oriented," Levinson says, "where Harold was 'Boom, boom, boom, boom, neat, not neat, you left out this control, da-da-da.' He was more into the detail. Mike preferred kind of steering the ship."[22]

Don Ganem, who arrived in the lab as a postdoc in 1980 and is now a faculty member in Microbiology and Medicine at UCSF, agrees. "Mike [can] synthesize across giant realms of territory and create elaborate and largely correct models of what's going on, rather than explain every detail of a model the way Harold likes to do. They have a giant overlap of skills intellectually, but these differences make them together really perfect. It was fun to watch."[23]

Both Bishop and Varmus, Ganem adds, "were incredibly well read, but the breadth of [Mike's] knowledge was truly encyclopedic. Harold's strong suit was being quick on the uptake. He instantly could grasp every ramification of something,

however remote." Ganem remembers meeting with Varmus regularly every Monday morning. "You never wanted to go in there unless you were certain you had considered every conceivable control, every conceivable implication." On a Sunday evening, "by thinking about it continuously," Ganem would sometimes recognize a subtle implication of one of that week's experiments. Then, on Monday, "You would show him the [result], and the first thing out of his mouth would be the [idea] you had spent all day Sunday trying to generate."[23] Such quickness demoralized some postdocs, but Ganem "found it inspiring."

Three subsequent discoveries underscored the Stehelin paper's inference that c-src performs a necessary function in animal cells. First, by further refining the same molecular hybridization approach, Deborah Spector in the Bishop-Varmus lab reported in 1978 that c-src is present in mammalian genomes also.[24] Subsequent work in other labs revealed src-related genes in multicellular animals from worms to people.

The second discovery came in 1978, from two laboratories, one led by Ray Erikson, the other by Varmus and Bishop.[25] Both showed that v-src acts as a protein kinase, which regulates the function of other proteins by transferring a phosphate from ATP to a specific amino acid in the target protein. As Art Levinson tells the story, he and Hermann Oppermann in the Bishop-Varmus lab had obtained an antibody to v-src and used it to show that v-src itself was the target of an unknown protein kinase—that is, v-src was phosphorylated.[22] Looking for that protein kinase he would add v-src to cell extracts, and kept finding radioactive phosphate transferred from ATP to a protein clearly smaller than v-src—50 kilodaltons, rather than 60. (One "dalton" is equal to the mass of a proton, so the protein was as heavy as 50,000 protons, 10,000 protons lighter than v-src.) Repeating the same result eventually led him to realize the radioactive band was an abundant 50-kilodalton protein, the anti-v-src antibody's "heavy" subunit. "I said, 'Holy shit, maybe [v-src] itself is the kinase, and it's phosphorylating the antibody! . . . Lo and behold," he adds, "that's exactly what happened."[22]

The third discovery was that v-src is not the only viral oncogene with a normal counterpart in animal cells. In 1980 Diane Sheiness in the Bishop-Varmus lab found that a messenger RNA in normal chicken and other avian embryos is closely related to the carcinoma-inducing gene of a different chicken retrovirus, not related to RSV.[26] In this case the viral cancer gene is now called v-myc, and the mRNA found in normal cells is said to be encoded by c-myc. Subsequent studies have discovered many additional virally transmitted cancer-causing genes, or oncogenes, all derived from normal cellular genes.

The discoveries of Bishop, Varmus, and their colleagues laid a firm foundation

for understanding the causes of cancer and treating it effectively. That foundation rests on a simple insight—every cancer is a genetic disease, caused by DNA mutations that alter proteins that normally regulate the growth and function of cells. The first discovered oncogene, v-src, is a mutant counterpart of c-src, the first proto-oncogene. C-src and c-myc were converted into mutant oncogenes accidentally, in the course of their kidnapping by a retrovirus. In most cases the cancer-causing mutation makes the proto-oncogene's protein product persistently active by removing a constraint that normally limits its activity. Moreover, the mutation producing an oncogene need not result from a viral intervention. Mutations that convert proto-oncogenes into active oncogenes can result from chemical mutagens, radiation, or failure of a replicating normal cell to correct a simple mistake in copying the DNA. As if that were not enough, mutations need not cause cancer by increasing activity of a growth-promoting protein, but instead can *reduce* activity of a tumor-suppressor protein. Products of "tumor-suppressor genes" normally suppress, rather than stimulate, uncontrolled growth and replication of animal cells.

Since v-src and c-src were discovered, many dozen oncogenes and tumor suppressor genes have been found in human cancers, and at least two hundred more in animals. While the numbers will continue to increase, each oncogene or tumor suppressor brings with it potential insights into the complex regulatory networks that control normal cells and cancers. The resulting complexity may seem daunting, but insights from these discoveries have already produced significant advances in treatment of cancer.

For instance, consider the fact that the v-src protein—like the products of c-src and many other proto-oncogenes and oncogenes—is a special kind of protein kinase. This particular kinase, along with dozens of its close relatives in cells, transfers a regulatory phosphate group to many different protein targets, but always to a single kind of amino acid, a tyrosine (one of the twenty different kinds of amino acid in proteins). Over the past thirty years, sharper genetic and molecular tools have dissected myriad regulatory pathways that hinge on such protein tyrosine kinases, or PTKs. Chemical PTK inhibitors dramatically improve life expectancy of patients with certain cancers. By inhibiting an aberrantly active PTK in cells of chronic myeloid leukemia, for instance, a drug called Gleevec can convert a fatal disease into a treatable chronic condition. Treatments directed at pathways defined by other oncogenes are being developed.

## Perspective

What influences converged to produce the explosive burst of discovery at UCSF in the 1970s? To glean hints, we can ask two kinds of questions about the oncogene story. First, what was special about the way Bishop and Varmus tackled c-src? Second, how did their local environment influence the outcome?

Scientists who knew Varmus and Bishop as searchers for v-src and c-src agree that they shared one quality that is critical for most important scientific discoveries—that is, a white-hot intensity of focus, unwaveringly directed at answering the question they have chosen to answer. More than 20 years later, Arthur Levinson, their postdoc in the early 1980s, still occasionally dreams that "I'm back in [Mike's] lab and I'm not serious enough, I've forgotten how to do things, I've forgotten how to pipette, and I'm just scared to death Mike will somehow not approve of my work ethic."[22] Varmus is also known for his legendary ability to focus on the task at hand. "You know when your conversation with Harold is over," says one friend. "It's when he turns around and starts working on his email."[27] Together the two lab chiefs set a challenging standard for working hard. "Mike and Harold . . . were passionate about the work," Art Levinson says. "It was pretty much flat-out science. . . .[P]eople would work 90, 100 hours a week, week in and week out."[22] A hard-working scientist as a UCSF postdoc and later at Genentech, Levinson tried hard not to become a target for one of Bishop's favorite comments, aimed at postdocs who might mention taking a private day off: "He would say, 'It's your career.'"[22] Still, to make a discovery as important and hard to grasp as c-src, intensity and a matchless "work ethic" are not enough. The same is true of the razor-sharp intelligence Bishop and Varmus also share.

Although these qualities are surely useful, my guess is that both men shared a more crucial gift—an unusually receptive imagination, similar to the receptivity toward new ideas and approaches we saw in Boyer (Chapters Four-Six). Precise and practical, their imaginations spent little time wandering romantically from cloud to cloud, but focused instead on tools and ideas that might furnish answers to key questions. Among other results of that receptive and practical imagination, I have cited the use of molecular hybridization to find the provirus and c-src, as well as showing that c-src preceded v-src by assessing the evolutionary distance between c-src genes in different bird species.[28]

At the time, Bishop says, many virologists did not think this way. Because molecular hybridization was technically hard to understand, "They didn't get it. They didn't find it persuasive."[1] Other virologists would have been happier with real DNA sequences, which are more straightforward identifiers for all sorts of genes, but no

sequences were available. Worse, although they deemed Darwin's theory of evolution correct, many of them didn't actively think about experiments in evolutionary terms. Bishop cites reactions to his and Varmus's evidence, presented at a meeting, that c-src is present in mammals as well as birds. "That made people raise eyebrows." After his talk, he says, "Up goes the hand of [a prominent virologist], and he says, 'You're trying to tell me that a chicken gene is in humans?' And I said, 'Yeah, and most of the rest of the chicken genome as well. Have you heard of Darwin?'"[1] By the late 1970s, most virologists (and biologists in general) would have felt sure that Darwin's ideas about evolution were correct, but that was a far cry from actually *using* Darwinian evolution to explain results of experiments. In this respect, Varmus and Bishop were very much in the vanguard.

Given the extraordinary abilities Bishop and Varmus brought to San Francisco, what did their new environment contribute to their success? Certainly neither left the east coast for UCSF because he harbored any illusions about the reputation of his new academic home or its reputation for high-quality scientific research. As with their predecessors—Herb Boyer, Bill Rutter, Holly Smith, and many others, all the way back to Julius Comroe—the attractions were not academic. Instead, they would live in a new world, close to shining blue water, Carol Doda, Beat poets, and the beckoning Sierra range, and far from the constraints and high-pressured fracas of Boston and Washington. The free-spirited openness of this new world in the 1960s played more important roles in shaping dreams and behavior of UCSF's scientists and administrators than most would like to admit, although it is true that such intangible influences are hard to define and still harder to document.

In the workplace, at least early on, Varmus and Bishop appear to have only dimly sensed the UCSF community's hankering for a more distinguished future. Instead, for both of them in those years, the real attractions were a quiet, island-like enclave of virologists and a benevolent patron. Although Bishop calls him a Renaissance patron, it is hard to class Leon Levintow—charming, modest, and bright as he was—with one of the Medici or a man like Pope Julius II. Instead of commanding the fealty and performance those luminaries demanded of Michelangelo and other artists, Levintow saw in Bishop, and later saw in Varmus, a rare brand of brilliance and drive that merited strong support. Levintow's critical contributions were to bring Bishop to UCSF and to ensure that Bishop and Varmus got resources they needed from the dean and from Microbiology. Some needs were material—e.g., a brief initial contribution from Levintow's NIH grant to help Bishop until his first grant application was funded or temporary postdoctoral support paid by the dean's office. Equally important, the offer of a faculty position in Microbiology, along with Bishop's and Levintow's recogni-

tion of his research contributions, probably helped to rescue Varmus from his "little trough of concern" after arriving in San Francisco. Even Bishop, who can appear almost infinitely confident, recalls with joy the praise of his Department chair, Ernest Jawetz, for a lecture he gave to medical students.[29] In effect, Levintow helped to fashion a snug protective cocoon around his two protégés, encouraging their experiments and shielding them from the exigencies of administrative responsibility and anxiety about advancement or resources.

The welcoming patronage accorded Bishop and Varmus contrasts sharply with Microbiology's apparent lack of interest in Herb Boyer, who was ignored by Jawetz, confined to poor-quality lab space outside the new Health Science towers, and furnished scant material, psychic, or intellectual support. Still, albeit in different ways, UCSF gave Boyer, as well as Bishop and Varmus, one invaluable commodity—a liberal dose of benign neglect. Freedom from the hierarchy and academic pyramids they left on the east coast gave all three license to do pretty much what they wanted, because they could feel that "no one was keeping score." No one knew or cared what Boyer did, so he kept plugging at restriction endonucleases. Levintow unobtrusively noted the rapid progress of Bishop and Varmus and offered quiet, sometimes useful, but never directive advice. Sometimes the advice was not useful, as when he suggested Bishop shelve reverse transcriptase or joked (years later) to Varmus that that the normal src protein was an insubstantial gas.[3]

Nearly every scientist needs sustained input from other scientists. Boyer had to wait five years for that kind of input, which came when Biochemistry's new growth furnished him opportunities to work with Howard Goodman and talk to Gordon Tomkins (Chapter Four). Later, he profited hugely from productive collaborations with Stanley Cohen and Art Riggs (see Chapters Five and Six). From the beginning, Bishop and Varmus got input from the local virology group, from peers outside like David Baltimore and Howard Temin, and in collaborations with Peter Vogt that contributed critically to discovering src. But their most crucial advantage, in comparison to Boyer and everyone else, came from their long, close collaboration with one another.

Long-sustained collaborations between two scientists are extremely rare—rare enough, in fact, to have triggered the wry surmise that one of two such collaborators must be a saint![30] The Bishop-Varmus collaboration lasted for about 10 years, so that one or both may qualify for sainthood. But what did the collaboration contribute to their remarkable discoveries? We like to imagine magical complementation between personalities, but I agree with Art Levinson, their onetime postdoc, who feels that neither scientist depended "absolutely 100 percent upon [the] other. Both on their

own would have done really, really well. But one plus one, I would say, was two. I don't think it was three."[22] Still, two really is twice one! As Levintow suggested, the story would not have unfolded in the same way without either Varmus or Bishop.[8] Certainly, it would have unfolded at a slower pace.

The collaboration's success, more human than saintly, depended on special qualities of both scientists, combined with a more than a dash of help from their UCSF environment. Varmus and Bishop may be unusually driven and competitive, but they are also perceptive judges of ability and promise, able to take a long view of complex problems. Each of these task-oriented scientists was smart enough to place an appropriately high (but distinctly unsaintly) value on the other's ideas and abilities, and to recognize that success in tackling a difficult challenge could depend on working together. Having made that judgment, it seems likely that they correctly sensed a need to harness their competitive drives, to prevent them from interfering with the task.

Such a harnessing strategy—conscious or unconscious, perhaps never explicitly acknowledged by either partner—helps to explain their otherwise quite extraordinary apparent abstention from talking, one-on-one, about the science they were doing. I suspect this strategy allowed them to bypass or ignore competitive interference with essential communication about complex questions, tasks, and plans closest to both their hearts. In this scenario each collaborator's need for the other's prowess could best be satisfied in the buffering presence of other participants, where they could hash out critical questions about experiments and revel in the clash of alternative ideas without raising the more personal issues often triggered by overt disagreement in a one-on-one situation.

Given their competitive natures and the sanctified behavior long-term collaboration may require, it seems unlikely that their collaboration would have lasted 10 years without an invaluable (if largely inadvertent) contribution from UCSF and Leon Levintow. This was the insulating buffer that surrounded their island enclave on HSE-4. The cocoon protected the delicate relation between Varmus and Bishop not only by providing resources and promotions, but also by reducing their interactions with the rest of the UCSF community and limiting opportunities for recognition by UCSF's powers-that-be—and consequently preventing local recognition and a large, eager audience from kindling the kind of competitive striving that often dooms long-term scientific collaborations. I have presented the cocoon as if UCSF and Levintow constructed it without help from Bishop and Varmus, whereas a real cocoon is created by an insect's larval stage and maintained by the pupa while it transforms into a mature adult. While Bishop tends to describe his privileged existence in HSE-4 as his patron's creation, it is not clear to what degree he, and later Varmus, contributed to

maintaining the cocoon. At the least, they appear not to have chafed at its confinement or sought escape from a situation that was much to their advantage.

The cocoon's buffering effect, hardly noticed by the campus as long as its protection lasted, became obvious when it broke open, triggering separation of the apparently indissoluble Bishop-Varmus team.[31] Just as the cocoon was constructed without conscious intent on the part of the institution or its leaders, the eventual breach in its protection was similarly unintended. Concentrating on their research, for almost a decade both Varmus and Bishop preserved fairly low profiles on campus, Varmus's perhaps a bit lower than his older colleague's. Although Levintow and the virology community—at UCSF and outside—may have recognized Varmus and Bishop as coequals scientifically, deans and chancellors knew only that Varmus had come to work in Bishop's lab, and was working with Bishop. So, when the directorship of the Hooper Foundation, a research institute on campus, became available in 1979, the institution decided to reward excellence by asking Bishop to take the position.

The problem, Varmus says, did not stem from his interactions with Bishop, their scientific collaboration, or their relative reputations among scientific peers. Instead, Varmus wrote later, offering Bishop the Hooper directorship was a problem because UCSF's leaders "tended to deal with [Bishop] as the senior partner almost exclusively. His elevation to an institute directorship would solidify this aspect of our relationship and make me feel even less visible within my home base. I did not desire autonomy, dominance, or greater resources of my own. I wanted parity."[31] The virology community had imagined that UCSF wasn't keeping score, but now the scorekeepers—doing their jobs properly—were busy trying to make their scientists happy.

With Bishop's new position came an entire research floor in one of the Health Science towers. Offered a lab on that floor, Varmus declined because he felt that would move him even farther from parity. Thus the partners split in part because of their own remarkable success, which brought Bishop the directorship and simultaneously suggested to both him and Varmus "that we both could do this on our own."[3] Soon UCSF asked Varmus to renovate and supervise an entire research floor. This was HSE-4, where the Bishop-Varmus lab had nestled comfortably for more than a decade. Once administrative diligence fractured the cocoon, the two moths separated, each fluttering off on its own.

The Bishop-Varmus collaboration was rare in its intensity, effectiveness, and duration, as well as in its participants and their extraordinary abilities. Still, extreme cases can teach lessons applicable to the rest of us. This lesson goes something like this: *competition and cooperation are often both essential for creative innovation, but collaboration may thrive best under conditions that mitigate overt competition*

*between the collaborators*. Today, collaborations like that of Varmus and Bishop would be difficult to sustain for nine productive years, at UCSF and other leading institutions. Instead, two such obviously brilliant individuals would be constantly subjected to stimuli that pull young scientists in different directions—repeated bids to apply for (and receive funding from) national awards; scientific journals bidding for prestige-seeking authors; the institution's emphasis on independence of each researcher; ubiquitous, ever-attentive audiences of striving, competitive peers; the university's push for favorable publicity, more often (and more conveniently) focused on an individual than on a partnership or team.

Each of these environmental influences is understandable and even laudable, but they ineluctably distract attention from thinking and experiments. Some young scientists are distressed by failure to receive this or that national award, others by well-meant advice—correct, by the institution's standards—that their remarkable collaborative accomplishments don't show sufficient evidence of "independence." In 1970 UCSF could afford to leave its micro-environments alone, so the HSE-4 cocoon remained secure. Nowadays, an institution bent on enhancing innovation needs to harness its leaders' vision, tact, and imagination to manage the fertile but inevitable tension between collaboration and competition. The task is subtle and essential, but not easy.

Chapter Nine

# Barbarian at the Gate

## Naming the Citadel

In April, 1982, three sentences by Stanley Prusiner ignited what he later termed a "firestorm" of controversy.[1] Then a neurologist and an obscure UCSF researcher, he had struggled for eight years to identify the infectious agent responsible for transmitting a trio of fatal neurological diseases—scrapie in sheep, kuru and Creutzfeldt-Jakob disease (CJD) in humans—from one animal to another. After summarizing his own work in a review article, he wrote three remarkably un-inflammatory sentences:

> Because the dominant characteristics of the scrapie agent resemble those of a protein, an acronym is introduced to emphasize this feature. In place of such terms as "unconventional virus" or "unusual slow virus-like agent," the term "prion" (pronounced *pree-on*) is suggested. Prions are *protein*-aceous *in*fectious particles which are resistant to inactivation by most procedures that modify nucleic acids.[2]

The deadpan tone and surfeit of passive verbs are surely deliberate, but the performance raises a host of questions. Why did many scientists vehemently oppose his dubbing the scrapie agent a "prion"? Why did Prusiner risk eliciting such a reaction? Did he make a mistake, or did he kindle the furor on purpose?

When I began writing this book, I knew something about Prusiner's research, but rather less about the man himself. From our early years at UCSF, I could recall only an enthusiastic young man with a bushy head of dark-brown hair. In nearly four decades as colleagues, Prusiner and I had spoken directly only a few times.[3] Years later, when I sought to interview him, he said he was extremely busy running his complex research program and writing a memoir about his life and work. We would talk, he said, in eight or nine months. A year later, the interview had not taken place. During that year, I interviewed several people about him and his work. Each had strong opinions about Prusiner, which often differed sharply from those of other interviewees, but all agreed that his unique personality is an indispensable key to understanding his scientific achievement. Thus I was disappointed by Prusiner's written refusal to

give me an interview. From talking with people I had already interviewed, he wrote, he felt that "the information you have now should be sufficient, and I don't believe I would be able to offer you anything more substantive. Maybe more to the point, I have resisted doing interviews of the sort you want for 25 years and not regretted my decision once."[4]

Prusiner's choice not to talk with me has required me to draw his portrait—unlike those of most people in this book—entirely by triangulating testimony from others. This has not proved easy. Several people, including some who said they were acting at Prusiner's request, refused to discuss him with me.[5] Among the disparate views of his character and motivation offered by others, one motif recurs again and again. William Mobley, a former UCSF faculty member in the same department as Prusiner, said it most simply. Sometime in the late 1980s, Mobley recalls, he told Prusiner that he admired his tenacity and ability to take aim at a problem, even if what he did put him at odds with others. Then he added, "Stan, you're a gold-plated barbarian! Once you're sure you want to do something, you do it no matter what others think." Prusiner, he thought, was "a little taken aback, but I think he saw my admiration was real. The truth is, nobody but a gold-plated barbarian could have accomplished what he did."[6]

As triangulations accumulate, supplemented by a modicum of guesswork, we shall begin to understand the Prusiner puzzle. His rare combination of tenacity, determined focus, and an exotic, almost barbaric refusal to care what others think explains a great deal, including his steadfast unwillingness to be interviewed. This chapter, describing Prusiner's history before the firestorm, shows how that unusual combination led to his bold prion-naming manifesto in 1982. The subsequent chapter, taking up the story later that year, recounts the extraordinary discoveries that led to a Nobel Prize in 1997. Together, the two chapters raise questions about creative science and how it can be thwarted or promoted by an individual discoverer's unique personality and by his world around him, including the host institution, colleagues and competitors elsewhere, and even world events far beyond the lab.

## Finding a Citadel to Conquer

Prusiner grew up in America's heartland. His paternal grandfather, the family's first American citizen, emigrated from Moscow in 1896 to join a small community of other Russian Jews in Sioux City, Iowa. In 1942, Stanley B. Prusiner was born in Des Moines, Iowa. The bare bones of his biography can be found in short autobiographical accounts[7] he wrote after receiving a Nobel Prize. When he was ten, the family moved to Cincinnati, Ohio, where his father could find a better position as an

architect. A good student, he "found high school rather uninteresting," but enjoyed his college years (1960-1964) majoring in chemistry at the University of Pennsylvania. Next he entered medical school, also at the University of Pennsylvania, where he inclined more toward research than toward clinical medicine. After a research project as an undergraduate, in medical school he worked in the lab of a famous biophysicist at the medical school, Britton Chance, who used light and fluorescence to characterize living tissues and tumors. In Chance's lab and in a Swedish lab during much of his last year in medical school (1967-1968), Prusiner studied heat production by specialized fat depots of the Syrian golden hamster, a rodent that would play a major role in his later prion work. Deciding to devote his life to biomedical research, he obtained an NIH fellowship, a yellow-beret position like those Michael Bishop and Harold Varmus took a few years earlier. The fellowship would begin in 1969, after a year of internship (now called the first year of medical residency). Although "not enthusiastic" about spending a year working every other night, he applied for an internship in Holly Smith's Department of Medicine at UCSF, beginning in July 1968. He "managed to survive [the internship] because San Francisco was such a nice place to live."[7] Aside from that indication that Prusiner found the Bay Area attractive, I have not been able to discover his reason for choosing a city so distant from his home and his medical school.

The young intern made strong impressions on teachers and colleagues. Curtis Morris, then director of the department's Clinical Research Center, remembers Prusiner as "a very bright fellow[, who] . . . didn't hesitate to disagree with me or anybody else, and . . . enjoyed pointing out logical issues, [saying] 'You've misread that. You misunderstand that. This can't be.'" This argumentative style "bothered a lot of people, including some of his fellow house officers," but Morris thought that Prusiner—"always interested in mechanisms"—would "challenge and argue, not because he didn't think you knew what you were talking about but because he was trying to learn himself. He was trying to work through the argument, . . . by disagreeing."[8] Morris, who liked the young man and later became a good friend, says he "also recognized that [Stan] was not a very comfortable person with himself" and "didn't feel comfortable with a lot of people." Why this was the case Morris doesn't know, but "I think he thought, very early on, 'I've got to be something. I'm not going to be pushed around.'"[8]

One of Prusiner's fellow residents, Morris Schambelan, remembers him as a serious, passionate young doctor who could fight hard and effectively for his patients. With considerable difficulty, for instance, Prusiner arranged a kidney transplant for a poor black man whose end-stage renal failure would have killed him without this

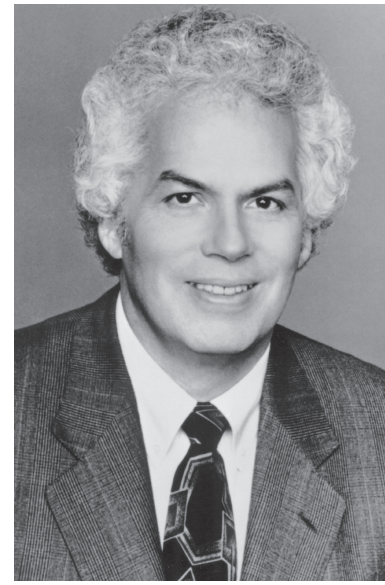
intervention. Similarly brave passion could also lead him to launch brash criticism at a senior authority figure—most memorably on the occasion of a seminar by a visiting expert on metabolic diseases. Garbed in his white intern's uniform, Prusiner listened a while, then rose from the audience to demonstrate on the blackboard that the speaker's account was dead wrong.[9] Such upstart behavior surprised many and shocked a few. Like all medical schools, this one—despite its location in a town notorious for attracting hippie flower children rebelling against their parents' culture—took itself very seriously.

Prusiner appears to have enjoyed the role of *enfant terrible*. In 1969, during his exit interview from internship, he told Holly Smith, the Chief of Medicine, "I'm going to walk to the center of the Golden Gate Bridge, and take this stethoscope"—which he withdrew from his uniform pocket—"and throw it as far as I can, into the Bay!" Dryly, Smith responded, "Stan, I think that would be an excellent idea."[10]

Prusiner spent the next three years at the NIH, working in the laboratory of Earl Stadtman, a biochemist famed for his pioneering studies of enzymes and metabolism.[11] Studying bacterial enzymes that make and break down glutamine, Prusiner learned how to purify proteins and to appreciate the crucial importance of accurately assaying the activity of the protein he was trying to identify—knowledge that would later stand him in good stead. He was also exposed to the so-called "Stadtman Way," whose key ingredients were hard work, absolute integrity, and rigorous analysis of experiments in order to rule out every possible interpretation except the correct one.[11]

During his last year at the NIH, Prusiner decided that his research career would focus on the brain. He considered two possible routes into science. The simpler one would have involved a fellowship in a lab focused on basic neurobiology, but Prusiner chose a more circuitous path, apparently contrary to his preference for science over clinical medicine—that is, he would pursue residency training in clinical neurology, as "a better route to developing a rewarding career in research."[7] This choice crucially set the ambitious young man's future course. A subsidiary choice—to take his neurology residency training at UCSF, rather than elsewhere—may have been determined by meeting Sandy Turk, a math teacher in San Francisco whom he married in 1970.

Robert Fishman, chair of UCSF's Neurology department, was not sure he wanted to accept Prusiner into his residency program. An outstanding clinician, Fishman had been recruited to San Francisco six years earlier from Columbia, in the wake of John Saunders's deposal as chancellor. For him, clinical neurology and teaching were nearly sacred duties. Prusiner's preference for research over both these duties triggered serious doubts for Fishman, but two scientifically oriented faculty he had recruited earlier, Ivan Diamond and Howard Fields, strongly supported Prusiner's



Stanley B. Prusiner, discoverer of prions. In 1982 his lab identified the protein that transmits an infectious neurodegenerative disease in an unprecedented new way. Photo taken in 1991.

candidacy, as did Holly Smith. On his recommendation, Smith scribbled a summary: "Prickly, but worth it."[10] That succinct judgment proved correct, but it cannot have been easy to be sure what would become of this young, occasionally combative outsider, smart and capable but uncomfortable with himself and with the world around him—and clearly bent on conquering it.

Fishman accepted Prusiner into the residency in 1972, but in subsequent years would repeatedly present obstacles to Prusiner's progress. Their conflicts reflected both Fishman's devotion to clinical excellence and his managerial style—withering wit combined with demanding standards of behavior and performance. (Both the wit and the standards, I suspect, were patterned after his own mentors at Columbia.[12]) "Bob ran his department through criticism," Fields says. "If you were a good scientist, then you were not a good clinician; if you were a great clinician, you didn't do anything in the lab . . . [Y]ou'd walk into his office, and you'd walk out feeling bad because you weren't living up to his expectations."[13] In addition, Prusiner came to feel that Fishman had little understanding of and no real interest in biochemical mechanisms of disease.[8]

Prusiner began the residency in July, 1972. Two months later, he admitted a woman patient who had experienced progressive memory loss and difficulty performing routine tasks. He was surprised to learn that the woman was dying from Creutzfeldt-Jakob Disease, a devastating but fortunately rare disorder that produces multiple holes in the brain, making it look like a sponge. CJD, a "spongiform enceph-

alopathy" (sponge-like brain disease), was thought to result from infection by an as yet unidentified slow virus. It closely resembled two other Transmissible Spongiform Encephalopathies (TSEs, for short). In the late 1950s Vincent Zigas and Carleton Gajdusek had discovered kuru, which was transmitted by cannibalism among the Fore people of New Guinea. The other disease, scrapie, was first described in European sheep 200 years earlier. Each disease had been transmitted—with incubation periods that lasted months or even years—by injecting brain extracts from affected people (who died of CJD or kuru) or from sheep (dead from scrapie) into the brains of monkeys or rodents.[14]

"Captivated" by the strange properties of the elusive "slow virus" of scrapie, Prusiner read avidly about TSEs. During the two years of his neurology residency, he learned that the agent appeared remarkably resistant to X-rays, chemicals, and enzymes that destroy most proteins, RNA, or DNA. Moreover, unlike an ordinary virus, it managed to kill the host without producing an immune response or inflammatory reaction. Finally, the disease—or at least susceptibility to it—was inherited. Some sheep strains resisted scrapie infection, but others were sensitive. Identifying the scrapie agent and determining its structure, Prusiner felt, would make a "wonderful project."[7]

Prusiner began with a single guiding principle—the only way to identify the scrapie agent and learn how it caused disease was to purify it biochemically, using its unique physical properties (size, electrical charge, etc.) to separate it from other components of diseased tissue. Fundamentally, this was the approach he learned in the Stadtman lab. But applying this strategy to scrapie made his quest a risky one. Measuring an enzyme's activity required only a few hours' work, but assaying the scrapie agent required injecting it into the brain of a mouse (or another animal) and then waiting at least six months, and sometimes a year, for the infection to kill the animal. It would take a very long time to know whether any individual purification step worked, and therefore to plan the next step. If one approach didn't succeed, could Prusiner afford to wait many more months to design an alternative? Where would he find the money to pay for maintaining large numbers of infected animals while he waited for some of them to die?

Prusiner recognized the daunting assay problem from the beginning. "I had no clever idea about how to circumvent [it, but] . . . I did think that after working with the scrapie agent . . . I might eventually be able to develop [a satisfactory] assay."[1] Hindsight may show that he managed to do exactly that, but his choice poses an enigma. Knowing how hard the problem would be, where did Prusiner find the courage to tackle it? Similarly, as trials and setbacks accumulated afterward, how did he

summon the persistent, stubborn resolve to keep on trying?

This fledgling barbarian had chosen to capture a citadel of science's empire that would prove nearly impossible to conquer. Without the requisite gold-plated armor—the accoutrement that dazzled Mobley later—he risked appearing irretrievably odd to the empire's regular citizens. In the workaday research world of the 1970s, pursuing the agent of so rare a disease seemed at best an odd windmill-tilting exercise and, even odder, dangerous to an ambitious man's career. One person considering a position in Prusiner's lab was advised to stay away from slow viruses because "Other people who have tried this field have failed. It's very risky."[15] Instead, scientists should tackle more significant problems, like cutting and splicing DNA or finding oncogenes, and in simpler systems (e.g., plasmids for Boyer and Cohen, tumor viruses for Varmus and Bishop).

## Slow Progress, Followed by Disasters

In 1974, during his residency's last year, Neurology offered Prusiner a position as assistant professor, with a small lab on the seventh floor of Health Sciences East (HSE-7). The offer replayed the themes of 1972—Fishman's continuing doubts, opposed by research-oriented faculty who strongly supported the new hire. For years the department would remain divided into two factions, with Fields, Diamond, and Prusiner arrayed on one side and more clinically oriented faculty on the other. Fishman oscillated, siding first with one group, then with the other.[13]

Once on the faculty, Prusiner had to cope with the classical, constantly recurring challenges of academic research—funding, lab personnel, and experiments. The first challenge was to obtain grant support from the NIH. In 1975 Prusiner was awarded two grants, one focused on glutamyl transpeptidase (about $40,000 per year, supporting extensions of his previous work in the Stadtman lab at the NIH), the other on "slow brain diseases" (about $60,000 per year, to support his studies of the pathogenesis of TSEs).[16] As a beginner in the scrapie field, he faced serious competition from established researchers, including Carleton Gajdusek, who had just taken a position at the NIH in Bethesda and would soon (in 1976) receive a Nobel Prize for discovering the transmission of kuru. Still, in that year Prusiner received further substantial support for his scrapie project—an award from the Howard Hughes Medical Institute (HHMI).[16] Holly Smith, an HHMI advisor, probably helped nudge the award toward Prusiner, as he helped the HHMI to support Herb Boyer and other UCSF faculty in the 1970s.

The NIH and HHMI probably didn't suffice to cover the costs of caring for research animals, plus laboratory expenses and salary support for postdocs and a cou-

ple of technicians. (By 1977, the lab would house two or three postdoctoral fellows and two technicians, and occupy approximately one fourth of HSE-7.[17]) By the early 1980s, and perhaps earlier, Prusiner spent a good deal of the time on the road, giving seminars and seeking funds from foundations and private sources to support his scrapie research.[18] According to one of Prusiner's collaborators in later years, fund-raising "was one of Stan's great skills. He could spin straw into gold, and routinely did."[19]

Another spur to travel was the need to recruit good postdocs to the laboratory. He made no pretence of wanting graduate students in his lab, on the ground that they could not be as productive as young people who had already learned to do research. Prusiner's talks about his scrapie research surely attracted a number of postdocs. These talks—as I remember from hearing an early version of the scrapie story, sometime in the 1970s—were not only forceful and clear, but also compelling and downright evangelical.

Quite soon, however, Prusiner's failed attempt to attract one potential postdoc showed that he would have to skirmish against forces besides the chair of his department. Finishing his PhD at Caltech in 1974, Robert Rohwer had been offered a postdoctoral position to study scrapie in Gajdusek's laboratory at the NIH. Hearing that Prusiner planned to tackle scrapie in San Francisco, he asked for an interview. At the interview, Rohwer remembers expressing discomfort with Prusiner's affiliation with Neurology, but feeling somewhat assured when "he told me that he was going to have a joint appointment in biochemistry." A bit later, he says, a friend in UCSF's Biochemistry department told him, "No, there's no way we're going to give him an appointment in this department. Forget that. He's lying to you." From then on, Rohwer distrusted Prusiner.[20] At that point Prusiner was a fledgling faculty member, unschooled in academia's genteel propensity for hinting yes while leaning toward no. He may have been misled, and my guess is that he genuinely believed Biochemistry would give him a joint appointment. Rohwer thought of Prusiner as a neurologist rather than a biochemist or molecular biologist. "He didn't strike me as an intellectual, not the kind of person that I was used to dealing with at Caltech."[20] Prusiner came to feel that this kind of snobbery afflicted his home institution also. Howard Fields thinks that much of UCSF's scientific establishment—including the Biochemistry department, virologists (especially Mike Bishop), and faculty in the Neuroscience Graduate Program—ignored and disdained Prusiner for years.[13] (Biochemistry did offer Prusiner a joint appointment in 1976, two years after his interview with Rohwer, but made him only a Lecturer, rather than an Assistant Professor. The department waited twelve more years, until 1988, to award him a joint appointment as Professor of Biochemistry.)

In addition to fund-raising and recruiting postdocs, Prusiner worked hard at launching his research program. Everyone who knew him then agrees that his focus on this effort was phenomenal, in the lab and out. According to one early postdoc, Prusiner "worked all the time." In the lab during the day he planned experiments with technicians and other co-workers. At night, "[H]e would go home with a backpack full of books or magazines, [or] journal articles. . . . I'm sure that's what he did every night, until he just conked out."[21] Prusiner responded to the NIH's failure to fund his first scrapie application by setting up a collaboration with two experienced TSE investigators, William Hadlow and Carl Eklund, at the NIH-funded Rocky Mountain Laboratory in Hamilton, Montana. Hadlow and Eklund, he wrote later,[7] "taught me an immense amount about scrapie" and helped him jump-start a bold research program explicitly aimed at devising a viable method for purifying the scrapie agent.

The first substantial result of this collaboration, published in 1977, began by stating the problem: "The scrapie agent has defied isolation and identification."[22] Its oddball physicochemical properties, slow replication in host animals, lack of detection by host defenses, and resistance to inactivation by heat, formaldehyde, or large doses of ionizing radiation all suggested that the agent "is a novel infectious entity" and had led other investigators "to speculate that the agent may not contain a nucleic acid [i.e., RNA or DNA], but may be composed only of carbohydrate, or possibly protein." Moreover, difficulties in purifying the agent reflect "the inconvenient [that is, almost impossibly slow and prohibitively expensive] titration assay in mice." Each theme—unusual biochemistry, peculiar interactions with host tissues, resistance to inactivation, the dauntingly arduous assay, and the possibility that the agent was a protein without a nucleic acid core—would intrigue Prusiner and trigger controversy with his competitors for years.

Progress at first was at best incremental—advances came in tiny steps, and the correct path forward remained hard to see. In this first paper on the subject, Prusiner reported experiments on the scrapie agent in spleens of infected mice.[22] (After injection into the brain, the agent accumulates first in the infected animal's spleen and later, in much larger amounts, in the brain.) Spleen homogenates of infected mice, usually treated with a detergent to dissolve cell membranes, were spun in plastic tubes (centrifuged) at high speeds for various periods of time, allowing particles of different sizes and densities to fall to different positions in the tube. Fractions of the centrifuged fluid were then separated by drawing fractions first from the top of the tube and then proceeding to the bottom. Finally, each fraction was diluted appropriately and assayed for scrapie agent. For the assay, a small amount of fluid from each dilution was injected into the brains of six mice. After waiting up to a year for the

animals to show signs of brain disease, the amount of scrapie agent was reported as the ID50 ("infectious dose-fifty per cent") in a standard volume. The ID50 "titer" is a multiple of the number of doses in such a volume that reproducibly infects 50% of injected mice.

The results indicated (albeit not at all precisely) the size of the scrapie agent, which sedimented in the centrifuge at a position similar to that of ribosomes, the cellular machines that translate mRNA sequences into amino acid sequences of proteins (see Chapter Six). The incremental "good news" was that—contrary to suggestions by other labs—the scrapie agent need not associate with cell membranes. But the conclusion was resolutely optimistic—"it will be possible to isolate the scrapie agent."[22]

During the next year, two papers presented further small advances. One showed that the scrapie agent could be separated from ribosomes.[23] The second reported that a carefully designed series of centrifugations produced a fraction, termed "P5," which contained a decreased amount of cell protein, along with a substantial portion of the total amount of scrapie agent initially harvested from the infected animals.[24] In comparison to the starting spleen homogenate, the ratio of scrapie agent titer to total protein in the P5 fraction was twenty-fold higher—a significant enrichment, but nowhere near the degree of enrichment necessary to produce a "pure" preparation of the scrapie agent.

In further experiments, the P5 fraction was centrifuged under several different conditions—for instance, with and without detergent (to assess the agent's water solubility); through gradients of increasing concentrations of sucrose, an inert sugar, or after placing a "cushion" of high-density sucrose at the bottom of the tube (for information about sizes and densities of scrapie particles). The results suggested that some scrapie particles (those of the smallest apparent size) were tinier than any known animal virus.[24] Surprisingly, in some gradients similar amounts of scrapie agent were found at virtually every sucrose density, suggesting that the agent in solution could take on a wide range of densities and sizes, perhaps by aggregating with itself or with other particles in the tissue extract.

Exposing the P5 fraction to elevated temperature (e.g., 37 °C instead of the usual 4°C) for 30 minutes before centrifugation produced an even bigger surprise. The scrapie agent was not inactivated, but the higher temperature induced most particles of the agent to form large aggregates, many-fold bigger than the small apparent sizes seen at 4°C.[24] This behavior, unusual for most proteins and nucleic acids, suggested to Prusiner and his colleagues that the scrapie particle is hydrophobic. In other words, its surface—like those of fats, waxes, or oil droplets—"hates" water and prevents it from dissolving easily in water. In contrast, most (hydrophilic) carbohydrates, pro-

teins, and nucleic acids "love" water and dissolve in it readily. The scrapie agent's hydrophobicity, they suspected, might help to explain its mysterious properties—its apparent tendency to aggregate, and also why it was so hard to purify, resisted heat inactivation, liked to associate with membranes, and failed to elicit strong immune responses.[25]

These real but still incremental advances must have frustrated Prusiner, but he had known from the beginning that depending on such a slow and expensive method for detecting the scrapie agent would create big problems. Something had to change, and it did. First, an unexpected setback jolted the lab—the NIH decided to stop funding scrapie work at the Rocky Mountain Lab, a move that would leave Prusiner very much on his own—a barbarian general with no horse (not even a mouse) to ride. Tackling the problem directly, he moved the mouse experiments to the Bay Area and tested the possibility of switching the assay to a different small animal, the Syrian hamster. Hamsters might prove useful because they die sooner than mice—two or three months after getting a brain injection of scrapie agent rather than after six to twelve months.

Most of Prusiner's mouse experiments had been performed at the Rocky Mountain Lab, where the animals' brains were injected with extracts prepared in biochemical experiments performed in San Francisco. Now, watching scrapie-injected hamsters with their own eyes in San Francisco, Prusiner's technicians—Pat Cochran and a recent recruit, Darlene Groth—made two key observations.[17] First, "clinical" signs of scrapie in the hamster—startling easily, head-bobbing, difficulty righting themselves when placed on their backs—did not just appear earlier than in the mouse, they also appeared more reproducibly from animal to animal. Second, injecting hamsters with higher titers of scrapie agent consistently induced earlier signs of disease (sometimes as soon as fifty-five days after injection) and the animals died earlier. Conversely, animals injected with lower titers of the agent got sick later and died later.

These observations gave Prusiner and his team the idea of setting up and testing a new incubation-time assay—less expensive and much faster than the previous (death end-point) assay—for measuring the scrapie agent titer in hamsters. Careful comparison showed that measuring the time of disease onset after injection of different dilutions of scrapie agent could estimate the ID50 as reliably as waiting to count the percentage of dead animals. The incubation-time method accelerated experiments enormously.[26] Prusiner later estimated that the acceleration was about 100-fold, because instead of injecting 60 mice and waiting a year to estimate an ID50, the lab could assess a high-titer sample with four animals in seventy days.[27]

The new technician experienced these big changes in the Prusiner lab first-hand.

Groth, a blonde woman of twenty-four with an AB in Biochemistry from UC Berkeley, joined the lab in 1977 and has worked there ever since.[17] She told her husband she was working for a "really energized and enthusiastic" boss, who might be headed for a Nobel Prize. At first she and Cochran would meet with him to plan experiments on a whiteboard in his office, and would send extracts from experiments to Rocky Mountain Labs. Along with the shift to hamsters the lab grew—comprising twelve people on HSE-7 by 1979, plus animal caretakers in a Prusiner-controlled animal facility across the Bay.[28] (Housing new people also required more space—a persistent theme. In the 1970s Prusiner persuaded Fishman to assign to him part of the HSE-7 lab of a fellow faculty member, at the time on sabbatical leave.[29] Eventually Prusiner's lab would take over the entire floor.)

Soon the animal assay effort became an efficient, well-oiled machine, churning out scrapie agent assays at a rate much faster than at the Rocky Mountain Lab, and indeed faster than in any other scrapie lab in the world. Working with Cochran and Groth, Prusiner planned and supervised the design of every animal assay. The two technicians kept very close tabs on the actual experiments, where meticulous bookkeeping was needed to keep track of experiments that involved first dozens, then hundreds, and eventually thousands of animals.[30] The animal operation functioned on its own, without direct participation by postdocs, transient lab citizens whose involvement in this lab-within-the-lab would inevitably reduce efficiency and accuracy.

Sometime in late 1978 or 1979, as the new operation shifted into high gear and began producing exciting new information at a phenomenal rate, Prusiner received devastating news, in two installments. He learned that Neurology might not promote him to tenure in 1980, the year he would complete his first six years as an assistant professor. In addition, after paying for his scrapie research since 1976, the HHMI would not renew its support after 1981, when his funding would be cut off. I do not know how he first heard either piece of bad news, although Stephen DeArmond, a pathologist who has collaborated with him since the mid-1980s (see below), says Prusiner showed him a letter from Bob Fishman, "saying something like, 'It's not appropriate for a neurologist to be working on a sheep disease, and maybe you should work somewhere else.'"[31] The HHMI does not make public its reasons for terminating or maintaining support.

Coming one after the other, these adversities must have hurt and discouraged Prusiner. Still, the tenure threat may not have surprised him, because Fishman's doubts about Prusiner were obvious to other Neurology faculty, including Diamond and Fields. Curtis Morris, who often discussed Fishman with Prusiner in those early years, says the two "were like oil and water. . . . Stan didn't respect his brain. Bob was

not in a position to really evaluate the science that Stan was doing, and Stan knew that." In the other direction, "Fishman really didn't know what to make of Stan, but he thought Stan was very aggressive, and very self-centered, and wanted more and more all the time." Morris says he advised Prusiner, "He's your boss. You've got to make your peace with him. And he is going to allow you to be successful, but you have to contain yourself a little bit." Moreover, he adds, Prusiner "had a free run. [H]e had the basic materials of what he needed to work with. . . . I said, 'You've got to understand, Stan, you're in a position now to do what you want to do.' I made that point with him over and over."[8]

Fortunately for both Prusiner and UCSF, in 1980-81 the tenure threat was averted. Prusiner's supporters, including Morris, Fields, and Diamond, all advised Fishman that refusing tenure to Prusiner would not be justified. All agree, however, that the decisive support for Prusiner came from Holly Smith, chair of the Department of Medicine. As the story goes, Smith told Fishman the young scientist had a great idea, and if he were to prove right—something no one could predict for sure—he would get a Nobel Prize. Smith takes no special credit for turning Fishman around, but admits to joining others in supporting Prusiner.[10] I suspect that Smith, an immensely powerful figure, knew how to apply quiet but critical pressure at just the right time.

## "You Name it, You Own it."

Termination of the HHMI's support posed a critical problem, which would not go away. Despite having developed a powerful new weapon (the hamster incubation-time assay), the barbarian would soon lose the wherewithal to maintain the siege, feed his animals, and pay his soldiers. Without substantial new funds to support the lab, the whole project would quietly cease. His relatively small NIH grant for the scrapie work had terminated back in 1978, and he had probably (I am guessing here) received relatively small gifts from a few donors to supplement his HHMI support, which was soon to disappear.[32] Such a threat would have induced many excellent scientists to yield to adversity and head off in a different direction. Instead, Prusiner sought the necessary money elsewhere, in gifts from private sources known to support research.[33] One of the first to furnish substantial support was RJ Reynolds, a tobacco company that followed the advice of Fred Seitz, a physicist who had just retired (in 1978) as President of the Rockefeller University. Beginning in 1980, Reynolds would support Prusiner's scrapie research to the tune of $3.75 million, or $417,000 per year over the next nine years.[34] (In 2010 dollars, this would amount to $1.09 million per year—enough to maintain a fair-sized biochemistry lab, but perhaps not enough to foot the bill for the Prusiner lab's animal-care costs at the same time.[35])

Julius Krevans helped to shepherd the Reynolds gifts in Prusiner's direction, and probably helped him to garner support from other sources as well. (Krevans was dean of UCSF's School of Medicine until 1982, when he became chancellor; see Chapter Three.) After receiving his first donation from Reynolds, Prusiner began to receive substantial support from the Sherman Fairchild Foundation as well.[34]

In the years before the HHMI cut off his funding, Prusiner had not purified the infectious agent, but had published a small number of papers describing its biochemical behavior in stringently refereed journals—one paper in 1977, two in 1978, and none in 1979.[36] Then the pace accelerated, with four such papers on the scrapie agent from the Prusiner lab in 1980, the year of HHMI's unfavorable decision, and four more in 1981.[37] (Of these eleven papers, Prusiner was listed as first author on ten. This high proportion was virtually unique in comparison to most faculty researchers, because published research is usually performed and written up primarily by students and postdocs. Then and later, however, Prusiner wrote many of the papers from his lab.)

Taken together, these eleven papers presented two complementary sets of incremental advances. In the first set, Prusiner's lab tested a large number of treatments, including exposure to chemicals, enzymes, heat, ultraviolet light, and X-rays. Repeatedly, ID50 titers were not affected by treatments that selectively degrade or destroy nucleic acids, while treatments that selectively destroy proteins did reduce the amount of scrapie agent. Although the weight of these results favored protein over nucleic acid as an essential component of the agent, no single treatment by itself proved conclusively that the agent must contain the former and lack the latter.

The second set of advances began by confirming that the agent was hydrophobic and tended to aggregate, assuming a wide range of sizes, from that of a tiny protein to that of a bacterium, as Prusiner noted later.[27] These advances also uncovered two different approaches that purified the scrapie agent more than 100-fold, relative to the starting hamster brain homogenate. Most gratifyingly, enhanced purity of the agent intersected with attempts to distinguish between nucleic acid *vs*. protein as its essential components. In earlier trials, a protein-degrading enzyme, proteinase K (abbreviated PK in this chapter), had reduced the ID50 of scrapie agent only weakly or not at all. As purification improved, however, PK proved useful in two complementary ways. First, treating brain extracts for thirty minutes with PK at an intermediate stage of purification left infectivity intact, but removed many irrelevant brain proteins, further augmenting the scrapie agent's purity (ID50 titer, relative to protein content) at later stages in the procedure. Second, prolonged PK treatment (three hours) of the most purified preparations effectively destroyed most of the agent, thereby establish-

ing that the agent contained at least one essential protein component, which resisted PK degradation better than most proteins but became PK-susceptible after irrelevant competing proteins were removed. (Even after purification, the agent remained resistant to enzymes that degrade nucleases.)

In retrospect, we can surmise that the slow pace of these advances, in combination with the startling inferences Prusiner seemed on the verge of drawing, contributed to the HHMI's negative decision. In the project's first four years of HHMI support, the scrapie agent had been purified no more than perhaps 30-fold, and none of the chemical or enzymatic treatments had unequivocally established the agent's chemical nature. Worse, Prusiner seemed to flirt with an unimaginable inference—that is, the agent might include no nucleic acid whatever. In his very first substantive scrapie paper, published in 1977, he had referred to this notion as "speculation" by other labs.[22] By 1980, the RNA genome of Rous sarcoma virus had unlocked genetic secrets of a cancer and Genentech, maker of recombinant hormones, had issued an initial public stock offering for $35 million. Few dared entertain any inference contradicting the growing consensus that every puzzle in biology would be explained by a combination of genes, DNA, and RNA.

By the end of 1981 purification had further improved, however, and flirtation would have been a mild term for Prusiner's bold dalliance with the heretical notion that the scrapie agent was primarily, and perhaps *only*, a protein. The dalliance became public knowledge in April 1982, during the "firestorm" triggered by his review article in *Science*.[2] This review's first six pages described evidence—for the most part already published—for the scrapie agent's hydrophobicity, its tendency to aggregate, and its possibly tiny size. It added that some fractions of partially purified agent suggested that it migrated as a particle of less than 50,000 daltons—that is, large enough to include a protein, but with little remaining room for a core of nucleic acid. Most important, Prusiner listed six different enzymatic or chemical treatments that indicated the presence of at least one essential protein in the infectious agent, and described the agent's resistance to several treatments known to destroy nucleic acids.[38] Then came the verbally insipid but nonetheless incendiary naming of the prion—"*protein*aceous *in*fectious [particle] resistant to inactivation by most procedures that modify nucleic acids."[2]

The name certainly did arouse opposition, even in the Prusiner lab and before publication. Postdocs in the lab at the time expressed disagreement with Prusiner's choice to name the agent a prion. Paul Bendheim says[15] he initially suggested "*prian*, for proteinaceous infectious agent," but Prusiner later told him, "I changed it to prion." Another postdoc, Frank Masiarz, left the lab in December 1981 to join Chi-

ron, Bill Rutter's new company. Four years later, however, he was described as having quit after Prusiner decided to publish the prion-naming review, on which would have served as co-author. "I wanted it to be a very critical overview in terms of the possibilities for the structure of the agent," Masiarz was quoted as saying. "By creating the name prion, [Prusiner] clearly wanted to push the entire interpretation in the direction of a protein-only agent. I said there's no point in creating a name for something that we don't even know exists yet."[39]

Outside the lab, many competitors in the small community of scrapie researchers strongly opposed Prusiner's bold public challenge, finding it not just wrong, but also genuinely offensive. The review "rankled everyone in the field," says Robert Rohwer. "For me personally," he says, "it was shocking that *Science* would publish something . . . so kind of crassly exploitive." The scientific evidence was poorly documented and described in the review, he thinks—"assertions and . . . kind of pseudo data [with] nothing to back it up." Then the review "jump[ed] to the conclusion that it was, therefore, a protein and couldn't be anything else." Finally, he concludes, "Declaring that this stuff from henceforth would be known as prions was just a bit much . . . . [T]he word prion itself was offensive to a lot of people simply because it . . . was such a contrived thing."[20]

On the issue of whether a prion really contained *only* protein, Prusiner's review carefully hedged its bet by admitting that "current knowledge does not allow exclusion of a small nucleic acid within the interior of the particle."[2] Rohwer felt that Prusiner "was just redefining viruses—you know, proteins and nucleic acids mixed up together in little chunks . . . . [T]his wasn't a real clear definition of anything."[20]

As a scientist working in a very separate field, I don't remember having any opinion about the propriety of Prusiner's review. Now, knowing that the term prion appears in every scientific paper on scrapie-related diseases,[40] I admire him for saying what he thought. In a censorious mood, I might wish Prusiner had stated that he was using the new name in order to propose a bold hypothesis—that a protein is *both necessary and sufficient* to transmit scrapie and scrapie-related diseases.[41] This alternative course could have incurred a bigger risk, compared to straddling the fence in order to "emphasize" the agent's protein component, and it isn't certain that a more clearly stated hypothesis would have spurred further experiments by others in the field. Moreover, weighing the consequences of his actions, Prusiner may have suspected that *Science* would not publish such a bold hypothesis in any case. He may even have judged that stating a mere hypothesis might not incite the kind of outraged contempt he wanted.

In fact, controversy incited by the review proved enormously useful to Prusiner.

He surely knew what would happen, despite his disingenuous later claim[1] that he "never imagined the irate reaction of some scientists to the word 'prion'—it was truly remarkable!" In contrast, one postdoc, Paul Bendheim, recalls that "Stan said, 'We need to come up with a new name for the scrapie agent.' I don't know if he said it there or to me privately at another point, but he said something to the effect, and it's true, 'If you name it, you own it.'"[15] A second postdoc, David Bolton, was later quoted as recalling that his boss told him and others in the lab, "If we coin a new term for it, [and] draw people's attention, . . . we're going to get money."[39] That prediction was accurate (see Chapter Ten). The idea that Prusiner kindled the firestorm on purpose fits with his account to a reporter four years later. The reporter snookered him into reckless braggadocio, and then printed it.[39] Describing a newspaper story that preceded the *Science* article, Prusiner told him, "They put my picture and prions in the upper left-hand corner of the front page on Friday. Reagan was on the right. And everyone in the world played catch-up on Saturday. *The New York Times* was very upset. So was everybody else. That kind of thing did more than anything I could ever do. The prion became a household word among biologists immediately. They didn't even have to read *Science*.'"[39]

Public boasting—this was published in *Discover* magazine—is guaranteed to trigger holier-than-thou responses from scientists. The feelings behind his bragging are easy to understand. Prusiner's resolute dedication to understanding scrapie had met with a major cutoff of funding and threatened refusal of academic tenure. Having overcome those obstacles, he was consumed by a provocative and exciting idea and sure it would prove correct. In his early forties, this young man—like many successful people—took himself very seriously. His gloating betrayed a degree of naïveté and a lamentable inability to laugh at himself, but it was excusable, perhaps even inevitable. I am sorry, however, that the response to his boasts taught Prusiner to refuse future in-depth interviews.

The *Discover* piece harped on the heresy of Prusiner's protein-only idea, and he himself later termed it "truly heretical."[1] Perhaps so, but Prusiner escaped the fate of most heretics because—as one of his collaborators reminded me, the difference between heresy and science is simple—"the scientist does the experiment."[19] Besides, as a label heresy fails to evoke Prusiner's explosive combination of pugnacity, willingness to follow clues wherever they may lead, and unrelenting eagerness to meet any challenge. Rather, it is more helpful to consider Prusiner in the years before 1982 as a young barbarian rebel-in-training, preparing himelf to raid the citadel. Some UCSF scientists and competitors like Rohwer may have already written him off as an untutored barbarian, but he didn't fully embrace barbaric rebellion until April 1982.

Naming the prion before fully identifying it, he told the world he had taken charge of a new infectious agent and was ready to defend his hegemony against all comers. The enfant terrible of 1969 had finally become a rebel proclaiming victory from just outside the gates, even before his siege engines were ready. The unspoken barbaric message, sensed by a teacher during Prusiner's internship, was stark—"I've got to be something. I'm not going to be pushed around."[8]

The next chapter tells how the barbarian made good his promise and earned gold-plated armor—first by routing enemies in pitched battle, but eventually by defeating a much mightier foe, in the form of a nearly insoluble scientific riddle.

Chapter Ten

# Marching into the Citadel

## In Golden Armor

The autoradiogram trumpeted good news, loud and clear. After four months in Stanley Prusiner's lab, David Bolton had just the result he hoped for. Elated, the twenty-eight-year-old postdoc wrote "February 5, 1982" on the film and slowly began to realize what his finding meant. To the uninitiated, the autoradiogram (illustrated on next page) conveys a murky message, at best, but Bolton saw, with welcome clarity, a protein that must be a part of the scrapie agent itself. He would soon transmit the news to his labmates, his lab chief, and the editor of a scientific journal—"This changes everything."[1]

The illustration shows eight vertically arranged stacks, or "lanes," of horizontal black bands and splotches that run from top to bottom of the image. Lanes 3, 4, and 5 contain the splotches (white arrows) that most excited Bolton, because they represent extracts from brains of hamsters killed sixty days after injection with scrapie agent. In contrast, splotches at this level are conspicuously absent in two other lanes (6 and 7), which represent brain extracts from hamsters injected with a salt solution.[2] Horizontal splotches show the locations of radioactively tagged proteins, separated from other proteins by size (big at the top of a lane, small at the bottom), and detected on sensitive film. (An autoradiogram detects small amounts of radioactivity, just as an X-ray film detects X-rays.)

Bolton's revealing splotches reflected eight years of hard slogging and incremental advances by Prusiner's lab, followed by a few weeks of his own preliminary experiments. In this end-game, two hard-won advances by Prusiner and his technicians played essential roles. First, as we saw in Chapter Nine, the scrapie agent proved relatively resistant to degradation by proteinase K, so that exposing partially purified extracts to this enzyme for a short period removed most other proteins but left the scrapie agent (and its protein) intact. Second, applying a new wrinkle to the sucrose gradients took advantage of the agent's hydrophobicity and tendency to form large aggregates.[3] The lab's switch to the incubation time assay in hamsters (Chapter Nine)

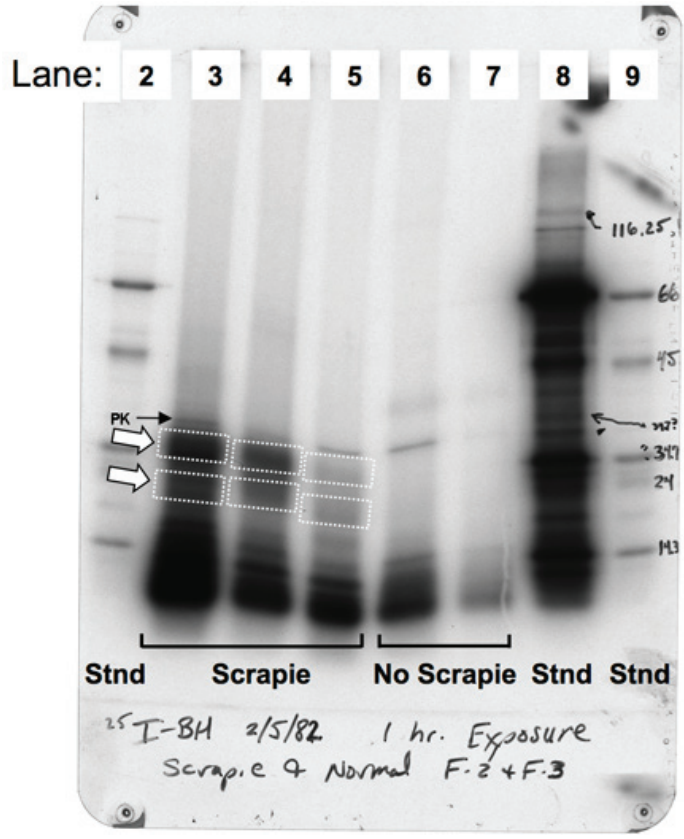



Figure 3: First identification of the prion protein

Proteins tagged with radioactive iodine ($^{125}$I) were subjected to an electrical field that made them migrate from the top to the bottom of a porous gel, in eight "lanes," numbered 2-9. The size of pores in the gel dictated that small proteins migrated faster and further toward the bottom, while larger proteins migrated less rapidly. The scrapie agent protein shows up as blurry horizontal bands (white arrows, outlined in white dashed boxes) in lanes 3, 4, and 5, which contain partially purified proteins from scrapie-infected hamster brains. (These lanes represent fractions from sucrose gradients performed on extracts from two different hamster brain preparations—lane 3 is from one preparation, while lanes 4 and 5 are separated fractions obtained from a different preparation. Both 3 and 4 contain more scrapie protein than does fraction 5.) Such bands are absent in lanes 6 and 7, which contain proteins subjected to the same purification procedure, applied to extracts from brains not injected with scrapie agent. Lanes 2, 8, and 9 contain protein standards, with relative sizes, in kilodaltons, scribbled on the right-hand side. PK (and the black arrow) indicates the location of proteinase K, which had been added in the course of purifying the brain extracts, as described in the text.

made both advances possible, because it allowed testing and devising refinements to a long series of gradually improving purification schemes.

In late 1981, Prusiner had asked Bolton to take over the project of Frank Masiarz, a postdoc who was leaving to work in Bill Rutter's company, Chiron. In Masiarz's lab notebook, Bolton found hints of radioactively labeled bands that were present in scrapie samples but not in extracts of normal brain. Masiarz had noticed the same hints, but doubted they were real because he couldn't reproduce them. Bolton spent January 1982 testing biochemical tricks designed to make the bands easier to see and more reproducible.[4] The first truly revealing experiment (shown in Figure 3) compared brain extracts subjected to the purification procedure for the scrapie agent, but derived from either scrapie-infected or normal (uninfected) hamster brain. The tricks worked—two protein bands in scrapie samples were absent from normal brain extracts. Compared to standard proteins of known size (see Figure legend), the bands migrated close together, with apparent sizes between 27,000 and 30,000 kilodaltons (that is, the weight of 27-30,000 protons).

Prusiner was traveling at the time, perhaps on one of the money-raising tours that kept the lab going, so Bolton announced the good news to Paul Bendheim and Michael McKinley, other postdocs in the lab. "You'd better really make sure you've got this, before you tell Stan," they told him.[1] He repeated the experiment several times, with exactly the same result. Soon after Prusiner returned from his trip, Bolton began writing a paper, which they planned to submit for publication in *Science*, a prestigious journal.

Bolton remembers that Prusiner told him that Eleanore Butz—the tough, no-nonsense editor who shepherded most biology papers through the review process at *Science*—"didn't want to publish this paper if it was not important." Prusiner asked him to "tell her what you think you've found." As he remembers the telephone conversation, Butz "started to ask me a question, and I said, 'Look, this protein is a part of the agent,' and I told her that this changes everything. 'If this is right, and I think it is, then everything changes from here, because now you have a molecular handle to identify this agent.'" Afterward, Prusiner told Bolton, "I don't know what you told her, but whatever you did was the right thing. They decided to publish the paper."[1] Entitled "Identification of a Protein that Purifies with the Scrapie Prion," it came out in September, 1982—five months after Prusiner's shocking prion manifesto, described in Chapter Nine.[2]

The manifesto had announced that something called a prion causes scrapie, kuru, and CJD. Although published eight weeks or so after Bolton saw the autoradiogram shown in the figure, the manifesto had been submitted to *Science* before Bolton's

exciting experiment, and did not mention it. Naming the prion and proclaiming the pathogenesis of three fatal brain diseases shocked competitors and proved useful for Prusiner, even though the triumph was not backed up by irrefutable evidence. But identifying a protein in the scrapie agent in fact made real triumph possible. Bolton's new finding truly did change everything. Deep beneath the citadel's walls, Prusiner had begun to dig a tunnel. More digging, plus a few well-placed explosives, and the walls would topple.

Naming the prion and identifying a prion protein did indeed presage a remarkable increase in research funds (see accompanying graph). From near zero in 1980, Prusiner received $794,957 from the NIH and $698,481 in non-federal funds in 1985, for a total of $1.493 million. In 1975 dollars, his funding in 1985 would have amounted to about $708 thousand, or four times his yearly grant funds in 1975—and approximately half his yearly grant funds in 2010 (again in 1975 dollars; see the graph's caption for details).

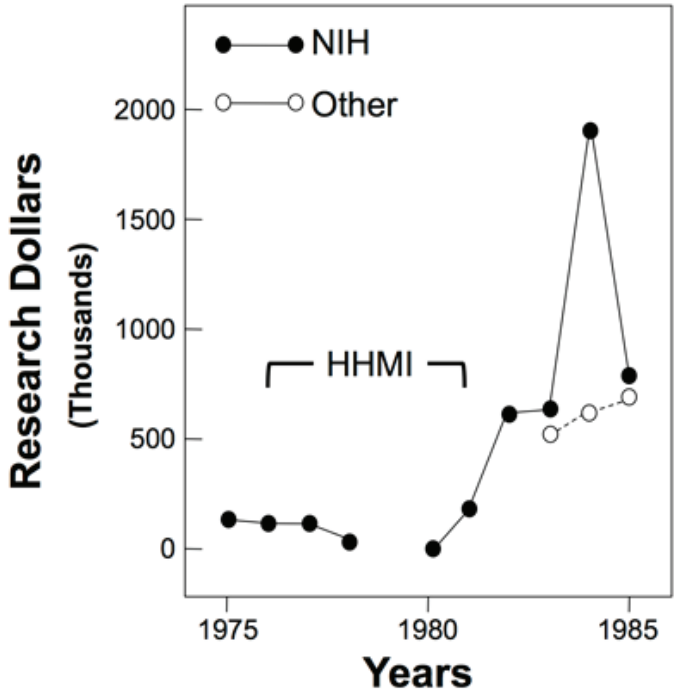
For Prusiner and his lab, 1982 was a banner year. "You name it, you own it!" would prove an accurate prediction.

## Glitches, Then a Riddle

The naming may have proved useful for grant purposes, but the autoradiogram's bands furnished irrefutable evidence for a protein component in the scrapie agent. The protein was initially called PrP27-30, meaning "Prion Protein, 27-30 kilodalton form." More important—as Prusiner could guess but could not yet be certain—PrP27-30 would furnish an indispensable molecular bulldozer for pushing aside otherwise impassable barriers to discovering an unprecedented way of transmitting infectious disease. Over the ensuing three years, those barriers began to shift. Later, when they finally fell aside, PrP27-30 would suddenly pose a baffling, unexpected challenge.

Immediately after finding the prion protein, the first step was obvious but by no means easy. Prusiner set his technicians to purifying larger amounts of the protein, to be used in making antibodies against PrP27-30—antibodies invaluable for detecting prions in tissues and cells from animals and patients. He first assigned Paul Bendheim, a postdoc, to isolate an anti-PrP27-30 monoclonal antibody—that is, an antibody made by a clone of immune cells from a mouse. After a year or so, Bendheim was joined by a second postdoc, Ron Barry, a budding immunologist.

Although considered more suitable for making antibodies against tiny amounts of protein (all the lab had available at the time), the monoclonal approach didn't work. So Bendheim tried a more traditional alternative approach, immunizing rabbits and looking for polyclonal antibodies—that is, antibodies produced by many




Graph 3: Research funds awarded to Prusiner, 1975-1985

As indicated by the filled circles, Prusiner's first NIH grants were awarded in 1975 and funding continued through 1976 and 1977, but official NIH Reports indicate that he received very little NIH support in 1978 and none in 1980. (The figure for 1979 could not be found.) Beginning in 1981 his NIH funding resumed and grew substantially thereafter. The one-year peak in 1984 reflects a Jacob Javits Award of almost a million dollars (money that could be used in later years, as well). The years (1976-1981) in which he received funds from the HHMI are indicated, but that organization does not reveal dollar amounts of support to individual researchers. He probably received additional non-federal funds before 1983, but UCSF was able to provide figures only for that year and thereafter (open circles). This money includes grants and gifts from the RJ Reynolds Tobacco Company, various foundations, and private donors. His total research funding in 1985 was about $1.49 million, or approximately ten-fold greater than his NIH grant funding in the 1970s. For the record, Prusiner received $6,890,418 and $5,144,767 from the NIH in 2009 and 2010, respectively. In 1975 dollars, this would amount to approximately $1.3 or 0.93 million, respectively—approximately twice as much as in 1984 or 1985. Funding in the intervening years is not shown, but was certainly substantial. [Values in 1975 dollars are computed by using the Biological Research and Development Price Index (BRDPI), which is described in Chapter Two, reference 13.]

different immune cells, but directed against one immunizing target protein. "Naively," he imagined that "If I could give a real hefty wallop [of PrP27-30] to a rabbit or two, let's see what happens." So he used most of the PrP27-30 he had, and "Lo and behold, we got this polyclonal serum."[6] As expected, the antibody reacted with the bands of PrP27-30 purified from scrapie-infected hamster brain, but not with similarly treated extracts from uninfected brain.[7] It also detected microscopic structures, characterized as "amyloid plaques," in slices of infected hamster brain, but not in normal brain.[8] Early on, Bendheim found that the antibody also detected a 34-kilodalton protein in homogenates of normal hamster brains.[6] (This finding was not published at the time, but was later confirmed by independent experiments in the laboratory of Stephen DeArmond, Prusiner's pathologist collaborator in studying scrapie pathogenesis.[9] The protein in normal brain would prove crucial.[10])

As a neurologist, Bendheim wondered whether the anti-PrP27-30 antibody would detect a protein in human brains of patients with CJD (Creutzfeldt-Jakob Disease). So he paid a visit to David Kingsbury, a collaborator of Prusiner's who worked with autopsied human brains at the Oakland Naval Hospital, across San Francisco Bay. That visit led to exciting results. The procedure for purifying PrP27-30 from hamsters also worked to purify the protein from CJD brain, and the CJD protein cross-reacted with anti-PrP27-30 antibody. The presumed CJD prion resembled hamster prions in molecular size and relative resistance to digestion with proteinase K, and also aggregated to form amyloid-like rods like those the Prusiner lab had described in PrP purified from hamsters.[11] Two papers, published in prestigious journals, provided the first immunologic and biochemical evidence for similar prion proteins in both scrapie and the human disease.[12]

To Bendheim's surprise, however, Prusiner—who had been out of town when he first talked with Kingsbury—"was not happy that I had started this collaboration without him initiating it."[6] As a result, he says, Prusiner asked his administrative assistant to "draw up a letter of understanding that I would not commence any new experiments or research activities during the remainder of my time [in the lab]. I thought, well, that's a weird thing to request. . . . [H]e clearly was unhappy that I had started these, quote/unquote, independent projects."[6]

To me and (I imagine) to many academic researchers, Prusiner's request does appear unusual. Rather than applaud Bendheim for taking the initiative, he reinforced control over someone he considered a potential loose cannon. Two other Neurology faculty (Howard Fields and Daniel Lowenstein, a Neurology department faculty member who worked for a period in Prusiner's lab) admire Prusiner as a scientist and colleague, but agree that he exerted unusually comprehensive control over the

research of every member of the lab. "[A] lot of people in his lab wound up really being upset with him," Fields says. "They felt their contribution wasn't fully recognized. The reality is that Stanley ran his lab as *his* lab. . . . [I]t was all his project, he conceived it, people did what he told them to. He was not into building other people's careers. He wanted to get people to do the projects that he wanted and then to go on, and he wasn't looking to develop his own competitors."[13] Lowenstein tells a similar story.[14] Ivan Diamond, one of Prusiner's allies in Neurology, stresses a subtly different point, that Prusiner felt if postdocs learn from their lab chief how to think they will be equipped to pursue their own interests afterward.[15]

Fields adds that Prusiner sometimes "alienated people who wanted to build their careers by being in his lab and then going out and establishing their own research careers. Stan didn't think that there was anybody who really could do that well in this area."[13] One former postdoc put it directly. When David Bolton was leaving the lab, he says, Prusiner advised him to leave prions and study atherosclerosis.[1] Later, after he and Bendheim began working on prions in New York, Prusiner forbade Michael McKinley, who was still in his lab, to talk with either of them, because they were potential competitors. (Ironically, Bolton says, one reason they got their new research positions was that the department chair in New York disagreed with Prusiner about scrapie pathogenesis and mistakenly judged that hiring them away from San Francisco would somehow interfere with Prusiner's future prion research.[1])

Darlene Groth has worked with Prusiner for thirty-three years, first as a lab technician and now as his lab manager. From her perspective, his goal was always simple—to keep the research moving ahead. His lack of willingness to nurture his postdoc's careers caused "a lot of resentment," she says, but he feels that postdocs "should go out and get their own job, that they should do it on their own and be independent and not be nurtured by him." Prusiner, she notes, "is patient and nurturing with new postdocs that come in, perhaps until a point when he realizes whether they're going to sink or swim. . . . But he comes to a certain point where he decides for their career whether they should continue on or go. [T]here will be times where he . . . knows it's time to stop [a project], which sometimes frustrates people. He'll say, 'No, we don't want to spend any more time doing that. It's not going anywhere. We don't have money for that.' It's like in industry when they say all of a sudden, we're not going to pursue this anymore." Prusiner is not a cold, calculating boss, she feels. "It's hard on Stan sometimes . . . . [P]eople think he's harsh, but actually he is a very compassionate person. I see that quite a lot in him."[16]

The salient point about Prusiner's handling of his postdocs, I think, is that he worked hard to exercise meticulous control of both ideas and experiments, and that

kind of control made him more effective in every endeavor. Accounts of his weekly lab meetings describe exchange of data and opinions among postdocs, followed by Prusiner's recording of major points and findings on a whiteboard.[17] (The whiteboard was sometimes photographed to make sure the record was not lost; in later years the recording was performed electronically by the board itself.) Later Prusiner would meet separately with one or two postdocs to direct and design experiments to exploit and extend the new findings. Precise, thorough supervision of every postdoc ensured tight coordination and minimal wasted effort, but left little room for independent thought, as Bendheim discovered when he initiated a collaboration on Creutzfeldt-Jakob Disease. In essence, Prusiner's policy of tight control simply extended an approach that worked elsewhere—in reserving to himself first authorship (and actual writing) of most scientific reports, in close supervision (and eventually design and construction) of his own animal-care facility, and even in choosing to name the prion himself. Not surprisingly, several postdocs are said to have felt that Prusiner slighted or took credit for their intellectual contributions. No one, however, would say this for direct attribution.

Prusiner's steadfast refusal to play the role of a collegial, nurturing academic would continue to irritate many colleagues at UCSF and to alienate some scientists who studied TSEs (Transmissible Spongiform Encephalopathies; see Chapter Nine) elsewhere. Robert Rohwer, a former postdoc of Carleton Gajdusek, argued strongly that the so-called prion was really a small virus under another name. As its title indicates, Rohwer's 1984 paper presented evidence that the "scrapie infectious agent is virus-like."[18] In addition to other discrepancies in Prusiner's evidence, he argued that proper comparison of scrapie infectivity with that of several known viruses showed that the scrapie agent was perhaps a bit smaller than most viruses, but similar to one or more of them in its relative resistance or susceptibility to chemical inactivation.

Prusiner's uncollegial style offended Rohwer, for whom "living in the Prusiner world was an extremely unpleasant thing." For instance, he says, publishing papers became difficult for scientists whose views differed from Prusiner's because "if you weren't on his side, you got blackballed." It also became hard to put together scientific conferences including researchers from both the scrapie-is-a-prion and scrapie-is-a-virus factions. Further, he says, Prusiner refused to supply others with reagents his own lab had developed. Finally, Rohwer found it "incomprehensible" that Prusiner "would train people in a field and then forbid them to work in it."[19]

Undeterred by opposition, Prusiner pushed full-speed ahead, and in a significantly new direction. Until this point he had relentlessly pursued one essential goal, isolation of a scrapie agent molecule. This had meant resolutely shunning the kind

of elegant molecular biology that enthralled many colleagues, who spent the early 1980s learning to clone genes and cut and splice DNA. With the scrapie molecule finally in hand, he moved quickly to exploit its potential for isolating a gene. By this time a few labs had developed technology for isolating cDNAs for specific mammalian genes, beginning with complete or partial amino acid sequences of the corresponding proteins. (A gene's cDNA is complementary to the mRNA transcribed from that gene, and ribosomes translate the mRNA make the corresponding protein; see Chapter Six.)

Finding a gene for the scrapie agent protein would require using the genetic code (Chapter Four) to guess the DNA sequence from that protein's amino acid sequence. For the crucial first step, obtaining the amino acid sequence, Prusiner chose a first-rate collaborator, Lee Hood. The Hood lab, at Caltech in Pasadena, CA, led the world in applying the fiendishly demanding new technology for determining amino acid sequences from tiny protein samples, comparable to the amounts of scrapie agent the Prusiner lab had isolated. Bolton accompanied Prusiner in a visit to Caltech to outline the project, but then Prusiner asked Darlene Groth, rather to Bolton's chagrin, to replace him in shepherding protein samples to Pasadena and monitoring the sequencing effort.[1] Rather quickly, Hood's lab determined the sequence of 15 or so amino acids from one end of PrP27-30.[20] Later they obtained sequences from other parts of the protein as well.[21]

Subsequent steps (skipping technical details) involved "reverse-translating" the amino acid sequence to create short DNA sequences that would hybridize (by base-pairing) with the PrP27-30 cDNA.[22] To isolate the actual cDNA, Prusiner reached much farther than Pasadena—all the way to Charles Weissmann, an outstanding molecular biologist in Zurich, Switzerland. Working together, the Weissmann and Prusiner labs isolated a cDNA encoding PrP27-30 from scrapie-infected hamster brains.[21]

UCSF in the early 1980s was a world leader in gene cloning technology, so why didn't Prusiner find a collaborator in San Francisco? The answer is simple—he didn't trust or respect the local experts. Many in UCSF's tight community of molecular biologists and virologists disliked Prusiner's personal style and feared contaminating their laboratories with a brain-destroying infectious agent. Worse, most felt sure he was dead wrong to suspect that the scrapie agent was just a protein, free of nucleic acid. In a recent hallway conversation, one of Biochemistry's old guard laughed and agreed: "Remember when Stan talked at our weekly department lunch—and everybody thought he was an idiot!" Rightly, Prusiner chose not to collaborate with people who thought that way.

Identifying the scrapie-agent gene must have been immensely satisfying, but it was followed by not one, but *three* unexpected results—all included in the paper, published in 1985.[21] First, the scrapie gene was not derived from a foreign invader, because normal (uninfected) hamsters expressed the same gene in their brains and many other tissues. Second, normal and scrapie-infected hamster brains contained similar amounts of mRNA encoding PrP. This more or less ruled out the hypothesis that scrapie resulted from accelerated transcription of a gene that normally remains dormant in brains of uninfected animals. Finally—as Bendheim had found earlier, but not published, and as DeArmond's lab was finding, in independent experiments—antibodies against PrP27-30 detected a slightly larger protein (33-35 rather than 27-30 kilodaltons in size) in both normal and scrapie-infected brain. Infected brains contained somewhat more PrP33-35 than did brains of uninfected hamsters. Proteinase K converted PrP33-35 from scrapie-infected brains to a size corresponding to PrP27-30, but completely destroyed PrP33-35 from normal brains.

The fact that PrP is a host protein surprised everyone, including Prusiner. The published paper noted that these observations could explain why the agent did not elicit immunologic or inflammatory responses in infected host animals—that is, the scrapie agent, composed primarily of a host protein, escaped such host responses because animals do not detect their own proteins as foreign.[21] But the authors could not easily explain the physical differences between the PrP molecules in normal and infected brains. In the latter PrP appeared to be slightly more abundant, protease-resistant, and folded into a different conformation or state of aggregation that might reflect subtle (and as yet undetected) mutations or DNA rearrangements, post-translational modification (e.g., phosphorylation or removal of amino acids by a protease), or association with an undetected second molecule present in either infected or normal brain.

The authors expressed similar puzzlement about whether scrapie infectious particles consist exclusively of some kind of modified PrP or of PrP associated with a nucleic acid. The fact that PrP is encoded in the host genome, they wrote, "would seem to support the argument for a scrapie-specific nucleic acid." For the scrapie protein to be infectious, even when devoid of nucleic acid, would require that its apparent structural difference from normal PrP (whether caused by mutation or something else) somehow renders "the 'normal' PrP protein infectious[, in a process that is] catalyzed by the modified protein itself, but not by the unmodified form."[21] In other words, the infectious PrP would have to convert normal (un-infectious) PrP molecules into the infectious state, in effect making more of itself from an otherwise harmless protein population in the host. Finally, the authors raised the possibility—in

their view unlikely—that infectious PrP somehow is associated with an undetected (but nonetheless pathogenic) virus, present in numbers much smaller than the number of PrP molecules.

## Solving a Riddle and Raising More Questions

After overcoming obstacle after obstacle, Prusiner now faced a much tougher challenge—an unanswerable scientific riddle. How does a normal cellular protein transmogrify itself into an infectious agent able to destroy the brain of a host animal or human? The ensuing decade would produce a satisfying and immensely surprising answer, different from what Prusiner or anyone else had expected. The arduous trek to that answer was conducted in a UCSF environment vastly different from the small world that gave birth to recombinant DNA, Genentech, oncogenes, and prions. As PrP and its cDNA posed a new riddle and transformed Prusiner's research program, his NIH funding increased and other scientists began responding more hospitably to his work. An international mad-cow epidemic, soon involving prion infection and dozens of human deaths from a new variant of CJD, suddenly made the brain's conversion of normal PrP into an infectious pathogen more than a topic for obscure scholarly debate. Prusiner's scientific memoir, soon to be published, will probably tell these stories in the rich detail they deserve. Here I'll focus on how Prusiner and his colleagues unraveled the formidable scientific riddle.

As always in science, the first task was to state the problem clearly. To do so, Prusiner and his colleagues coined two new acronyms. $PrP^{C}$ (the *C*ellular PrP in normal brains) clearly differed from $PrP^{Sc}$ ("PrP-*Sc*rapie," the protease-resistant, infectious PrP found in scrapie-infected brains), although antibodies raised against the latter protein detected its normal counterpart and both proteins appeared to be encoded by the same gene. That "appeared to be" was a crux of the scrapie puzzle. In the mid-1980s, none of the evidence absolutely *proved* either that $PrP^{C}$ and $PrP^{Sc}$ are encoded by the same gene, or that $PrP^{Sc}$ is both necessary and sufficient for infection. Prusiner strongly suspected both statements would turn out to be correct, although for the moment any mechanism proposed to explain scrapie infection still had to be hedged about with caveats. For us, peering through the retrospectoscope, certain facts make the rest of this story easier to understand, although they were not unequivocally established until later. Indeed, a single gene does encode both $PrP^{C}$ and infectious $PrP^{Sc}$, which contain the same amino acid sequence and are identical in size (i.e., the protein previously called PrP33-35). PrP27-30, the truncated prion protein (also infectious) whose partial amino acid sequence led to cloning the PrP cDNA, is shorter because proteinase K (added during the purification procedure)

lopped almost seventy amino acids from the front end of $PrP^{Sc}$.[23]

Fortunately, the sequence of a short string of amino acids from PrP27-30 gave Prusiner the PrP cDNA at just the right time in the explosive early development of molecular genetics. By the mid-1980s molecular biologists had begun to open wide gates to exploring mammalian genes. Scientists could clone, sequence, or alter any specific gene, and could also map its chromosomal location. They were rapidly learning to construct transgenic mice that differed from normal littermates only with respect to a single pre-designated gene. Over the next two decades, Prusiner would energetically exploit this lucky timing, which rewarded his unusual talent for keeping his eye on the ball and resisting the siren call of molecular biology until the right moment.[24] Sticking to the task of purifying the scrapie agent bore fruit just in time to take advantage of the DNA revolution.

Cloning the PrP gene soon produced the first signpost pointing toward a molecular genetic understanding of prion disease. Two mouse strains differed markedly in scrapie incubation times after the agent was injected into their brains—one developed scrapie eight months after injection, the other strain after only four months. Host strain differences in susceptibility to scrapie had been previously described in mice and were genetically analyzed in sheep twenty years earlier.[25] Now the Prusiner lab could ask whether the mouse gene responsible for the difference encoded $PrP^{C}$. In 1986 they reported that the $PrP^{C}$ gene and the gene controlling incubation time were closely linked and possibly identical in mice.[26] Later, the $PrP^{C}$ gene, located on mouse chromosome 2, was found unequivocally to control scrapie incubation times.[25]

If the PrP gene could determine incubation time in mouse strains, it might account for differences between host species and even for genetic predisposition to human prion diseases. Prusiner quickly recruited a cadre of bright postdocs to explore these possibilities, and they made three key discoveries. In 1989, they reported that differences at a few amino acid positions in host PrP produce reliably different scrapie incubation times—not only in different mouse strains, but also in mice *vs*. hamsters.[26] That year they showed that inheritance of certain PrP mutations makes humans likely to develop Gerstmann-Straussler-Scheinker syndrome, or GSS, a rare disorder which causes ataxia (impaired control of body movements) and spongiform encephalopathy.[27] And in 1993, Charles Weissman's lab and the Prusiner lab separately reported that mice totally lacking the PrP gene cannot be infected with scrapie.[28]

For years, Prusiner's opponents had cited evidence for different prion strains as virtually conclusive evidence that scrapie was transmitted by a virus, because to their minds a "strain" meant a virus with an altered nucleic acid sequence. They based this claim on the fact that scrapie-containing extracts from different animals (or strains

of animals) can transmit scrapie with different clinical characteristics or different incubation times in genetically identical host animals. By showing that prion strains reflect differences in the PrP gene, Prusiner's lab removed the need for postulating a virus.

Here a mouse strain engineered to express two similar but different prion genes—one from mouse, the other from hamster, provided an important lesson.[29] Given the choice between converting these two slightly different $PrP^{C}$ molecules into $PrP^{Sc}$, injected scrapie prions from diseased mice specifically chose to convert molecules like themselves (that is, endogenous mouse $PrP^{C}$) into $PrP^{Sc}$, while injected hamster $PrP^{Sc}$ chose to act on the endogenous hamster $PrP^{C}$. Incubation time was long (mouse-like) in the first case, and much shorter (hamster-like) in the latter. Thus the prion "strain" (hamster *vs*. mouse) chose to convert $PrP^{C}$ with its own sequence into $PrP^{Sc}$. The strain injected also dictated different locations and microscopic appearances of spongiform encephalopathy, even though the host animals were identical.

Another strong argument that viral nucleic acid is not required for prion infection came from studies of Fatal Familial Insomnia (FFI), a very rare form of human inherited prion disease in which inability to sleep precedes the onset of dementia.[25] PrP genes of FFI patients carry mutations different from those found in GSS. Moreover, infectious prions from FFI and GSS patients induce different patterns of brain pathology and different neurological defects when injected into brains of specially genetically engineered mice expressing a chimeric PrP containing sequences derived from both human and mouse genes.[25] Again, different amino acid sequences in $PrP^{Sc}$ from two donors induced clinically distinct disorders, but this time by attacking the same host $PrP^{C}$. The "strain difference" clearly stems from different structures of the infectious prion.

Thus the weight of the evidence now strongly suggested that the prion protein, *acting without input from an infectiously transmitted viral genome*, induces brain degeneration by causing replication of more infectious prions from a normal precursor protein, $PrP^{C}$. Armed with acronyms and new facts, we can succinctly phrase the question Prusiner now had to solve: How does $PrP^{Sc}$ convert $PrP^{C}$ into more $PrP^{Sc}$? Given the facts—including some he suspected, which now are known to be correct—the infectious process must somehow alter the structure of PrP without removing any of its amino acids.

How does that happen? In seeking an answer, Prusiner may have decided to follow Sherlock Holmes's favorite dictum—"When you have eliminated the impossible, whatever remains, however improbable, must be the truth." Indeed, several explanations, varying in plausibility, had been ruled out, leaving a conundrum impossible to

solve without more real facts.[30] So, in the latter 1980s and early 1990s he and his colleagues began to explore a thoroughly heretical notion—that the *distinctive shape* of an individual $PrP^{Sc}$ molecule (or a clump of such molecules) somehow induces $PrP^{C}$ molecules to assume a similar shape. Such a "templating" process could produce exponential increases in $PrP^{Sc}$.

Prusiner had mentioned this possibility, without making much of it, in his 1982 prion-naming paper.[5] There he cited a mathematician's theoretical proposal, in 1967, that a kind of templating might account for self-replication of the scrapie agent.[31] In the intervening years no one had presented a shard of evidence for or against this notion, although Prusiner and his postdocs had ruminated about the templating hypothesis for years. Similarly, Carleton Gajdusek—who has been quoted as claiming that he and Prusiner privately agreed that scrapie was transmitted by a "protein-only" infection—developed but never tested a related (but subtly different) "crystallization" theory, in which tiny crystals of $PrP^{Sc}$ serve as a nidus for crystallizing $PrP^{C}$ molecules, which take on the shape of $PrP^{Sc}$.[32]

The main obstacle to further advance in this direction was that Prusiner, Gajdusek, and most other investigators of prion diseases knew almost nothing about how proteins fold into distinctive 3D structures. Early on, Bruce Alberts was the only UCSF faculty member who did know more about proteins and was also willing to talk with Prusiner. In the late 1970s he invited Prusiner to present his work at his lab's weekly group meetings. For several years they met monthly for lunch, where Alberts tried "to focus him on thinking molecularly and making contacts with experts in protein folding." Protein templating "was an obvious possibility, even though it was unprecedented," Alberts says. "My main role was to say that the fact that it was unprecedented didn't mean anything. He should try to figure out exactly what was happening."[33]

Those early conversations may have pushed Prusiner toward experimental tests of the templating hypothesis. If so, those tests were long delayed. In 1991, as Alberts recommended, Prusiner asked Fred Cohen, a young MD-PhD in UCSF's Department of Medicine, to collaborate on structural analysis of prions. Despite his keen interest in understanding how proteins fold into unique states, Cohen might have said no. Many hard-core biochemists at UCSF, he remembers, would have advised him, "Don't waste your time with that guy. You and I have better things to do." Alberts, unlike his colleagues, did urge him to meet Prusiner—and he is glad he did.[34]

When they did talk, Cohen felt that Prusiner suspected "you could come up with an answer with just a protein," but how that scenario might actually work was not at all clear. As for templating, "some of the discussions took place in my office, because

the idea was so heretical that neither of us really wanted to speak about it outside the room." The core issue was that details of actual mechanism were completely lacking. But as they talked, he became intrigued. "The great thing about Stan is he is totally focused on figuring out the experiment that will get the answer. He is a free thinker with respect to the things he is willing to test, [and] a hawk when it comes to actually proving that it's right or wrong. So, rather than talk a bunch about this, [we would] purify the cellular isoform of the prion protein and do experiments [to] see what the hell it looked like."[34]

Those who don't know what any protein looks like should review the lesson we learned in Chapter Four. Every protein is a linear string composed of linked amino acids, twenty chemical building blocks whose different shapes reflect chemically distinctive "side chains," which stick out from the string of blocks and guide three-dimensional folding of the mature protein, following well-understood rules.[35] The large number of variables makes it hard to predict a folded 3D structure reliably from amino acid sequences, but reliable prediction of the folded structures of some short sequences is possible. Such short sequences tend to be arranged in either of two ways. In one of these, called the "α helix," side chains project away from the center of a coiled spiral. The second, a semi-straight linear strand with side chains alternately pointing in opposite directions, often forms a flat "β sheet" of strands folded next to one another, side by side. In addition, short loops connect a helices and b strands to one another.[35]

By 1991, the 3D structure of $PrP^{C}$ remained unknown, but X-ray analysis of scrapie agent fibers had shown that protein molecules in amyloid-like $PrP^{Sc}$ aggregates were folded into a distinctive structure, largely composed of β sheets.[25] Cohen, Prusiner, and their colleagues first predicted and eventually determined the 3D structure of $PrP^{C}$.[36] Years of hard work and elegant analysis produced a coherent scenario that outlines, in some detail, how $PrP^{Sc}$ probably bends $PrP^{C}$ into a shape that replicates $PrP^{Sc}$. While "native" $PrP^{C}$ is composed mostly of α helices, a long floppy stretch of amino acids near its starting end does not normally fold into an easily identifiable 3D structure.[36] Experimentally, exposure to certain chemicals can push it to form either α helices or β sheets. In the brain, it appears that $PrP^{Sc}$, mostly composed of β sheet, binds to this floppy region of $PrP^{C}$ and bends it to form more β sheet. The complex details boil down to a simple story—$PrP^{Sc}$ acts as a template to re-shape $PrP^{C}$ into a replicating infectious prion, destroying normal brain in the process.[37]

Prusiner's evidence still failed to convince all the critics, even as late as 1997, when he received the Nobel Prize in Medicine or Physiology for discovering prions.

At that point, many in the prion field, including Bob Rohwer,[19] felt the facts still did not rule out some kind of virus in the pathogenesis of scrapie and other TSEs. If the infectious prion protein is both necessary and sufficient for transmission of infection, they argued, it should be possible to make infectious prions that contain *only* synthetic PrP, made in bacteria.

Experiments in the twenty-first century have met that stringent criterion. Fittingly, the first big step in this direction began with the recombinant $PrP^{C}$ made by Prusiner, Cohen, and their colleagues. Amyloid rods made from this recombinant $PrP^{C}$ were injected into mice that had been engineered to express large amounts of transgenic (but apparently normal) $PrP^{C}$. After a long incubation period (380-660 days), these animals developed a scrapie-like encephalopathy, and their infected brains produced prions that would transmit the disease to other animals.[38] Since then the experiment has been repeated in different ways. For instance, one of Prusiner's former postdocs, Ilya Baskakov, collaborated with Robert Rohwer to show that recombinant (bacteria-produced) hamster prions, exposed to homogenates of normal hamster brain, can transmit (after serial passage through other hamsters) a scrapie-like disease.[39]

Finally, it has been possible to produce scrapie from recombinant $PrP^{C}$ without exposing it to any kind of brain extract.[40] This experiment was based on a procedure called *P*rotein *M*isfolding *C*yclic *A*mplification (PMCA), in which normal brain homogenate is mixed with a small "seed" of scrapie agent (that is, $PrP^{Sc}$ from the brain of a scrapie-infected animal) and then subjected to extremely vigorous agitation (called "sonication"). The sonicated mixture is then serially diluted and the sonication-with-homogenate procedure repeated several times, but without adding any further scrapie agent "seed." In 2010, Kim *et al.* reported a significant modification of this procedure. They performed nine rounds of PMCA, but added, in place of brain homogenate, recombinant $PrP^{C}$ (made in bacteria) at every cycle. The original seed of scrapie agent had been diluted 100 trillion-fold, enough to guarantee that it could not directly induce scrapie. Nonetheless, the PMCA-treated recombinant $PrP^{C}$ produced scrapie when injected into hamsters. This experiment (in combination with multiple control experiments) proves that $PrP^{Sc}$, derived from recombinant $PrP^{C}$, is both necessary and sufficient for transmission of scrapie.[40]

Once solved, a truly fertile scientific question begets more fascinating questions. For instance, the recent experiments with recombinant $PrP^{C}$ do rule out a virus and do show that the protein-only hypothesis is correct. But, as Rohwer points out,[19] the ratio of infectivity to number of $PrP^{Sc}$—produced either in test tubes or by infecting animals—is very small: that is, approximately 1 infectious dose per 10,000 to

100,000 protein molecules. Thus either $PrP^{Sc}$ is a very inefficient pathogen, or $PrP^{Sc}$ preparations include a tiny subpopulation of infectious particles with characteristics that differ in unknown ways from the bulk of $PrP^{Sc}$ molecules. Finding the correct explanation poses a fascinating scientific challenge.

Further questions abound, along with provisional answers. How is any TSE—scrapie, kuru, FFI, GSS, or CJD—propagated within the brain? Work by several labs, including a long-term collaboration between DeArmond and Prusiner, suggests that $PrP^{Sc}$, released from individual neurons at their synapses with target neurons, transforms (previously) normal $PrP^{C}$ on the surfaces of target neurons into more $PrP^{Sc}$. The target neuron then internalizes the newly made $PrP^{Sc}$ on its surface and transports it to a downstream synapse. This kind of slow spread through the brain suggests how kuru, CJD, FFI, and GSS can differ so much from one another, even though all are caused by transformation of nearly identical proteins into $PrP^{Sc}$. Differences between TSEs are likely to reflect subtle variations in ways neurons get infected in different parts of the brain, as well as the multi-step transport of $PrP^{Sc}$ within different neurons. We still do not know precisely how mutations alter folding, but it seems likely that different neurons could do better or worse jobs of preventing (or promoting) aberrant folding of certain mutant $PrP^{C}$ molecules into $PrP^{Sc}$ molecules.

Prusiner and other investigators are intrigued by similarities between TSEs and several non-infectious but equally enigmatic and devastating neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's chorea.[41] Like TSEs, these other diseases can be inherited and fail to trigger immune responses or inflammation. In each case, moreover, neurodegeneration is associated with deposition of clumps of aggregated, mis-shapen protein molecules, which often assume an amyloid-like structure. The clumped protein is not PrP, but is different for each disease. Is there something about the brain that promotes aggregation of misshaped proteins? How do the changes in protein shape occur in each case? In each disease, does a "seed' of malformed protein induce normally shaped protein to change its conformation? Does protein clumping *cause* neurodegeneration, or does it protect the host (however inadequately) by sequestering toxic particles of malformed protein? Can answers to some of these questions point the way to treatment or prevention of these diseases, two of which (Alzeheimer's and Parkinson's) afflict many more people than prion diseases do?

Astonishingly, prions are not confined to mammals, or even to multicellular organisms. Budding yeast—yes, the single-celled creature that causes bread to rise—makes, harbors, and transmits prions. Although unrelated to mammalian $PrP^{C}$, yeast prions also are proteins encoded by defined genes. Unlike mutant genes, however,

prion-dependent physiologic changes can be transmitted from one yeast to another *by protein alone*. Yeast prions (like $PrP^{Sc}$) induce shape changes in host proteins encoded by the same gene, and accumulation of aggregated prions affects the yeast's physiology. Yeast prions, like those of mammals, come in different strains, and their structures may open avenues to deeper understanding of how an aberrantly folded protein can persuade its normally folded counterpart to change its shape.[42] Whether the changes induced by yeast prions constitute a disease or a disorder, however, is unknown—in some cases the prion-induced change may even be beneficial, suggesting that prions may represent a protein behavior, genetically programmed in a wide variety of organisms, that evolution has sometimes found useful.[43]

## Perspective

An amazing achievement by any measure, the discovery of prions stands out even in comparison to other Nobel-quality science. One measure of the difference is how dauntingly hard a task that discovery proved to be, all the way from the beginning to the endgame. It was difficult to persuade other scientists that the question was worth asking, and equally hard to find money to start and complete the work. For years, biochemical experiments yielded information at a grindingly slow rate. Early findings—even those that later proved crucial—failed to meet anyone's expectations or to fit into a rational framework. Once PrP was isolated and its gene identified, the protein-only hypothesis gathered momentum. After first promising to yield its secrets to molecular genetics and genetically engineered mice, the problem transformed itself into a tantalizing riddle in the arcane field of protein folding, and raised myriad new questions.

The prion ranks as an outstanding discovery not just because the problem was hard to solve, but even more because its solution was totally unexpected and without precedent. Recombinant DNA, Genentech, and oncogenes grew out of competition among well-funded groups of dedicated scientists who could build on each other's achievements because they shared similar knowledge, laboratory tools, experimental strategies, and ultimate goals. As a result, these discoveries were keenly anticipated beforehand and, once announced, readily understood and applauded by competitors in each group. In contrast, during his first decade of scrapie research Prusiner was scorned by a few and ignored by others at his home institution, and vociferously opposed by some TSE investigators. His opponents adhered to the notion that scrapie was caused by a "slow virus," principally because they couldn't imagine an infectious agent that didn't replicate its own genome. A prominent faction among them remained loath to entertain the prion concept for two decades—even after the

PrP gene was cloned, strong evidence supported the protein-only hypothesis, and Prusiner received his Nobel. His prickly behavior may have exacerbated opposition to Prusiner's ideas, but the opposition arose and persisted for simpler reasons—he began by contradicting the orthodox view, persisted stubbornly in apparent error, strived harder and more effectively than others to determine whether he was right, and then refused to apologize for being right.

This book has posed two questions about each discovery it describes. Why did this individual scientist make the discovery? Why did the discovery take place at UCSF? Let us focus first on Stanley Prusiner, the wildest of all possible wild cards, whose quirky, difficult personality drove every step of his discovery. At the outset he focused on precisely the right problem, purifying the scrapie agent. After overcoming multiple obstacles to identify the prion protein, he turned the same single-minded tenacity and determination toward the molecular genetics of prions and then toward the templating hypothesis. When problems arose, he tackled and solved them—by creating a faster, more accurate assay for the scrapie agent, designing experiments on many thousands of rodents in efficient facilities he planned and built himself, and, at every stage, persuading government and foundations to support his projects, year after year, to the tune of many millions of dollars. Virtually no one in any academic research laboratory, with or without a Nobel Prize, matches Prusiner's ability to organize such an immense, tightly coordinated, and well-funded research program. Indeed, it is hard to imagine anyone better suited to the dauntingly difficult problem he chose to tackle.

Tenacity, determination, problem-solving, and meticulous control of his lab's entire effort—essential as such abilities may be to the success of every outstanding scientist—do not account for the sheer originality of Prusiner's prion discovery. In addition, he relied on a remarkable faith in his own intuitive judgment about every choice, whether among employee candidates, advisers, collaborators, projects, scientific questions, or minor and major research strategies. "More than any other scientist I know, Stan is guided by his own internal gyroscope," says another Nobel Prize winner, Michael Brown.[44] That gyroscope was operating way back in the early 1970s, he says. Of eight postdocs (including Brown and Prusiner) who worked at that time in Earl Stadtman's lab, seven studied the bacterial enzyme that makes glutamine. Prusiner, the sole outlier, focused instead on the enzyme that degrades glutamine.

The same gyroscope dictated many crucial decisions Prusiner made over the years, all contrary to received wisdom. For instance, his choice to begin a scientific career by taking a neurology residency, in preference to research in a neuroscience lab, furnished him with the impetus for his life's work—his first patient with

Creutzfeldt-Jakob Disease. Investigating scrapie, a sheep disease of little interest to physicians, and then concentrating exclusively on the apparently impossible task of isolating the scrapie agent, represented choices many other ambitious young scientists dared not make—choices that cost Prusiner dearly before they eventually bore fruit. Other such choices included favoring the lab over clinical neurology, a preference that alienated his department chair; devising an innovative, absolutely necessary new assay for scrapie infection; naming prions before the evidence proved they really existed; fearlessly turning to foundations and a tobacco company for funding, when NIH funds proved inadequate and the Howard Hughes Medical Institute (HHMI) backed out; patiently waiting to isolate a protein before leaping into recombinant DNA and molecular genetics; venturing into the terra incognita of protein folding to explore the templating hypothesis. Without trusting his internal gyroscope, Prusiner would never have discovered the unexpected mechanism by which prions propagate themselves—an unprecedented case of infection without transmission of a gene.

The formidable combination of determination, tenacity, tight control, and unwavering internal gyroscope led to an extraordinary discovery, but it also caused trouble. Postdocs felt poorly used by Prusiner, his department chair tried to get rid of him, the biochemists and molecular biologists disdained him, and many scientists in his new field found him insufferable. It didn't help that he sought to control every interaction in great detail, showed zero interest in teaching, made no secret of his adverse judgments about colleagues and competitors, and evinced little curiosity about any aspect of biology not directly germane to his own concerns.

By the time William Mobley knew him, Prusiner had become the quintessential "gold-plated barbarian," reveling in triumph over naysayers who had scorned him for decades.[45] At the outset they saw him as barbaric primarily because he was a mere neurologist, and therefore not their kind of intellectual. But his innate barbarism almost certainly had deeper roots. He always saw the world from the outside, I suspect, wherever he might be. From the first Prusiner never showed any knack for masquerading as a "collegial" academic, perhaps because he judged it would not help him accomplish what he wanted to do. Collegial scientists—Mike Bishop or Bruce Alberts, for instance—are often highly successful. But for discovering the prion, as Mobley astutely suggests, Prusiner's gold-plated barbarity was essential. The problem would not have yielded easily to collegial collaboration, because solving it was technically and conceptually intractable and at the same time forbiddingly expensive. A brilliant "nice guy" might have been able to imagine the prion, but would not have shocked the world by naming it so early, nor could he have solved the prion's enigmas without the force of personality that bent to Prusiner's will hosts of postdocs, technicians,

administrators, individual donors, and foundations, as well as UCSF itself.

Single-minded self-concern tends to repel people who value collegiality, kindness, generosity, tolerance of disagreement, and willingness to help and teach. But extraordinarily creative individuals often lack one or more of these estimable qualities, and their genius, personal flaws, and achievements can be inextricably intertwined—think of Edward Teller, Michelangelo, Beethoven, Lyndon Johnson, and many more. The world repeatedly produces barbarians, individuals we often dismiss as purely destructive agents. But sometimes these barbarians create change the world badly needs.

Why did the prion discovery happen at UCSF? It is tempting to exclaim, with a resigned shrug, that this was simply because Prusiner chose to start his research career in San Francisco. Such a shrug recognizes both Prusiner's commanding personality and UCSF's adverse responses to him, including that of a Department chair who wished to deny him tenure, as well as antipathy, derision, and lack of interest on the part of colleagues in Biochemistry and Neuroscience. But that explanation omits several of UCSF's contributions to his success. Let us divide these into categories with different timing—before or after 1982, when Prusiner named the prion and his lab found PrP.

In his first eight years on UCSF's faculty, Prusiner was helped and supported by several people. From the beginning, Curtis Morris, a faculty member who had befriended him during his internship, years earlier, gave him regular advice and moral support. In addition, two Neurology faculty, Ivan Diamond and Howard Fields, served as valuable allies in departmental skirmishes. Several prominent face cards in UCSF's deck proved especially helpful. One was Holly Smith, who memorably recommended him for a residency position in Neurology—"Prickly, but worth it," he wrote. Smith also helped him land five years of crucial support from the HHMI and, even more critically, helped to reverse his chair's negative judgment about Prusiner's suitability for tenure. Another was Julius Krevans, who helped Prusiner meet and persuade a number of donors, including the RJ Reynolds Tobacco Company, to give him crucial financial support at critical times in his career, both before and after 1982. A third face card figure, Bruce Alberts, played a helping role in urging Prusiner to explore the templating hypothesis. (I'm not certain whether this occurred before 1982, afterward, or in both periods.)

Also in this early period, Prusiner received a quite different kind of help. Crucially, *no one dictated to Prusiner what research he should do, or how he should do it*. As Curtis Morris told him, he "had a free run. . . . I said, 'You've got to understand, Stan, you're in a position now to do what you want to do.'"[46] This freedom from su-

pervision may appear to be only a negative advantage, and it was surely inadvertent on UCSF's part—as it was for Boyer, Bishop, and Varmus also—but for all four it was invaluable. In their early years, UCSF provided each of them lab space and a faculty position, and their work was funded by the NIH or (for Prusiner) other sources. Beyond such material needs, these resolutely independent thinkers, all self-starters, required little or nothing in the way of scientific advice or direction from above. In Prusiner's case, as Fields saw it, "He was the major scientist in the Neurology department. He was recognized by Holly Smith. And he had tremendous ambition and he had a great idea, [which] was just barely doable. Here's Stanley with nothing and nobody, and he did it. But the reason he could do it, I think, was everybody was new. There wasn't an establishment that could say, 'No, the institution is bigger than you.'"[13] (In fact, however, Smith and others, including Fields, had to work hard to prevent Prusiner's chair, Fishman, from demonstrating the exact opposite—i.e., that the institution was bigger than Prusiner. Fishman *tried* to tell Prusiner what research he should be doing, but Prusiner refused to listen and the institution backed him rather than Fishman.)

After 1982, both Prusiner and UCSF were different. As his work became highly respected and well funded, it also began to raise questions—in cell biology, pathology, biochemistry, protein folding—he could not answer on his own. By that time, UCSF had become a major research center. In this new situation, and with just the right timing, Prusiner was able to tap critical intellectual resources. Two new UCSF collaborators—Stephen DeArmond in the pathogenesis of scrapie and human prion diseases, and Fred Cohen, in protein structure and folding—made especially important contributions. In addition to this useful intellectual support, Chancellor Krevans and other UCSF leaders allowed Prusiner's lab to occupy all of HSE-7 and—more important—allowed him to build his own animal facility at Hunters Point, south of San Francisco. For constructing the new facility and paying its employees, he had to obtain much of the money on his own, but it was a sign of unusual flexibility on UCSF's part to allow one faculty member to build and monopolize his own animal facility.

In summary, Stanley Prusiner made an immensely original and important discovery—an unprecedented new mode of infection and pathogenic mechanism, with implications that extend not only to human diseases, but also into fundamental biology. The discovery depended upon the extraordinary conjunction of a nearly insoluble problem and an unstoppable genius determined to solve it. Prusiner did so, with a modicum of good luck but mainly because of his organizational ability, intellectual tenacity, and capacity for entertaining—and for critically testing—ideas others considered impossible, beside the point, or just plain laughable. All this took place in an

institutional milieu that erected obstacles in his way, but nonetheless proved flexible enough to allow his research to move forward. Along the way Prusiner's prickly, often rebarbative personality hurt some individuals and alienated others, but his trust in his own intuition, combined with determination to test those intuitions, carried the day.

Without that golden armor, the barbarian could not have discovered the prion or earned the glittering prizes he sought so long.

Chapter Eleven

# Something in the Water?

## Can We Bottle It?

"How did UCSF become a leader in biomedical research so quickly, right out of the blue?" Did the off-hand tone mask real puzzlement, I wondered, or did the questioner think something in San Francisco's water was responsible? In fact, David Baltimore posed the question in mock-apology for the large number of UCSF researchers on the roster of speakers he had recruited for a symposium in the late 1980s.[1]

This book began with a promise to answer the same question. Stories of key academic leaders and innovative scientists at UCSF in the 1970s then raised further questions. Do the individual lives of the academic leaders and discoverers in laboratories explain the burst of successive discoveries at UCSF over such a brief period? What do they tell us about how UCSF became a major biomedical research center? Did the academic leaders and their nascent biomedical enterprise play direct, essential roles in generating the burst of discovery?

The answer to the last question is "No." To me this came as a surprise, because it contradicted UCSF's creation myth, to which I subscribed. The leaders and discoverers did come to UCSF at much the same time, and attracted by the same magnet—that is, the prospect of freedom from inherited constraints for both the institution and innovative scientists. But those face card leaders did not recruit or foster the early progress and discoveries of the first three discoverers. In reality, they decisively affected the early progress of only the wildest of the wild cards, Stanley Prusiner, when he faced threats to his academic tenure and funding. Unlike the others, Prusiner was hired in 1974, well after the leaders arrived and established themselves.

If the myth is wrong, then what did cause those discoveries to burst forth? What was in that water, after all? Briefly, I think the "water" supplied three essentials: (1) opportunity for adventures at a new frontier; (2) freedom to apply skill and passionate effort to asking questions, without unnecessary constraints from funding sources, supervisors, and pressures for quick results or conformity to prevailing views; (3)

material resources adequate to the task. The present chapter will pull together the evidence that these ingredients brought four remarkable wild card scientists to San Francisco and allowed them to make their discoveries.

In its turn, this chapter's answer bears heavily on another set of questions I asked at the beginning of this book. Why is it so much harder in the twenty-first century to foster scientific innovation comparable to that of researchers on the Parnassus campus forty years ago? Can institutions and their citizens intentionally create more bursts of discovery? The answer to the latter question, I shall contend, is yes. Fundamental scientific research aims to transform understanding of the natural world, a transformation that has always required seekers of new knowledge to devote themselves unremittingly to change. Now, however, academic leaders must make precisely the same commitment, because developments over the past forty years have made bold, decisive leadership absolutely necessary. In the succeeding and final chapter, we shall examine key elements of commitment and leadership necessary to make those changes happen.

## "Change was in the air, anything was possible"

What evidence supports the notion that something special about UCSF attracted and promoted the success of extraordinary young scientists and outstanding academic leaders? Are we not instead parsing a random array of coincidence that chanced to bring these individuals together at a particular time and place? Historical analysis can never conclusively prove that events did not occur by chance, but in this case that appears unlikely, for a simple reason—while the individuals in this story differ in many ways, both the magnet that drew them to San Francisco and their paths to discovery are strikingly consistent.

Certainly our wild card discoverers presented very different personas, ranging from Boyer's deceptively laid-back relaxation to Prusiner's ambition and passionate self-regard, or from Prusiner's unvarnished bluntness to Bishop's elegant diction and cultivated vocabulary. Their scientific strategies also varied dramatically, from the precise, logical, detailed, and intellectually diverse Varmus to the gruff, no-nonsense, know-what-you-need-to-know Prusiner. Moreover, aside from the Bishop-Varmus collaboration, their separate research projects rarely touched one another, even in minor ways. Their attitudes toward teaching ranged from Prusiner's frank disdain to Boyer's rueful admission that he wasn't very good at it, or Bishop's pride in delivering an exciting lecture to medical students. Their relations to institutions also varied. Boyer had little to do with administration at UCSF, and retired from academia at age fifty-two. Prusiner minimized his contribution to university polity, focusing almost

entirely on his own research. Bishop and Varmus similarly gave little to their community early on, but later made outstanding contributions—Bishop as an academic leader and later as chancellor at UCSF, Varmus as director of the NIH, then as head of the Memorial Sloan-Kettering Cancer Center, and now as head of the National Cancer Institute.

Despite their differences, our discoverers shared consistent similarities. Driven, intense self-starters, all trusted their own intuitions and sought challenges off the beaten track. Before joining UCSF's faculty, all four learned to do research in excellent laboratories—one at Yale, the three MDs as yellow berets at the NIH—where they mastered the newest technical approaches for tackling basic genetic and molecular questions in bacteria or viruses. Each formulated a clear research goal and strategy before coming to UCSF, and each stuck to that strategy for years afterward. When they arrived at UCSF, they all navigated well below the institution's radar for a while, because at first almost no one seemed to notice them or their research. Thus his department chair and even Bill Rutter ignored Boyer until after he and Cohen had invented recombinant DNA technology, Bishop and Varmus were locally unheralded (outside the virology community) for years, and Prusiner was underestimated or frankly disapproved by many, including his department chair. Finally, they expressed very similar reasons for choosing UCSF in preference to renowned research centers in the eastern US. All wished to focus primarily on their research and not to bother overmuch with teaching, but they did not choose UCSF because of its excellence as a place to do science—excellence it could not claim at the time, in any case. Instead, they chose it because San Francisco and California offered more freedom and more fun—sunshine, Carol Doda, Beat poets, fishing in the San Francisco bay—compared to east coast cities. More important, to achieve his research goal, each sought to avoid close supervision by older and more established scientists, intense local competition, and what Bishop calls the promotion "pyramid"—in other words, the principal academic constraints they observed in eastern biomedical research centers.

Compared to the discoverers, most of the face card leaders came to UCSF with already established careers, but shared with our four laboratory discoverers similarly strong ambitions and drive, backgrounds in laboratory research, and reasons for being attracted to UCSF. Of the eight most prominent leaders—Izzy Edelman, Richard Havel, Julius Comroe, Holly Smith, Bill Rutter, Gordon Tomkins, Julius Krevans, and Bruce Alberts—six were MDs, but almost all (Krevans is the sole exception) were trained also in experimental biology laboratories, in areas that included radioisotope technology, lipid biochemistry, molecular biology, protein chemistry, and cardiopulmonary physiology. Self-starters who trusted their own judgment, they also

chose UCSF because it offered freedom from constraints prevailing on the east coast. Compared to the wild cards, these individuals were older and often more specific and articulate about what they were trying to escape—including, for example, anti-semitism and "Red-baiters"[2] (Isidore Edelman), being passed over for a major position by an arrogant, conservative institution (Holly Smith), dictatorial department chairs (Bill Rutter), or institutional opposition to ideas for academic change (Julius Comroe). Each dreamed of making real contributions to the future—e.g., Edelman's burning desire to start his own lab, Comroe's eagerness to develop a first-rate research institute, Rutter's desire to develop his own department, and Smith's *summa*: "A perfect storm, a perfect opportunity. . . . All those things came together. Change was in the air, anything was possible."[2]

If we hope to shape our institutions to promote future innovation and creativity, it will help to identify UCSF's contributions to the creativity of its wild card discoverers four decades ago. As we have seen, most of UCSF's positive influences on this cohort of scientists were neither premeditated nor coordinated. As an institution, it paid little attention to choosing or recruiting these four men. Boyer's postdoctoral adviser was writing a book with UCSF's Microbiology chair, who needed a faculty member to teach bacterial genetics. Bishop's lab mentor at the NIH retired to UCSF and promptly recruited him. Varmus received cold welcomes at two schools nearby, and crossed the San Francisco bay because a surly Berkeley virologist mentioned Leon Levintow and Mike Bishop—who, to their credit, hired him forthwith. Prusiner completed his Neurology residency in a department that then hired him with great trepidation.

Once these individuals arrived on the scene, UCSF made little effort to welcome or nurture them. Boyer was excluded from his department's best research facilities and ignored by his chair. Prusiner was given a small lab and his chair told him, a few years later, to adopt a different project and look for a job elsewhere. Neither the school nor their department had much to do with Bishop and Varmus, a situation that suited them very nicely. Luckily, their patron, Leon Levintow, served as a conduit for resources from the dean, and provided the protective cocoon on HSE-4 that shielded them from most of UCSF in their early years. Such treatment made it easier for Boyer, Bishop, and Varmus to operate in the lab freely, without interference from anybody. Characteristically, Prusiner grasped similar freedom on his own, initially without help from anyone.

So what did UCSF offer these researchers? In those days, the institution's first responsibility to researchers was to meet their material needs. They had adequate labs because Clark Kerr's decision to make UCSF the University's premier venue for bio-

medical teaching and research resulted in construction funds for two Health Science Research towers, with invaluable space for expanding laboratory research. From their first days on the faculty, Prusiner, Bishop, and Varmus all worked in those towers, and later in the 1970s Boyer's lab moved to HSE from an older research building. Thus this entire saga derives in no small measure from the fateful decision, in about 1960, to build capacious new lab facilities at Parnassus.

In addition, all four received enough money to support their research. Three of the four—Boyer, Bishop, and Varmus—received their money from the NIH. As we saw in earlier chapters, NIH funds for research rapidly increased in the 1960s and 1970s, and after lagging behind for years, UCSF in 1970 caught up with its leading competitors and even surpassed them. The accelerated increase in UCSF's NIH funds depended both on new lab space in the Health Science towers and on the resignation of John B. deC. M. Saunders as chancellor in 1965. Kerr's ouster of Saunders, urged by UCSF's leaders, including Comroe and Smith, removed a formidable roadblock. The largest immediate benefit of removing that roadblock went to NIH grantees in Comroe's Cardiovascular Research Institute and to researchers in clinical departments, but the flow of NIH money to faculty in basic science departments increased also. The NIH was less generous with Prusiner, whose early finances were shaky until he found funding from non-government sources, with the help of Holly Smith and Julius Krevans.

What about the intellectual environment around these young scientists when they made their breakthrough discoveries? Nowadays UCSF rightly takes pride in its close-knit community of basic scientists, who freely share ideas and reagents and collaborate directly. In their early years at UCSF Bishop and Varmus had each other and some input from local virologists, but key members of their real intellectual community (e.g., people like David Baltimore and Howard Temin) were scattered across the continent. In his first four years at UCSF Boyer interacted relatively little with anybody outside his own lab, although after 1970 he got to know Howard Goodman and Gordon Tomkins, recently arrived members of the Biochemistry department. For his first decade at UCSF, Prusiner was intellectually isolated—as his inclination and personality would have made him, one suspects, almost anywhere on the planet. At the outset, Boyer, Prusiner, and the Bishop-Varmus team worked pretty much on their own, and profited only later from interactions with other UCSF scientists.

Campus leaders did little to foster the early research of these young scientists. Clark Kerr deserves a good deal of credit for setting the stage, but the outstanding leaders who came to UCSF in the 1960s exerted almost no direct impact on Boyer, Bishop, and Varmus in their early years. Rutter and the other leaders appeared on the

scene after or at virtually the same time as they did, had nothing to do with their hiring, and knew little about them until their work had already attracted national recognition. Prusiner, as in other respects, is different. He joined the faculty in 1974, later than the other three, and Holly Smith gave him crucial help in obtaining HHMI funding and in his struggle to get tenure in Neurology. Similarly, Dean Krevans helped Prusiner garner research funds from non-federal sources in the 1980s, which enabled him to expand his research despite a shortfall in NIH funds. Otherwise, aside from Rutter's organization of Biochemistry, many of these leaders' key impacts on basic biomedical research at UCSF would not become most evident until the 1980s (see below).

So far it appears that most of UCSF's material and intellectual contributions to the creative accomplishments of these four scientists were negative or inadvertent. They came to UCSF not because it sought them out, but because the California air seemed to promise freedom and change. They owed their new lab space to Clark Kerr's prescient wisdom and most of their ample research support to the growing generosity of the NIH. Even intellectually, their local environment appears to have played a decidedly minor role.

## Wild Cards Need to Think Wildly

Is that all there is to it? Just give four very bright, ambitious young men labs and a certain amount of money, and watch the discoveries burst forth? No, these four scientists did not get there entirely on their own. While their ambitions and innate abilities did much to determine the outcome, UCSF did make a critical contribution—by giving to each the freedom to focus his passion, curiosity, and formidable skills on a challenging question. To watch that freedom at work, let us recall how our wild cards actually made their discoveries.

Boyer brought to San Francisco a first-rate idea, which no one found very interesting. Ignorant (like everyone else) of the many potential varieties of restriction endonuclease, he repeatedly failed to find the right enzyme, but eventually identified EcoRI by testing hundreds of *E. coli* isolates from a big clinical laboratory. Other labs showed that DNA cleaved by EcoRI had sticky ends, and he determined their base sequence. Then, diverging from his original goal, he used EcoRI as his first stepping stone to embark on a series of extraordinarily fruitful voyages of discovery, each propelled by combining his own ideas with the complementary ideas of a collaborator. The first—with Stan Cohen, who knew about bacterial resistance to antibiotics and how to get naked plasmids into bacteria—showed how to cut, recombine, and propagate defined pieces of DNA. This became the linchpin of recombinant DNA

technology. The second—with Art Riggs, who knew a lot about the lac operon and synthetic DNA—produced the first DNA linker sequence and showed that chemically synthesized DNA was as amenable to clever recombination experiments as any other DNA. The third—with Robert Swanson, who knew how to start and grow a company—extended the collaboration with Riggs and his colleagues to clone recombinant, synthetic DNA encoding insulin in bacteria. Together, they made Genentech as the world's first successful biotech company. Boyer was smart, but his essential skills also included willingness to persist in exploring an unfashionable idea and, once he identified EcoRI, to switch gears and turn his attention to cutting and splicing genes. Most important, he showed an uncanny ability to recognize the value of other people's ideas and combine them with his own, making a product better than either contribution by itself. UCSF's contribution to each advance (as usual in those days) was inadvertent but still essential—it left him free to persist in tackling restriction enzymes, and then to craft collaborations with anyone whose ideas he thought might complement his own.

Bishop and Varmus, each on his own, latched onto the idea that tumor viruses could open the way to understanding the molecular genetics of cancer—a powerful idea that had energized very smart scientists around the world, and almost no one at UCSF. But once they got together in a small enclave of virologists on HSE-4, the pace rapidly accelerated. Two superb scientists proved better than one, especially in tackling a demanding but supremely logical problem ideally suited to their skills and inclinations. They complemented one another not by contributing disparate but complementary styles, ideas, or temperaments, but because they worked out a way to harness two powerful competitive urges and two razor-sharp minds into an effort that succeeded much more rapidly than either protagonist could have managed by himself. UCSF's contribution was the hospitable cocoon of HSE-4, combined with its inadvertent neglect of two very ambitious and capable individuals, which left their competitive urges free to combine and discover the first cancer gene. Their collaboration thrived as long as UCSF left the cocoon to itself, and promptly broke down when deans and chancellors proffered a big reward to one collaborator, but not the other.

Prusiner came onto the Neurology faculty after a patient's neurodegenerative disease made him wonder why and how that disease is contagious. Was it a virus? If so, could it be purified, identified, and eventually understood and even treated? Many people in the field and at UCSF considered the whole idea unfeasible, and especially so for a clinically trained neurologist, unversed in viruses or other microbes. But Prusiner brought to the problem assets no one else could match. First, he saw that the crucial initial task was to purify the scrapie agent, in order to find out what it is and

does. His other invaluable assets included dauntless determination, narrowly focused but intense curiosity, and the ability to ignore criticism as well as the seductive lure of recombinant DNA research. At least at the outset, this young man did not need, and probably would not have heeded, intellectual or any other contribution from collaborators. What Prusiner did need was a big lab, money to support postdocs, and freedom to do exactly what he wanted, his way. Here UCSF's contributions were not all inadvertent, but their intents were undeniably mixed. On the one hand, Prusiner's personality and style elicited abundant adverse criticism from his chair, many biochemists, and some virologists, and at the beginning few UCSF faculty wanted to risk contaminating their labs (and perhaps their minds) with the scrapie agent. On the other hand, several UCSF leaders had the foresight, early on, to protect him when it looked as if he would have a hard time getting tenure and to help him find nonfederal funds when the NIH well seemed to run dry. The upshot of UCSF's contributions to Prusiner, messy as they were, was that he got a lab, material resources, *and* freedom to do the work he wanted to do. It's impossible to guess how Prusiner would have fared early on in other biomedical research centers, but he surely would have found the going difficult in many outstanding institutions, for the same reasons that caused him difficulties at UCSF.

Each wild card's skills, style, and temperament were strikingly fitted to the problems he chose to tackle. Boyer's openness to others' new ideas was ideal for his remarkably fertile collaborations with Cohen, Riggs, and Swanson. Bishop and Varmus had the right intellectual chops to tackle the logical intricacies of cancer viruses and the right personalities to spur one another to rapid discoveries. And Prusiner's combination of determination, insight, self-trust, and organizational ability made him precisely the right scientist to unravel the knotty and almost impenetrable problem of scrapie.

The essential freedom UCSF gave each of these four young scientists was nothing more or less than the opportunity to choose and solve a problem perfectly suited to his ability. Details differ, of course. Thus Boyer was allowed to follow his nose and tackle a long and difficult problem for many years, and then to choose new goals for which he was suited, as well as the right collaborators. Bishop and Varmus were freed from pressures that would have made it hard for two remarkably competitive individuals to cooperate so effectively. Finally, although the outcome was a close-run thing, when push came to shove UCSF found the flexibility to give Prusiner the freedom to do the experiments he needed to do. Each wild card was enabled to think and act wildly, in his own particular way.

Note also that after taking faculty positions, two of our wild cards required a

long time to reach their first truly exciting goals—seven years before Boyer (with Cohen) spliced antibiotic resistance genes into plasmids and eight years before Prusiner's lab identified the protein component of PrP. These time periods substantially exceed those required for tenure decisions (six years, usually) and NIH grant renewals (four or five). When a problem is tough, real progress can take considerably more than a jiffy.

The idea that freedom from constraints can constitute an essential condition for discovery is by no means universally recognized. In our present culture, such a contribution can sound hyper-subtle and counter-intuitive, and creative innovation is more often attributed to market competition than to freedom of ideas and opportunity, although the actual evidence points strongly in the other direction.[3] Nonetheless, bustling busy-ness and pressure to "get it off the desk" (or lab bench) throttle creative thinking. James Watson's *The Double Helix* shows that he and Francis Crick were free to think about DNA because their time and energy were not fettered by supervisors demanding immediate results.[4] Elsewhere he commented, slyly: "It is necessary to be *slightly under employed* if you are to do something significant."[4] In the twenty-first century, as in the twentieth, concerns about laziness and lack of productivity can make us forget that creative individuals rarely profit from external pressure, which often thwarts their effort rather than spurring it. As one scientist put it, "Scientific research is like a porcelain egg. Catch it too tightly, and it shatters, and you have nothing."[5]

For a host of reasons, now the research of beginning scientists is squeezed harder and harder every day. In particular, freedom to follow one's nose in the lab is very hard to come by—a point we shall return to later. Forty years ago UCSF's basic science departments provided that freedom for several reasons: the faculty lacked authority figures inclined to tell young recruits what to do in the lab (these recruits, especially Prusiner, would have paid little attention in any case); NIH grants were more generous and easier to get than now, and their administration not so burdensome; basic science teaching duties in the medical school were not onerous; most young faculty were in the tenure track, so their salaries did not have to come from grants (a substantial advantage in comparison to salary arrangements at some schools, then and now). To be sure, UCSF provided this freedom with as little planning and foresight as it applied to recruiting or fostering the progress of the four discoverers. Passive and inadvertent as these contributions were, each of the four recipients followed his curiosity precisely where it led him, and that made a huge difference.

In essence, UCSF's most important contribution to these young pioneers was to fulfill the promise that drew them to San Francisco, by furnishing them a straightfor-

ward opportunity for real adventure. Remember Izzy Edelman's wry defense of UCSF as a provincial medical school, back in 1955—"Think of it this way. There's nowhere to go but up!"[2] In fact, he was specifying the central element of real adventure, which begins with a frontier wilderness that challenges the adventurer to explore it. In their different ways, all the wild cards responded to the lure of adventure. So did the face card leaders. Attracted to an unformed school in a land of opportunity, they enjoyed the fruits of a parallel promise—the opportunity to transform an insignificant medical school into a powerful instrument for change.

The burst of discovery we have examined suggests clear inferences about institutions and creative research. We shall explore their implications for future creativity in the next chapter, but they bear summarizing now. To kindle similar discoveries, an academic biomedical research center must accomplish four essential tasks: First, it must *create, find, or take advantage of a frontier that welcomes adventurous pioneers*—that is, a social and economic environment that welcomes and promises change. For UCSF forty years ago, San Francisco and California made this contribution. Second, the academic research center must *choose young scientists likely to make original discoveries*. In the 1960s and 1970s, our four discoverers chose themselves, in order to escape from constraining environments elsewhere. The third task is to *provide to the scientists it recruits the material resources and personnel necessary to do the job*. In our case, these contributions were made by Clark Kerr, a growing NIH, and economic prosperity. Finally, the institution must *free scientists to follow their curiosity wherever it leads*, limited as little as possible by other duties, unreasonable time pressures, or senior figures who think they know what they should be doing. UCSF did this inadvertently, for the most part, but also by offering its faculty well-supported tenure track salaries and modest teaching duties and by responding flexibly to crises, most notably with Prusiner.

## Obstacles to Innovation

Simply re-creating research centers designed to mimic UCSF of the late 1960s will not kindle bursts of discovery, just as twenty-first century California can no longer attract risk-taking pioneer settlers. In either case, prospective pioneers would be gravely disappointed. The problem is not that today's pioneers are more sophisticated—although they are. Or that we cannot find institutions where research matches UCSF's mediocre quality in the 1960s—these abound. Or that experimental biology is not still asking fascinating questions. How does the brain think? Can we persuade a skin cell to make a kidney? How does a single cell become a wolf, or a tree, or a girl? Rather, the task of creating an environment *de novo* that will attract and nourish sci-

entific pioneers has become fiendishly difficult. For the same reason, highly respected biomedical research centers struggle to maintain the creativity of forty years ago. We can't go home again, because too much has changed.

However much we wish to generate bursts of discovery comparable to UCSF's in the 1970s, two classes of obstacles make it impossible to reconstruct in the twenty-first century the environment that cradled those discoveries. One class is exciting, the other mundane and dangerous, but we can afford to ignore neither. The exciting obstacles reflect profound changes in the methods and complexity of experimental biology, largely triggered by discoveries depicted in this book—recombinant DNA, commercial biotechnology, and understanding of the molecular basis of cancer and other diseases. Together these discoveries have converted the cottage industry of experimental biology into a densely interdependent enterprise. Gone are the days when Boyer could devote four years to finding the right bacterial restriction enzyme, entirely on his own. Or when Bishop and Varmus could busily seek the first oncogene while sequestered in their HSE-4 cocoon, without needing to clone or sequence genes or delve into arcana of computational biology, protein structure, or high-tech microscopy. Instead, now an investigator choosing to study any biological process must almost always master new fields or technologies. To gain insight into a human disease, she may need to analyze genes of yeast, flies, or worms, assess effects of a gene mutation in a transparent fish, or fold her mind around a chemical mechanism at the level of atoms and shapes of tiny macromolecules.

Such versatility became necessary when the DNA revolution laid bare the underlying unity of all forms of life and furnished tools for exploiting it. Different organisms have adapted multiple mechanisms to perform every task, so each task at first appears unique—but then often turns out to use an unfamiliar mechanism already analyzed by someone else. The implications for individual scientists are exemplified by one of our wild card scientists, the only one whose career we followed beyond the birth of the DNA revolution. At that pivotal turning point, Stanley Prusiner's prion discovery suddenly required him to find collaborators who could decipher an amino acid sequence and clone a gene. Later he needed colleagues able to dissect scrapie's spread in the brain and to imagine how PrP folds itself into a dangerous new shape. In this new century, no biologist can solve a significant problem without borrowing essential knowledge, reagents, or technology from someone else. Faced with such opportunities, and with the burdens that accompany them, creative young experimental biologists need plenty of help. Collaborations, already essential for some of our wild cards in the 1970s, have become absolutely essential for most experimental biologists in the twenty-first century.

That's the exciting part, but we must also address thoroughly mundane obstacles, which are more formidable. Even more than the DNA revolution, ongoing changes in our and other modern societies have profoundly altered the organization, social interactions, economic pressures, and day-to-day practice of experimental biology in academia. The gawky youth of 1970—bright-eyed, nimble, curious, ready for anything—has settled into paunchy middle age, stiff-jointed, muscle-bound, and struggling to augment his income. Harsh as it sounds, the caricature summarizes the disturbing facts.

First, success has made biomedical research bigger and more expensive (see Table[6]). That expansion was well on its way by 1970, when NIH gave out more than $700 million in research grants, including $12.3 million (supporting 168 grants) to investigators at UCSF. Thirty-eight years later, in 2008, UCSF was awarded 899 NIH research grants, amounting to $448 million, a 36.4-fold increase—or 5.2-fold, in constant dollars. Even the constant-dollar increase represents substantial changes in complexity, both for the institution administering the grants and in the work environment of the average scientist supported by an NIH grant. As compared to 1970 the average experimental biologist at UCSF nowadays has a larger lab and supervises more students and postdocs—fifteen or so, *vs*. five to eight earlier.[7] She often supports her lab with three or more NIH grants, whereas in 1970 one or two usually sufficed. Worse, today's investigators must apply for grants more often, because the rate of success in the review process is much reduced. Of all grants reviewed by the NIH in 2009, only 23% were funded, *vs*. twice that proportion (46%) in 1966, the year Herb Boyer arrived at UCSF.

For young researchers, the consequences are often disastrous, leading one angry senior commentator to conclude that "the present funding system in science eats its own seed corn," by turning them into bureaucrats and by favoring older and more senior scientists "who know how to raise money for a big [research] group," produce many papers, and appear effective without being either innovative or efficient.[8] The anger is real, and I suspect the substance is at least partly correct, if hard to document. Certainly today's scientists are forced to spend more time seeking money and writing applications and less doing or thinking about experiments. They feel as if they must run a small business, in addition to—or instead of—focusing on scientific questions, innovative experiments, and their students. Rather than satisfy discoverers' thirsts for adventure, we offer bitter lessons in careerism, focused on techniques for telling older scientists what they want to hear.

Part of the problem is that availability of "soft money" from the NIH is converting many research universities into exploiters rather than supporters of scientific

## Table 4: NIH Funding, Then and Now[6]

| Research Grants | | Then (1970) | Now (2008) | Fold Change* |
|---|---|---|---|---|
| To UCSF | Number | 168 | 899 | + 5.4 |
| | $ (Millions) | 12.3 | 448 | + 36.4 (5.2)† |
| NIH Total | $ (Millions) | 708 | 20,129 | + 28.4 (4.0)† |
| **Applicant Success Rate** | | **Then (1966)** | **Now (2008)** | **Fold Change*** |
| | Reviewed (no.) | 9343 | 26093 | + 2.8 |
| | Awarded (no.) | 4250 | 6087 | + 1.4 |
| | Successful (%) | 46 | 23†† | - 2.0 |
| **Age of Grantees (%)** | | **Then (1980)** | **Now (2003)** | **Fold Change*** |
| | 35 or less | 23 | 3 | - 7.7 |
| | 40 or less | 51 | 38 | - 1.3 |
| | 41-55 | 39 | 39 | (None) |
| | 56 or more | 10 | 23 | + 2.3 |

*Fold increases, indicated by a plus sign (+), are calculated by dividing the value for 2008 or 2003 by the value for the corresponding earlier reference year. Fold decreases, indicated by a minus sign (-), are calculated by dividing the earlier by the later value.
†Fold changes in constant dollars, shown in parentheses, are calculated using the Biological Research and Development Price Index (see reference 17, Chapter Two).
††Because it depends on the number of grants reviewed, this value may be artificially high. In recent years reviewing grants has become such an onerous task that the NIH has significantly increased the percentage of "triaged" grants (turned down by reviewers without formal review). I do not know whether triaged grants are included in the number of grants reviewed, but suspect many more grants are triaged now than in earlier years.

investigators. In a recent editorial in *Science*,[9] Bruce Alberts notes that "the NIH actually rewards institutions for paying faculty salaries with unguaranteed 'soft money' from research grants by providing increased overhead payments." (Each grant brings to the institution not only salary and dollars for actual research, but also "overhead" or "indirect costs" to defray institutional expenditures for administration and facilities. These extra costs, which account for a substantial fraction of total grant funds

paid out, are negotiated with the institution.) Consequently, Alberts adds, "any institution that draws on its own finances to pay its professors . . . must not only use its own funds but also loses the overhead on the salaries that it would otherwise accrue. [And, because] the NIH will reimburse institutions for the cost of new research buildings, paid out as overhead charges on research grants as the building depreciates over several decades, . . . advocates for expansion . . . argue that the costs will eventually be borne in large part by the U.S. government." He points out that such reliance on the federal government poses "a huge risk" to universities. "Because the NIH budget cannot increase at a high enough rate to pay for the ever-expanding US biomedical research enterprise, each institution is betting that the faculty in its new facilities will outcompete those at other institutions for the limited research grants available"—thereby pitting universities against one another "in a process that resembles an arms race." As a result, Alberts says, these university and NIH policies subject individual investigators to pressures that make real research difficult. "Biomedical scientists are spending far too much effort writing grant applications and reviewing those of others, leaving precious little time to do what they should be doing: reading the scientific literature and thinking deeply about their research and teaching."

In the latter years of the twentieth century, when NIH grant expenditures doubled, the universities' use of soft-money made sound business sense, because it helped to buy more "arms" (facilities) and pay more "soldiers" (investigators). But in the twenty-first century NIH grant funds have stopped increasing, and the arms race Alberts described begins to look more like a real war. Federal largesse will not increase as it once did, so this war is likely to produce substantial casualties. Universities may well decide to treat many of their troops as dispensable, because they are paid primarily with the NIH's money, and a soldier who falls in the line of duty (that is, fails to obtain the next grant) can be replaced by another from the next crop of young recruits, also paid on soft money. It is possible that such alarmist worries will prove unjustified, even though the threat looks real to thoughtful people like Bruce Alberts. I would feel better if the nation's biomedical enterprise showed signs of recognizing a need to insure itself and its investigators against the possibility that optimistic expectations may not be realized.

These problems are scary enough, but two other trends disturb me just as much. The first is the progressive graying of experimental biologists who run their own labs. Increasingly, young people spend longer times in graduate school, serve as postdocs in one lab after another, and go on to work as lieutenants of a "Principal Investigator" in a larger lab. Finally—assuming they have not left science for another kind of job—they find independent positions as faculty or working in biotech when they are

in their late thirties or early forties. In addition, academia adheres to a research model in which experiments are performed almost exclusively by students and postdocs. Every year this model produces a new population of ambitious young people, but they face scarce opportunities for independent research. The result is stark: in 1980, almost a quarter of all NIH grantees were younger than 35, but by 2003 the percentage in that age group had dwindled to 2.3%. Reciprocally, NIH grantees older than fifty-five increased from ten to 23% (see Table[6]). Truly creative science is largely done by the young, but the graying trend systematically prevents young minds from working on their own ideas at their most original, innovative age.[10] Instead, beginning scientists explore old ideas in the labs of older scientists, further entrenching the gray-haired scientists but wasting young talent.

In a parallel trend, the innovative potential of grant proposals suffers both from the ever-decreasing likelihood of getting funding for a proposed project and from the NIH's reviewing process. The reduced rate of successful applications (see Table) induces applicants—perhaps especially those who are just starting out—to spend huge amounts of energy trying to second-guess reviewers, and so to ask questions and propose experiments they think will please a review panel. The review panels augment this trend, by assigning greater value to proposals that are better supported by preliminary data and more likely to produce results that agree with prevailing views. Thus, as one might expect, awards go preferentially to established laboratories who propose more scientifically conservative projects—at the expense of more innovative, riskier proposals from less well known scientists. Deploring this trend, some senior scientists have worked to devise alternative grant evaluations designed to enhance innovative science, but the NIH, like an enormous ocean liner, is hard to turn. The graying of grantees also favors conservative grant proposals, because young applicants, who might otherwise be willing to risk being wrong, now work instead for elders who tell them what questions to ask and which disease or treatment to study—and then garner the credit for their findings. More broadly, the high proportion of older scientists among grantees tends to nudge standards of reviewing agencies in scientifically conservative directions.

In addition, I suspect that now, in comparison to 1970, a higher proportion of NIH awards goes to support projects that fall under narrow rubrics supported by a vocal minority. Such minorities may include relatives—often senators and congressmen—of patients suffering from a particular disease, as well as passionate supporters or opponents of particular approaches (e.g., using animals or stem cells) or research aims (basic *vs*. applied). As a result, an agency sometimes defines its research priorities narrowly or even re-directs an ongoing project. (Three years ago, I sat in a

meeting where the NIH's representatives directly coerced researchers into altering the goals of an ongoing multi-lab project.) Oversight by Congress and NIH administrators has a legitimate place in decision-making in a democracy, but the resulting pressures inevitably limit the ability of a creative scientist to follow a question wherever it may lead, even when some colleagues consider the project wrong or poorly conceived. Boyer's colleagues may have shown little interest in his early work on restriction enzymes, but this was better than the overt opposition such an apparently unexciting topic might elicit today. Similarly, Prusiner wasn't forced to obey his department chair's strictures against studying scrapie, a sheep disease. Freedom and innovative discovery are tightly linked.

Senior colleagues, even when they are excellent scientists, can hinder the creative thinking of others in subtle ways. They cast long shadows even if they refrain from explicitly directing the work of their young colleagues—shadows that limit others' creativity by (inadvertent) intimidation, occupying excessive lab space, monopolizing scarce resources, or unduly influencing a department to pursue a particular intellectual direction. Older colleagues can exert immensely positive influence as well, as we saw with Gordon Tomkins and, in a different way, with Leon Levintow. This book's wild cards were relatively free from either kind of influence because UCSF's research enterprise was nascent and the future leaders were not yet firmly ensconced in their new positions.

Although "Science, the Endless Frontier" still beckons, both research centers and young scientists today face teeming obstacles to creativity and innovation.[11] The central problem is straightforward. Any useful, essential, and successful human entity—e.g., in our particular context, an academic department, a university, or an NIH program—risks crippling its capacity for innovation because it is constrained by norms and procedures required to maintain momentum of a complicated effort. A considerable portion of the US automobile industry has suffered from precisely this malady, while other companies—Genentech comes to mind—found ways to grow and prosper because they rewarded innovation rather than stifling it. The difficult challenge in experimental biology is this: how can a huge and successful but entrenched establishment embrace and augment the individual freedom to operate that is necessary to attract, retain, and nurture the best wild card researchers, as well as the best face card leaders? In 1970 UCSF's wild cards could manage without first-class leadership because institutional constraints were weak. Today's challenge—make no mistake about it—cannot be met without bold, insightful, and courageous leaders who learn how to restrict or modify growing constraints on individual creativity.[12] We shall return to this issue in the next chapter.

Before proceeding, I must mention one large and increasingly ominous class of obstacles to innovation—the increasingly widespread political, economic, and social forces that work to hinder scientific creativity at every turn. Senators and presidential candidates distrust evolution and actively oppose stem cell research. Urban poverty and inadequate public schools waste young minds, while prejudice and the fear of raising (or even maintaining) taxes severely hampers necessary improvement of K-12 science education. Brilliant, well-educated men and women—including hordes of our brightest college students—overwhelmingly choose careers in the financial industry, rather than in science. Fear and pervasive distrust of universities, intellectuals, and scientific research persuade loud know-nothing groups—and, as a result, ordinary citizens—to oppose whooping cough vaccination, water fluoridation, and even electric cars.

Acting together, these trends are robbing the US of its position as the world's leader in science and technology. Chinese, Indian, and European scientists who would have flocked to work in US labs twenty years ago now often find equally good (or even better) support and training outside of North America. US scientists are beginning to seek opportunities in other countries. The US's relative deficit in innovative technology and science was documented in a thorough and thoughtful analysis, published in 2007 and revisited in 2010.[13] In 2007 it noted that among the world's nations the US is in sixth place in competitiveness based on innovation, and in fortieth place in the rate of innovative change over the past decade. The followup report in 2010 concluded that the problem is getting worse. It recommended a doubling of funds devoted to research and development in physical sciences and engineering (funds for biomedical research doubled in the latter part of the twentieth century), along with major changes to improve K-12 education in mathematics and science. Implicitly, the report appeared to conclude that biomedical sciences are well funded, which may well be correct. As we have discussed, however, it looks as if innovation by today's muscle-bound basic biomedical research effort lags far behind the money invested in it. Fortunately some US leaders, within and outside the scientific community, are working to reverse these ominous trends. Certainly scientists and other concerned citizens should support them, but for now this book will focus on critical problems closer to home.

## Parnassus and Mission Bay, 1985-2010

Because the next chapter will outline proposals for experiments testing changes designed to foster innovation in research universities, it may be useful to consider briefly one example of such change in a university setting. In this example, leadership

played a much more significant role than it did in fostering the progress of our wild card researchers in the early 1970s. To that end, I shall briefly describe how experimental biology at UCSF responded to changes induced by the DNA revolution in the twenty-five years that followed the burst of discovery in the 1970s.

Relative to other biomedical research centers, in 1985 basic science departments on the Parnassus campus enjoyed competitive advantages. Vibrant, beautiful San Francisco helped in recruiting new faculty, despite the exorbitant cost of housing. A more substantial plus was the guaranteed base salary the University of California paid to faculty in basic science departments, relieving them from having to pay large fractions of their own salaries from NIH grants—a relief not available to faculty at many private universities. Perhaps more important, the spiritual legacy of Gordon Tomkins and the recruiting skills of Bill Rutter had created a superb Biochemistry department replete with first-class scientists in molecular biology, cell biology, and protein structure.

Disadvantages were evident also. Materially, funds from state coffers did not come close to the support some schools routinely receive from their alumni—a situation that persists today. More critically, aside from Biochemistry UCSF's basic science departments had little to boast about, with the bright exceptions of Bishop and Varmus in Microbiology and several rising stars recruited to Physiology's neuroscience program by Zach Hall. Intellectual stagnation in the other departments had long been abetted by a dearth of leadership, low standards for recruiting new faculty, meager dean's office support, and lack of cooperation among departments.

Fortunately, two converging forces performed the jiu-jitsu trick of converting these disadvantages into advantages. The first was the profound transformation of experimental biology initiated by the DNA revolution Boyer and Cohen had kindled in the 1970s. By the early 1980s, distinctions between pharmacology, anatomy, physiology, and biochemistry had become outdated guides for identifying and solving biomedical puzzles or for discovering new treatments. Instead, scientists could now apply a common set of experimental tools to understanding mechanisms and functions shared by cells and molecules of yeast, flies, worms, mice, and humans.

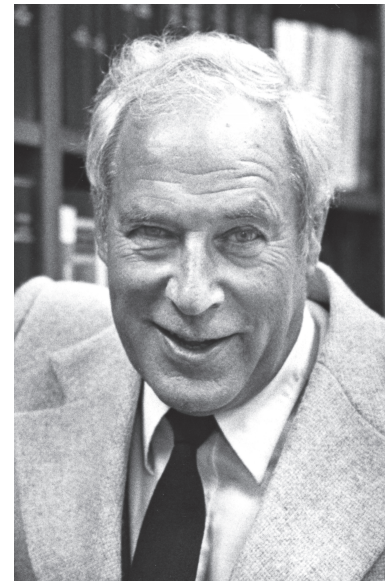
The second force depended on the penetrating vision and deft political skills of leaders who saw what had to be done and knew how to make it happen. Attracted to UCSF at the same time and for the same reasons as our wild card discoverers, the key leaders included Holly Smith, Bruce Alberts, and one of the original wild cards, Mike Bishop. They were assisted by people they recruited to the Parnassus campus, as well as intellectual progeny of Gordon Tomkins. Such helpers included less senior scientists who replaced previous department chairs or led new graduate programs—e.g.,

individuals like Zach Hall, me, and Keith Yamamoto (the latter two were Tomkins's protégés). Collectively, all these leaders recognized the new methods and goals of experimental biology, held their research and that of others to a high standard, and were committed to fostering the careers of innovative young scientists.

Their main goal was to rejuvenate the medical school's stagnant basic science departments, a goal they hoped to accomplish by creating a joint graduate training effort, called the Program in Biological Sciences, or PIBS.[14] I don't know who generated that idea, but it soon became a powerful mechanism for erasing the old slate and crafting a new dispensation. Acting together, four elements made PIBS a success—a solid reason for its existence, a fortunate influx of funds, strong support from the medical school and the Biochemistry department, and a decisive shift of administrative and financial power from basic science departments to a new umbrella entity dominated by first-rate scientists.

Intellectually, the new graduate program explicitly recognized the newfound unities of fundamental biology and of biomedical research. In turn, this new direction helped Holly Smith, the *eminence grise* of previous critical transitions at UCSF, to persuade the Markey Foundation to donate $13 million for founding PIBS. The money would have proved much less useful without the far-seeing cooperation of Rudi Schmid, whom Smith had recruited earlier to the Department of Medicine. A gastroenterologist and pioneering investigator of liver function, Schmid replaced Julius Krevans as the medical school dean in 1983. (I presume he was Smith's candidate for the deanship.) The new dean surprised everyone with an act unprecedented in any dean's office—that is, he assigned all the Markey money to PIBS, to be administered by its Steering Committee. Schmid's office received not a dime of the money, and even supplemented Markey-derived startup funds for new basic science faculty with money from its own coffers. Without additional help from a second quarter—Biochemistry, UCSF's only nationally recognized basic science department—PIBS would have been doomed. Luckily, Alberts and several of his faculty saw substantial benefits in attracting excellent researchers to other departments. In addition, Biochemistry generously provided PIBS with excellent academic leaders (e.g., Yamamoto and Marc Kirschner to head its Molecular Biology and Cell Biology tracks) and administrative staff.

PIBS was an innovative pioneer. At the time most biomedical graduate training throughout the US was supervised separately by multiple departments, just as it had been at UCSF. Despite the DNA revolution, departments everywhere jealously guarded their prerogatives and their faculty saw sharing scarce resources and access to coveted students as grievous threats to their power. In this light, the fourth



Rudi Schmid, student of bilirubin metabolism. Hired by Holly Smith to head a division of gastroenterology, Schmid later became Dean of the School of Medicine and helped basic science departments establish a common graduate program and improve the quality of their research. Photo in the 1980s.

bulwark of PIBS's success appears even more surprising and essential for its success. Administratively, the new program transferred substantial power from the five basic science departments to scientists on its own Steering Committee, made up of newly appointed department chairs plus outstanding scientists who directed graduate research tracks (e.g., cell biology, molecular biology, and neuroscience) within PIBS.

The Steering Committee, with Mike Bishop as its elected chair, controlled finances, faculty appointments, and graduate training. Specifically, it decided how and where Markey money would be spent (e.g., in startup funds for new faculty, lab renovations, and equipment purchases) and also set standards for graduate courses and examinations. Crucially, the Committee held effective veto power over departmental appointments of new faculty, because a new faculty appointee not approved for PIBS membership by the Committee could not receive Markey startup money or train first-rate PIBS graduate students. Hiring faculty had been the prerogative of departments, but now a department chair hired a new faculty member with advice and consent from PIBS, and search committees for new faculty would always include PIBS faculty from multiple departments. (In theory, a department was free to administer its own graduate program and hire faculty of any quality or specialty it chose. But doing so without PIBS's cooperation, and thereby that of other departments, would mean giving up access to Markey funds and PIBS students, as well as losing useful allies in other departments.)

Crafting and maintaining a new regime in which departments worked together

was not easy. Change triggered inevitable protest and opposition from old-guard faculty. In Biochemistry some worried about losing their cozy aura of exclusive excellence, while older faculty in other departments worried about losing their students. Fortunately, however, the dean's office and Alberts, Bishop, and others worked hard to institute necessary change and help lagging departments improve their research efforts.

For instance, as the new chair of Pharmacology in 1984, I had found a tiny faculty comprising one superb neuroscientist, a couple of excellent teachers, and a dominant faction of less gifted individuals united mainly by opposition to the new chair. My naïve zeal would never have managed to effect change without continuing help from outside. The dean's office supported reassignment of lab space and long-overdue renovation, furnished positions to recruit six new assistant professors, and supplemented PIBS's Markey funds to attract excellent scientists. PIBS faculty in other departments, including Biochemistry, worked long and hard to help me find, choose, and sign up the best candidates. Together we devised an excellent graduate course in cell biology, followed by a full-fledged new cell biology graduate program. Later, another university tried to steal one of my department's best new faculty members, whose lab space was less than adequate. I lacked the wherewithal to counter the competing offer, so PIBS and the new chair of Physiology, Zach Hall, came to the rescue, supplying necessary funds and temporary lab space, respectively.

Beginning in 1985, these new forces transformed UCSF's experimental biology community in exciting ways. New chairs in the four other basic science departments recruited young researchers that matched in quality those Rutter and Alberts brought to Biochemistry. Together, Anatomy, Microbiology, Pharmacology, and Physiology hired outstanding neuroscientists, biochemists, geneticists, developmental neurobiologists, and cell biologists. Brilliant, lively, and imaginative, these young scientists made remarkable discoveries, attracted superb graduate students into PIBS programs, and later assumed leadership positions at UCSF and elsewhere.[15]

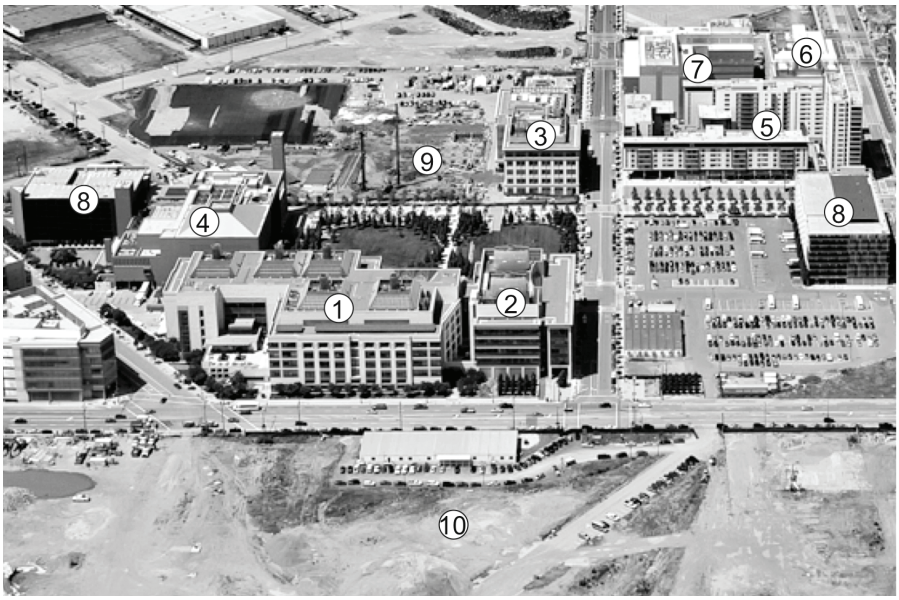
The increasing size and strength of the basic science community created its own problems. Clinical departments increasingly felt, with some justification, that undue deference to laboratory research was causing Dean Schmid, his successors in the dean's office, and the entire campus to neglect their pressing need for space and support for their own innovative programs. As research programs of their new faculty expanded, basic science departments found themselves caught in a "space crunch." Abated for some time by retirements of older faculty and piecemeal renovation of the ageing facilities in the Health Science towers, the crunch finally forced the campus to think about substantial expansion of its research facilities. In the 1990s UCSF sought

second sites for potential expansion, looking east of the San Francisco bay, south of the city, or near the Golden Gate Bridge. Eventually the focus turned to Mission Bay, the last undeveloped corner of San Francisco, more than 300 acres of former railroad property that were unused except for a ramshackle set of warehouses and a golf driving range.

Plans for the Mission Bay campus gradually became reality. Hoping to attract people and commerce to the surrounding area, the property developers and the city donated 43 acres of land to the University. From state sources, philanthropy, and settlement of a lawsuit against Genentech, UCSF cobbled together the necessary funds to start building a multi-billion-dollar project.[16] In 2003, a cohort of faculty from the Parnassus campus, plus some new recruits, moved into labs in Genentech Hall, the first research building. Since then, the Mission Bay campus has added (or will soon add) five additional buildings dedicated to basic and translational research, a center for conferences and recreation, campus housing, parking facilities, and specialty hospitals for children, women, and cancer patients.[17] As predicted, the area has attracted research institutes, biotech companies, restaurants, condominiums, and other businesses.

Just as success created problems for UCSF and its experimental biology community two decades ago, continuing expansion and cleaving UCSF into two campuses three miles apart inevitably generated significant difficulties. For instance, crowding that accompanied the space crunch brought scientists together with clinicians and other scientists more frequently and conveniently than is now the case. Separation of the campuses, with a twenty-minute bus ride between them, makes it hard to maintain interactions between research groups or researchers and clinicians. Even within the Mission Bay campus, growth in the number of scientists and their greater distances from one another, plus assigning researchers into separate buildings because of their specific expertise, may tend to fragment a research enterprise where discovery often depends on combining disparate insights to see problems in a new way.

These problems will be mitigated by construction of hospitals across the street from the Mission Bay research labs and by plans for integrating clinicians and translational research and basic biologists on the new campus. And, most fortunately, the curiosity of UCSF's basic scientists shows no sign of turning away from fascinating new questions posed by human biology in health and disease. Meanwhile, excellent laboratory scientists remain on the Parnassus campus, including outstanding immunologists, investigators of infectious disease, and others whose research is tied closely to clinical medicine. They maintain an excellent graduate training program in Biomedical Sciences, called BMS. (BMS was founded at Zach Hall's instigation when




UCSF's Mission Bay campus in 2010. The first buildings completed on the new site were laboratory research buildings: (1) Genentech Hall; (2) Byers Hall; (3) Arthur and Toni Rembe Rock Hall. In addition, the site includes: (4) the William J. Rutter Center, which houses recreation and conference centers; (5) residential housing for students and faculty; (6) the Helen Diller Family Cancer Research Building; (7) the Smith Cardiovascular Research Building (home of the CVRI); (8) two parking garages; (9) the future site of the Neurosciences Research Building, currently under construction; (10) the future site of the UCSF Benioff Children's Hospital, the UCSF Women's Specialty Hospital, and the UCSF Cancer Hospital, where construction is now under way. All the buildings shown were completed between 2003 and 2010, when the photo was taken.

he was still Physiology chair, a decade before the move to Mission Bay. BMS and PIBS dominate graduate training at Parnassus and Mission Bay, respectively, although some faculty and students in each program work in labs at the "other" campus.)

UCSF's progress in experimental biology over the past 25 years is exemplified by creation of new graduate programs and by organizing a new research campus at Mission Bay. As creative acts, founding PIBS and BMS and constructing the new campus required the same mix of vision and courage that produced the ground-breaking discoveries by our wild card scientists. Neither of the institution's later creative acts could have happened in the absence of two influences, which began much earlier:

one of these was the effort, skill, and vision of a small group of powerful leaders, the other a major transformation of UCSF's institutional culture.

The key leaders, who served from 1985 to about 2005, included—among others—Holly Smith, Bruce Alberts, Julius Krevans, Mike Bishop, Rudi Schmid, and Haile Debas. In addition, Zach Hall and Keith Yamamoto—working, respectively, out of the offices of the chancellor and the medical school dean—planned the Mission Bay campus and arranged the transition from Parnassus. Strikingly, seven of the eight came to San Francisco as far back as the 1970s, the period when the UCSF's burst of discovery took place. Indeed, six belonged to the group of wild cards and face cards focused on in this book. Thus the attractions that brought our wild cards to UCSF and the conditions that kindled the resulting burst of scientific innovation also brought to Parnassus the cadre of people who would lead the charge for decades to come.

Closely intertwined with the development of new leadership, the transformation of institutional culture was more complex and just as important. Essentially, UCSF's separate and sometimes overtly opposed clinical and research enterprises gradually formed a genuine partnership that made each partner stronger. The process began when basic science departments crossed the bay to join the Parnassus campus, but sharing a campus would not have sufficed. At least eight key developments bound the partners together: (1) construction of hospital and research facilities, abetted by UC's president and Regents, and by increased NIH funding for research; (2) Medicare, which reimbursed costs of patient care for many more patients and allowed recruitment of first-rate clinicians; (3) early replacement of the old guard (Saunders and his allies) by new leaders (Comroe, Reinhardt, Smith, Rutter, Krevans) who valued both research and patient care; (4) early models of research excellence, furnished first by Comroe's CVRI and then by Rutter's rejuvenated Department of Biochemistry; (5) increasing cooperation between clinical and basic science departments (e.g., via the dean's tax, Jack's Club, etc.; see Chapter Three); (6) the fundamental scientific discoveries of Boyer, Bishop, Varmus, Prusiner, and many others, which opened avenues to understanding disease mechanisms and developing effective new therapies; (7) birth of Genentech and the biotech industry; (8) continually increasing NIH funding for UCSF, which allowed new construction, faculty growth, and development of broad-based basic and clinical research programs.

The success of experimental biology at UCSF owes a great debt to clinical medicine, and not only for the fascinating scientific problems posed by patients and their diseases. This book's saga omits almost completely the parallel story of the growth and accomplishments of UCSF's efforts in advancing patient care, clinical research,

teaching medical students, and training young physicians and other health professionals outside the basic science world—that is, the enormous framework in which my smaller story developed. Including that framework would have required a much longer and more complex book than this one. Moreover, the relation between the larger story and the smaller saga I chose to tell is real but limited. It is real in the sense that many individual leaders (Havel, Edelman, Smith, Krevans, and Schmid) came to UCSF over the same time period and actively contributed to progress in both experimental biology and the clinic. Fortunately for me, I could limit my story because in the early 1970s our wild card researchers operated largely without interacting with the clinical, teaching, or other missions of UCSF.

I have stressed the essential roles of skilled and visionary leaders, as well as a powerfully cooperative institutional culture because they were responsible for difficult but essential changes that adapted experimental biological research to a new era. Along with many other UCSF faculty, I was thrilled to participate in and profit from that culture, in which it seemed that the efforts of clinicians and experimental biologists could synergize to make a new world. But neither leaders nor institutional cultures last forever—nor should they, because institutions and their human denizens must adapt to and exploit changing conditions. In the twenty-first century, biomedical research institutions cannot return to a time when discoverers and leaders could comfortably manage without one another, as they did forty years ago. Neither can those institutions preserve in amber the leaders and culture that were so effective in 1985. Instead, the next and final chapter will detail challenges posed by the ongoing transformation of institutional cultures at the NIH, UCSF, and other medical schools and research institutes. Chapter Twelve will consider possible ways future leaders may help to ignite further bursts of discovery that match or surpass that described in this book.

Chapter Twelve

# Hatching Innovation

## Incubate, Don't Squeeze

About fifty years ago, Clark Kerr wrote that American universities were rapidly transforming themselves from walled enclaves of high culture into large "multiversities" charged not only with teaching the young but also with the new task of creating knowledge necessary for economic progress.[1] Since then, by providing these institutions with federal money and generous support from private sources, the US has made biomedical research one of the leading producers of such knowledge. Now, however, our knowledge industry is in decline. Objective, comprehensive analysis shows that the US generates innovation in science and technology more slowly than five other countries, and the rate is not accelerating.[2] This chapter will focus on possible ways to reverse the innovation lag in experimental biology. That lag is due not to lack of money invested by society, but rather to increasing inertia in the system at every level—at the NIH, in biomedical research centers, in recruiting the young, in rewarding seniors, and in individual laboratories. Increasingly, innovation is stifled by channeling young scientists into lock-step career paths, by progressive graying of established researchers, and by rewarding conventional research goals and strategies at the expense of innovation. Tightly squeezed, research's porcelain egg is cracking. How long before it shatters?[3]

We have focused on influences that kindled a remarkable burst of biomedical innovation in one small institution forty years ago. Now, when every biomedical research center strives to kindle innovation, no leader would dream of re-creating conditions that prevailed at UCSF in 1970—and not only because the DNA revolution and subsequent advances in our scientific knowledge make this a foolhardy task. The even more critical difference between 1970 and now is today's biomedical research behemoth, funded by government, foundations, and industry. The behemoth's billions support thousands of established researchers, who are able, productive, and certain that our biomedical research effort is the best in the world—although they vigorously complain that the behemoth does not funnel enough money into their own labs.

It is one thing to describe past discoveries, but quite another to prescribe with confidence changes that will guarantee new discoveries. Rather than masquerade as omniscient seer or revolutionary zealot, in this chapter I shall prescribe more modestly, proposing experimental changes in national research policy and practice at the NIH and in biomedical research institutions.

One central principle guides my proposals. *Genuinely creative research is endlessly fertile, each innovative discovery carrying within it the seeds of further discoveries, different and unexpected. Consequently, conditions and policies conducive to discovery must also change, often quite rapidly.* Institutions cannot promote discovery by mimicking UCSF in 1970. Indeed, the national and institutional policies and practices of 1985—or, I argue, even 2010—will not suffice to kindle discovery now. We can do so only if we first recognize that innovative discovery is much more like a real fertilized egg than the man-made porcelain version. Not squeezing the porcelain egg may preserve it forever, but it will remain stubbornly sterile. Fertilized real eggs, with luck and gentle incubation, produce live progeny and myriad descendant generations, with lineages as wondrously different as dinosaurs, emus, and soaring red-tailed hawks. Cracking porcelain triggers a twinge of regret, but repeated failure to hatch live eggs quenches limitless possibility and risks devastating loss.

## Scope and Strategy

As Chapter Eleven showed, the forces limiting biomedical innovation are both widespread and immensely complex. Some originate at levels far removed from the biomedical research enterprise itself, involving national and international economies, social trends, and national politics, as well as education (K-12 as well as colleges, universities, and graduate schools) and the technology industry. Within the biomedical research enterprise, constraining forces arise, reinforce, and occasionally counteract each other at many levels, including the health care colossus (federal, state, private, insurers, hospitals, health providers, patients and patient advocates, etc.); the pharmaceutical and biotech industries; charitable foundations; the NIH; a host of rapidly growing biomedical research centers, institutes, medical schools, and their myriad administrators; graduate training programs; scientific journals; and the researchers themselves, with their scientific societies, competing areas of expertise, hierarchies of privilege and power (technicians, students, postdocs, adjunct faculty, tenured principal investigators), and symbiotic and adversarial relations with all levels of the larger biomedical enterprise.

Within the biomedical research world each level of constraining force is growing larger, more monolithic, and less able to change and adapt to new problems. Each

tends to plan only for the short-term future, apparently trusting that the longer term will simply replicate the past—despite abundant evidence that the professed goals and the very fabric of biomedical research explore, promote, and promise change. Constraints on innovation are further reinforced by interlocking relations between levels. Thus, for instance, both cooperation and ongoing battles between investigators, the biomedical research centers where they work, and the NIH itself tend to perpetuate present policy, whatever it may be, and make change even more difficult.

Given the size and inertial complexity of present constraints on innovation, my proposals for change will be limited, focused, experimental, and (I hope) potentially feasible, rather than radical, universal, and frankly impossible. The guiding principle, outlined above, is that discovery begets change and new discoveries, so that the research enterprise itself must also learn how to change. From this are derived four secondary principles: *focus*, *leadership*, *flexibility/diversity*, and *heterogeneity of institutional cultures*.

The first principle is simple: we must choose our battles carefully. This means, for instance, focusing on innovation as more critical than on "ordinary good science," which does not alter fundamental understanding but clarifies and extends prevailing paradigms. Extending those paradigms is undeniably useful, but fading innovation poses a more urgent problem and may prove more amenable to change, because it involves fewer scientists and so can fight on a narrower front.

Second, we need to recognize that change in the biomedical behemoth—small or large, at the level of laboratories, departments, universities, the NIH, and other institutions—critically requires courageous, dedicated leadership. Despite science's itch for new facts and innovative interpretations of nature, when push comes to shove scientists stick to established custom, just like other academics. Still, superb leaders can engineer difficult transformations, as we saw at UCSF with Saunders's ouster and the later reorganization of PhD programs in basic science departments. As with discoveries, institutional change must carry within itself seeds of self-renewing future change, even when its agents can foresee neither the future problem nor its solution. In the 1960s and early 1970s, UCSF attracted agents of change to its laboratories and also to its corridors of power. It owed this good fortune to conditions we cannot easily conjure up in the twenty-first century—economic prosperity, abundant resources, location in San Francisco, and UCSF's fresh, unfinished state, ripe for transforming pioneers. For this reason, superb leadership is now even more essential than before, to attract innovative young minds and to shape environments conducive to their talents.

Third, wherever possible we should work to increase flexibility and diversity

of goals, individual choice, and previously rigid policies and practices within the research enterprise. Pitched battle against the behemoth and its well-entrenched vested interests will fail. Instead, we should push for local, incremental change that increases choices available to investigators and research administrators.

Fourth, in order to increase flexibility, diversify research opportunities, and effect policy change we should take advantage of important cultural differences between individual institutions and their component parts. I offer two examples. First, the cultures of leading biomedical research centers often differ remarkably. Fred Cohen, a former colleague and now a venture capitalist committed to innovative science and technology, says that biomedical research centers at one end of a broad spectrum organize their research into "multiple solar systems around a myriad bright stars"—which they can do because they are supported by "arbitrary amounts of cash" from private and federal sources. These institutions tend to hire already shining stars from other institutions by offering generous resources, many slots for assistants and staff, and the opportunity to become the center of a solar system that may convey them in any direction they choose. At UCSF and a few other schools at the other end of the spectrum, he says, "collaboration is the right thing to do, junior faculty members are given resources much closer to senior faculty members than not, [and] the gradient or the pyramid isn't [as steep as elsewhere]. In a world of finite resources, that cultural set point can maintain a broad-based innovative set with a lot of enthusiasm."[4]

To this I would add that institutions at the solar-system end of the spectrum provide "planets" with little direct support, instead expecting them to function as efficient but replaceable enhancers of the brightness of the star around whom they revolve. In contrast, collaborative institutions with less steep pyramids are more able to welcome and cherish new colleagues, nurture their development, and thereby become more hospitable to youth and change. Thus, when solar-system institutions with the opposite bias attract established scientists from places like UCSF, the star's departure frees up a faculty position and resources for outstanding new talent.[5] Institutions at both extremes, and many in between, can prove highly successful, but those that welcome youth and change are probably better bets to overcome increasingly formidable obstacles to innovation.

Separate cultures within an institution present both challenges and opportunities. Consider, for instance, the history of relations between clinical medicine and basic sciences at UCSF. Between the move of basic science departments from Berkeley San Francisco in the mid-1950s and the end of Krevans's deanship in 1982, it may have appeared, as Krevans argues, that UCSF had seamlessly woven clinical and basic research into a single fabric.[6] Over the ensuing twenty-five years, however, the seam

re-appeared, and by 2010 began to reveal ragged tears.[7] Now the chancellor's office urges that UCSF transform itself into a center of "human biology" and emphasize patient-oriented "translational" research—that is, research aimed at translating basic discoveries into clinically relevant diagnosis and therapy. Over-built labs at Mission Bay and recent flat-lining of the NIH's grant budget make it harder to fund recruitment of new faculty into a small but increasing number of empty labs. Basic scientists worry that the institution will fill these empty labs with alien faculty from clinical departments.

Like all culture wars, this local skirmish is more complex and nuanced than a paragraph can convey. The local fight is fuelled by a growing national and federal push to "translate" basic knowledge into diagnosis and treatment of disease—a push that critically affects NIH research grant awards and institutional priorities. Both locally and nationally, however, the war involves important issues and jostling for key resources. Legislators, administrators, and scientists perceive that the public cares more about cures than about biological mechanisms, so that the way to increase NIH funding of research is to claim that we will speed "translation" of knowledge into patient care. Sadly, animosities, rhetoric, and hot air on both sides obscure the much more urgent need to foster genuinely innovative research, which lags on both sides of the clinical-basic divide.

More than 30 years ago, after he retiring from directing the Cardiovascular Research Institute, Julius Comroe began to think about "how to get the most for the medical research dollar and how to . . . generate medical breakthroughs." Using what he called a "retrospectroscope," he took a hard look at how recent life-saving biomedical advances—that is, recent in the mid-1970s—had come about.[8] A vast number of important advances, he found, began in research that had little or nothing to do with practical usefulness of any kind, and even so-called "applied" research often produced results that were most valuable in contexts not imagined by the researchers who did the original experiments. His analysis of many cases indicated that neither "basic" nor "applied" research, alone, would do the trick. Instead of EITHER/OR, Comroe came firmly down on the side of BOTH/AND.[8]

The reason for choosing BOTH/AND is that tight links connect all branches of life sciences, making almost every fundamental discovery relevant to human life. Comroe's point is re-affirmed by the stories of recombinant DNA, oncogenes, and prions. Thus, for instance, Stan Cohen was taking a sophisticated approach to answering an "applied" and medically important question, How do bacteria inherit resistance to multiple antibiotics? In contrast, Herb Boyer was asking, How does a restriction enzyme recognize a specific DNA sequence?—an arcane "basic" ques-

tion that fascinated him more than it did most of his colleagues. Who could have predicted that this particular combination of applied and basic questions would lead to recombinant DNA technology, which changed all experimental biology and now underlies most modern methods for diagnosing and treating disease? Similarly, who knew that identifying the first oncogene would open avenues to detailed understanding of the genetics of cancer and to effective canceer therapies? Or that a brash young neurologist, upbraided by his chairman for studying an obscure sheep disorder, would discover a new mode of transmission for infectious disease and a templating process that helps to explain many neurodegenerative diseases?

At the time of those discoveries, and for a few years afterward, it seemed possible to imagine that investigating fundamental mechanisms in nature would always be linked indissolubly to research important for human needs, clinical and otherwise. Now researchers begin to think, instead, that the entire biomedical research enterprise should veer sharply toward basic or applied ("translational") research, and so array themselves as soldiers in separate armies on opposite sides of a gradually deepening chasm. Nonetheless, it seems to me that the truly critical issue for biomedical science is how to recruit and support creative young scientists at every level and in every department, basic or clinical. To do so, we must emphasize innovative potential and accomplishment and curtail arguments over less critical issues—whether a researcher sees patients, has an MD, or studies the kidney, the brain, cells, molecules, or microbes. Rather, we must ask, and keep on asking, "Will this person's work either advance understanding of life or sharpen tools for fighting disease?" Both are equally crucial.

## Can the NIH Change its Ways?

The NIH controls all the most powerful and obvious levers for effecting innovation, but also serves as one of the principal obstacles. Its huge size, entrenched bureaucracies, increasing dominance by non-scientists and older scientists, and present funding policies and practice vigorously resist attempts at effective intervention, executive or legislative. Experimental attempts to promote innovation, devised by thoughtful scientists and NIH leaders, have been strongly opposed by inertial resistance. One early experiment, for instance, was a small but provocative change in NIH guidelines for judging grant proposals. In addition to the usual criteria, reviewers are now explicitly required to consider promise for innovation a positive factor in judging proposed projects. Whether or not the new criterion influences actual funding, this apparently innocuous change elicited the following urgent warning to grant applicants, issued on the web by one of NIH's institutes:[9]

Don't Propose Too Much or Be Too Innovative
Our Advice: Be Careful How Much You Propose and How Innovative You Are

"Innovation can be tricky to factor into your proposal," the warning continues. "Beware of Being Too Innovative. It can be harder to gain acceptance if your ideas are far outside the mainstream, especially if you are less experienced. But innovation is a review criterion, so you do want to show how you will break new ground."[9] Juxtaposed with clear evidence that scientific innovation in the US is falling behind that of other countries, this advice appears ludicrous.[2] The wrenching irony, however, is that the advice is largely correct. To get grant money, successful applicants must learn to disguise strikingly new and innovative ideas as clever but straightforward variations on the same old tunes.

Several ongoing experiments by the NIH are designed to increase the likelihood of funding first-rate research by creative scientists. These include: *K99 awards*, which promote transition from training to faculty status by funding two years of supervised research in an established lab, plus three years of independent research in a faculty position; n*ew policies for reviewing grant applications of young investigators,* which designate young and first-time applicants for individual research grants for special consideration by reviewing committees; *Pioneer Awards*, to applicants of any age with bold new research plans: *New Innovator Awards*, for highly creative young investigators; *Transformative RO1* grants, for funding "destructive," paradigm-breaking ideas of applicants of any age; the *Early Independence Award Program*, recently announced by the new NIH director, which in 2011 will provide five years of funding to ten unusually creative investigators who transfer to faculty status almost immediately after obtaining their doctoral degrees, at an institution they choose.[10] These new approaches introduce useful wrinkles in the review process, like shorter applications and face-to-face meetings with reviewers. They will identify some outstanding researchers with appealing ideas, but the number of individual investigators benefitting from the new awards will be few and the review process remains subject to prevailing conservative pressures. These experiments nibble at the edges of the innovation problem, but fail to tackle it head-on.

Bolder, more potentially effective reforms are needed. One reform, proposed by Bruce Alberts in *Science* magazine, is that the NIH require that individual scientists eligible for NIH grants receive substantial salary funding (e.g., 50% or more) from the institution where they work.[11] This requirement would counter temptations to academic institutions to recruit faculty researchers supported largely or entirely by

NIH grants and to over-build research facilities with indirect cost payments from the NIH. I am willing to bet that the NIH will give Alberts's proposal short shrift, however, because it threatens crucial vested interests of so many large biomedical research centers. Alberts says he has heard positive responses from scientist colleagues, and has discussed the issue with NIH officials. Eight months after his proposal was published, however, the NIH has not responded to it officially.

The NIH might pursue other routes to changing research practices in labs dependent on its funds. By preventing funded investigators from keeping PhD students and postdocs in their labs for many years, it could perhaps reduce the age at which individual young scientists receive their first grant. It might even require that permanent employees, rather than students or postdocs, make up a certain fraction of the experimental researchers in every lab. Even less likely, it could explicitly favor applications by young investigators, in comparison to their seniors. Such measures would be opposed by many, driven by selfishness, of course, but also by legitimate concerns (e.g., fairness).

One other potential measure might help substantially to enhance efficiency of lab research and reduce built-in advantages of senior investigators compared to their younger colleagues. It would also reduce the ever-growing flood of grant applications—most of which will never be funded—that presently plagues the NIH. For established investigators (those who have already received five or more years of NIH support), this measure would replace RO1 grants with "Investigator Grants." (RO1 grants are presently awarded to investigators to support separate projects, so that a single investigator's lab is often supported by multiple RO1 grants. In 2010, 26,752 RO1 grants supported individual investigators, for a total of $10.6 billion. RO1 grants accounted for 59% of all NIH grants and 49.5% of the total NIH grant budget.[12]) I propose, instead, that all established NIH investigators be funded by a single "Investigator Grant," rather than one or more separate RO1 grants. (This idea, which is not new, was suggested to me by my UCSF colleague, Ronald Vale. He bears no responsibility for the form in which I have cast it, however.[13])

More specifically, applications by these established investigators (every seven years, say, following their first grant) would be judged primarily on their research track record in the previous grant cycle. The main criterion for judging an investigator's track record would be the perceived significance of innovative discoveries, relative to total research expenditures (and number of participating lab personnel) over the same time period. More significant innovation would be rewarded with more funds in the next cycle, but only if the investigator managed her lab in a way that maximized productivity (per dollar) of each person in the lab. (Beginning investiga-

tors, applying for their first grant, would be judged on future promise; their grants would be for seven years also, depending on NIH's evaluation of progress in year four.)

While maintaining merit-based competition as essential policy, these changes would also enhance individual accountability of each established investigator, the relative value and efficiency of their lab personnel, and—if funding criteria are followed correctly—indirectly increase funds available for supporting proposals by beginning investigators. Established investigators could focus more easily on long-term projects and would no longer write six proposals in order to get funding from three. Some of their labs would become smaller, while all—if the funding criteria are followed correctly—would conduct their research more efficiently, because it would no longer appear as useful to throw large numbers of personnel at a problem, hoping one or two will hit paydirt.

Such Investigator Grants could have deleterious effects, of course. Indeed, they might actually reduce innovation if, for instance, an unusually innovative senior investigator with a marvelous idea has to wait for the next grant cycle to increase her funding, rather than (as she can now) propose an exciting new project and hope to receive new funds within 9-12 months. Established investigators will fervently marshal this and other arguments against the plan, but changes in the economic environment could make it more attractive. Indeed, in the face of prolonged, substantial reductions in overall federal grant funds, some version of Investigator Grants might preserve productivity among established investigators while indirectly releasing funds for young investigators, an especially vulnerable and (for the future) valuable subpopulation.

## What Can Institutions Do on their Own?

As noted earlier, one major obstacle to innovation in academia is the slow, lock-step career pathway an aspiring young researcher must endure before becoming an independent investigator. A second is the relentless pressure—imposed by current funding policy and practice on all investigators—to tackle a circumscribed set of conservative, less risky goals. While both obstacles reflect strong forces outside individual institutions, including the NIH and other funding sources, academia contributes significantly to strengthening the same obstacles, and the heterogeneity of research cultures among academic institutes and schools offers opportunities for change. Displacing obstacles at the level of individual institutions requires battle on two critical fronts—hiring the right scientists and leaders, and fashioning the right environment.

In the first battle, hiring innovative leaders or scientists, the initial problem is

to attract the interest of ideal candidates, because reputation for scientific excellence is not enough. An institution seeking to foster creative science must find or devise a magnet that draws creative individuals together. Reliance on the magnet of its surroundings, as at UCSF forty years ago, will not suffice. Instead, for pioneers the most powerful magnet is the prospect of real adventure. Young pioneers will be drawn to a community of adventurous minds and to leaders committed to nourishing innovative scientists. Exciting colleagues trump material resources and illustrious senior scientists, every time.

When the candidate pool contains the right innovative scientist, the next task is to identify that individual. Maestros of this art—like Holly Smith, Bill Rutter, Bruce Alberts, and Keith Yamamoto—recognize that the task of hiring new leaders or new scientists is immensely important and demanding, requiring hard work and vast energy. Rather than pick the best applicant in a narrow field, they devote hard work and intense energy to finding the best anywhere, evaluate them from every angle, elicit a range of opinions, and never rush to judgment just in order to fill a position now rather than later. They consider every candidate's goals, experimental plan, passion, energy, self-trust, and focus. The successful candidate's experimental plan should promise true innovation, new to the institution and to science. Finally, the best judges try to recruit scientists who will build a viable community of cooperating individuals. Superb scientists may exhibit personal quirks and eccentricities, but social skills are often essential for success. Biology's increasing apparent complexity makes scientific loners less effective than they once were.

How can hiring practices at one institution mitigate the graying of the experimental biology community, which strengthens obstacles to its creativity? Increasingly, new faculty assume their first independent research position when they are much older than in the past—averaging in 2010 nearly 10 years older than in 1970.[14] Individual institutions can recognize and mitigate this graying, even if actions of one or a few institutions cannot strike at the problem's deep roots, as the NIH might. For instance, we should push our graduate students and postdocs out of the nest earlier. Developing the skills of an independent scientist should not require six years working for a PhD, plus postdoctoral stints that take even longer. In addition, we should do everything we can to find the best bright-eyed, bushy-tailed younger scientists before years of toil for one of us inevitably dull their sharp young minds. Joe DeRisi, an outstanding molecular biologist who joined UCSF's faculty in 1999, at the age of twenty-nine, proposes to subject a radical notion to experiment, by advertising faculty positions with an explicit statement that postdoctoral work is not required. The risks are real, but he says UCSF has the necessary resources to mitigate the risk, in

the form of established faculty committed to developing outstanding young scientists.

It is absolutely critical for institutions to recruit judges who will recognize and foster young individuals' capacity for innovation. (Many claim to "know it when they see it"—until they don't—as I know from sad personal experience.) As compared to young scientists, potential leaders are older, with better-documented track records—and consequently more savvy about academia and sometimes more prone to adhere to established practice and received wisdom. As a result, excellent administrators may maintain large, efficient projects but remain hostile or indifferent to change—characteristics undesirable in leaders for research enterprises constantly in need of new ways to renew themselves. Leaders fully able to meet that recurring challenge do exist. Eager to act as agents of change, they may be chafing under the supervision of authoritarian leaders, like Bill Rutter, have been passed over by an elite institution, like Holly Smith, or after reaching professional maturity in innovative institutions, may assume leadership at home or carry the message elsewhere.

If an institution is to preserve or enhance its capacity for change, its leaders—whether imported or home-grown—must combat powerful forces that act to suppress innovation. Financial support from the NIH, industry, or charitable foundations tends to be awarded preferentially to established scientists, sometimes at the expense of originality. Recall that both the NIH and private sources turned down Prusiner's grant requests early in his career and began to shower him with funds only after he showed unmistakable signs of success. Reviewers for prominent scientific journals and for the NIH and other funding agencies naturally favor researchers whose ideas agree with their own, and consequently tend to approve well-documented work of established scientists in preference to ground-breaking proposals from younger scientists. As Bruce Alberts pointed out, soft-money support and other NIH policies also work against innovation, by forcing investigators to write and review grants rather than actually doing research.[11] These policies also offer institutions dangerous incentives to create cadres of scientists who work for other scientists but may later find themselves quite dispensable. Finally, every institution's imperative to preserve itself fosters hide-bound internal bureaucracy and a bias for retaining ageing leaders. For all these reasons, innovative biomedical research centers must find, support, and reward leaders who are willing and able to effect change. Because scientific discovery creates and depends on change, innovative institutions must continually transform themselves or fall into decline.

In addition to hiring excellent creative scientists, leaders of such institutions must also nurture nascent discovery and shield it from hazard. In the 1970s it was easier for young scientists to identify and exploit exciting opportunities, because that era's

smaller, simpler institutions (NIH, other funding sources, and universities) were less able (or inclined) to smother their initiative and creativity. Now leaders must nurture innovation in positive ways, and at the same time play essential protective roles, in which they mitigate hindrances to discovery imposed by other faculty, institutional norms, and sources of research funding. Both positive and preventive leadership roles are more critical now than ever before.

One rule is absolutely critical: leaders must make sure that every faculty appointee enjoys the freedom to conduct independent research. The appointee's research is not to be dictated or supervised by others, and must entail no obligation to collaborate with or otherwise serve interests of senior faculty. Even more important, every effort must be made to minimize pressure on all investigators to do what others do. Unfortunately, leaders often respond to the same ubiquitous, insidious pressures as their protégés, and these pressures can represent worthwhile goals, albeit in a different context. Sources of pressure include perceived relevance (translational research or a fashionable "basic" question); glamour of a field; and exciting approaches not appropriate for the question at hand (remember Prusiner's critical choice of biochemical purification over molecular genetics). In some ways the hardest freedom to guarantee is freedom to pursue a truly difficult problem, even when multiple approaches have produced only incremental advances. Remember that the searches of Boyer and Prusiner for EcoRI and PrP, respectively, required long timeframes not usually tolerated by funding sources and biomedical research centers today. Seekers of exciting but hard-to-achieve goals need protection from pressure to "hit the ground running." Instead, leaders should monitor progress carefully and—if the goal is important and (eventually) feasible—somehow provide the encouragement, time, and freedom of action required to reach it.

Note that these essential leadership contributions are preventive. Our narrative of discoveries in an earlier time included only one key preventive action exerted by leaders—persuading Prusiner's chair not to turn him down for tenure. Now, however, perhaps the leaders' most essential task is to defend and preserve the freedom of innovative researchers to tackle and solve difficult problems. To do so, they may be called upon to counteract deleterious impacts of faculty and other leaders of the institution itself, as well as external agents (NIH, regulators, etc.).

In listing these essential contributions first, I have been assuming that the research institution will have done its best to *provide adequate material resources* for research, including salary, lab facilities, start-up support for postdocs and students, equipment, and consumable supplies. These traditional institutional contributions vary from one institution to another, but startup funds for a new assistant professor

nowadays may amount to as much as $1 million.

Less important but still significant leaders' contributions to innovative investigators include: (a) promoting cooperation and collaboration when they can lead to innovation; (b) imposing light teaching loads on young faculty, freeing their roving imaginations to focus on creative experiments (seniors are usually better equipped than beginners to teach without losing their grip on the lab); (c) funneling available funds in directions that enhance innovation (e.g., to supplement an existing grant or support a new research direction); (d) providing ready advice and moral support when it can do the most good (even the very best young scientist will need guidance in making decisions about hiring, teaching, publication, and grant applications).

None of this long sermon is irrelevant, impractical, nor self-evident. At my own and other institutions, we violate these admonitions more frequently than we like to admit, with unfortunate consequences. The reasons are partly systemic, but can reflect plain poverty of imagination or simple selfishness. (A young scientist at another university told me that assistant professors there did not seek advice from senior faculty, because taking advice from any one senior invariably triggered overt hostility from others!) But I have also seen leaders heed these admonitions, to excellent effect, and received invaluable help myself from many older scientists, including Gordon Tomkins.

Imaginative, savvy leaders in the twenty-first century can promote creative innovation comparable to the examples recounted in this book. The task will not be easy, but I am convinced it can be done.

## Experiments in Promoting Innovation

Before I outline my own favorite proposal for fostering innovative discovery, let me first recount the story of an important precursor experiment. This is the Janelia Farm Research Campus of the Howard Hughes Medical Institute (HHMI), located near Washington, DC, in northern Virginia. Completed in 2006, the campus's organization and style are modeled after Cambridge's Lab of Molecular Biology and AT&T's Bell Laboratories. Its founding director, Gerald M. Rubin, expressly planned a venue for experimental biology conducted in an environment free from funding criteria biased toward predictable outcomes and influences that distract from innovative research—including directing a large laboratory, forgoing hands-on experiments, running a small business, writing grants, teaching, and committee duties. The leading precedent seems to have been the lab in which Rubin himself was trained—that is, the Molecular Biology Lab in Cambridge, England during the regime of Max Perutz.[15] Janelia Farm's forty or more independent investigators work on six-year contracts,

direct small groups (no more than six students or postdocs), and receive salaries and research support entirely from the HHMI (no grants from the NIH or other sources). The facility's scientists are chosen for scientific ability and promise, and their progress is regularly assessed by Rubin and committees of first-rate scientists appointed by the HHMI. The new institute's research focuses on how neuronal circuits process information and on new methods for analyzing images of functioning cells.

Because Janelia Farm welcomed its first scientists in 2006, it is too early to judge whether this attractive alternative research model will—as its founders hope and I predict—effectively promote creative, innovative research. As an experimental model, however, Janelia Farm has two defects, which would have been almost impossible to avoid in one small experiment. The approach is well designed, but too expensive for most private funding sources or universities to contemplate—the HMMI may be the only exception. Thus it cannot serve as a valid model for major changes in biomedical research funding at the national level. Moreover, its relatively small size confines Janelia Farm to a limited range of research targets and precludes exploring alternative strategies, more adaptable to a national context, for promoting creative innovation.

The project has a third defect, technical but also important: while it is a real experiment, no one is keeping careful records of what happens there—how decisions are made, how the new scientists and their mentors think about their jobs, how invited candidates are chosen, how they decide to accept or turn down Janelia Farm positions, and what eventually becomes of those who accept and those who don't. Twenty years on, such information could prove immensely useful, but the HHMI has not set aside money for maintaining such records or for systematic interviews with participants. In the Janelia Farm experiment no one is keeping a lab notebook, as Rubin ruefully admits.[16]

Although I admire the Janelia Farm idea, these defects reduce its relevance to the urgent need for a nationally effective approach to fostering innovation in experimental biology. The chief obstacle, of course, is that the NIH behemoth would inevitably balk at the notion of funding separate institutes *as institutes* (e.g., small versions of Janelia Farm), because it (and the national ethos) considers judging competing applications from individual scientists both more efficient and more equitable. Moreover, small institutes certainly present dangerous opportunities for incompetence and even corruption.

On the other hand, it seems likely—at least to me—that a small number of model "innovation incubators" embedded within major research universities could avoid those dangers and foster innovation. Now I shall outline a different version of the

Janelia Farm experiment, funded privately and by the NIH. Like Janelia Farm, it is designed to deal with the twin problems of lock-step career paths and pressure on beginning scientists to follow the crowd. This experiment would ask whether creative minds thrive better in an environment where a director and a small steering committee evaluate, advise, and distribute financial support to each scientist, without intervention by committees and a bureaucracy somewhere else. What might these innovation incubators do? How might they integrate into the larger biomedical enterprise? How should they be funded?

The central idea requires founding several (less than ten) model incubator institutes, each supporting the research of five to ten *young* investigators, all chosen, advised, and evaluated by senior scientists known for their ability to pick and foster progress of younger colleagues. Each model incubator institute, embedded within a different leading research university, would focus on fundamental investigation targeting an important biologic function or disease, including but not confined to either pathogenesis or treatment. Examples could include cognition, neurodegenerative disease, parasitic infections, metastatic cancer, synthetic biology, nutrition and metabolism, cardiovascular disease, and many others. (Note, by the way, that these examples are neither purely "basic" nor strictly translational, but located squarely between the two.) Each young investigator would be receive support for a limited period (maximum of eight or ten years, with formal NIH review at about five years) and would then be expected to return to the existing system (universities, institutes, industry, etc.). In every case, almost all salary and research support would go to "incubated" young investigators, except for partial salary for the incubator institute's director and perhaps administrative staff, plus minor salary support for members of a steering committee. Incubator funds would not support research of the director or committee members. Their research would instead remain quite separate from that of "incubated" scientists, none of whom would work "for" any supervisor, including the director. Each incubator institute should be guaranteed to last for at least 20 years—time enough to launch the careers of several generations of young scientists and to evaluate the results.

Appointing first-rate individuals to direct and serve on steering committees of these incubators will test the notion that careful selection of the right young scientists, followed by persistent, long-term interaction with peers and senior investigators—*face-to face, as goals are formulated, experiments performed, and results evaluated*—can enhance both innovation and evaluation of individual progress. The experiment will also ask whether stable financial support to first-rate young scientists, administered by wise, knowledgeable seniors *on the site*, is a viable option for

subsets of academic research. We need to know whether this strategy better promotes achievement of long-sought but difficult goals, in comparison to the formally fair but unavoidably less intimate and chancier method of peer review by committees unfamiliar with the applicant or the work.

Each model incubator's general goals would resemble those of Janelia Farm, but their administrative arrangements would vary in details that might affect the likelihood of promoting innovation or potential for later expansion and adaptation. These varying arrangements would help to identify approaches that could or should be applied eventually to more investigators and bigger research problems, and so would explore diverse possible funding models and policies, varying in: (a) *career track* (e.g., contract, tenure-track, and hybrid models for teaching, graduate education, financial support, academic advancement, and ultimate transition to "ordinary" faculty status; (b) *lab organization and support* (e.g., five to eight workers per lab, supported entirely by incubator funds or by combining such support with more conventional sources); (c) *general funding* (various combinations of NIH funds and investment by biomedical research centers, using funds from alumni donations and private sources); (d) *review, advice, and accountability* (e.g., various mixes of incubator director and expert advisors, with different modes of oversight by local and NIH-appointed external committees); (e) *collaboration* (modern biologists collaborate freely and often, but sometimes struggle to get credit for work done with others—a problem that may be approached in different ways by different incubators). Differences among the institutions in which the incubator programs are embedded will add other variations to the experiment, because different institutions and their directors will inevitably approach the challenge of judging and fostering the progress of bright young investigators in different ways.

Readers from several large biomedical research centers will recognize aspects of local programs at various institutions that provide beginning scientists—often called "Fellows"—a lab and modest support for independent research, before they move on to a formal career. These differ from the proposed "incubators," however, in several ways, including level and duration of support, administration, and overall purpose.[17]

Can we find the right leaders for these incubators? Trusting disbursement of funds to a single director or a small committee runs the very real danger that inadequate leadership may spend the funds unwisely and/or selfishly, wasting resources that would otherwise be awarded to competing individual applicants. The danger can be reduced by choosing host institutions and directors with high standards of research excellence, followed by careful oversight from the host institution and the NIH. I do not know how easy it will be to find the right directors for such incuba-

tors, but my limited experience in a single institution has allowed me to know at least a dozen individuals I would deem suitable. My guess is that today every leading biomedical research center supports at least three similarly skilled potential directors, each in her or his fifties or early sixties.

The cost of a program of this size is substantial but not prohibitive. Let us estimate (generously) that supporting a beginning scientist costs an average of $1 million per year (less at the outset, more as time goes on), plus $3 million in startup and renovation costs. The yearly cost for five such centers, each supporting (at steady state) eight young investigators, would come to about $55 million—approximately 0.27% of the NIH's 2008 grant budget, and less than 12% of NIH grants to UCSF in that year.[18, 19]

I am confident that the productivity and value of research incubated in these incubators would at least match those funded by extant grant mechanisms. But will the mini-experiment produce genuinely creative innovation? To find out, it will be necessary to compare research results of "incubated" scientists with those of an appropriate control group—e.g., a larger cohort who receive continuous NIH support, awarded by the usual mechanisms, during the same period. It will also be essential to define "innovation" before the experiment begins, perhaps by preparing a benchmark list of the fifty "most innovative discoveries" in experimental biology over the past fifty years, and asking whether the rate of similarly ranked innovative discovery in "incubator" or control labs matches or surpasses it. (Rates of discovery would be adjusted, of course, to correct for the different time periods and numbers of investigators involved.) Whether the incubators' discovery rate does or does not surpass previous rates, without documenting the experiment in the equivalent of carefully maintained lab notebooks we can only guess why the incubators did or did not achieve their goal. Accordingly, the experiment's budget must include funds for maintaining careful records, plus interviews, surveys, and analysis by personnel not directly employed or hired by the institutes themselves. Properly kept "lab notebooks" for this experiment might prove especially critical in judging different approaches to preventing the most obvious danger of small incubators—that is, poor local leadership and oversight, resulting in incompetent or flawed performance, owing to weak leaders, cronyism, etc.

Let us imagine that the experiment produces positive results—that is, overall, or in its variations, the experiment produces genuinely important innovations at a rate not likely to occur by chance. It may not be possible to be sure of such a result for fifteen to twenty years, although a dozen might suffice to indicate a likely outcome. If the outcome is strongly positive, how should academia and the NIH respond? Pre-

sumably, it would be obvious that the program should be expanded, to the point of providing similar incubator environments for many or most beginning investigators in "basic" experimental biology—with, of course, attendant require major changes in both research universities and NIH funding mechanisms. Obstacles and difficulties would abound, but the ultimate rewards in terms of creativity and innovation would be worth it.

What about investigators "hatched" from the incubators, or the larger number of "controls" who survive and prosper in the NIH grant mêlée while the experiment is in progress? If incubators turn out to be useful, should more senior investigators also work in locally administered incubator-like environments? This option, I suspect, would not prove attractive to the NIH or to its successful grantees. Moreover, the incubator-based approach may not be a good way to judge or reward savvy veteran investigators, who know the ropes and will be tempted to wangle unwarranted support from a local supervisor—an easier task, perhaps, than getting it from a distant NIH committee.

The truth is that we cannot know the future of experimental biology or predict how our successors will behave. For biomedical research to work efficiently, however, it will have to find a creative balance between freedom and competition. Innovation clearly requires both, but each brings dangers in its train. Innovation thrives best when the innovator is free to choose among questions, range across multiple possibilities, and sniff out the way to an answer. Innovators always compete, of course, but the best of them create their own competitive drive and often do their best when not under direct duress. On the other hand, all scientists profit from some degree of overt competition, and every worthwhile task runs some risk of failure. Current trends in NIH funding and organization of research centers increase the intensity of competition for beginners while (comparatively) relieving competitive pressures on established senior scientists. To preserve a creative balance between freedom and competition, I propose reversing the present situation—that is, reducing competitive pressure on beginners and shifting more of it onto senior researchers. Such a shift could give the young more freedom to develop and sharpen the motivation of their seniors.

## Farewell

Today many biomedical scientists share forebodings about their future—which, as always, are heightened by financial pressures like those resulting from the recent economic downturn. The real problem, however, is less a shortage of money than a woeful lack of thoughtful planning for the future. For this lack scientists must share the

blame with politicians and US citizens. Our mutual focus on immediate gains rather than future consequences has constructed a muscle-bound research establishment that funnels resources preferentially to established scientists and big laboratories, at the expense of strong support for young people. Similarly, it has crafted a training system for PhD students and postdocs that supplies cheap labor to established laboratories and curtails opportunities for beginners.

No one in 1970 could have predicted the subsequent accomplishments of biomedical research. Similarly, at present we cannot know what the next four decades will bring. A historian forty years from now may view this book's forebodings and proposals for change as prescient and useful, or dismiss them as nostalgia for a vanished past. My own principal worry is that my proposals are too small and too late to be effective. Not only do they fail to deal with many critical problems that bedevil funding and administration of biomedical research, but the scope of my main proposal is minuscule, even in relation to the limited goal of enhancing innovation, both in funding (less than 0.3% of the NIH grant budget) and in the number of scientists it would directly affect (a few dozen). Its greater defect may be political. The proposal's immediate beneficiaries would be a tiny cadre of young scientists with no political clout in academia, and it will be opposed by powerful vested interests, including proponents of both "basic" and "translational" research, older scientists who seek greater power and more support for their own labs, devotees of specific organ systems, or enthusiasts for immediate cure of ageing, cancer, diabetes, and heart attacks.

The problem of increasing and maintaining scientific innovation is nonetheless real, and failure to tackle it is dangerous. Fortunately, the fundamental human need for adventure will never go away. Back in the 1960s and 1970s, we watched the primordial scene of a white-coated leader inviting a potential young recruit to look at the view from Parnassus. It was the recruit's invitation to participate in a great adventure that drove all the innovative discoveries we described. A few very bright young scientists, along with leaders who were to recruit the next generation, accepted that invitation. Guiding them through travails and triumphs, their pioneering ambition, determination, and courage created fertile new knowledge and an innovative institution.

Their stories inspire hope and warn us against dangers. Some of us may emulate them directly. Others, I hope, will help their successors to do so.

# Notes and References

## Introduction

[1] As chairman of UCSF's Department of Medicine, Holly Smith took many potential faculty members to see the view of San Francisco from the roof of Moffitt Hospital (Smith, LH Jr, Interview). I do not know how many UCSF faculty used this strategy to attract new recruits, but in the 1970s I tried it on potential recruits for postdoctoral training in my laboratory. Sometimes it worked.

[2] The UCSF faculty member, Holly Smith, remembers this conversation with Herbert Evans during a walk down Parnassus Avenue in the 1960s (Smith, LH Jr, Interview.)

[3] I recounted the story of my life and work at UCSF in a memoir (HR Bourne. *Ambition and Delight*. 2009).

## Chapter 1. Nowhere to Go But Up!

[1] RJ Havel, Oral History, 1995.

[2] Joseph LaDou, "The Sponge Case." A History of UCSF. UCSF Library and UCSF Department of Anthropology, History, and Social Medicine. Web 7 May 2009.

[3] "1899-1918. Early Academic Programs and Teaching Hospitals. The Impact of Full-time Pre-Clinical Faculty." In A History of UCSF. UCSF Library and UCSF Department of Anthropology, History, and Social Medicine. Web. 7 May 2009.

[4] "People. Herbert McLean Evans (1882-1971)." In A History of UCSF. UCSF Library and UCSF Department of Anthropology, History, and Social Medicine. Web. 7 May 2009.

[5] IL Chaikoff focused on metabolic responses to hormones, use of radioactive tracers to study intermediary metabolism, and the synthesis of cholesterol and adrenal and thyroid hormones. (See SF Cook, GS Gordan, and HB Jones, "Israel Lyon Chaikoff, Physiology: Berkeley." In Memoriam. Calisphere, University of California. Web. 7 May 2009.) Choh Hao Li, a Chinese-born biochemist, determined the first amino acid sequences of peptide hormones, including the pituitary hormone that stimulates production of cortisol. Later, in 1967, Li would move to UCSF to head the Hormone Research Laboratory. (See LL Bennett, D Chung, H Papkoff, and EL Way, "Choh

Hao Li, Biochemistry: San Francisco." In Memoriam. Calisphere, University of California. Web. 7 May 2009.)

[6] Two distinguished scientists in this period served as directors of a research institute on the Parnassus campus, the Hooper Foundation. Appointed in 1914, the Hooper's first director, George H. Whipple, would later receive a Nobel Prize for discovering liver extract as therapy for pernicious anemia. Dean of the UC Medical School from 1920 to 1921, Whipple left to organize a new medical school in Rochester, New York, after recruiting the next director of the Hooper, Karl F Meyer, from Berkeley. Meyer discovered the virus that causes western equine encephalitis and the bacterial cause of parrot fever. (See "George Whipple." Wikipedia. Web. 7 May 2009; JB deCM M Saunders and EB Shaw, "Karl Friedrich Meyer, Pathology; Microbiology; San Francisco." In Memoriam. Calisphere, University of California. Web. 7 May 2009.)

[7] This was the German-born Jacques Loeb, whose acerbic remark is quoted in reference 3, above. I have not been able to recover the first citation of this quote.

[8] In the 1920s, the Rockefeller Foundation offered to defray the dollar costs of reconsolidation, but only if clinical teaching were transferred to Berkeley. In response, the San Francisco clinicians successfully persuaded the University Regents not to accept the offer, in effect saying that if anyone were to move, it should be the basic science faculty.

[9] C Kerr, Memoir, Volume 1, 2003. The best account of Kerr's presidency of UC is his own superb two-volume memoir/history. The first and larger volume, cited here, deals with what Kerr considered the good things he accomplished at UC. The second volume describes his handling of the Free Speech Movement in 1964-65 and the subsequent repercussions, including his firing by Ronald Reagan.

[10] C Kerr, *The Uses of the University*, 1963.

[11] One rare book story is redolent of a medical and academic world very different from UCSF now. In the summer of 1963, Robert Schindler (a recent chair of the Department of Otolarygology at UCSF) was about to enter UCSF as a medical student. Awarded a medical history fellowship, he worked in the UCSF library under the tutelage of Professor Chauncey Leake, a bibliophile, pharmacologist. and medical administrator. On a trip to Italy, Herbert Evans, the chair of anatomy in Berkeley and a fellow bibliophile, bought for the library a priceless old book, René Laennec's treatise on his invention of the stethoscope, *De l'Auscultation Mediate* ("On Indirect Listening"). The book's margins showed scribbled comments in Laennec's own handwriting and blotches presumably made by Laennec's pen. Shortly thereafter, John Saunders purchased another copy of the book (also in Italy) and donated it to the library also. Leake showed Schindler copies of the book, in which Laennec's purported notes and blotches were identical, indicating that both, sadly, were fakes. "Saunders

too has a very discerning eye for medical rarities," Leake said, adding, "Just goes to show you, Bob, not everything is as it seems." Emails to the author from Robert Schindler, April 22, 2009 and March 31, 2010.

[12] From Reports on NIH Research Grants for the year(s) mentioned.

[13] I Edelman, Oral History, 1996.

[14] JH Comroe, *CVRI: The Early Years*. In: RJ Havel, editor. Cardiovascular Research Institute, 1983.

[15] A decade earlier, in 1946, Comroe had been an assistant professor of Pharmacology at the University of Pennsylvania for 10 years, despite his growing reputation as a teacher and investigator. Against his chair's advice, he took the chair of Pharmacology in an affiliated school, the Graduate School of Medicine (GSM) of the same university, also in Philadelphia. Comroe hoped to expand his new department, along with the school, in response to the need for further education of young physicians returning from the war. To this end, he worked hard, and successfully, but by 1956 it was becoming clear that the University of Pennsylvania was ready to close down the GSM, in part because applications from World War II veterans had dwindled. A few years earlier, Comroe had been passed over for the vacated chair of Physiology back in the University's mainline medical school. Overall, as a biographical memoir by his colleagues puts it, Comroe "was seeing new opportunities." (See SS Kety, RE Forster. *Julius H. Comroe, Jr 1911-1984, A Biographic Memoir*: National Academy Press, 2001.)

[16] LH Smith Jr, Interview, 2009.

[17] This, along with many other quotes from Smith in this chapter, unless otherwise noted, are from LH Smith Jr, Oral History, 1994 and 1995.

[18] Email from LH Smith Jr to the author, April 27, 2009.

[19] The multiple reasons listed in this paragraph come from references sixteen and seventeen, above. The one actual quote is from reference seventeen.

## Chapter 2. Deciding a Future

[1] LH Smith Jr, Oral History, 1994 and 1995.

[2] Smith, LH Jr, Interview, 2009.

[3] I. Edelman, Oral History, 1996.

[4] Clark Kerr, Oral History, 1994.

[5] JH Comroe, *CVRI: The Early Years*. In: RJ Havel, editor. Cardiovascular Research Institute, 1983.

[6] From official reports of NIH Research Grants for the year(s) mentioned.

[7] F Larragueta, Interview, 2009.

[8] The story of Gil Gordan, Leon Goldman, and Saunders's subsequent "blacklisting" of faculty opposed to him is described in Clark Kerr and Morton Meyer, Oral History, 1995. The same story is also detailed by Comroe in a later note he dictated for the "Saunders notebook," described in reference 10, below.

[9] Clark Kerr and Morton Meyer, Oral History, 1995.

[10] JH Comroe Jr, "Saunders" notebook. This notebook has been preserved, along with Comroe papers on other subjects, by Richard J. Havel. Although most of Comroe's notes, and some letters in the file, are not dated, I have been able in most cases to infer their timing from their content.

[11] In order of their appointments, the new chairs included Alexander Margulis in 1963 (from Washington University, St. Louis, in Radiology); Bert Dunphy in 1964 (from Harvard and Oregon, in Surgery); L.H. Smith, Jr. in 1964 (from Harvard, in Medicine); Melvin Grumbach in 1966 (from Columbia, in Pediatrics); and Robert Fishman in 1966 (also from Columbia, in Neurology).

[12] JH Comroe's "Saunders" notebook contains a Xerox copy of this letter to Kerr, along with a note in Comroe's hand naming those who attended the meeting with Wellman, but had not signed the original letter. The signers included Comroe, Smith, Dunphy, Reinhardt, Edelman, and Sokolow, plus Stuart Cullen (anesthesia), Robert H. Featherstone (Pharmacology), A. Simon (Psychiatry), and R. Margulis (Radiology). Nonsigners who attended the meeting were Ernest Page (Obstetrics and Gynecology) and Ernest Jawetz (Microbiology).

[13] The BRDPI is a price index that indicates how much the NIH budget must increase in order to preserve purchasing power for laboratory research. It is calculated by the Office of Science Policy Analysis of the National Institutes of Health. Web 18 August 2010.

[14] C Kerr, Memoir, Volume 2, 2003.

[15] Administrative records, School of Medicine's Dean's Office, AR-92-83, Carton 7, Special Collections, UCSF Library. The AAMC report is quoted in a footnote to Clark Kerr and Morton A. Meyer, Oral History, 1995.

[16] Kerr remembered (see references four and nine above) that the blue-ribbon committee included Lowell T Coggeshall, Dean of the Division of Biological Sciences at the University of Chicago, as well as Robert H Ebert, who was Dean of Harvard Medical School, 1965-77. (Ebert is the man who took the chair of the Department of Medicine at the Massachusetts General Hospital—a job for which Holly Smith was passed over.)

[17] San Francisco Examiner, January 19, 1965.

[18] David Perlman, San Francisco Chronicle, February 10, 1965.

[19] C Kerr, Memoir, Volume 1, 2003.

[20] KM Ludmerer, Time to Heal, 1999.

## Chapter 3. New Brooms

[1] The account of this meeting at Jack's Restaurant is based on an interview with Leon Levintow in 2009, and an e-mail message from him on May 25, 2009.

[2] LH Smith Jr, Interview, 2009.

[3] A few years after coming to UCSF, a rhinoplasty gave Rutter a more ordinary-looking nose.

[4] WJ Rutter, Oral History, 1992.

[5] WJ Rutter, Interview, 2009.

[6] EE Penhoet, Interview, 2009.

[7] WJ Rutter papers, UCSF Library.

[8] I Edelman, Oral History, 1996.

[9] For a fuller descriptions of Tomkins's career and personality, see: Ames, Gordon M Tomkins (1926-1975). In: Litwak G, editor. *Biochemical Actions of Hormones*. New York: Academic Press. pp. xvii-xxxvi. 1977; or HR Bourne, *Ambition and Delight*, 2009.

[10] WJ Rutter, letter to SC Cullen, November 1, 1967, Rutter papers.

[11] LH Smith Jr, letter to WJ Rutter, November 3, 1967, Rutter papers.

[12] LH Smith Jr, letter to WJ Rutter, November 20, 1967, Rutter papers.

[13] James A Spudich, Interview, 2009.

[14] Comments in this sentence are from: JA Spudich, Interview; 2009; R Cooke, Interview, 2009; C Guthrie, Interview, 2009; and R Kelly, Interview, 2009.

[15] R Kelly, Interview, 2009.

[16] C Guthrie, Interview, 2009.

[17] KR Yamamoto, Interview, 2009.

[18] In part, the new willingness of medical schools to accept Jews as students and faculty members may have reflected changes in social attitudes following the Holocaust in World War II. More practically, as KM Ludmerer indicates in *Time to Heal* (1999), increasing federal funds for research and patient care meant that medical schools competing to attract the brightest students and researchers could ill afford to continue ignoring bright, dedicated young people like the Jews they had regularly rejected in the 1940s.

[19] JR Krevans, Interview, 2009.

[20] From official reports of NIH Research Grants for the year(s) mentioned.

[21] JR Krevans, Oral History, 2005.

[22] KM Ludmerer, *Time to Heal*. 1999.

[23] This calculation is based on the Biomedical Research and Development Price Index, calculated by the Office of Science Policy Analysis of the National Institutes of Health. Web 26 June 2009.

[24] CB Wilson, Interview, 2009.

[25] The rate of rise of UCSF's research grants was less during Krevans's tenure than in the previous eleven years, in which NIH grants rose at a rate never seen since (see reference 20, above). If we correct dollar values for inflation of the costs of doing research, using the BRDPI (see reference 23), UCSF's research grants increased from $7.2 million in 1971, when Krevans became Dean, to $11.9 million in 1982, when he moved to the Chancellor's office—an increase, in constant dollars, of 65% in eleven years. Relatively speaking, this was much slower than during the previous 11-year period (1960-70), when UCSF's grants rose by 247%, from $1.9 to $6.6 million per year (also in constant 1950 dollars). The difference between the two periods largely reflects the fact that total NIH dollars devoted to research grants also increased more slowly in the later period than in the earlier one—that is, by 41% in 1971-82, vs. 143% in 1960-70, in constant 1950 dollars. As compared to total annual NIH expenditures for research grants, UCSF's research grants increased by larger percentages in both periods.

[26] In 2008, UCSF received $448 million in research grants from the NIH (see reference 20, above). In constant 1950 dollars (corrected by the BRDPI, cited in reference 22, above), this would be $34 million, or 2.22% of the total of $1.525 million awarded in NIH grants during 2008. In 1982, UCSF's research grants amount to 2.1% of the NIH dollars paid in research grants. Earlier, however, in 1960, UCSF's research awards were 1.2% of the total NIH grants awarded for research.

## Chapter 4. Bizarre Little Binary Point

[1] J Gitschier, Wonderful Life: an interview with Herb Boyer, *PLoS Genet* 5(9): e1000653. doi:10.1371/journal.pgen.1000653, September, 2009.

[2] Boyer says that the subsequently discovered EcoRI activity, found in a freshwater bacterium rather than in a clinical infection, showed biochemical properties significantly different from those his graduate student found. See reference 1, above, and HW Boyer, Interview, 2009.

[3] Such interviews include the interview in reference 1, above, plus: HW Boyer, MIT Oral History, 1975; HW Boyer, Interview, 2009; and HW Boyer, UC Oral History, 1994.

[4] HW Boyer, Interview, 2009.

[5] HW Boyer, MIT Oral History, 1975.

[6] The idea seemed straightforward at the time, but wasn't. First Boyer would use a battery of different mutation-causing chemicals, called mutagens, in order to produce more frequent mutations in the arabinose operon, and in ways that differed from mutagen to mutagen. He would use genetic tricks to localize ("map") each chemically induced mutation to a specific gene in the arabinose operon, and eventually to a specific site within each gene. Deciphering the genetic code would be accomplished by using chemical mutagens to repair mutations made by other mutagens. Unfortunately, how this would happen was by no means clear.

[7] Chapter Six will describe how amino acid sequences of newly made protein chains are specified by the base sequences of "messenger RNA" polymers, which are copied (by base complementation, just as DNA is replicated) from DNA sequences of chromosomal genes and transported out of the cell's nucleus. For a lucid and detailed understanding of the genetic code, DNA, and RNA, see HF Judson, *The Eighth Day of Creation*, 1979.

[8] The series of four papers includes: W Arber, D Dussoix. Host specificity of DNA produced by Escherichia coli. I. Host controlled modification of bacteriophage lambda. *J Mol Biol* 5: 18-36, 1962; D Dussoix, W Arber. Host specificity of DNA produced by Escherichia coli. II. Control over acceptance of DNA from infecting phage lambda. *J Mol Biol* 5: 37-49, 1962; W Arber, S Hattman, D Dussoix. On the Host-Controlled Modification of Bacteriophage Lambda. *Virology* 21: 30-35, 1963; D Dussoix, W Arber. Host Specificity of DNA Produced by Escherichia Coli. Iv. Host Specificity of Infectious DNA from Bacteriophage Lambda. *J Mol Biol* 11: 238-246, 1965.

[9] HW Boyer, UC Oral History, 1994.

[10] Meselson and Yuan found that a restriction enzyme from K-strain E. coli required adenosine triphosphate (ATP) and S-adenosylmethionine to show activity. (See M Meselson, R Yuan. DNA restriction enzyme from E. coli. *Nature* 217: 1110-1114, 1968.) By adding these reagents, Boyer was able to measure restriction enzyme activities in K12 and B/r strains.

[11] Boyer's lab found that the enzyme they were testing cut each lambda DNA molecule at a single site. Then they specifically tagged one end of the cut DNA to identify the specific base that would be present if all the lambda DNA molecules were cut at the very same site. To their surprise, they found all four bases (A, T, G, and C) in equal amounts. Although they thought these results were spurious results of unsuccessful experiments, another lab eventually showed, by electron microscopy of clipped lambda fragments, that the enzyme really did cut each DNA molecule at a different site. This kind of restriction enzyme does bind to a single short sequence of DNA, but then leaves the initial binding site by using ATP to migrate along the DNA chain in order to clip at a randomly selected site elsewhere.

[12] Boyer's lab determined how many genes are required to encode the strain-B restriction and modification enzymes, and mapped these genes to specific sites in the bacterial genome. See HW Boyer, BC Carlton. Production of two proteolytic enzymes by a transformable strain of Bacillus subtilis. *Arch Biochem Biophys* 128: 442-455, 1968; D Roulland-Dussoix, HW Boyer. The Escherichia coli B restriction endonuclease. *Biochim Biophys Acta* 195: 219-229, 1969; HW Boyer, D Roulland-Dussoix. A complementation analysis of the restriction and modification of DNA in Escherichia coli. *J Mol Biol* 41: 459-472, 1969.

[13] HindII clipped in the middle of a six-base sequence, G T (T or C) | (A or G) A C (with the clip site indicated by "|"). The complementary DNA strand, of course, would be C A (A or G) | (T or C) T G. See HO Smith, KW Wilcox. A restriction enzyme from Hemophilus influenzae. I. Purification and general properties. *J Mol Biol* 51: 379-391, 1970; TJ Kelly, Jr, HO Smith. A restriction enzyme from Hemophilus influenzae. II. *J Mol Biol* 51: 393-409, 1970.

[14] RN Yoshimori, A genetic and biochemical analysis of the restriction and modification of DNA by resistance transfer factors, PhD thesis, University of California, Northern Regional Library Facility, Richmond, CA, 1971.

[15] For an especially graceful exposition of the extended analogy between experimental manipulation of DNA and editing manuscripts, see SS Hall, *Invisible Frontiers*, 1987. This marvelous book describes the DNA revolution, including Boyer's experiments with recombinant DNA, as well as the exciting three-way race to express the insulin gene, which involved Genentech, the biotech company Boyer founded, the labs of Bill Rutter and Howard Goodman at UCSF, and the lab of Walter Gilbert at Harvard.

[16] More specifically: In the mid-1970s, Fred Sanger in England and Maxam and Gilbert at Harvard developed useful experimental protocols for reading the sequence of fairly long stretches of DNA rather rapidly, which of course revealed the translated sequence of corresponding proteins. By the 1980s it became possible to make recombinant and precisely re-engineered proteins in bacteria and other organisms, and even to inactivate or insert genes into intact mice. The Human Genome Project and its many progeny have now read and recorded, base by base, the entire gene dictionaries of humans, many species of bacteria, and diverse organisms in between.

[17] P Berg, JE Mertz, Personal Reflections on the Origins and Emergence of Recombinant DNA Technology, *Genetics* 184:9-17, 2010. Two of the three laboratories were in Stanford's Biochemistry Department. Berg and Mertz primarily review Janet Mertz's work in Paul Berg's lab and, to a lesser extent, that of Peter Lobban, in Dale Kaiser's lab. A third lab, not at Stanford, entertained a similar idea (RH Jensen, RJ Wodzinski, MH Rogoff, Enzymatic addition of cohesive ends to T7 DNA. *Biochem Biophys Res Comm* 43:384-392, 1971). In their review, Berg and Mertz fail to mention a fourth

effort, in their own backyard. Sgaramella, in the Department of Genetics at Stanford, showed that DNA cleaved by EcoRI could be linked back together by a DNA ligase that was unable to link a blunt-ended DNA to another blunt ended DNA. He inferred that EcoRI must cleave DNA in a way that leaves overhanging sequence on both ends of the cut. See V Sgaramella. Enzymatic oligomerization of bacteriophage P22 DNA and of linear Simian virus 40 DNA. *Proc Natl Acad Sci USA* 69: 3389-3393, 1972.

[18] The enzyme, terminal deoxynucleotidyl transferase (TdT) will add chains of a single nucleotide to one of the two DNA strands when provided with a single nucleosidyl triphosphate (a base, attached to ribose and and three phosphates). TdT "knows" which strand should be linked to the new building block because the complementary strands in a helical DNA fragment spiral in opposite directions and thus present chemically different groups—termed 3' and 5', to indicate different positions on the ribose portion of each building block—for attaching the phosphate group that will link one building block to the next. For non-specialist readers, these details are beautifully described in HF Judson. *The Eighth Day of Creation: Makers of the Revolution in Biology*. New York: Simon and Schuster, 1979.

[19] This result was published in 1972. See DA Jackson, RH Symons, P Berg. Biochemical method for inserting new genetic information into DNA of Simian Virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli. *Proc Natl Acad Sci USA* 69: 2904-2909, 1972.

[20] JE Mertz, RW Davis. Cleavage of DNA by R 1 restriction endonuclease generates cohesive ends. Proc Natl Acad Sci USA 69: 3370-3374, 1972. Of the two Proceedings papers reporting that EcoRI generates cohesive ends, the one by Mertz and Davis presented more comprehensive evidence. The other paper—by Sgaramella, cited in reference 17, above, and published in the same issue of the Proceedings—showed that one kind of DNA ligase could link together SV40 DNA fragments cut by EcoRI but could not link DNA fragments cut in a way that left "blunt ends" without overhanging sequence. (A different variety of ligase could repair cuts of either kind.) This indicated that EcoRI cleaves DNA to produce sticky ends.

[21] DNA was layered at the top of the gel, and fragments driven down it under the influence of an electrical field. Big DNA fragments would migrate down the gel more slowly than small ones. The idea was fine, but the Boyer lab couldn't get the procedure to work in a reproducible way.

[22] J Hedgpeth, HM Goodman, HW Boyer. DNA nucleotide sequence restricted by the RI endonuclease. *Proc Natl Acad Sci USA* 69: 3448-3452, 1972.

[23] If you are puzzled by the notion of how nature could create two strands running in opposite directions, think about the nucleotides that make up each strand. Each nucleotide is an asymmetrical chemical, with front and back ends that differ from one

another. In the triplet sequence, CAT, the "front" end of nucleotide A attaches to the back end of the next nucleotide in the sequence (T), while A's "back" end attaches to the front end of C. The complementary sequence, which we write GTA, is "really" ATG, with a front end at G and a back end at A. To a properly twisted mind, the picture is crystal clear.

[24] SN Cohen, UC Oral History, 1995.

[25] The song, "Only You," was overshadowed by a different song, of the same name, which Cohen judges better than his. Still, it furnished royalties that helped him pay for part of his education. See Cohen's UC Oral History, cited in reference 24.

[26] SN Cohen, Interview, 2009. Cohen thinks the two may have met briefly before this time. His letter of invitation can be found appended to his UC Oral History, cited in reference 24.

[27] According to Cohen's oral history, cited in reference 24, Cohen was quoting from an interview of Falkow, by Charles Weiner, on May 26, 1976 and February 26, 1977, MIT Oral History Program.

## Chapter 5. The DNA Revolution is Born

[1] HW Boyer, Interview, 2009.

[2] HW Boyer, UC Oral History, 1994.

[3] SN Cohen, UC Oral History, 1995.

[4] SN Cohen, AC Chang, HW Boyer, RB Helling. Construction of biologically functional bacterial plasmids in vitro. *Proc Natl Acad Sci USA* 70: 3240-3244, 1973. Some colonies sensitive to both antibiotics contained two different plasmids, one conferring resistance to each antibiotic. The same result was seen even if the DNA had not been treated with EcoRI and ligated. This is because a single bacterium exposed to the transformation procedure can sometimes take up two plasmids.

[5] J Gitschier, Wonderful Life: an interview with Herb Boyer, *PLoS Genet* 5(9): e1000653. doi:10.1371/journal.pgen.1000653, September, 2009.

[6] AC Chang, SN Cohen. Genome construction between bacterial species in vitro: replication and expression of Staphylococcus plasmid genes in Escherichia coli. *Proc Natl Acad Sci USA* 71: 1030-1034, 1974.

[7] Morrow may have explained to Boyer that the immature eggs of female frogs have "amplified" (increased the amount of) their ribosomal DNA by approximately 1,000-fold, relative to the rest of their DNA. The increase of ribosomal DNA allows the oocyte, once it is fertilized, to make the huge numbers of ribosomes required for synthesizing new protein during the first stages of amphibian development. (Ribosomes are large RNA-protein machines for making new protein molecules, based on sequences

of messenger RNAs.) In addition, ribosomal DNA contains a relatively high proportion of two bases (G and C), a property that makes it float more easily than ordinary frog DNA in solutions of cesium chloride, providing a ready means for separating homogeneous ribosomal DNA. See IB Dawid, DD Brown, RH Reeder. Composition and structure of chromosomal and amplified ribosomal DNA's of Xenopus laevis. *J Mol Biol* 51: 341-360, 1970.

[8] P Berg, UC Oral History, 1997. According to Berg, Morrow "never told me anything about this. He kept telling me that he was delayed in finishing his thesis because of computer problems, or this problem . . . . I had no idea. People in the lab knew, but nobody said anything to me about it. Eventually, the experiment was done, and John came to me and told me about it. I almost kicked him out of the lab, I was so furious. He was using me and lying to me about what was delaying his departure. In fact, surreptitiously, he had gone off-- He had every right to do that, but at least he could have been upfront about it . . . . The[n] our department went off on its retreat. He remained behind, [b]ut because I thought the experiment was so terrific, I called him and invited him to come and give a talk to the department on this experiment. . . . The point is, I did not know about that experiment when it was being conducted. I regard it as one of the critical experiments in the whole evolution of the DNA cloning technology."

[9] More technically, they mixed EcoRI-cleaved DNA fragments from pSC101 DNA with similarly cleaved fragments of frog ribosomal DNA, and used DNA ligase to link together whatever fragments might have stuck to one another in the mixture. They then used the stably linked DNA to transform a population of tetracycline-sensitive E. coli, and selected colonies that grew in the presence of tetracycline. All such colonies would have incorporated pSC101's replication and tetracycline-resistance genes, but it was necessary to screen individual colonies separately to identify those plasmids that also carried inserted fragments of ribosomal DNA. See JF Morrow, SN Cohen, AC Chang, HW Boyer, HM Goodman et al. Replication and transcription of eukaryotic DNA in Escherichia coli. *Proc Natl Acad Sci USA* 71: 1743-1747, 1974.

[10] Falkow's account is quoted in SS Hall, *Invisible Frontiers*, 1987.

[11] These more exacting criteria are described in the paper cited in reference 9. Briefly, the ribosomal DNA-containing recombinant fragments shared with native rribosomal DNA a higher buoyant density, assessed by centrifuging it in solutions of cesium chloride. In addition, radioactively tagged native ribosomal DNA hybridized to putative ribosomal DNA fragments in the appropriate plasmid extracts, but not to DNA of pSC101 or plasmids that did not contain fragments of the size expected for EcoRI fragments of ribosomal DNA.

[12] KR Yamamoto, Interviews, 2008-2009.

[13] Cohen's Oral History describes how McElheny found out about the work and called him for an interview. The Times story was McElheny, V. Animal gene shifted to bacteria: Aid seen to medicine and farm. *New York Times*. May 20, 1974. David Baltimore, then at the Massachusetts Institute of Technology, would share a Nobel prize in 1974.

[14] N Reimers, UC Oral History, 1997.

[15] See JF Gibbons, "The role of Stanford University: A Dean's Reflections," in C-M Moon et al, *The Silicon Valley Edge*. 2000, pp. 200-217.

[16] The patent's claims were unusually broad, but in fact the procedure it described proved broadly applicable to DNA throughout biology. Reimers and his Stanford and UCSF colleagues put a very low price on the patent-based licensing fees for recombinant DNA technology, in order to allow commercial applications but also (I suspect) to reduce the likelihood of suits aimed at negating application of the patent to biotechnology companies, big and small.

[17] P Berg, JE Mertz, Personal Reflections on the Origins and Emergence of Recombinant DNA Technology, *Genetics* 184:9-17, 2010.

[18] M Singer, D Söll. Guidelines for DNA hybrid molecules. *Science* 181: 1114, 1973. For Boyer's description of this meeting and its discussions, see HW Boyer, MIT Oral History, 1975.

[19] Cited in reference 9, above.

[20] This sentence, the tale of its removal from the final version of the paper, and Cohen's conversation with David Baltimore are in SN Cohen, UC Oral History, 1995, which is cited in reference 3, above.

[21] P Berg, UC Oral History, 1997.

[22] Specifically, as stated in his Oral History, cited in reference 3, Cohen felt that recombinant experiments should be allowed if—like the experiments he and Boyer had first reported—the antibiotic resistance gene was not novel to the bacterium used for the experiments.

[23] P Berg, D Baltimore, HW Boyer, SN Cohen, RW Davis et al. Letter: Potential biohazards of recombinant DNA molecules. *Science* 185: 303, 1974. The signers included Berg, Baltimore, Boyer, Cohen, Ronald W. Davis, Richard Roblin, James D. Watson, Sherman Weissman, and Norton D. Zinder. The statement regarding plasmids reads as follows: "Construction of new, autonomously replicating bacterial plasmids that might result in the introduction of genetic determinants for antibiotic resistance or bacterial toxin formation into bacterial strains that do not at present carry such determinants; or construction of new bacterial plasmids containing combinations of resistance to clinically useful antibiotics unless plasmids containing such combinations of antibiotic resistance determinants already exist in nature."

[24] P Berg, D Baltimore, S Brenner, RO Roblin, MF Singer. Summary statement of the Asilomar conference on recombinant DNA molecules. *Proc Natl Acad Sci USA* 72: 1981-1984, 1975.

[25] A borrowed version of Oscar Wilde's delicious apothegm about truth. See *The Importance of Being Earnest,* Act I, 1995.

[26] Smith shared the 1978 Nobel Prize in Medicine or Physiology with Werner Arber (who discovered the restriction-modification system in bacteria) and his Hopkins colleague, Daniel Nathans (who used the enzyme Smith discovered as a selective and precise molecular scissors to cut and characterize pieces of DNA).

[27] My account of the octanucleotide's synthesis, along with quotes from Boyer on the repressor binding site experiments with Art Riggs, come from unpublished portions of Jane Gitschier's extensive interview of Boyer in 2009. A short version of their conversation is published (cited as reference 5, above). Both Gitschier and Boyer generously permitted me to include in this chapter additional information and quotes taken from the full interview. The Roche scientist responsible for making the octanucleotide was Alex Nussbaum.

[28] SS Hall, *Invisible Frontiers*, 1987.

[29] In 1965, Francois Jacob and Jacques Monod, along with Andre Lwoff, received the Nobel Prize for genetic studies of the regulation of ß-galactosidase production in bacteria.

[30] Arthur D Riggs, UC Oral History, 2005.

[31] K Itakura, UC Oral History, 2005. Itakura's account differs slightly from that of Riggs. While Riggs remembers that Itakura's application letter stated that he had actually synthesized the lac operator DNA, Itakura says that at this point in his postdoctoral work (with SA Narang, at the National Research Council of Canada, in Ottowa), in Toronto, he had only adapted the new phosphotriester method for synthesizing DNA and shown that it was 10 times faster than previous methods. In fact, Itakura says, he accomplished the actual synthesis in six or seven months of the following year (1973-74), which he had to spend in Ottowa awaiting resolution of a visa problem, although he had accepted the job offer in California.

[32] Riggs remembered (UC Oral History, cited in reference 30) that he was talking with Gilbert, who had just described blunt-end cloning with T4 DNA ligase in a seminar at City of Hope. "We were sitting around, and . . . the light bulb went on, and I said, 'Hmm, Wally, if we made double-stranded DNA with a restriction enzyme site in it, could we use your enzyme to blunt-end ligate or join it to, for example, the lac operator?' Wally thought about it for a second and he said, 'Yes.'"

[33] Heyneker's strategy for detecting the repressor binding sites depended on the fact that he inserted the plasmids into normal E. coli. Growing on glucose, he knew, normal

E. coli would make no b-gal, because the available repressor protein (10-20 repressor molecules per bacterial cell) was more than enough to assure repressor occupation of its DNA binding site. Introduction and replication of the plasmid, however, would cause the host bacterium to accumulate 30 copies of the plasmid genome, and therefore 30 copies of the repressor binding site. Because this excess was more than enough to soak up all the lac repressor protein in an E. coli cell, the cell should start making plenty of β-gal. Cells that make β-gal can be distinguished from untransformed cells (which do not) by the enzyme's ability to convert a chemical compound, nicknamed X-gal, into a product that turns β-gal-containing colonies blue. Blue colonies, then, would tell him that the experiment worked. For details, see the resulting publication: HL Heyneker, J Shine, HM Goodman, HW Boyer, J Rosenberg et al. Synthetic lac operator DNA is functional in vivo. *Nature* 263: 748-752, 1976.

[34] The quote from Boyer is in SS Hall, *Invisible Frontiers*, 1987.

[35] HL Heyneker, UC Oral History, 2002.

[36] WJ Rutter, Interview, 2009.

[37] KR Yamamaoto, Interview, 2009.

[38] Zach W Hall, Interview, 2009.

[39] See, for instance, YC Kim, JC Grable, R Love, PJ Greene, JM Rosenberg. Refinement of Eco RI endonuclease crystal structure: a revised protein chain tracing. *Science* 249: 1307-1309, 1990.

[40] At a minimum, such a list would include several ideas mentioned in Chapters Four and/or Five, including: (1) Noel Bouck's suggestion that he had been looking at the phenomenon studied by Albers and his colleagues—the remark that acquainted Boyer with restriction enzymes in the first place; (2) the idea that antibiotic resistance plasmids often encoded restriction enzymes; (3) sticky ends, which he knew about from many sources, including Gordon Tomkins; (4) the specific discovery that EcoRI cleaves to make sticky ends, which Berg told him about, and which accelerated his drive to sequence the EcoRI site and recombine DNA fragments; (5) the entire collaboration with Cohen; the idea to clone frog ribosomal DNA, offered by a graduate student, John Morrow; (6) magically useful staining of DNA fragments by ethidium bromide (7); and Art Riggs's proposal to use synthetic DNA to bind the lambda repressor, which led to development of the first DNA linker sequence. Chapter Six will describe three more: (1) the collaboration with Robert Swanson, a brilliant entrepreneur, to found Genentech; (2) the notion of cloning the insulin gene from a man-made synthetic DNA sequence; (3) Riggs's suggestion that somatostatin should serve as a test-case for using synthetic DNA to clone a gene.

[41] SN Cohen, Interview, 2010.

[42] The quote is from the interview with Cohen cited in reference 41, above. Besides the

elitism of Berg and his colleagues in Biochemistry at Stanford, Cohen also pointed out the special impetus that came from learning in Hawaii, all at once, that Boyer's EcoRI recognized six bases and left cohesive ends, and his sudden insight that the enzyme would cut his plasmid's genome at a few well-defined sites. In addition, I would add that young scientists often hesitate, with reason, to collaborate with established seniors because senior authors tend to get most of the credit for the results of such collaborations.

## Chapter 6. A Transforming Harvest

[1] S Hughes. Making Dollars Out of DNA: The first major patent in biotechnology and the commercialization of molecular biology, 1974-1980. *Isis* 92: 541-575, 2001. Hughes writes that these ideas were recorded in notes of conversations with Boyer in 1974, taken by an assistant to Neils Reimers, head of Stanford's Office of Technology Licensing (OTL), as part of OTL's investigation of the feasibility of writing the patent application they filed in November 1974. The assistant met separately with Stanley Cohen, who would almost certainly have suggested similar applications, but Hughes found no notes of this meeting in OTL records.

[2] From a memo by a different assistant to Reimers, who recorded a conversation with Boyer in August, 1975. The memo, found in the OTL archives, is quoted by Hughes in the article cited in reference 1, above.

[3] In March 1976, well after the August 1975 note recording his interest in using synthetic DNA to make hormones, the Boyer lab began its collaboration, described in Chapter Five, with Itakura and Riggs, in which their synthetic lac operator DNA was recombined with his synthetic EcoRI cleavage site. Boyer may have gotten the germ of the idea for using synthetic DNA from Riggs earlier, although Riggs was not able to pinpoint precisely when he and Boyer first talked about recombining their synthetic DNA sequences. (See Riggs's Oral History, taken in 2005, which is cited in reference 10, below.)

[4] Robert A. Swanson, UC Oral History, 1997.

[5] HW Boyer, UC Oral History, 1994.

[6] P Berg, UC Oral History, 1997.

[7] See references 4 and 5, above.

[8] HW Boyer, Interview, 2009.

[9] The advantage stemmed partly from a moratorium on recombinant DNA experiments in Cambridge, imposed by the Cambridge City Council, and partly from extra-stringent cautionary measures required for experiments with human cDNAs, measures that required the Harvard-based cloning team headed by Wallace Gilbert to cross the

Atlantic and perform their experiments in a special high-safety facility in England. (See SS Hall, *Invisible Frontiers*). Thus the advantage for Genentech accrued despite the company's decision to follow guidelines laid down by the NIH's committee, RAC. Ironically, RAC guidelines did not apply explicitly to synthetic DNA, but Genentech abided by them anyway.

[10] Somatostatin was best known for its ability to turn off signals triggered by growth hormone. Excessive responses to growth hormone are found only in one very rare disease, pituitary giantism (also called acromegaly)—a tiny market, indeed.

[11] Arthur D Riggs, UC Oral History, 2005.

[12] The first experiment failed because it used a recombinant DNA encoding a short sequence of only 10 irrelevant amino acids upstream of somatostatin's fourteen amino acids. The resulting protein was made, but was so short that it was degraded immediately by enzymes in the bacteria. To avoid this problem, the next DNA construct appended the somatostatin DNA downstream of almost the entire sequence of a large bacterial protein, β-galactosidase. The large protein, including its tiny somatostatin tail, successfully resisted degradation by host bacteria, so that the experimenters could isolate it and cleave somatostatin from it. For further details, see the oral histories of Boyer and Riggs, cited in references 5 and 11, respectively, as well as the published paper (K Itakura, T Hirose, R Crea, AD Riggs et al. Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin. *Science* 198:1056-1083, 1977).

[13] The Nobelist was George Wald. Crimson Staff and Anthony Y Strike, Council extends DNA experiment ban; Wald, Meselson debate gene research. *Harvard Crimson,* September 30, 1976. Web November 13, 2009.

[14] KR Yamamoto, Interview, 2009.

[15] HW Boyer, Interview, 2009.

[16] The official preferred that this comment not be directly attributed.

[17] Quoted from the 1954 yearbook of Derry Borough High School, in: James D. Watson (with Andrew Berry), *DNA: The Secret of Life*. Random House, New York, 2003.

[18] HL Heyneker, UC Oral History, 2002.

[19] P Berg, J Mertz, Personal Reflections on the Origins and Emergence of Recombinant DNA Technology. *Genetics* 184:9-17, 2010.

[20] The amino acid sequences of cow and pork insulin are similar but not identical to that of human insulin, but both products can induce allergic reactions in very small numbers of patients. Despite the low incidence of problems with animal insulins, the academics and Genentech imagined that synthesizing insulin in bacteria would be cheaper than getting the hormone from animals grown on farms, so that human insulin would take over the market. Although it turned out that the bacterial route was

not cheaper, human insulin ultimately captured a substantial fraction of the insulin market, nonetheless.

[21] Incident and quote from SS Hall's book, *Invisible Frontiers*, cited in reference 26, below.

[22] Axel Ullrich, UC Oral History, 1994 and 2003.

[23] WJ Rutter, Interview, 2009.

[24] JF Morrow, SN Cohen, AC Chang, HW Boyer, HM Goodman et al. Replication and transcription of eukaryotic DNA in Escherichia coli. *Proc Natl Acad Sci USA* 71: 1743-1747, 1974.

[25] The nucleotides of RNA (ribonucleic acid) complement DNA because their bases are for practical purposes identical to those of DNA, but RNA's nucleotides contain a sugar (ribose) that contains an extra oxygen atom not present in DNA (deoxyribonucleic acid). Three of the bases in RNA (A, C, and G) are identical to the corresponding bases of DNA. In RNA, however, thymine (T) is replaced by uridine (U). Note, by the way, that mRNA is only one of several kinds of RNA, which perform other tasks in the cell.

[26] The two chains are encoded as a larger protein, pre-proinsulin, in which chains A and B (with twenty-one and thirty amino acids, respectively) are connected by a short linker sequence. Pancreatic islet cells make pre-proinsulin, trim it down to separate A and B chains, and fold the two chains together, connected to one another by multiple disulfide bonds.

[27] Incident and quote from SS Hall, *Invisible Frontiers*, cited in reference 8, below.

[28] SS Hall, *Invisible Frontiers*, 1987.

[29] Following the usual nomenclature rules, pBR322 and pMB9 are designated by numbers, preceded by letters based on the names of their creators. ("BR" corresponds to Bolivar and Rodriguez, "MB" to Mary Betlach; "p" stands for plasmid.) Both plasmids contained genes for resistance to ampicillin, and both were engineered to accumulate in large numbers in transformed bacteria. In addition, pBR322 contains a tetracycline-resistance gene that includes an EcoRI site. Insertion of any EcoRI-linked DNA into that site interrupts the gene and abolishes tetracycline resistance. Thus the principal advantage of pBR322 over pMB9 is that the former plasmid allows cloners easily to detect and discard tetracycline-sensitive colonies, which contain plasmids without DNA inserts. More specifically, colonies that grow in the presence of ampicillin are likely to contain a plasmid, and the plasmids of ampicillin-resistant colonies that are also sensitive to tetracycline are likely to contain DNA inserted into the pBR322's EcoRI site. In his ill-fated pBR322 experiment, Ullrich found five plasmid-containing colonies that met these criteria, and Shine determined that two of the five contained insulin cDNA inserts. (For an account of the whole story, see Hall's book,

*Invisible Frontiers*, cited in reference 28, above.)

[30] A Ullrich, J Shine, J Chirgwin, R Pictet, E Tischer et al. Rat insulin genes: construction of plasmids containing the coding sequences. *Science* 196: 1313-1319, 1977.

[31] N Wade. Recombinant DNA: NIH rules broken in insulin gene project. *Science* 197: 1342-1345, 1977.

[32] Senator Stevenson chaired the Senate Subcommittee on Science, Technology, and Space. Notes in Boyer's UC Oral History (cited in reference 9, above) provide more details about the hearing, and SS Hall's book, cited in reference 28, presents a lively account of Rutter's and Boyer's testimony.

[33] In the article cited in reference 1, above, Sally Hughes describes other steps in the gradual acceptance of recombinant DNA as a legitimate way to make products for medical or agriculatural use. In addition to the growing realization that the new technology was actually safe, the changes included Genentech's successes with somatostatin, insulin, and growth hormone, as well as a 1980 Supreme Court Ruling (in Chakrabarty) that an organism can be patented, plus approval, soon thereafter, of patents for the Boyer-Cohen recombinant DNA technology. In effect, it became clear that recombinant DNA furnished opportunities for companies and even universities to make money. The prospect of profit trumped inchoate fears, as it usually does.

[34] L Villa-Komaroff, A Efstratiadis, S Broome, P Lomedico, R Tizard et al. A bacterial clone synthesizing proinsulin. *Proc Natl Acad Sci USA* 75: 3727-3731, 1978. This paper presented evidence that at least one plasmid produced actual insulin protein (preproinsulin, actually), as a "fusion protein" with part of the penicillinase marker in pBR322. That is, the preproinsulin cDNA was inserted at a site in the penicillinase gene and bacteria carrying that plasmid make the "front part" of penicillinase linked (or fused, in molecular biology jargon) to preproinsulin. This protein would not have become a commercially viable product, even if the insulin portion had been human, rather than rat.

[35] Even today, contaminating mRNAs still occasionally disrupt carefully planned experiments. On the one hand, mRNAs are exquisitely sensitive to degradation by cellular enzymes, but on the other hand labs often make a lot of it, and also make large quantities of the corresponding cDNA. A smidgen of the contaminating "wrong" mRNA may be reverse-transcribed to produce a wrong cDNA, or cDNA isolated in a previous experiment can contaminate a tube or a glass pipet and stealthily slip itself into the cloning site intended for a cDNA encoding a different protein. No one knows precisely what happened to produce the unhappy result in Porton, England.

[36] Rutter says he tried hard, but had trouble getting Goodman promoted, and that Goodman was consequently "super mad at me." Goodman, he says, "taught more people the details of . . . molecular biology, DNA based technology, than anybody in

the department," but getting him promoted was difficult because referees (i.e., writers of letters evaluating suitability for promotion) from outside the Department saw many papers Goodman co-authored with others, including different senior researchers. "We knew how valuable his contributions were, but sometimes people outside did not." (Quotes are from the Rutter interview cited in reference 23, above.) One of Rutter's Biochemistry faculty, Christine Guthrie, says (Interview, 2009) that Rutter "tried adamantly to keep me from getting tenure," and would have succeeded if other faculty, including Bruce Alberts, had not come to her aid.

[37] George B Rathmann, UC Oral History, 2003.

[38] Most of my information about Rutter's departure from the Biochemistry chair comes from interviews with Keith Yamamoto in 2009. Other faculty confirmed the fact that Biochemistry faculty opposed the notion of their department chair starting a company.

[39] See PH Seeburg, J Shine, JA Martial, RD Ivarie, JA Morris et al. Synthesis of growth hormone by bacteria. *Nature* 276: 795-798, 1978; DV Goeddel, HL Heyneker, T Hozumi, R Arentzen, K Itakura et al. Direct expression in Escherichia coli of a DNA sequence coding for human growth hormone. *Nature* 281: 544-548, 1979.

[40] The litigation led to Seeburg's sensational account of a New Year's Eve foray, along with Axel Ullrich, his fellow employee at Genentech, to reclaim DNA encoding human growth hormone from a freezer in Goodman's lab at UCSF. The suit and its legal ramifications are well described in: M Rimmer, Genentech and the Stolen Gene: Patent Law and Pioneer Inventions, Bio-Science Law Review, Pharmalicensing.com. Web December 7, 2009. http://pharmalicensing.com/public/articles/view/1070133214_3fc8efde16011/genentech-and-the-stolen-gene-patent-law-and-pioneer-inventions

[41] Berg received 50% of the award, "for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA." Gilbert and Sanger, a British scientist, shared the remaining 50%, "for their contributions concerning the determination of nucleic acids."

[42] J Gitschier, Wonderful Life: an interview with Herb Boyer, *PLoS Genet* 5(9): e1000653. doi:10.1371/journal.pgen.1000653, September, 2009.

## Chapter 7. Lone Wolf and Literary Maeven

[1] JM Bishop, *How to Win the Nobel Prize*, 2003.

[2] JM Bishop, Interview, 2010.

[3] Four of these became leading experimental biologists, including Howard Berg (chemotaxis—that is, bacteria swim toward food sources), John Menninger (biochemis-

try of protein synthesis by ribosomes), John Dowling (biochemistry of vision), and Lubert Stryer (biochemistry of vision). The first three dropped out of medical school to become PhD students, leaving Stryer, like Bishop, to complete medical school.

[4] More specifically, a different tRNA molecule corresponding to each amino acid conducts it to the correct place in the growing protein chain by finding the corresponding word (a specific triplet of bases, corresponding to the amino acid) in mRNA. The tRNA accomplishes this feat by using a unique triplet of bases in its own sequence to recognize and bind transiently but specifically to the complementary base triplet in the mRNA, which is positioned by the ribosome at just the right place and time to ensure incorporation of the tRNA's cargo, the correct amino acid. tRNA, called s-RNA (for "soluble RNA") by others, was discovered by Hoagland and his colleagues in 1958. (See MB Hoagland, ML Stephenson, JF Scott, LI Hecht, PC Zamecnik. A soluble ribonucleic acid intermediate in protein synthesis. *J Biol Chem* 231: 241-257, 1958.)

[5] The amino acid was $^{35}$S-methionine. Unfortunately, after Bishop had made the amino acid he lost two thirds of the product by dropping it on the floor. "I was so upset that I went over to the sink and broke five of the ten 500 milliliter graduated cylinders Elmer owned," he says. (Quote from interview cited in reference 2, above.)

[6] At the time this failure was mysterious (Bishop Interview, reference 2 above), because neither Bishop nor anybody else had imagined that the RNA genome of an RNA virus might not be the coding strand. Later he and others showed that the RNA genomes of certain viruses do encode proteins, while others (like Sindbis) are copied into a base-complementing coding RNA strand, and still others—like Rous sarcoma virus, the retrovirus on which Bishop and Varmus later focused their initial effort—are reverse-transcribed into complementary DNA, which in turn serves as a template for cellular mechanisms to transcribe into new viral RNA genomes.

[7] The "yellow beret" epithet may have begun as a pejorative term, emphasizing the lack of bravery of researchers in contrast to the courage of "green berets" in military Special Forces. From the Korean War early in the 1950s until the end of the Vietnam War in the 1970s, male physicians who were not disabled were required to serve in the armed forces for two years following their clinical training. At different times, Bishop and Varmus (like the author of this book) were accepted into the research associate program at the NIH, which was an extension of the program in which Richard Havel participated in the 1950s (see Chapter One). The program gave research training to several hundred outstanding young MDs during the Vietnam War. Many of these men went on to become leaders in academic medicine throughout the US, and now refer proudly to their yellow berets.

[8] Briefly, the three species of RNA included: (a) single-stranded genomic RNA of the vi-

rus itself, called the "positive" strand because another lab had shown it to be the coding strand, translated into viral protein on ribosomes. (Note that the genomic RNA of Sindbis is the "negative" or non-coding strand—a possibility Bishop didn't imagine when he worked in the Pfefferkorn lab.) (b) The second was a double-stranded RNA helix (analogous to the DNA double helix), formed by base complementation between the positive and negative strands. Strikingly, Bishop and Koch found that this double-stranded RNA is itself infectious. (c) The third was an ill-defined fraction Bishop called (correctly, as it turned out) "replicative intermediate"— the negative RNA strand in the process of being transcribed to form positive strands of genomic RNA for incorporation into new viruses.

[9] H Varmus, *The Art and Politics of Science*, 2009.

[10] HE Varmus, Interview, 2009.

[11] I Pastan, Interview, 2010.

[12] The first collaboration (see Chapter Five) between Art Riggs and Herb Boyer depended on incorporating into the genome of E. coli multiple copies of a short DNA sequence (the lac operator) that binds a protein called the lac repressor. This collaboration (see HL Heyneker, J Shine, HM Goodman, HW Boyer, J Rosenberg et al. Synthetic lac operator DNA is functional in vivo. *Nature* 263: 748-752, 1976) took place eight years after Pastan's telephone call to Varmus, although it is described in a previous chapter of this book.

[13] In fact, Perlman and Pastan used a close chemical relative of cyclic AMP, which was known to enter animal cells (unlike cyclic AMP itself) and to mimic cyclic AMP's characteristic effects. See RL Perlman, I Pastan. Regulation of beta-galactosidase synthesis in *Escherichia coli* by cyclic adenosine 3',5'-monophosphate. *J Biol Chem* 243: 5420-5427, 1968.

[14] Base complementation was first inferred from the DNA double helix of Watson-Crick and later worked out in many labs. In the DNA case, A associates with T, and C with G. In the case of RNA, T associates with a chemical relative of T, called U, for uridine.

[15] The actual measurement was performed as follows. The probe's DNA strands (which of course complement one another nicely) would first be separated (e.g., by heating or chemical treatment) and then tightly attached to a piece of filter paper, in such a way that they would be unable to re-associate with one another but could associate with any mRNA sequence in a bacterial extract that contained a long enough stretch of complementary base pairs. If mRNA in the extract had been obtained from E. coli cells previously incubated with radioactive uridine, the amount of radioactivity bound to the filter paper would reflect the amount of complementary mRNA sequence in the bacteria.

[16] Temin had presented effects of pharmacological inhibitors that block RNA transcription from DNA, plus some molecular hybridization experiments—evidence many others in the field (like Bishop and Varmus) thought weak and inconclusive. See HM Temin. The Effects of Actinomycin D on Growth of Rous Sarcoma Virus in Vitro. *Virology* 20: 577-582, 1963; HM Temin. The Participation of DNA in Rous Sarcoma Virus Production. *Virology* 23: 486-494, 1964; HM Temin. Homology between Rna from Rous Sarcoma Virous and DNA from Rous Sarcoma Virus-Infected Cells. *Proc Natl Acad Sci USA* 52: 323-329, 1964.

[17] Bishop tried to isolate conditional Sindbis virus mutants in the Pfefferkorn lab, almost a decade earlier, but in that case the conditional defect was supposed to prevent viral replication. In the Pfefferkorn lab, the replication procedure itself stopped working. In contrast, the conditional mutation Bishop sought in San Francisco would have left viral replication intact, but would have prevented formation of cancer foci in infected cells.

[18] A viral "clone" is a population of viruses that are all descended from one virus particle. The RSV clone Bishop furnished to Martin "bred true," in the sense that all its progeny appeared to infect and cause cancer, just as their parent did. If Martin had not begun with a cloned RSV, he could not be sure that the virus he called a conditional mutant was not just a variant virus contaminating the virus population he started with.

[19] GS Martin. Rous sarcoma virus: a function required for the maintenance of the transformed state. *Nature* 227: 1021-1023, 1970.

[20] Pure RSV virions were treated with a mild detergent to loosen their protein coats and mixed with nucleoside triphosphates of G, C, A, and T, in which T was radioactive. Bishop's mistake was to follow the advice of a visiting expert that he should add cold (non-radioactive) deoxyTTP (dTTP) to raise the total dTTT concentration. In his RNA-to-RNA transcription experiments with polio virus, he had used straight radioactive UTP, undiluted with cold UTP, in order to get plenty of radioactivity incorporated in the RNA product of the reaction. In this case, the expert said, he should add cold dTTP also, because DNA polymerases needed a higher concentration of substrate.

## Chapter 8. A Rare Partnership

[1] JM Bishop, Interview, 2010.

[2] Charles Carman served briefly as acting Dean of the School during a period in 1970, between the departure of Stuart Cullen to the Department of Anesthesia and the arrival of his successor, Dean Julius R. Krevans.

[3] HE Varmus, Interview, 2009.

[4] HM Temin. The Effects of Actinomycin D on Growth of Rous Sarcoma Virus in Vitro. *Virology* 20: 577-582, 1963; HM Temin. The Participation of DNA in Rous Sarcoma Virus Production. *Virology* 23: 486-494, 1964; HM Temin. Homology between Rna from Rous Sarcoma Virous and DNA from Rous Sarcoma Virus-Infected Cells. *Proc Natl Acad Sci USA* 52: 323-329, 1964.

[5] The filter hybridization procedure is detailed in reference 15 to Chapter Seven.

[6] Modern molecular biologists would apply a process called restriction analysis. Distances between cleavage sites susceptible to specific restriction enzymes differ reproducibly at every location in every genome, making it easy to recognize almost any specific genomic sequence. First, each of several different restriction endonucleases is used to cut genomic DNA into reproducible small pieces. Then the experimenter asks whether a radioactive probe (representing the gene or sequence he seeks) binds to and "lights up" a small number of those pieces, and whether those pieces fall into a unique and well-defined pattern of different sizes. Such a pattern defines the existence of a specific piece of genomic DNA whose base sequence complements that of the radioactive probe.

[7] Briefly, genomic double-stranded DNA is isolated from a cell or tissue sample, sheared into short pieces (about 400 bases long), and heated in solution to a temperature that causes them to separate from one another. The solution is then allowed to cool to a new, lower, temperature, at which the separated complementary strands float about in solution and eventually reanneal with one another, at a rate that depends directly on their concentrations and on the degree of base complementation between their sequences. The reannealing rate is slowed, however, if each separate strand must also search through many different strands of poorly complementing DNA before each finds the "right" sequence. To determine the rate of reannealing, the experimenter stops the process at appropriate times in order to assess how much of the single-stranded DNA he started with has reannealed to form double-stranded DNA.

Before the reannealing process started, Varmus and his colleagues would add to this mixture a tiny amount of the radioactive DNA probe. Because molecules of the single-stranded probe "look for" the few complementary sequences of proviral DNA among the myriad pieces of genomic DNA, they anneal to those proviral sequences at a rate that depends directly on their concentration, modified by the other variables listed above. Thus the rate of reannealing can be used to "count" the number of proviral DNA copies in a tube of genomic DNA prepared from normal or RSV-infected cells. At various times after reannealing has begun, the number of probe molecules (lures) that have snagged a proviral sequence (the right fish) is determined by placing a portion of the reannealing mixture into test tubes with crystals of hydroxylapatite,

a poorly soluble chemical. Double-stranded DNA, including reannealed pieces of the radioactive probe, binds to the hydroxylapatite, while un-annealed single-stranded DNA (radioactive and otherwise) is soluble. Soluble and insoluble fractions are separated in a centrifuge, and their radioactivity counted. Based on the times required for reannealing, compared to times required for standard DNA mixtures to reanneal, the experimenters can reliably detect and count the number of proviral DNA sequences per animal genome. Although effective, the actual procedure is tedious because it requires precisely timed pipetting of reannealed mixtures and subsequent handling (including several pipetting and washing steps, dilution with various other reagents, and centrifugation) of multiple separate tubes and reagents.

[8] L Levintow, Interview, 2009.

[9] Indeed, genomes of animals not susceptible to infection by this virus family should not contain sequences complementary to RSV—and in fact neither human DNA (from a cultured cancer cell line) nor fish DNA (from salmon sperm) contained such sequences. See HE Varmus, RA Weiss, RR Friis, W Levinson, JM Bishop. Detection of avian tumor virus-specific nucleotide sequences in avian cell DNAs. *Proc Natl Acad Sci USA* 69: 20-24, 1972.

[10] HE Varmus, PK Vogt, JM Bishop. Integration of deoxyribonucleic acid specific for Rous sarcoma virus after infection of permissive and nonpermissive hosts. *Proc Natl Acad Sci USA* 70: 3067-3071, 1973.

[11] HE Varmus, RV Guntaka, WJ Fan, S Heasley, JM Bishop. Synthesis of viral DNA in the cytoplasm of duck embryo fibroblasts and in enucleated cells after infection by avian sarcoma virus. *Proc Natl Acad Sci USA* 71: 3874-3878, 1974.

[12] Sarcomas are malignant tumors of connective tissues (bone, muscle, and fibrous tissue). Their tissue origins, appearance under the microscope, and clinical characteristics distinguish them from another common class of malignant tumor, the carcinomas, which arrive from epithelial tissues (e.g., organs like skin, the gut, lung, kidney, breast, etc.).

[13] GS Martin. Rous sarcoma virus: a function required for the maintenance of the transformed state. *Nature* 227: 1021-1023, 1970.

[14] Both Bishop and Varmus have written books that furnish elegant expositions of the state of knowledge (and ignorance) prior to their discoveries. See JM Bishop, *How to Win the Nobel Prize*, 2003; H Varmus, *The Art and Politics of Science*, 2009.

[15] PH Duesberg, PK Vogt. Differences between the ribonucleic acids of transforming and nontransforming avian tumor viruses. *Proc Natl Acad Sci USA* 67: 1673-1680, 1970. The deletion mutants lacked approximately 2,000 of the 9,000 or so nucleotides found in normal RSV.

[16] They first used reverse transcriptase to prepare small fragments of radioactive DNA

complementary to the RNA genome of normal RSV (that is, DNA essentially identical to the lure used in fishing for proviral sequences in cells.) They would then mix these radioactive fragments with a large excess of non-radioactive td mutant RNA, allow the DNA and the td RNA to anneal with each other, and separate the DNA/RNA duplexes from the remaining single-stranded radioactive fragments. Single-stranded radioactive fragments, unable to hybridize with td RNA, should represent the v-src portion of normal RSV and would consequently furnish just the probe they sought. (See D Stehelin, RV Guntaka, HE Varmus, JM Bishop. Purification of DNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. *J Mol Biol* 101: 349-365, 1976.) In his memoir, cited in reference 1 above, Bishop notes that at the time preparation of the probe merited publication in a stringently reviewed journal. Later, making such probes became much easier, so that the probe's preparation would be described as part of the (presumably) more exciting experiment it was designed for.

[17] This conversation probably took place sometime in 1975, before publication of the probe paper but five years after Varmus joined Bishop in San Francisco.

[18] D Stehelin, HE Varmus, JM Bishop, PK Vogt. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 260: 170-173, 1976.

[19] A pioneer of what he called the "molecular clock," Wilson used DNA sequences to detect and quantify evolutionary similarities and differences between different species, within a species, or between any given gene and its precursors and descendants in evolution. For Stehelin's paper, Wilson was kind enough to estimate phylogenetic distances between different bird species, based (at that time) on the fossil record and on distances assessed by antibodies to various proteins. For instance, he estimated that the turkey and emu lineages diverged from that of the chicken 40 and 100 million years ago, respectively.

[20] JM Bishop, *How to Win the Nobel Prize*, 2003.

[21] Stehelin's paper does not mention "fidelity," a later term presumably adopted by more sentimental scientists. Instead, Stehelin incubated the duplexes at different temperatures and assessed the temperature at which half the duplex had melted. (Separation was determined by relative tendency to associate with hydroxylapatite; see reference 7, above.) By this measure, a rather high temperature was required to separate the src probe from proviral sequence—a high-fidelity duplex in which base-pairing was presumably perfect. This temperature was slightly (but reproducibly) higher than the temperature required to separate chicken c-src from the probe, indicating that chicken c-src is not identical to v-src. Duplexes between the probe and c-src DNA of other species melted at progressively lower temperatures, in accord with their esti-

mated relative phylogenetic distances from the chicken.

[22] A Levinson, Interview, 2009.

[23] D Ganem, Interview, 2009.

[24] DH Spector, B Baker, HE Varmus, JM Bishop. Characteristics of cellular RNA related to the transforming gene of avian sarcoma viruses. Cell 13: 381-386, 1978. Detecting mammalian c-src required refining the probe and the hybridization process. The c-src signal had not been detected in Stehelin's experiments, cited in reference 18, above.

[25] The discoveries were nearly simultaneous, but one came a bit earlier (MS Collett, RL Erikson. Protein kinase activity associated with the avian sarcoma virus src gene product. Proc Natl Acad Sci U S A 75: 2021-2024, 1978). Before he heard of the Collett-Erikson report, Arthur Levinson in the Bishop-Varmus lab found the same phenomenon, and published the result later (AD Levinson, H Oppermann, L Levintow, HE Varmus, JM Bishop. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. Cell 15: 561-572, 1978).

[26] DK Sheiness, SH Hughes, HE Varmus, E Stubblefield, JM Bishop. The vertebrate homolog of the putative transforming gene of avian myelocytomatosis virus: characteristics of the DNA locus and its RNA transcript. Virology 105: 415-424, 1980.

[27] This friend preferred not to be quoted by name. The story was confirmed by others.

[28] Others include Bishop's perceptive conjecture that reverse transcriptase might be part of the RSV virion (as in fact it proved to be, although his first experiments missed it) and his suggestion for using hydroxylapatite to separate double- from single-stranded nucleotide sequences in searching for the provirus and for genomic c-src sequences.

[29] After hearing Bishop lecture to medical students, Jawetz told him, "I can't believe what you did there." Jawetz had the reputation of being a first-rate teacher. (Quote from interviews cited in reference 1, above.)

[30] This suggestion came from Stanford's Arthur Kornberg. Tom Kornberg, his son and a faculty member at UCSF, quoted his father to this effect.

[31] H Varmus, The Art and Politics of Science, 2009.

## Chapter 9. Barbarian at the Gate

[1] SB Prusiner, Autobiography, Nobel Prize in Physiology or Medicine 1997. Web 9 June 2010.

[2] SB Prusiner. Novel proteinaceous infectious particles cause scrapie. *Science* 216: 136-144, 1982.

[3] On one occasion in the 1980s, I remember, Prusiner graciously complimented me about a talk I had presented at a national meeting, where he was also a speaker. Our next encounter, sometime in the late 1980s or early 1990s, involved the Cell Biol-

ogy Graduate Program at UCSF, of which I was an organizer. Prusiner expressed a desire to be accepted as a faculty member of the program, but made it clear that he did not intend to take graduate students into his lab or teach in cell biology courses. I responded that his research was certainly of the quality we sought in accepting faculty, but that we were also serious about wanting faculty who cared about teaching graduate students. The third encounter, in 2003 or thereabouts, involved the Institute for Neurodegenerative Diseases at UCSF, which Prusiner founded. As chair of an academic review committee, I criticized aspects of the Institute and suggested several changes, which he did not like.

[4] In February or March 2009, I first contacted Prusiner's secretary to ask for an interview. On March 31, he replied that I could talk to him on May 7, 2009. In that brief encounter he told me he was writing a scientific memoir, and would be available for an interview in early 2010. In two brief conversations since May 2009, he said he was still thinking about it. The turndown came in an email message, dated April 5, 2010 (written in response to yet another email request from me). His response follows:

> Dear Henry,
>
> Thanks for your note.
>
> I have given a lot of thought to your request and also spoken with several people at UCSF whom you have interviewed regarding my laboratory's activities. I feel that the information you have now should be sufficient, and I don't believe I would be able to offer you anything more substantive.
>
> Maybe more to the point, I have resisted doing interviews of the sort you want for 25 years and not regretted my decision once. I am not sure that I want to change my approach at this time.
>
> I hope you can understand my feelings in this matter. Best of luck with your book. I look forward to reading it when it becomes available.
>
> With very best wishes,
>
> Stan

[5] After discussing with Prusiner my invitation to interview them, two individuals—both of whom value him as a friend—decided not to talk with me. A third individual, whose opinions I suspect to be less positive, was advised by friends to communicate with me very circumspectly, so as to avoid possible reprisals from Prusiner. It is impossible to know for sure whether (or in what specific regards) other people I interviewed harbored similarly unspoken reservations. Triangulation is a catchy term, but the process is not as straightforwardly geometrical as it sounds.

[6] W Mobley, interview, 2010. Mobley is now chair of the Department of Neurology at the University of California, San Diego School of Medicine.

[7] Most biographical information (and some quotes of Prusiner's writing, except where

otherwise indicated) come from either or both of two very similar accounts: (a) the Nobel autobiography cited in reference 1, above. (b) Stanley B Pruissner [sic]—Father of Prions, Monday, June 11, 2007, *Biogene*. Web 3 June 2010.

[8] RC Morris, interview, 2010.

[9] M Schambelan, interview, 2010. Schambelan is now Emeritus Professor of Medicine at UCSF.

[10] LH Smith Jr, Interview, 2009.

[11] The Stadtman Way: A Tale of Two Biochemists at NIH. Office of NIH History/ Web 11 June 2010. As described in Chapter Three, UCSF had unsuccessfully courted Stadtman to chair its Department of Biochemistry a few years earlier.

[12] I spent two years (1965-66 and 1968-69) as a resident in internal medicine at Columbia, and had ample opportunities to observe clinical teachers in both medicine and neurology. At that time the teaching tradition was extremely elitist and hierarchical, often characterized by acerbic criticism and intolerance of impolite or unpolitic speech, opinions, and behavior. Like many others, I welcomed the transition from an uptight east-coast school like Columbia to the freer, more democratic style that prevailed in a west coast institution (UCSF).

[13] H Fields, interview, 2010.

[14] The early history of research on TSEs is nicely recounted in several chapters of the two-volume "green book" (SB Prusiner, and WJ Hadlow, eds. *Slow Transmissible Diseases of the Nervous System*, New York, Academic Press, 1979), which was the result of a symposium in honor of William J Hadlow, a leader of Rocky Mountain Laboratories. Here I am relying primarily on the chapters by Carleton Gajdusek (volume I, pp. 7-36) and E Beck and PM Daniel (volume I, pp. 253-270).

[15] P Bendheim, Interview, 2010.

[16] The NIH awards are listed in official Reports of NIH Awards for 1975-1978. The HHMI does not publish the amounts of its awards.

[17] D Groth, Interview, 2010.

[18] Several individuals vaguely recalled that in the early years Prusiner received some gifts to support his work from local sources in San Francisco, but I was unable to find records of such gifts or their use for years prior to 1982. In the mid-1980s, as described in Chapter Ten, Prusiner did garner considerable amounts of support from non-Federal sources, including gifts and awards from companies and foundations.

[19] F Cohen, Interview, 2010.

[20] RG Rohwer, Interview, 2010.

[21] R Mamelok, Interview, 2010.

[22] SB Prusiner, WJ Hadlow, CM Eklund, and RE Race. Sedimentation properties of the scrapie agent. *Proc Natl Acad Sci USA* 74: 4656-4660, 1977. The scrapie agent did

not need to be associated with membranes, as shown by the fact that it sedimented to the same position in the tube in both the presence and absence of a detergent (deoxycholate), which destroys membranes. It was not associated with ribosomes because heating the brain extracts before centrifugation destroyed ribosomes without affecting the agent's sedimentation behavior.

[23] SB Prusiner, WJ Hadlow, CM Eklund, RE Race, and SP Cochran. Sedimentation characteristics of the scrapie agent from murine spleen and brain. *Biochemistry* 17: 4987-4992, 1978. During incubation at 37 or 80° C, enzymes in the tissue extracts chewed up ribosomal RNA, but the scrapie agent survived just fine.

[24] SB Prusiner, WJ Hadlow, DE Garfin, SP Cochran, JR Baringer, RE Race, and CM Eklund. Partial purification and evidence for multiple molecular forms of the scrapie agent. *Biochemistry* 17: 4993-4999, 1978. In most of their previous centrifugations, including those performed to produce the P5 fraction, every fraction of the centrifuged fluid, from the top to the bottom of the tube, contained the same amount of sucrose. In the sucrose gradient experiments, the agent collected in the P5 fraction was centrifuged through a linearly increasing concentration of sucrose, which often ranged (from the top to the bottom of the tube) between 15 and 30% sucrose, where "%" indicates the ratio of the weight of sucrose to volume of fluid.

[25] Carbohydrates and most proteins are hydrophilic (water-loving, so that they dissolve readily in water) and aggregate more readily at cold temperatures. It was well known, however, that exposed surfaces of particles that tend to aggregate more readily at higher temperatures are usually hydrophobic.

[26] SB Prusiner, DF Groth, SP Cochran, FR Masiarz, MP McKinley, and HM Martinez. Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. *Biochemistry* 19: 4883-4891, 1980; SB Prusiner, SP Cochran, DE Downey, and DF Groth. Determination of scrapie agent titer from incubation period measurements in hamsters. *Adv Exp Med Biol* 134: 385-399, 1981.

[27] SB Prusiner. Prions. *Scientific American* 251: 50-59, October 1984.

[28] The lab's first animal experiments used UCSF's animal facility at the Parnassus campus, but that arrangement proved unsatisfactory because the facility lost animals or mixed them up. Consequently, the animal experiments were transferred first to the Naval Biological Lab in Oakland, CA, and then to space owned by UC Berkeley on Fourth street in Berkeley, CA. In the 1990s Prusiner's animal operation was transferred to a dedicated facility built at Hunters Point in San Francisco. D Groth, Interview, 2010.

[29] I Diamond, interview, 2010.

[30] Each treatment or injection was precisely recorded, as well as each rodent's state of

health for weeks and months, with careful monitoring for signs of scrapie, as well as timely harvesting and tracking of brain and other tissues that would be used in further experiments. At its peak, by the 1990s, the Prusiner animal facility was keeping track of experiments on 55,000 animals, and now (in 2010) it studies about 20,000 animals. This number, Groth says (Interview, 2010), is larger than the sum of all other experimental animals throughout UCSF.

[31] S DeArmond, Interview, 2010.

[32] I asked UCSF, under the California Public Records Act, to furnish information about gifts and grants to Prusiner in this period, but it appears that no records of gifts to him have been retained from years prior to 1982. In later years he did begin to garner substantial funds from non-Federal sources, in addition to the NIH, as described in Chapter Ten. See also reference 18, above.

[33] In 1998, an abbreviated version of Prusiner's Nobel address (SB Prusiner. Prions. *Proc Natl Acad Sci USA* 95: 13363-13383, 1998) listed—in addition to the NIH, the National Science Foundation, International Human Frontiers of Science Program, and the American Health Assistance Foundation—gifts over the years from the HHMI and eight other non-government sources, including the Sherman Fairchild Foundation, the Keck Foundation, the G. Harold and Leila Y. Mathers Foundation, the Bernard Osher Foundation, the John D. French Foundation, R. J. Reynolds, National Medical Enterprises, and Centeon.

[34] Krevans told me, in very general terms, that he strongly supported Prusiner, but gave no information about negotiations with donors or about specific gifts (Interview, 2009). Prusiner's published papers repeatedly acknowledge generous (but quantitatively unspecified) gifts from multiple foundations. Remarkably, the best available public documentation of any of these gifts—specifically, those from RJ Reynolds—can be found in the extensive publication of confidential tobacco industry documents on the web (University of California, San Francisco, Legacy Tobacco Documents Library, web 23 June 2010). This website is sponsored by the UCSF Center for Tobacco Control Research and Education, directed by Stanton A Glantz at UCSF. Searching this site for RJ Reynolds and Prusiner produces a 25-page list of documents, including applications from Prusiner, notices of money awarded at various times, and reports of "site visits" by Fred Seitz and others to talk about the scrapie with Prusiner and Krevans. Statements in the text are supported by the following specifc documents: Examples include: $3,747,500 given to SB Prusiner, 1980-88, in Biomedical Research Contibutions (MRC) 1976-89; Seitz reports to an RJ Reynolds official on spending a morning discussing Prusiner's research with Prusiner and Krevans, Letter from F Seitz to HC Rohmer, November 18, 1980.

[35] Conversion of 1980s dollars to 2010 dollars was calculated by using the Biological

Research and Development Price Index, or BRDPI, a price index indicates how much the NIH budget must increase in order to preserve purchasing power for laboratory research. This index, which I also used in Chapter Three of this book, is calculated by the Office of Science Policy Analysis of the National Institutes of Health. Web 22 June 2010.

[36] These are the papers cited in references 22, 23, and 24.

[37] SB Prusiner, MP McKinley, DF Groth, KA Bowman, NI Mock, SP Cochran, and FR Masiarz. Scrapie agent contains a hydrophobic protein. *Proc Natl Acad Sci USA* 78: 6675-6679, 1981; SB Prusiner, DF Groth, MP McKinley, SP Cochran, KA Bowman, and KC Kasper. Thiocyanate and hydroxyl ions inactivate the scrapie agent. *Proc Natl Acad Sci USA* 78: 4606-4610, 1981; SB Prusiner, SP Cochran, DE Downey, and DF Groth. Determination of scrapie agent titer from incubation period measurements in hamsters. *Adv Exp Med Biol* 134: 385-399, 1981; MP McKinley, FR Masiarz, and SB Prusiner. Reversible chemical modification of the scrapie agent. *Science* 214: 1259-1261, 1981; SB Prusiner, DF Groth, SP Cochran, MP McKinley, and FR Masiarz. Gel electrophoresis and glass permeation chromatography of the hamster scrapie agent after enzymatic digestion and detergent extraction. *Biochemistry* 19: 4892-4898, 1980; SB Prusiner, DF Groth, SP Cochran, FR Masiarz, MP McKinley, and HM Martinez. Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. *Biochemistry* 19: 4883-4891, 1980; SB Prusiner, DF Groth, C Bildstein, FR Masiarz, MP McKinley, and SP Cochran. Electrophoretic properties of the scrapie agent in agarose gels. *Proc Natl Acad Sci USA* 77: 2984-2988, 1980; SB Prusiner, DE Garfin, SP Cochran, MP McKinley, DF Groth, WJ Hadlow, RE Race, and CM Eklund. Experimental scrapie in the mouse: electrophoretic and sedimentation properties of the partially purified agent. *J Neurochem* 35: 574-582, 1980.

[38] The review (see also reference 36, above) listed the following treatments indicating the presence of a key protein in the scrapie agent: degradation by proteases (proteinase K and trypsin), inactivation by carbethoxylation (using diethyl pyrocarbonate): three treatments used to denature protein but leave nucleic acids intact (sodium dodecyl sulfate, guanidinium thyocyanate, and phenol); inactivation by urea. Evidence arguing against inclusion of an essential nucleic acid in the agent included its resistance to various nucleases, alkali, and ultraviolet radiation, and prsoralens (chemicals that can penetrate the coats of some viruses and inactivate nucleic acids).

[39] Gary Taubes, The Game of the Name is Fame. But is it Science? First published in the December 1986 issue of *Discover*, and reprinted, with permission, by *Slate* (Web, 25 June 2010). Clearly biased against Prusiner, this article aims to discredit the prion theory and Prusiner himself. I have not been able to check the accuracy of the quotes

from Frank Masiarz, whom I was not able to contact. Paul Bendheim and David Bolton, however, said that quotes from them in the article are accurate.

[40] Indeed, the word appears in the title of a very recent paper (discussed in Chapter Ten), on which Rohwer is a co-author. N Makarava, GG Kovacs, O Bocharova, R Savtchenko, I Alexeeva, H Budka, RG Rohwer, and IV Baskakov. Recombinant prion protein induces a new transmissible prion disease in wild-type animals. *Acta Neuropathol* 119: 177-187, 2010.

[41] This criticism was first suggested to me by David Bolton (Interview, 2010).

## Chapter 10. Marching into the Citadel

[1] D Bolton, Interview, 2010.

[2] DC Bolton, MP McKinley, and SB Prusiner. Identification of a protein that purifies with the scrapie prion. *Science* 218: 1309-1311, 1982.

[3] The wrinkle is called "rate-zonal discontinuous gradient centrifugation" (SB Prusiner, DC Bolton, DF Groth, KA Bowman, SP Cochran, and MP McKinley. Further purification and characterization of scrapie prions. *Biochemistry* 21: 6942-6950, 1982). As acknowledged in that *Biochemistry* paper, the wrinkle was suggested by Juan I Korenbrot, a faculty member in UCSF's Department of Physiology, who did not work on scrapie. The rate-zonal discontinuous procedure differed from that used in previous experiments, where the sucrose gradients were linear, with a density that smoothly increased from the top to the bottom of the centrifuge tube. Instead, in the new procedure a 4-millileter "cushion" of 60% sucrose was placed at the bottom of the tube, and 32 milliliters of less dense (25%) sucrose was placed above it. The extract (4 milliters, containing detergent to prevent aggregation), containing partly purified scrapie agent and previously treated with enough proteinase K to destroy most cellular proteins, was placed on top of the 25% sucrose. Because neither sucrose solution contained any detergent, centrifuging small particles of the agent through the detergent-free 32 milliliters of 25% sucrose gradually stripped them of detergent, causing them to aggregate. Consequently, during centrifugation most of the agent formed large particles, which migrated all the way down the tube, to the high-density cushion, and stopped at the interface between the two sucrose concentrations. As a result, fractions 2 and 3, collected from this region of the tube (and tested in the autoradiogram shown in Figure 3; see Figure legend), contained most of the scrapie agent. In contrast, most of the remaining brain protein was not hydrophobic and did not aggregate, causing it to migrate in lighter fractions, above the 25/60% interface.

[4] Bolton's tricks were designed to make it easier to see small amounts of protein in autoradiograms of polyacrylamide gels. In such gels, in a process called electrophoresis,

an electrical field propels proteins to positions that correspond to their size. Before electrophoresis, the proteins were radiotagged with iodine-125 and dissolved in a detergent called sodium dodecylsulfate (SDS). One trick was to manipulate the chemical cross-linking of the polyacrylamide gel so that small proteins (around 30 kDa, as Bolton had estimated from the results in Masiarz's lab notebook) would migrate near the middle of the gel. The other trick—precipitating, washing, and then redissolving SDS-protein complexes before electrophoresis—reduced background (non-protein) radioactivity. (See paper cited in reference 4, above.)

[5] SB Prusiner. Novel proteinaceous infectious particles cause scrapie. *Science* 216: 136-144, 1982.

[6] P Bendheim, Interview, 2010.

[7] PE Bendheim, RA Barry, SJ DeArmond, DP Stites, and SB Prusiner. Antibodies to a scrapie prion protein. *Nature* 310: 418-421, 1984.

[8] Amyloid is a specially arranged protein aggregate, which stains with Congo Red and exhibits green birefringence when exposed to polarized light. Multiple different kinds of protein—including, but by no means limited to, PrP—can make up amyloid plaques.

[9] S DeArmond, Interview, 2010.

[10] Presence of antibody-detected protein in normal brain seems to contradict the published finding that the anti-Prp27-30 antibody detected Prp27-30 in preparations purified from infected but not from normal hamster brains. This was because (as described later in this chapter and detailed in reference 21, cited below), the published finding was based on extracts treated with proteinase K (PK), which rapidly digested PrP in normal brains but trimmed PrPSc to produce a proteinase-resistant fragment of 27-30 kDa. After the discovery of PrP-27-30 in PK-treated extracts of scrapie-infected brains had been published (reference 7 above), Bendheim found that the antibody could also detect a somewhat larger protein in unpurified extracts from normal brain, and a protein of about the same size in infected brain. His reason for not reporting the antibody- detected protein in normal brain, Bendheim says, was that "antibodies can play tricks on you"—that is, polyclonal antibodies can sometimes cross-react with proteins not closely related to the antigen used to make them. Somewhat later, Stephen DeArmond's lab (see reference 9, above) independently found that the antibody detected a protein in extracts of normal brain (not treated with proteinase K). DeArmond was not surprised, because at about the same time Prusiner's lab found an mRNA encoding a protein with the same sequence as that of the protein associated with the scrapie agent (as described later in the chapter and reported in reference 21).

[11] SB Prusiner, MP McKinley, KA Bowman, DC Bolton, PE Bendheim, DF Groth, and

GG Glenner. Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* 35: 349-358, 1983.

[12] PE Bendheim, JM Bockman, MP McKinley, DT Kingsbury, and SB Prusiner. Scrapie and Creutzfeldt-Jakob disease prion proteins share physical properties and antigenic determinants. *Proc Natl Acad Sci USA* 82: 997-1001, 1985; JM Bockman, DT Kingsbury, MP McKinley, PE Bendheim, and SB Prusiner. Creutzfeldt-Jakob disease prion proteins in human brains. *N Engl J Med* 312: 73-78, 1985.

[13] H Fields, interview, 2010.

[14] Lowenstein (D Lowenstein, interview, 2010) noted that when he was a postdoc in the Prusiner lab, his boss told him not to work at the San Francisco General Hospital on weekends, because "You're doing your postdoc. You should be in the lab." Prusiner, he says, "saw that anything that I was doing that might be taking me away from the lab was not in his best interest."

[15] Diamond (I Diamond, interview, 2010, and email message, October 24, 2010) also mentioned his "impression, at a distance, that everything in that program was being done for Stan's purpose. . . . [I]n Stan's program everything was clear that it was all toward the main project that he was working on. . . . [H]e wasn't so interested in getting someone to develop their own career under his guidance as much as he was interested in having them do hard work for the project he was on, and I believe he sincerely expected these people to profit by their experience with him and go on scientifically to do well."

[16] D Groth, Interview, 2010.

[17] Accounts of lab meetings came from Bendheim and Lowenstein (references 6 and 14 above).

[18] RG Rohwer. Scrapie infectious agent is virus-like in size and susceptibility to inactivation. *Nature* 308: 658-662, 1984.

[19] RG Rohwer, Interview, 2010.

[20] SB Prusiner, DF Groth, DC Bolton, SB Kent, and LE Hood. Purification and structural studies of a major scrapie prion protein. *Cell* 38: 127-134, 1984. The sequence had to be pieced together from several different overlapping sequences. These were created by the protein's previous exposure (during purification) to proteinase K, which "frayed" the extreme amino-terminus of the protein so that every sample contained a mixture of short fragments, each begun at a slightly different location near the "real" amino terminus.

[21] B Oesch, D Westaway, M Walchli, MP McKinley, SB Kent, R Aebersold, RA Barry, P Tempst, DB Teplow, LE Hood, et al. A cellular gene encodes scrapie PrP 27-30 protein. *Cell* 40: 735-746, 1985.

[22] In brief summary: using the genetic code (that is, the list of three-base codons that

encode specific amino acids; see Chapter Four), the amino acid sequence of a short segment of PrP27-30 was reverse-translated to create a theoretical best guess at the corresponding DNA sequence. "Best-guess" DNA fragments were then used to hybridize (by base-pairing) with the PrP27-30 cDNA, which was found in a "library" of cDNAs encoding proteins made in scrapie-infected hamster brains.

[23] More precisely, the PrP gene in the Syrian hamster encodes 254 amino acids. In normal cells both ends of the protein are trimmed, to produce $PrP^{C}$, which has 209 amino acids. PrP27-30 has about 142 amino acids, because approximately 67 amino acids have been trimmed off its front end by proteinase K. The number is approximate because proteinase K cuts imprecisely, sometimes cleaving off 66 or 68 amino acids, rather than 67. Note that "naked" $PrP^{C}$ or $PrP^{Sc}$ would have a much smaller molecular weight, of about 21 kilodaltons. The difference is that cells attach strings of sugar molecules to new $PrP^{C}$ molecules, and the sugars are not removed when $PrP^{C}$ is converted to $PrP^{Sc}$. Reviewed in SB Prusiner. Prions. *Proc Natl Acad Sci USA* 95: 13363-13383, 1998.

[24] Donald Ganem, a colleague at UCSF, pointed out to me (interview, 2009) how radical Prusiner's refusal to be lured into molecular biology actually was, in the UCSF context. The elegant intricacies of DNA, RNA, and molecular genetics fascinated and seduced many of the best young scientists, who also tended to be impatient with the painstaking drudge work of purifying proteins. If Prusiner had yielded to the siren call of molecular genetics early on, Ganem suggests, he might have become lost in the byways of virology and missed the point of scrapie altogether—as in fact many other scientists did.

[25] Briefly reviewed in SB Prusiner. Prions. *Proc Natl Acad Sci USA* 95: 13363-13383, 1998.

[26] The mouse experiments are described in A Carlson, DT Kingsbury, PA Goodman, S Coleman, ST Marshall, S DeArmond, D Westaway, and SB Prusiner. Linkage of prion protein and scrapie incubation time genes. *Cell* 46: 503-511, 1986. In a separate set of experiments (see M Scott, D Foster, C Mirenda, D Serban, F Coufal, M Walchli, M Torchia, D Groth, G Carlson, SJ DeArmond, et al. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* 59: 847-857, 1989), hamster scrapie protein ($Pr^{PSc}$) was injected into brains of transgenic mice that expressed the PrP gene of the Syrian Hamster (in addition to normal mouse PrP). These mice showed the shorter scrapie incubation time characteristic of hamsters. (The mouse and hamster PrP genes encode proteins that differ at a very small number of amino acid positions.)

[27] A specific mutation in the human PrP gene tracked with inherited ataxia (loss of normal control of body movements) and spongiform encephalopathy in two unre-

lated families. (See K Hsiao, HF Baker, TJ Crow, M Poulter, F Owen, JD Terwilliger, D Westaway, J Ott, and SB Prusiner. Linkage of a prion protein missense variant to Gerstmann-Straussler syndrome. *Nature* 338: 342-345, 1989.) Since this first publication. at least 30 different PrP mutations have been found in different families with GSS. (See SB Prusiner. Detecting mad cow disease. *Sci Am* 291: 86-93, 2004.)

[28] See DH Bueler, A Aguzzi, A Sailer, RA Greiner, P Autenried, M Aguet, and C Weissmann. Mice devoid of PrP are resistant to scrapie. *Cell* 73: 1339-1347, 1993; and SB Prusiner, D Groth, A Serban, R Koehler, D Foster, M Torchia, D Burton, SL Yang, and SJ DeArmond. Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. *Proc Natl Acad Sci USA* 90: 10608-10612, 1993.

[29] SB Prusiner, M Scott, D Foster, KM Pan, D Groth, C Mirenda, M Torchia, SL Yang, D Serban, GA Carlson, et al. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* 63: 673-686, 1990.

[30] No covalent modification (e.g., enzyme cleavage or attachment of a phosphate or some other molecule to $PrP^{C}$) could be found that might account for its transformation into $PrP^{Sc}$, nor did it appear that $PrP^{Sc}$ induced de novo synthesis of more $PrP^{Sc}$ on ribosomes. Searches for small nucleic acids that might induce synthesis of a protein that associates with $PrP^{C}$ to transform it into $PrP^{Sc}$ were not successful.

[31] JS Griffith. Self-replication and scrapie. *Nature* 215:1043-1044, 1967.

[32] R Rhodes, *Deadly Feasts. The "Prion" Controversy and the Public's Health*. 1997. On pages 161-162 of this book, Rhodes says Gajdusek recounted his "protein-only" agreement with Prusiner, in a conversation that took place in about 1980. Gajdusek's crystallization theory was not precisely the same as templating, however, because he was simply imagining that individual protein molecules could associate with one another to form large and growing polymers, analogous to ice or known polymeric proteins. But the individual components of a polymer need not change their structure very much, whereas a key element of the templating theory, as it eventually developed, is the dramatic shape change undergone by each individual monomeric $Pr^{PC}$ molecule when it becomes $Pr^{PSc}$. These changes might favor polymerization, to be sure, and surely were catalyzed by association of $Pr^{PC}$ with mis-shapen $Pr^{PSc}$, but polymers were not necessarily the active infectious agents.

[33] B Alberts, Interview, January 2010.

[34] F Cohen, Interview, 2010.

[35] For instance, greasy side chains tend to aggregate together, water-loving side chains tend to cover the surface of the folded protein, small or big side chains fit into smaller or bigger holes, and like charges repel one another while positive and negative charges attract each other. As noted in the text, $\alpha$ helices and $\beta$ strands can often be pre-

dicted, but structural prediction gets becomes harder when one tries to guess exactly how a set of predicted α helices and β-strands will nuzzle up to one another to form the stable 3D fold of a protein able to hold its shape and function in cells.

[36] See Z Huang, JM Gabriel, MA Baldwin, RJ Fletterick, SB Prusiner, and FE Cohen. Proposed three-dimensional structure for the cellular prion protein. *Proc Natl Acad Sci USA* 91: 7139-7143, 1994; and TL James, H Liu, NB Ulyanov, S Farr-Jones, H Zhang, DG Donne, K Kaneko, D Groth, I Mehlhorn, SB Prusiner, et al. Solution structure of a 142-residue recombinant prion protein corresponding to the infectious fragment of the scrapie isoform. *Proc Natl Acad Sci USA* 94: 10086-10091, 1997.

[37] For instance, as explained in greater detail in reference 25, it looks as if the transition of $PrP^{C}$ to $PrP^{Sc}$ does not happen easily unless a second (but still not identified) cellular component (provisionally termed "protein X") can bind to a site located in the other half of $PrP^{C}$ (that is, its α-helical portion). The X component makes the transition more likely, but is not absolutely required, in view of other evidence, cited in reference 40 below, showing that infectious prions can be made by adding a "seed" of $Pr^{PSc}$ to recombinant $PrP^{C}$ (made in bacteria), in the complete absence of other cellular proteins.

[38] G Legname, IV Baskakov, HO Nguyen, D Riesner, FE Cohen, SJ DeArmond, and SB Prusiner. Synthetic mammalian prions. *Science* 305: 673-676, 2004.

[39] N Makarava, GG Kovacs, O Bocharova, R Savtchenko, I Alexeeva, H Budka, RG Rohwer, and IV Baskakov. Recombinant prion protein induces a new transmissible prion disease in wild-type animals. *Acta Neuropathol* 119: 177-187, 2010.

[40] JI Kim, I Cali, K Surewicz, Q Kong, GJ Raymond, R Atarashi, B Race, L Qing, P Gambetti, B Caughey, et al. Mammalian prions generated from bacterially expressed prion protein in the absence of any mammalian cofactors. *J Biol Chem* 285: 14083-14087, 2010.

[41] See, for instance, B Frost, and MI Diamond. Prion-like mechanisms in neurodegenerative diseases. *Nat Rev Neurosci* 11: 155-159, 2010; and P Brundin, R Melki, and R Kopito. Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat Rev Mol Cell Biol* 11: 301-307, 2010.

[42] Y Ohhashi, K Ito, BH Toyama, JS Weissman, and M Tanaka. Differences in prion strain conformations result from non-native interactions in a nucleus. *Nat Chem Biol* 6: 225-230, 2010.

[43] LZ Osherovich, and JS Weissman. The utility of prions. *Dev Cell* 2: 143-151, 2002.

[44] M Brown, interview, 2010. In addition to the quotes in this section of the chapter, Brown suggested several of the more general ideas, but I have embellished, extended, and modified them in ways he might not recognize. Brown, an expert on scientific creativity, shared the 1984 Nobel Prize for Phyiology or Medicine with Joseph Gold-

stein, his long-standing research partner in studying control of cholesterol metabolism.

[45] W Mobley, interview, 2010. Mobley is now chair of the Department of Neurology at the University of California, San Diego School of Medicine

[46] RC Morris, interview, 2010.

## Chapter 11. Something in the Water?

[1] Baltimore, then director of the Whitehead Institute at the Massachusetts Institute of Technology, was introducing a Whitehead Symposium he had organized at MIT. I was one of five or six UCSF faculty among the 20-25 presenters at the symposium. Neither Baltimore nor I can recall his exact words on this occasion, but he confirms that he asked the question.

[2] Each of these quotes is presented in context in an earlier chapter. In Chapter One, Edelman lamented anti-communist "Red-baiters" who prevented him from getting a job he wanted (I Edelman, Oral History, 1996), and adjured Richard Havel not to worry about UCSF's provinciality. Holly Smith's aria (LH Smith Jr, Interview, 2009) is more extensively quoted in Chapter Two.

[3] Steven Johnson, *Where Good Ideas Come From: the natural history of innovation*. 2010. Johnson's intriguing book attempts to cover all kinds of innovation, especially but not exclusively in science and technology, and by no means limited to biology. The principal idea is that innovation comes from combining ideas and facts derived from different and previously (apparently) unrelated fields, and therefore critically depends on the free flow of information. He tackles the question of whether economic market forces spark innovation, and presents strong evidence that this is not the case. Instead, he documents the fact that "most of the paradigmatic ideas in science and technology that arose during the past century have roots in academic research"—where, he points out, information flows much more freely than in commerce or the market. In agreement with his emphasis on maximizing flow of information, he considers collaboration a major source of innovation, but does not explicitly consider the essential role (in my view) of freedom from constraints to the imagination, which requires the unfettered opportunity of individual minds to explore questions they choose with approaches they devise.

[4] James Watson describes their freedom from supervision in *The Double Helix*, published in 1968. The actual quote does not appear in that book, however, and I have not been able to pinpoint its source—although it is often attributed to Watson.

[5] The un-named scientist, probably an atomic physicist, was referring to a somewhat different problem. Quoted in a magazine article: Scientist's Warning. *Time*, October

29, 1945, he (or she) was protesting US government plans to place atomic energy under military control, with stringent security regulations that would hamper scientific communication. The specific protest, in a congressional hearing, opposed the May-Johnson bill, which was sponsored by President Harry Truman and subsequently passed. Re-quoted, with attribution to *Time*, in Paul Boyer, *By the Bomb's Early Light*. University of North Carolina Press, 1985, page 52.

[6] All values for the early and the most recent reference year in Table 1 were found in NIH reports on the web. Specifically:

(a) The number and dollar values of research grants awarded to UCSF or to all applicants in 1970 and 2008 come from Annual Reports of the NIH for those years.

(b) The NIH calculates success rates of research grant applications as the percentage of reviewed grants that are actually funded in a particular year. The numbers and percentages for 1966 (estimated) and 2008 (actual) are taken from a table of success rates for the years 1962-2008, issued by the NIH Office of Extramural Research and found on the web, August 24, 2010.

(c) The ages of successful NIH applicants in different age groups are found in Figure 1-2 of a book, *Bridges to Independence: Fostering the Independence of New Investigators in Biomedical Research*, National Academies Press, 2005. The NIH commissioned this report, which was authored by experts at the National Academies and was available on the web. August 24, 2010.

[7] These are my estimates, based on my own observations at the time. I have not been able to obtain the actual figures.

[8] PA Lawrence. Real Lives and White Lies in the Funding of Scientific Research. The granting system turns young scientists into bureaucrats and then betrays them. *PLoS Biology* 7(9): e1000197. doi:10.1371/journal.pbio.1000197. 2009. The author writes from a British perspective but his arguments resonate with my young colleagues in the US.

[9] B Alberts, Overbuilding research capacity [editorial], *Science* 329:1257, 2010. Alberts suggests that the NIH curtail this ominous trend by requiring universities to use their own funds to pay a substantial fraction of the salaries of their research faculty.

[10] Scientists used to say that youth was crucial in physics, but not important in biomedical research. That view is not correct. Among Nobel Prize winners from 1901 to 1992, 68% of winners in medically related fields began their Nobel-qualifying research before age 35, and the corresponding percentage of winners in physics was 74%. See PE Stephan and SG Levin. Age and the Nobel Prize Revisited. *Scientometrics* 28:387-399, 1993.

[11] This was the title of Vannevar Bush's report, commissioned by Franklin Roosevelt and published in 1945. As described in Chapter One, the report stimulated subse-

quent US government investment in science—including the NIH—beginning after World War II and continuing to the present.

[12] I am indebted to Keith Yamamoto for his generous willingness to educate me about many issues in this chapter. The indebtedness is especially strong in this paragraph on the central problem of supporting innovative research in large enterprises. Several of the ideas presented, including specific phrases, directly reflect Yamamoto's advice and help.

[13] Committee on Prospering in the Global Economy of the 21st Century. *Rising Above the Gathering Storm: Energizing and Employing America for a Brighter Economic Future*. National Academy Press, 2007. This 592-page book was sponsored by the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. It can be purchased or downloaded (free, as a pdf document) from the web (3 November 2010), at http://www.nap.edu/catalog/11463.html. The followup study, *Rising Above the Gathering Storm, Revisited*, is also available (free, as a 107-page pdf document) on the web (3 November 2010), at http://www.nap.edu/catalog.php?record_id=12999.

[14] This story is told in greater detail in my memoir, *Ambition and Delight. A Life in Experimental Biology*, 2009.

[15] Among these outstanding faculty, I list a few who were hired between 1985 and 1995. Biochemistry hired Cynthia Kenyon, a nematode geneticist who studies the genetics of ageing, and Joseph DeRisi, a molecular biologist who has pioneered genomic analysis of malaria parasites and viral diseases. Anatomy hired Cori Bargmann, a nematode geneticist, and Marc Tessier-Lavigne. Both are now at the Rockefeller University, where Bargmann is a professor and Tessier-Lavigne has just been elected President—following successful interim service as Chief Scientific Officer at Genentech. Microbiology hired Donald Ganem, a leading virologist, and Elizabeth Blackburn, who came to UCSF from UC Berkeley and recently shared a Nobel for her work on the biochemistry of telomeres. Pharmacology hired David Julius, who discovered vertebrate receptors for heat, cold, and touch; two outstanding cell biologists who study the cytoskeleton, including Ronald Vale, until recently chair of his department, and Timothy Mitchison, now professor at Harvard. Physiology hired Lily and Yuh Nung Jan, a couple widely acclaimred for their work on ligand-gated ion channels and neural development, and James Hudspeth, a leading expert on the cell biology of hearing and cochlear hair cells, also now at the Rockefeller University.

[16] For details of this suit, see reference 41 in Chapter Six.

[17] Completed research buildings include, in addition, one devoted to chemical and computational biology, one focused on developmental and basic neurobiology, and others housing cancer research and the Cardiovascular Research Institute and cardiology

outpatient clinics. A large additional research building, now under construction, will house more neurobiology labs, the Department of Neurology, and Stanley Prusiner's Institute for Neurodegenerative Diseases. Recently the university broke ground for construction of new pediatrics, women's, and cancer hospital facilities on 16th street, facing Genentech and Byers Halls.

## Chapter 12. Hatching Innovation

[1] C Kerr, *The Uses of the University*, 1963.

[2] See the report sponsored by the National Academies, referenced in reference 13, Chapter Eleven.

[3] See reference 5, Chapter Eleven.

[4] Quotes in this paragraph from F Cohen, Interview, 2010.

[5] UCSF lamented the departures of luminaries like Marc Kirschner and Tim Mitchison (among others) to Harvard, as well as three outstanding researchers to Genentech, the Rockefeller University (see reference 15, Chapter Eleven), and high offices at the NIH and the National Academy of Sciences (e.g., Harold Varmus and Bruce Alberts). In each case, the person who replaced the departed individual helped to rejuvenate UCSF's research enterprise and is still making important contributions.

[6] Chapter Three, above, presents Krevans's account of extensive cooperation between clinical and basic science departments. See JR Krevans, Interview, 2009, and Oral History, 2005.

[7] As we saw in Chapters One and Two, the move of basic science departments from Berkeley to join the clinical departments in San Francisco did little to heal the cultural breach between them, at least at first. But the breach was unable to withstand the potent combination of Julius Comroe, the CVRI, increasing NIH funds, new research facilities, and recruitment of clinical leaders with basic research training (Edelman, Havel, Comroe, Smith). After Comroe and his allies defeated Saunders, Smith and Edelman were able to recruit Bill Rutter as chair of Biochemistry. At about the same time Microbiology hired Boyer, Bishop, and Varmus, and later Neurology hired Prusiner. Strikingly, three of these four outstanding scientists were MDs, not PhDs. As the Biochemistry department, clinical departments, Comroe's (and later Havel's) CVRI, and our wild card researchers prospered, by the early 1980s it may have looked as if the cultural breach had almost healed. Over the next twenty-five years, from about 1985 to 2010, the breach gradually opened again. Many clinicians felt that Rudi Schmid, Krevans's replacement in the dean's office, was biased toward support of basic science research, as revealed by the rapid development of basic science departments (from which I benefitted, in the Pharmacology department), forma-

tion and exuberant success of PIBS, and perceived overcrowding of labs in clinical departments. Later, around the turn of the century, the breach became wider. Schmid was no longer on the scene, but UCSF's huge investment in new lab buildings at Mission Bay appeared to underline a continuing bias toward basic research and (some thought) freed up too little space at Parnassus for labs and other efforts of clinical departments.

[8] JH Comroe, *Retrospectroscope*, 1977.

[9] Advice to grant applicants from the National Institute of Allergy and Infectious Disease, under the heading: NIAID Research Funding: Part 4. Target Your Audience. Web 28 October 2010. I thank Robert Lehrer, Emeritus Professor of Medicine at UCLA, for bringing this advice to my attention.

[10] See F Collins. Scientists need a shorter path to research freedom. *Nature* 467: 635, 2010.

[11] B Alberts, Overbuilding research capacity [editorial], *Science* 329:1257, 2010. I spoke to Alberts in May 2011.

[12] From Table 101, NIH Research Grants, Awards and Total Funding by Grant Mechanism, Type, and Activity Code, Fiscal Years 2001-2010. NIH, Office of Extramural Research, Web May 4, 2001.

[13] Conversation between Ronald Vale, UCSF faculty member, and the author, at Mission Bay, UCSF, 2011.

[14] See *Bridges to Independence: Fostering the Independence of New Investigators in Biomedical Research*, National Academies Press, 2005. The NIH commissioned this report, by experts at the National Academies and available on the web. August 24, 2010.

[15] G Ferry. *Max Perutz and the Secret of Life*, 2007.

[16] Conversation with Gerald M. Rubin in Washington, DC , 2010.

[17] The UCSF Fellows Program, for instance, supports young scientists for five years of "sheltered independence" to pursue their own research. They are given lab space for two to four workers, and supported by extramural grants and University funds. At present UCSF supports seven fellows. Other schools have similar programs. The "incubators" I propose would provide greater support for a longer period, be formally monitored as an experiment sponsored by the NIH, and would incorporate deliberately designed differences in administration, supervision, and funding at different academic sites.

[18] If startup costs per supported investigator amount to $3 million and recur about every eight years, this would add $375,000 per year to the cost of supporting each incubated investigator, for a total of $1.38 million per year, per investigator—or, for five incubator institutes with eight investigators each, a total of $55 million per year.

[19] See Table 1 in Chapter Eleven.

# Sources

## Oral Histories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Herbert W Boyer, *Project on the Development of Recombinant DNA Research Guidelines*. Interviewer: Rae Goodell, the Massachusetts Institute of Technology (MIT) Oral History Program. Interview on May 20, 1975, in San Francisco. Transcript on file at MIT Archives, quoted with permission from MIT and H. W. Boyer.

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November 20, and December 4, 1995.

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Julius R Krevans, *Diversity in US Medical Schools*. Interviewer: Nancy M Rockafellar, The UCSF Oral History Program, Kalmanovitz Library, UCSF, San Francisco. Interviews in 2005.

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Groth, Darlene, Interview in her office, HSE-7 at UCSF, January 27, 2010.
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## Papers

JH Comroe, Jr, "Saunders." This thick loose-leaf notebook, two inches thick, was preserved among boxes of other papers from Comroe's office by his successor as head of the CVRI, Richard J. Havel. It contains a large number of undated notes, handwritten and typed letters, newspaper clippings, and other records. Few of the documents are explicitly dated, but the approximate dates of many can be inferred from their contents.

William J Rutter Papers, Archives and Special Collections, UCF Library. Altogether the papers fill 13 cartons. Every letter cited in this book belongs to the correspondence between Rutter and officials at UCSF, during Rutter's negotiations for the chair of the Department of Biochemistry. These letters are located in Subgroup 1, Series 1, Carton 1, Folder 1A, "Negotiations with UCSF."

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