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The Making of a Scientist II (Nobel Lecture)**

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I was born in Verona, Italy on October 6, 1937. Fascism, Nazism, and Communism were raging through the country. My mother, Lucy Ramberg, was a poet; my father, Luciano Capecchi, an officer in the Italian Air Force. This was a time of extremes, turmoil and juxtapositions of opposites. They had a passionate love affair, and my mother wisely chose not to marry him. This took a great deal of courage on her part. It embittered my father.

I have only a few pictures of my mother. She was a beautiful woman with a passion for languages and a flair for the dramatic (see Figure 1). This picture was taken when she was 19. She grew up,

Figure 1. A photograph of my mother, Lucy Ramberg, at age 19.

with her two brothers, in a villa in Florence, Italy. There were magnificent gardens, a nanny, gardeners, cooks, house cleaners, and private tutors for languages, literature, history, and the sciences. She was fluent in half a dozen languages. Her father, Walter Ramberg, was an archeologist specializing in Greek antiquities, born and trained in Germany. Her mother was a painter born and raised in Oregon, USA. In her late teens, my grandmother, Lucy Dodd, packed up her steamer trunks and sailed with her mother from Oregon to Florence, Italy, where they settled.

My grandmother was determined to become a painter. This occurred near the end of the 19th century, a time when young women were not expected to set off on their own with strong ambitions of developing their own careers.

My grandmother became a very gifted painter. Let me share with you a couple of her paintings, which also illustrate the young lives of her children. These paintings are very large, approximately seven feet by five feet. The first painting (Figure 2) is the center panel of a triptych depicting my mother and her two brothers Walter and Edward

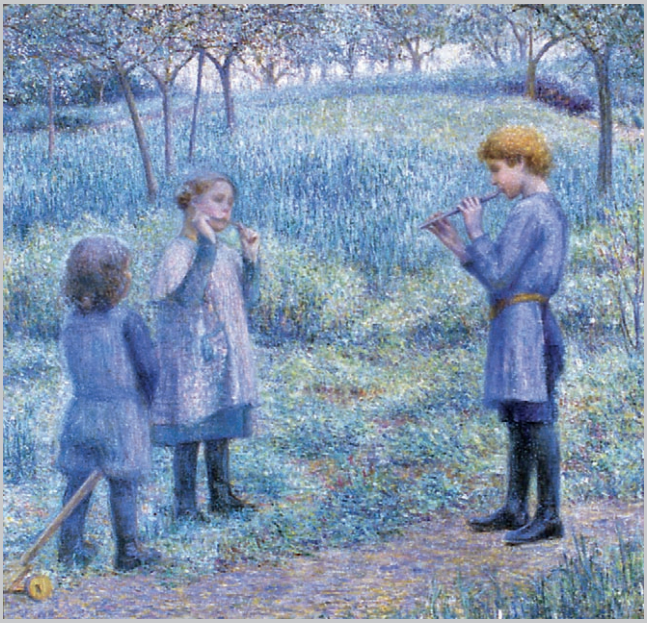

Figure 2. A painting done by my grandmother, Lucy Dodd Ramberg, of her three children, left to right, Edward, Lucy, and Walter. It was painted at their villa in Florence, Italy in 1913.

(both of whom became physicists) surrounded by olive trees at the villa in Florence. The influence of the French impressionist painters is evident. The second painting (Figure 3) is of my mother, age 8, and her younger brother Edward, age 6, having a tea party, again at the villa in Florence. Their father, the German archeologist, was killed as a young man in World War I. My grandmother finished raising her three children on her own by painting, mostly portraits, and by converting the family villa into a finishing school for young women, primarily from the United States.

My mother's love and passion was poetry. She published in German. She received her university training at the Sorbonne in Paris and was a lecturer at that university in literature and languages. At that time, she joined with a group of poets, known as the Bohemians, who were prominent for their open opposition to Fascism and

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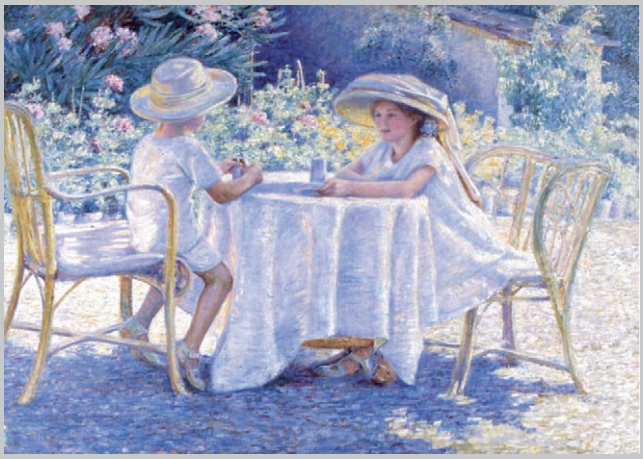

Figure 3. A painting by Lucy Dodd Ramberg of my mother, Lucy, and uncle Edward having tea at the villa in Florence, Italy (1913).

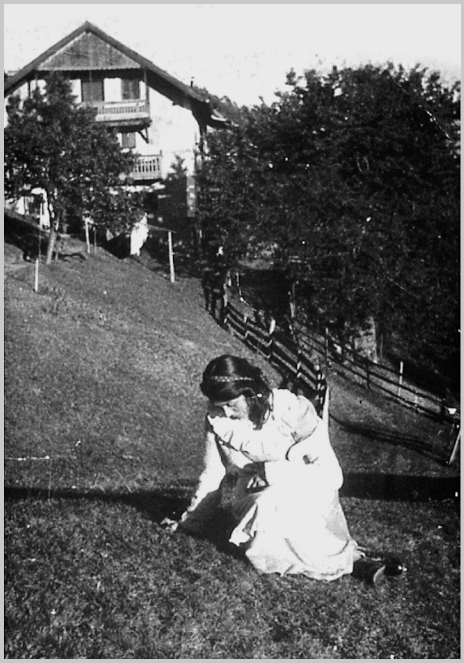

Figure 4. A photograph of the chalet where my mother and I lived in Wolfgrübben just north of Bolzano, Italy. In the foreground is my mother, Lucy.

Nazism. In 1937 my mother moved to the Tyrol, the Italian Alps. Figure 4 shows the chalet north of Bolzano, in Wolfgrübben, with my mother in the foreground. We lived in this chalet until I was $3^1/_2$ years old. In the spring of 1941 German officers came to our chalet and arrested my mother. This is one of my earliest memories. My mother had taught me to speak both Italian and German, and I was quite aware of what was happening. I sensed that I would not see my mother again for many years, if ever. She was incarcerated as a political prisoner in Germany.

I have believed that her place of incarceration was Dachau. This was based on conversations with my uncle Edward, my mother's younger brother. During World War II, my uncle lived in the United States. Throughout these war years, he made many attempts to locate where my mother was being held. The most reliable information indicated that the location was near Munich. Dachau is located near Munich and was built to hold political prisoners. My mother survived her captivity, but after the war, despite my prodding, she refused to talk about her war experiences.

Reporters from the Associated Press (AP) have found records that my mother was indeed a prisoner during the war in Germany. In fact, they have found records of German interest in my mother's political activities preceding 1939. In that year, they had her arrested by the Italian authorities and jailed in Perugia and subsequently released. However, the AP reporters did not find records indicating that my mother was incarcerated in Dachau. Though Germans were noted for their meticulous record keeping, it would be difficult now to evaluate the accuracy of the existing war records, particularly for cases where data is missing. It is clear, however, that exactly where in Germany my mother was held has not yet been determined. Regardless of which prison camp was involved, her experiences were undoubtedly more horrific than mine. She had aged beyond recognition during those five years of internment. Following her release, though she lived until she was 82 years old, she never psychologically recovered from her wartime experiences.

My mother had anticipated her arrest by German authorities. Prior to their arrival, she had sold most of her possessions and gave the proceeds to an Italian peasant family in the Tyrol so that they could take care of me. I lived on their farm for one year. It was a very simple life. They grew their own wheat, harvested it, and took it to the miller to be ground. From the flour they made bread, which they took to the baker to be baked. During this time, I spent most of my time with the women of the farm. In the late fall, the grapes were harvested by hand and put into enormous wooden vats. The children, including me, stripped, jumped into the vats and mashed the grapes with our feet. We became squealing masses of purple energy. I still remember the pungent odor and taste of the fresh grapes.

World War II was now fully under way. The American and British forces had landed in Southern Italy and were proceeding northward. Bombings of northern Italian cities were a daily occurrence. As constant reminders of the war, curfews and blackouts were in effect every night; no lights were permitted. In the night we could hear the drone of presumed American and British reconnaissance planes, which we nicknamed "Pepe." One hot afternoon, American planes swooped down from the sky and began machine gunning the peasants in the fields. A senseless exercise. A bullet grazed my leg, fortunately not breaking any bones. I still have the scar, which, many years later, my daughter proudly had me display to her third-grade class in Utah.

For reasons that have never been clear to me, my mother's money ran out after one year and, at age $4^1/_2$, I set off on my own. I headed south, sometimes living in the streets, sometimes joining gangs of other homeless children, sometimes living in orphanages, and most of the time being hungry. My recollections of those four years are vivid but not continuous, rather like a series of snapshots. Some of them are brutal beyond description, others more palatable.

There are records in the archives of Ritten, a region of the Southern Alps of Italy, that I left Bozen to go to Reggio Emilia on July 18, 1942. AP reporters exploring this history have suggested that my father

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came to the farm, picked me up, and that we went together to Reggio Emilia where he was living. I have no memory of his coming to the farm, nor of having traveled with him to Reggio Emilia. I have recently received a letter from a man who remembers me as the youngest member of his street gang operating in Bolzano, which is on the way to Reggio Emilia.

I did end up in Reggio Emilia, which is approximately 160 miles south of Bolzano. I knew that my father lived in Reggio Emilia and I have previously noted that I had lived with him a couple of times from 1942–1946, for a total period of approximately three weeks. The question has been raised why I didn't live with him for a much longer period. The reason was that he was extremely abusive. Amidst all of the horrors of war, perhaps the most difficult for me to accept as a child was having a father who was brutal to me.

Recently, I have also received a very nice letter from the priest in Reggio Emilia who ran the orphanage in which I was eventually placed. I remember him because he was one of the very few men I encountered in Reggio Emilia who showed compassion for the children and took an interest in me. I am surprised, but pleased, that after all these years he still remembers me among the thousands of children he was responsible for over the years. Further, I believe I was at that orphanage for only several months, the first time in the fall of 1945, after which I ran away, followed by a second period, in the same orphanage, in the spring of 1946. But his memory is genuine, for he recounts incidents consistent with my memories that could only have been known through our common experience.

In the spring of 1945, Munich was liberated by the American troops. My mother had survived her captivity and set out to find me. In October 1946, she succeeded. As an example of her flair for the dramatic, she found me on my ninth birthday, and I am sure that this was by design. I did not recognize her. In five years she had aged a lifetime. I was in a hospital when she found me. All of the children in this hospital were there for the same reasons: malnutrition, typhoid, or both. The prospects for most of those children of ever leaving that hospital were slim because they had no nourishing food. Our daily diet consisted of a bowl of chicory coffee and a small crust of old bread. I had been in that hospital in Reggio Emilia for what seemed like a year. Scores of beds lined the rooms and corridors of the hospital, one bed touching the next. There were no sheets or blankets. It was easier to clean without them. Our symptoms were monotonously the same. In the morning we awoke fairly lucid. The nurse, Sister Maria, would take our temperature. She promised me that if I could go through one day without a high fever, I could leave the hospital. She knew that without any clothes I was not likely to run away. By late morning, the high, burning fever would return and we would pass into oblivion. Consistent with the diagnosis of typhoid, many years later I received a typhoid/paratyphoid shot, went into shock, and passed out.

The same day that my mother arrived at the hospital, she bought me a full set of new clothes, a Tyrolean outfit complete with a small cap with a feather in it. I still have the hat. We went to Rome to process papers, where I had my first bath in six years, and then on to Naples. My mother's younger brother, Edward, had sent her money to buy two boat tickets to America. I was expecting to see roads paved with gold in America. As it turned out, I found much more: opportunities.

On arriving in America, my mother and I lived with my uncle and aunt, Edward and Sarah Ramberg. Edward, my mother's younger brother was a brilliant physicist. He was a Ph.D. student in quantum mechanics with Arnold Sommerfeld and translated one of Sommerfeld's major texts into English. Among Edward's many contributions was his discovery of how to focus electrons, knowledge which he used in helping to build the first electron microscope at RCA. Edward's books on electron optics have been published in many languages. During my visit to Japan to celebrate the Kyoto Prize, several Japanese physicists approached me to express how grateful they were for my uncle's texts from which they learned electron optics. Another achievement, of which he was less proud was being a principal contributor to the development of both black and white and color television. While I grew up in his home, television was not allowed. Figure 5 shows a photograph of my uncle working in his laboratory.

Figure 5. A photograph of my uncle Edward Ramberg working in his laboratory at RCA Princeton, New Jersey.

My aunt and uncle were Quakers and they did not support violence as a solution to political problems anywhere in the world. During World War II, my uncle did alternative service rather than bear arms. He worked in a mental institution in New Hampshire, cleared swamps in the south, and was a guinea pig for the development of vaccines against tropical diseases. After the war he settled in a commune in Pennsylvania, called Bryn Gweled, which he helped found. People of all races and religious affiliations were welcomed in this community. It was a marvelous place for children: it contained thick woods for exploration and had communal activities of all kinds—painting, dance, theater, sports, electronics, and many sessions devoted to the discussion of the major religious philosophies of the world. Every week there were communal work parties, putting in roads, phone lines, and electrical lines, building a community center and so on.

The contrast between living primarily alone in the streets of Italy and living in an intensely cooperative and supportive community in Pennsylvania was enormous. Time was needed for healing and for erasing the images of war from my mind. I remember that for many years after coming to the United States I would go to sleep tossing and turning with such force that by morning the sheets were torn

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and the bed frame broken. This activity disturbed my aunt and uncle to the extent that Sarah would take me from one child psychologist or psychiatrist, to another. These professionals were not very helpful, but the support of the community was. The nightly activity eventually subsided. There may be lessons to be learned from such experiences for the treatment of the children from Darfur, the Congo, and now Kenya.

Sarah and Edward took on the challenge of converting me into a productive human being. This, I am sure, was a very formidable task. I had received little or no formal education or training for living in a social environment. Quakers do not believe in frills, but rather in a life of service. My aunt and uncle taught me by example. I was given few material goods, but every opportunity to develop my mind and soul. What I made of myself would be entirely up to me. The day after I arrived in America, I went to school. I started in the third grade in the Southampton public school system. Sarah also took on the task of teaching me to read, starting from the very beginning.

The first task was to learn English. I had a marvelous third grade teacher. She was patient and encouraging. The class was studying Holland, so I started participation in class functions by painting a huge mural on butcher block paper with tulips, windmills, children ice skating, children in Dutch costumes, and ships. It was a collage of activities and colors. This did not require verbal communication.

I was a good, but not serious, student in grade school and high school. Academics came easily to me. I attended an outstanding high school, George School, a Quaker school north of Philadelphia. The teachers were superb, challenging, enthusiastic, competent, and caring. They enjoyed teaching. The campus was also magnificent, particularly in the spring when the cherry and dogwood trees were bursting with blossoms. An emphasis on Quaker beliefs permeated all of the academic and sports programs. A favorite period for many, including me, was Quaker meeting, a time set aside for silent meditation, and taking stock of where we were going. My wife and I sent our daughter to George School for her own last two years in high school so that she might also benefit from the personal virtues it promotes, and we think she has.

Sports were very important to me at George School, and physical activity has remained an important activity for me to this day. I played varsity football, soccer, and baseball, and wrestled. I was particularly proficient at wrestling. I enjoyed the drama of a single opponent, as well as the physical and psychological challenges of the sport. After George School, I went to Antioch, a small liberal arts college in Ohio.

At Antioch College I became a serious student, converting to academics all of the energy I had previously devoted to sports. Coming from George School, I carried the charge of making this a better, more equitable world for all people. Most of the problems appeared to be political, so I started out at Antioch majoring in political science. However, I soon became disillusioned with political science since there appeared to be little science to this discipline, so I switched to the physical sciences—physics and chemistry. I found great pleasure in the simplicity and elegance of mathematics and classical physics. I took almost every mathematics, physics, and chemistry course offered at Antioch, including Boolean algebra and topology, electrodynamics, and physical chemistry.

Although I found physics and mathematics intellectually satisfying, it was becoming apparent that what I was learning came from the past. The newest physics that was taught at Antioch was quantum mechanics, a revolution that had occurred in the 1920s and earlier. Also, many frontiers of experimental physics, particularly experimental particle physics, were requiring the use of larger and larger accelerators, which involved bigger and bigger teams of scientists and support groups to execute the experiments. I was looking for a science in which the individual investigator had a more intimate, hands-on involvement with the experiments. Fortunately, Antioch had an outstanding work-study program; one quarter we studied on campus, the next was spent working on jobs related to our fields of interest. The jobs, in my case laboratory jobs, were maintained all over the country, and every three months we packed up our bags and set off for a new city and a new work experience. So one quarter off I went to Boston and the Massachusetts Institute of Technology (MIT).

There I encountered molecular biology as the field was being born (late 1950s). This was a new breed of science and scientist. Everything was new. There were no limitations. Enthusiasm permeated this field. Devotees from physics, chemistry, genetics, and biology joined its ranks. The common premises were that the most complex biological phenomena could, with persistence, be understood in molecular terms and that biological phenomena observed in simple organisms, such as viruses and bacteria, were mirrored in more complex ones. Implicit corollaries to this premise were that whatever was learned in one organism was likely to be directly relevant to others and that similar approaches could be used to study biological phenomena in many organisms. Genetics, along with molecular biology, became the principal means for dissecting complex biological phenomena into workable subunits. Soon all organisms came under the scrutiny of these approaches.

I became a product of the molecular biology revolution. The next generation. As an Antioch college undergraduate, I worked several quarters in Alex Rich's laboratory at MIT. He was an X-ray crystallographer, with very broad interests in molecular biology. While at MIT I was also fortunate to be influenced by Salvador Luria, Cyrus Leventhal and Boris Magasanik, through courses, seminars, and personal discussions. At that time Sheldon Penman and Jim Darnell were also working in Alex Rich's laboratory. When placed in the same room, these two were particularly boisterous, providing comic relief to the fast moving era.

After Antioch, I set off for what I perceived as the "Mecca" of molecular biology, Harvard University. I had interviewed with Professor James D. Watson, of "Watson and Crick" fame, and asked him where should I do my graduate studies. His reply was curt and to the point: "Here. You would be fucking crazy to go anywhere else." The simplicity of the message was very persuasive.

James D. Watson had a profound influence on my career (see Figure 6). He was my mentor. He did not teach me how to do molecular biology; because of my Antioch job experiences, I had already become a proficient experimenter. Jim instead taught me the process of science—how to extract the questions in a field that are critical to it and at the same time approachable through current technology. As an individual, he personified molecular biology, and, as his students, we were its eager practitioners. His bravado encouraged self-confidence in those around him. His stark honesty made our quest for

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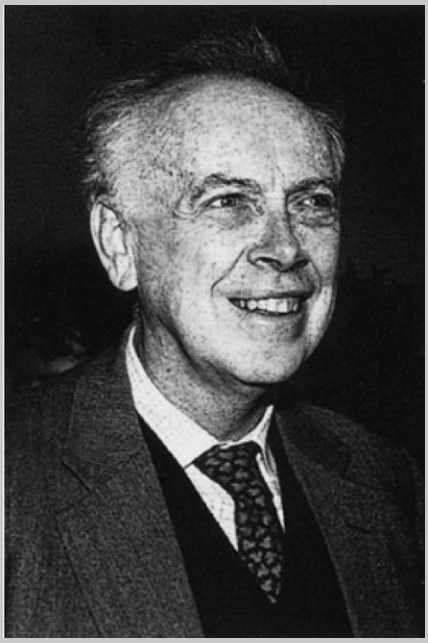

Figure 6. A photograph of James D. Watson.

truth uncompromising. His sense of justice encouraged compassion. He taught us not to bother with small questions, for such pursuits were likely to produce small answers. At a critical time, when I was contemplating leaving Harvard as a faculty member and going to Utah, he, being familiar with my self-sufficiency, counseled me that I could do good science anywhere. The move turned out to be a good decision. In Utah I had the luxury to pursue long-term projects that were not readily possible at Harvard, which, in too many cases had become a bastion of short-term gratification.

Doing science in Jim's laboratory was exhilarating. As a graduate student, I was provided with what appeared to be limitless resources. I could not be kept out of the laboratory. Ninety-hour weeks were common. The lab was filled with talented students, each working on his or her own set of projects. Represented was a mixture of genetics, molecular biology, and biochemistry. We were cracking the genetic code, determining how proteins were synthesized, and isolating and characterizing the enzymatic machinery required for transcription. At this time, Walter Gilbert was also working in Jim's laboratory. He was then a member of the physics department, but had also been bitten by the molecular biology bug. Jim and Wally complemented each other brilliantly, because they approached science from very different perspectives. Jim was intuitive, biological; Wally quantitative, with a physicist's perspective. They were both very competitive. As students, we received the benefit of both, but also their scrutiny. They were merciless, but fair. You had to have a tough hide, but you learned rigor, both with respect to your science and your presentations. Once you made it through Jim's laboratory, the rest of the world seemed a piece of cake. It was excellent training. Despite the toughness, which at times was hard, Jim was extremely supportive. He also made sure that you, the student, received full credit for your work. Despite the fact that Jim was responsible for all of the resources needed to run his laboratory, if you did all of the work for a given paper, then you were the sole author on that paper. Among all of the laboratory heads in the world, I believe that Jim Watson was among very few in implementing this policy.

The summer before I started graduate school, Marshal Nirenberg had announced that polyU directs the synthesis of polyphenylalanine in a cell-free protein-synthesizing extract. That paper was a bombshell! I decided I would generate a cell-free extract capable of synthesizing real, functional proteins. Jim's laboratory had started working on the RNA bacteriophage, R17. Its genome also served as messenger RNA to direct the synthesis of its viral proteins. That would be my message. The cell-free protein-synthesizing extract worked beautifully. Authentic viral coat protein and replicase were shown to be synthesized in the extract.[1] Further, the coat protein was functional, it bound to a specific sequence of the R17 genome, thereby modulating the synthesis of the replicase. To this day, the high affinity of the viral coat protein for this RNA sequence is exploited as a general reporter system to track RNA trafficking within living cells and neuronal axons. In collaboration with Gary Gussin, also a graduate student in Jim's laboratory, this system was used to determine the molecular mechanism of genetic suppression of nonsense mutations,[2] In collaboration with Jerry Adams, another graduate student in Jim's laboratory, the system was also used to determine that initiation of the synthesis of all proteins in bacteria proceeded through the use of formylmethionine-tRNA.[3,4] A similar mechanism is involved in the initiation of protein synthesis in all eukaryotic organisms. Finally, I used the same in vitro system to show that termination of protein synthesis unexpectedly utilized protein factors, rather than tRNA, to accomplish this end.[5,6] Jim Watson would later offer the very complimentary comment "that Capecchi accomplished more as a graduate student than most scientists accomplish in a lifetime." It was, indeed, a productive time, but it wasn't work; it was sheer joy.

While a graduate student in Jim's laboratory, I was invited to become a junior fellow of the Society of Fellows at Harvard. Being a

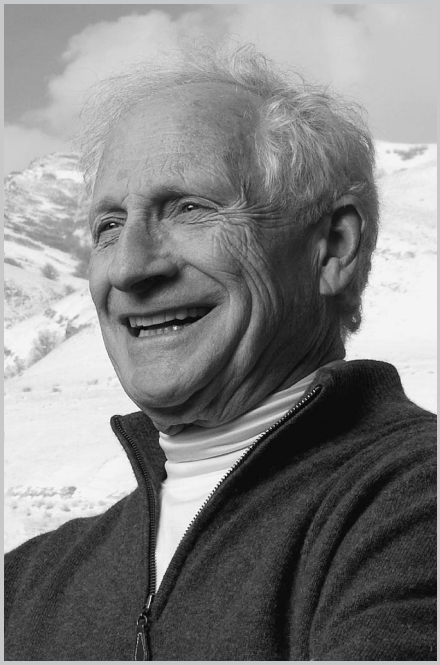

Figure 7. A photograph of Karl G. Lark.

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junior fellow was very special. The society's membership, junior and senior fellows, represented a broad spectrum of disciplines; all the members were talented, and most of them were much more verbal than I. Social discourse centered around meals, prepared by an exquisite French chef and ending with fine brandy and Cuban cigars. Frequent guests at these dinners were the likes of Leonard Bernstein. Surreal maybe, but also very special.

From Jim's laboratory, I joined the faculty in the Department of Biochemistry at Harvard Medical School, across the river in Boston. During my four years at Harvard Medical School I quickly rose through the ranks, but then, I unexpectedly decided to go to Utah. I was looking for something different. There were excellent scientists in the department I was in at Harvard Medical School, but the department was not built with synergy in mind. Each research group was an island unto itself. At that time, they were also unwilling to hire additional young faculty and thereby provide the department with a more youthful, energetic character. At the University of Utah, I would be joining a newly formed department that was being assembled by a very talented scientist and administrator, Gordon Lark (Figure 7). He had excellent taste in scientists and a vision of assembling a faculty that would enjoy working together and striving together for excellence. I could be a participant in the growth of that department and help shape its character. Furthermore, the University's administration, led then by President David P. Gardner, was in synchrony with this vision and a strong supporter. Gordon had already attracted Baldomero (Toto) Olivera, Martin Rechsteiner, Sandy Parkinson, and Larry Okun to Utah. After I arrived at Utah, we were able to bring to Utah such outstanding scientists as Ray Gesteland, John Roth, and Mary Beckerle. Utah also provided wide open space, an entirely new canvas upon which to create a new career (see Figure 8). These are views from one of the homes in Utah which I have shared with my wife, Laurie Fraser, and daughter, Misha. The air is clean, and I can look for long distances. The elements of nature are all around us. What a place to begin a new life!

Figure 8. Views from one of our homes in Utah and a photograph of my wife, Laurie Fraser, and daughter, Misha, just after she was born. Misha is now graduating from the University of California, Santa Cruz as an arts major.

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Preface

In 1996, as a Kyoto Prize laureate, I was asked to write an autobiographical sketch of my early upbringing. Through this exercise, shared by all of the laureates, the hope was to uncover potential influences or experiences that may have been key to fostering the creative spirit within us. In my own case, what I saw was that, despite the complete absence of an early nurturing environment, the intrinsic drive to make a difference in our world is not easily quenched and that given an opportunity, early handicaps can be overcome and dreams achieved. This was intended as a message of hope for those who have struggled early in their lives. As I have previously noted, our ability to identify the genetic and environmental factors that contribute to talents such as creativity are too complex for us to currently predict. In the absence of such wisdom our only recourse is to provide all children with the opportunities to pursue their passions and dreams. Our understanding of human development is too meager to allow us to predict the next Beethoven, Modigliani, or Martin Luther King.

The content of the autobiographical sketch was based on my own memories, on conversations with my aunt and uncle, who raised me once I arrived in the United States, and on conversations with my mother. Because of the added exposure resulting from the winning of the Nobel Prize, I have received letters from people who knew me in Italy during those formative early years. In addition members of the press have taken an interest in my story and have sought independent corroboration. An amazing and wonderful surprise is that they have discovered a half-sister of whom I was completely unaware. She is two years younger than I, and was given up for adoption before she was one year old. I am grateful for all of these new sources of information and revelation. Where appropriate, I will weave the new information into this retelling of my story.

Gene Targeting 1977–Present
Early experiments

My entry into what was going to become the field of gene targeting started in 1977. The size of my laboratory in Utah, devoted to this project, was modest: myself and two competent technicians—my wife Laurie Fraser, and Susan Tamowski. I was experimenting with the use of extremely small glass needles to inject DNA directly into nuclei of living cells. In the laboratory adjacent to ours, Dr. Larry Okun, a neuroscientist, was recording intracellular electrical potentials in cultured neurons from chick dorsal root ganglia. His apparatus for penetrating the cells nondestructively to measure these electrical potentials appeared to be ideal for conversion into a "microsyringe" to allow pumping of defined quantities of macromolecules, including DNA, into mammalian cells in culture. Larry (Figure 9) graciously helped me enormously with the process of conversion. I should further add that Larry Okun has been over many years, too many to count on one's fingers and toes, my favorite person to discuss science, politics, and trivia. But his rigorous insight into science, in particular, has been of immeasurable help to me throughout my tenure at the University of Utah. Having enticed me and my wife[7] to come to Utah from Boston in the first place, by organizing an unbelievably beautiful 10 day backpacking trip in the nearby Wind River Mountains of Wyoming, along a series of mountain lakes bursting with trout every evening, he owed us quite a bit, and he delivered. Once assembled, the injection apparatus (Figure 10) was quite effective, allowing me to do 1000 nuclear injections per hour of well-defined volumes of solution (in the range of femtoliters) containing chosen macromolecules.

Figure 9. A photograph of Lawrence M. Okun.

In 1977 Wigler and Axel showed that cultured mammalian cells deficient in the enzyme, thymidine kinase, Tk^-, could be restored to Tk^+ status by the introduction of functional copies of the herpes virus *thymidine kinase gene* (*HSV-tk*).[8] Although an important advance for the field of somatic cell genetics, their protocol—the use of calcium phosphate coprecipitation to introduce the DNA into the cultured cells by phagocytosis—was not very efficient. With their method, stable incorporation of functional copies of *HSV-tk* occurred in approximately one cell per million cells exposed to the DNA calcium phosphate coprecipitate.[8] It seemed that the low efficiency might be a problem of delivery. Most of the DNA taken up by the cells did not appear to be delivered to the nucleus, where it could function, but instead was destined for lysosomes, where it was degraded. I sought to determine whether I could introduce functional copies of the *HSV-tk* gene directly into nuclei of cultured TK^- cells using the microinjection apparatus described above. This procedure proved to be extremely efficient; one cell in three that received the DNA stably passed functional copies of the *HSV-tk* gene onto its daughter cells.[9] An immediate outcome of these experiments was that the high efficiency of DNA transfer that we observed by microinjection made it practical for investigators to use the same methodology to generate transgenic mice containing random insertions of exogenous DNA. This was accomplished by injection of the desired DNA into nuclei of one-celled mouse zygotes, with the resulting embryos allowed to come to term after transfer to the uterus of foster mothers.[10–14] The genera-

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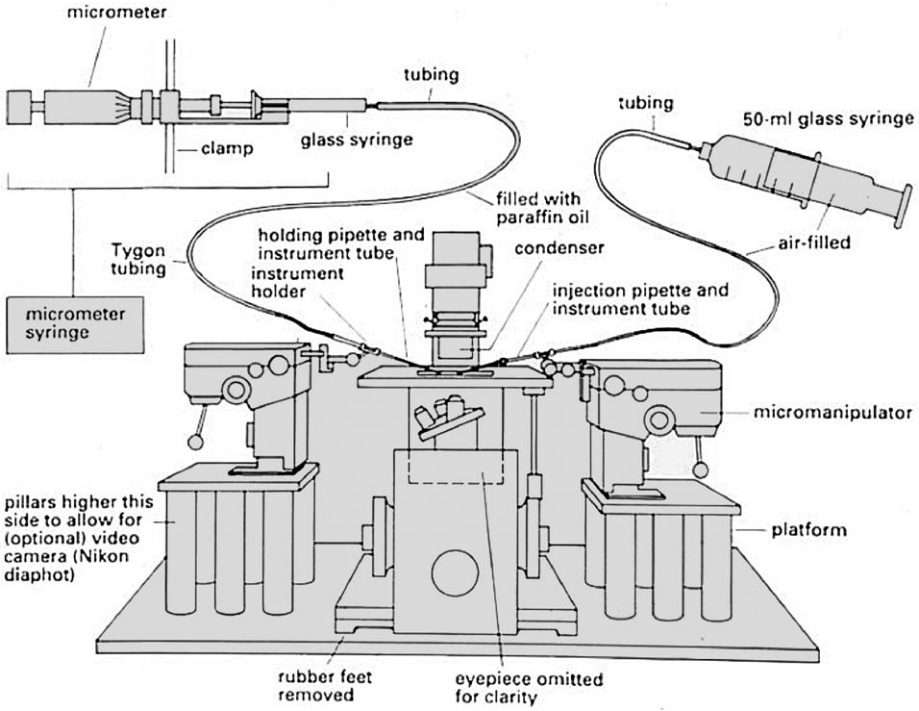

Figure 10. A schematic of the apparatus I used to inject DNA into nuclei of mammalian cells in culture. Micromanipulators are used to guide the needle, tip diameter 0.1 μm, containing the DNA solution into nuclei of living cells while being viewed through a light microscope.

tion of transgenic mice, in which chosen exogenous pieces of DNA have been randomly inserted within the mouse genome, has become a cottage industry.

However, I found that the efficient transfer of functional *HSV-tk* genes into the host cell genome required that the injected *HSV-tk* genes be linked to an additional short viral DNA sequence.[9] It seemed plausible to me that highly evolved viral genomes which, as part of their life cycle, resided in the host cell genome might contain bits of DNA sequence that enhanced their ability to establish themselves within the host cell genome. I searched the genomes of the lytic simian virus, SV40, and the avian sarcoma virus retroviral provirus for the presence of such sequences and found them.[9] When linked to the injected *HSV-tk* gene, these sequences increased the frequency with which TK^+ cells were generated by a factor of 100 over that produced by *HSV-tk* DNA injected alone. I showed that this enhancement did not result from independent replication of the injected *HSV-tk* DNA as an extra-chromosomal plasmid, but rather that the efficiency-enhancing sequences were either increasing the frequency with which the exogenous DNA was inserting itself into the host genome or increasing the probability that the *HSV-tk* gene, once integrated into the host genome, was being expressed in the recipient cells. The latter turned out to be correct. These experiments were completed before the idea of gene-expression enhancers had emerged and contributed to the definition of these special DNA sequences.[15] Further, the emerging idea of enhancers profoundly influenced our contributions to the development of gene-targeting vectors. Specifically, it alerted us to the importance of using appropriate enhancers to mediate expression of newly introduced selectable genes (used to select for successfully altered recipient cells), regardless of the inherent expression characteristics of the host chromosomal sites into which we were targeting those genes.[16]

Homologous Recombination

Although the ability to introduce exogenous DNA randomly into the host cell genome with very high efficiency by microinjection was itself extremely useful, the observation that I found most fascinating from these early DNA-injection experiments was that, when multiple copies of the *HSV-tk* plasmid were injected into a given cell, though many of them became randomly inserted into the host cell's genome, they would all be found at a single locus, as a highly ordered head-to-tail concatemer (see Figure 11).[17] This was the key observation and stimulus for the targeting project that followed.

It was clear, however, that this project would now progress more rapidly with the efforts of additional investigators. Fortunately, two very gifted postdoctoral fellows, Drs. Kim Folger Bruce and Eric Wong chose to join my group at this time (see Figure 12). It seemed that the highly ordered concatemers of exogenous genes found at the insertion sites could not arise by a random mechanism, but were likely generated either by replication of the injected DNA before insertion (for example by a rolling circle-type mechanism of DNA replication) or by

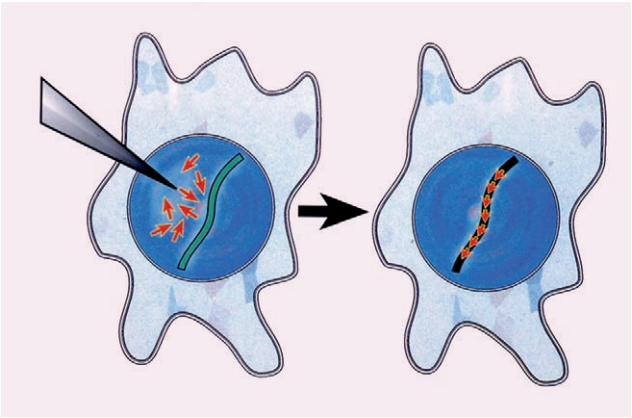

Figure 11. Formation of highly ordered head-to-tail DNA concatemers following introduction of multiple copies of the same DNA sequence into mammalian cell nuclei.

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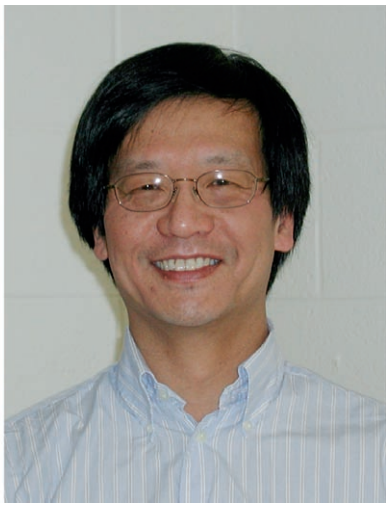

Figure 12. Photographs of Kim Folger Bruce and Eric Wong who worked in my laboratory from 1981–1985 and 1983–1986, respectively.

recombination was cell-cycle dependent, showing a peak of activity in early S phase; and that, although both reciprocal and nonreciprocal exchanges occurred, there was a distinct bias towards the latter.[22–24] These results contributed substantially to our choice of experimental design for the next stage of our quest–the detection of homologous recombination between newly introduced, exogenous DNA molecules and their endogenous chromosomal homologs in recipient cells.

In 1980, I submitted a grant application to the National Institutes of Health proposing to test the feasibility of such gene targeting in mammalian cells. These experiments were emphatically discouraged by the reviewers on the grounds that there might be only a vanishing small probability that the newly introduced DNA would ever find its matching sequence within the host cell genome, a prerequisite for homologous recombination. Despite the rejection, I decided to put all of our effort into continuing this line of research. This was a big gamble on our part. Aware that the frequency of gene targeting to homologous sites was likely to be low and that the far more common competitive reaction would be random insertion of the targeting vector into nonhomologous sites of the host cell genome, we proposed to use selection to eliminate cells not containing the desired homologous recombination events. One first test of gene targeting (Figure 14) used, as the chromosomal target, DNA sequences that we had previously randomly inserted into the host cell genome. Thus, the first step in this scheme required generating cell lines containing random insertions of a defective *neomycin-resistance gene* (*neo*^{r}) that contained either a small deletion or a point mutation in that gene. In the second step, the targeting-vector DNA also contained a defective *neo*^{r} gene, with a mutation that differed from the one present at the host cell target site. Homologous recombination, between the two defective *neo*^{r}

homologous recombination between the coinjected *HSV-tk* plasmids. We proved that they were generated by homologous recombination.[17] This conclusion was very significant because it demonstrated that mammalian somatic cells contain an efficient enzymatic machinery for mediating homologous recombination. The high efficiency of this machinery became evident from the observation that when more than 100 *HSV-tk* plasmid molecules were injected per cell, they were all incorporated into a single, ordered, head-to-tail concatemer.[17] These experiments were also the first demonstration of homologous recombination between cointroduced DNA molecules in cultured mammalian cells. From these results it was immediately apparent to me that if we could harness this efficient machinery to mediate homologous recombination between a newly introduced DNA molecule of our choice and the same DNA sequence in the recipient cell's genome, we would have the ability to mutate any endogenous cellular gene in cultured cells, in any chosen way. It was thus these experiments that provided us the incentive for vigorous pursuit of gene targeting in mammalian cells. Interestingly, these experiments were done prior to our hearing that gene targeting could be readily achieved in yeast.[18] The results derived from the analysis of mechanisms of gene targeting in yeast did, however, influence our thinking during subsequent development of gene targeting in mammalian cells.[19–21]

The next step in our quest for achieving mammalian gene targeting required our becoming more familiar with the homologous recombination machinery in mammalian cells, for example its substrate preferences and what were the most common reaction products resulting from homologous recombination. At this time Dr. Kirk Thomas also joined my research group as a postdoctoral fellow and became a critical contributor to our research (Figure 13). By examining homologous recombination between coinjected DNA molecules, we learned, among other things, that linear DNA molecules, rather than circular or supercoiled molecules were a preferred substrate for homologous recombination; that the efficiency of homologous

Figure 13. Photograph of Kirk R. Thomas who was in my laboratory from 1983–2002 first as a postdoctoral fellow and then as a Senior Scientist of the Howard Hughes Medical Institute.

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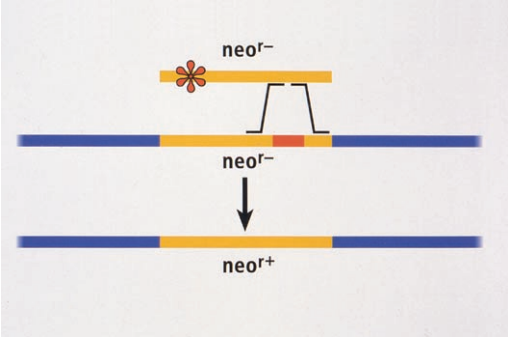

Figure 14. Regeneration of a functional *neo*^{r} gene by gene targeting. The recipient cell contains a defective *neo*^{r} with a deletion mutation (■). The targeting vector contains a 5′-point mutation (*). With a frequency of approximately 1 in 1000 cells receiving an injection of the targeting vector containing the point mutation, the chromosomal copy of *neo*^{r} is corrected with the information supplied by the targeting vector.

genes, one in the targeting vector and the second residing in the host cell chromosome, could generate a functional *neo*^{r} from the two defective parts and render the cells resistant to the drug G418, which is lethal to cells without a functional *neo*^{r} gene. Thus, successful gene targeting events would yield cells capable of growth in medium containing G418.

For the first step we generated recipient cell lines containing a single copy of the defective *neo*^{r} gene, cell lines containing multiple copies of the defective gene as a head-to-tail concatemer and, by inhibiting concatemer formation, even cell lines containing multiple defective *neo*^{r} genes as single copies inserted in separate chromosomes. These different recipient cell lines allowed us to evaluate how the number and location of the target sites within a recipient cell's genome influenced the targeting frequency. By 1984 we had good evidence that gene targeting in cultured mammalian cells indeed occurs.[25] At this time, I submitted another grant application to the same National Institutes of Health study section that had rejected our earlier proposed gene-targeting experiments. Their response was "We are glad that you didn't follow our advice."

To our delight, correction of the defective chromosomally located *neo*^{r} genes by homologous recombination with our microinjected gene-targeting vector occurred at an absolute frequency of 1 per 1000 injected cells.[26] This frequency was many orders of magnitude greater than the reversion frequency of the individual *neo*^{r} mutations by themselves. Furthermore, the frequency was not only higher than we expected, particularly considering that the extent of DNA sequence homology between the targeting vector and the target locus was less than 1000 base pairs, but the relatively high targeting frequency made it practical for us to examine a number of parameters influencing that frequency.[26] An important lesson learned from testing the different recipient cell lines was that all of the chromosomal target positions analyzed seemed equally accessible to the homologous recombination machinery, indicating that a large fraction of the mouse genome was likely to be manipulable through gene targeting.[26]

At this time, Oliver Smithies and his colleagues reported their classic experiment of targeting modification of the β-globin locus in cultured mammalian cells.[27] This elegant experiment demonstrated that it was feasible to disrupt an endogenous gene in cultured mammalian cells. Oliver and I pursued gene targeting independently. We had separate visions in mind and different approaches to its implementation. Through the years we have been extremely fortunate in our ability to share expertise and reagents, as well as enjoying each other's fellowship. That is not to say we were not competitive. Science is very competitive, and a high premium is placed on being first. Equally important, however, science is also a very communal enterprise in which all are dependent on past and concurrent contributions by many, many other investigators for advances and inspiration. Where would either Oliver's lab or mine have gotten without the ability to generate viable mouse chimeras, initially starting with mouse morulas, and then extending the technology to injected cells from the inner cell mass, EC cells, and ES cells into the preimplantation embryos? The contributions and progression of this technology by Mintz, Gardner, Stevens, Martin, and Evans are apparent,[28–34] providing just some examples of the many investigators whose efforts have been essential to our eventual ability to do gene targeting in living mice.

Having established that gene targeting could be achieved in cultured mammalian cells and having determined some of the parameters that influenced its frequency, we were ready to extend gene targeting to the living mouse. The low frequency of gene targeting, relative to random integration of the targeting vector into the recipient cell genome made it impractical to attempt gene targeting directly in one-celled mouse zygotes. Instead, it seemed our best option was to carry out the gene targeting in populations of cultured embryo-derived stem (ES) cells, from which the relatively rare targeted recombinants could then be selected and purified. These purified cells, when subsequently introduced into preimplantation embryos and allowed to mature in a foster mother, would be expected to contribute to the formation of all tissues of the mouse, including the germ line. Fortunately, at a Gordon Conference in the summer of 1984, when we were ready to initiate these experiments, I heard that Martin Evans had isolated from mouse embryos ES cells capable of contributing in just this way to the formation of the germ line and to do so at a reasonable frequency.[33,34] Martin's ES cells appeared to be much more promising in their potential to contribute to the embryonic germline than were the previously described embryonal carcinoma (EC) cells.[30,35]

Gene targeting in ES cells

In the winter of 1985, my wife, Laurie Fraser, and I spent a week in Martin Evans' laboratory learning how to derive and culture mouse ES cells, as well as how to generate mouse chimeras from these cells by their addition to recipient preimplantation embryos. Also instrumental in our learning these techniques were Dr. Elizabeth Robertson and Alan Bradley, a postdoctoral fellow and graduate student in Evans' laboratory, respectively. This is an excellent example of how science pro-

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gresses from the collective sharing of expertise and resources. We have always been grateful for Martin's generosity.

In the beginning of 1986, our effort thus shifted to doing gene-targeting experiments in mouse ES cells. We decided also to switch to electroporation as a means of introducing our targeting vectors into ES cells. Although microinjection was orders of magnitude more efficient than electroporation as a means for generating cell lines with targeted mutations, injections are done one cell at a time. With electroporation, we could introduce the gene-targeting vector into 10^7 cells in a single experiment, easily producing large numbers of cells containing targeted mutations, even at the lower efficiency. In addition, it was apparent to us that, as a technology, electroporation would be more readily adopted by other laboratories, relative to microinjection, thereby making gene targeting more user friendly to more scientists.

To rigorously determine the quantitative efficiency of gene targeting in ES cells as well as to evaluate the parameters that affect the gene targeting frequency, we chose as our target locus the *hypoxanthine phosphoribosyl transferase* (*hprt*) gene. There were two reasons for this choice. First, since *hprt* is located on the X chromosome, and the ES cell lines that we were using were derived from a male mouse, only a single *hprt* locus had to be disrupted in the recipient cells to yield hprt$^-$ cell lines. Second, a good protocol for selecting cells with a disrupted *hprt* gene existed, based on the drug 6-thioguanine (6TG), which kills cells with a functional *hprt* gene.[36] The strategy we used was to generate a gene-targeting vector that contained an *hprt* genomic sequence that was disrupted by insertion of *neo*^{r} in one of the gene's exons (Figure 15). The exon we chose, exon 8, encodes the active catalytic site for this enzyme. Homologous recombination between this targeting vector and the endogenous *hprt* locus would generate hprt$^-$ ES cells resistant to growth in medium containing both 6TG (killing cells with untargeted hprt$^+$ loci) and G418 (killing cells lacking the inserted *neo*^{r} gene, as described above). All cell lines generated from cells selected in this way, had lost *hprt* function as a result of the targeted disruptions of the *hprt* locus.[16] Thus, the *hprt* locus provided Chuxia Deng (Figure 16),

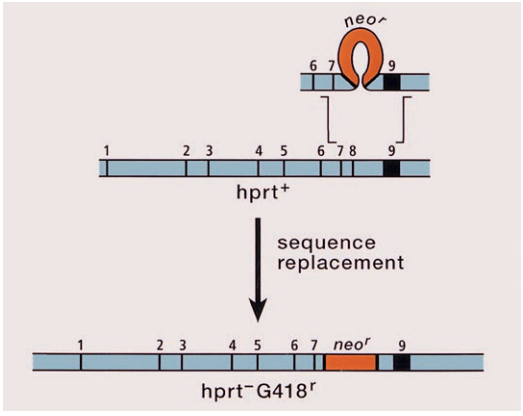

Figure 15. Disruption of *hprt* gene by gene targeting in mouse ES cells. The targeting vector contains genomic sequences from the mouse *hprt* gene disrupted in the eighth exon by *neo*^{r}. After homologous pairing between the vector and the cognate sequences in the endogenous *hprt* gene of the ES cell genome, a homologous recombination event replaces the ES cell genomic sequences with vector sequences containing the *neo*^{r} gene. The resulting cells are able to grow in medium containing the drugs G418, which kills cells without an inserted functional *neo*^{r} gene, and 6-TG, which kills cells with an undisrupted functional *hprt* gene.

Figure 16. A photograph of Chuxia Deng who worked in my laboratory as a graduate student from 1987–1992.

then a graduate student in our laboratory, an ideal locus for further study of the parameters that influenced the targeting efficiency.[37–39]

Because we foresaw that the *neo*^{r} gene would probably be used as a positive selectable gene for the disruption of many genes in ES cells, it was essential that its expression be mediated by an enhancer that would function in ES cells, regardless of the expression status of the target locus. Here our previous experience with enhancers proved of value. We knew from those experiments that the activities of promoter-enhancer configurations are very cell-type specific. To encourage strong *neo*^{r} expression in ES cells, we chose to drive its expression with a mutated polyoma virus enhancer that had been selected for strong expression in mouse embryonal carcinoma cells, which we presumed to be similar to mouse ES cells.[16,40] Subsequently, the strategy described above, namely using a *neo*^{r} driven by an enhancer that allows strong expression in ES cells independent of chromosomal locations, has become the standard for the disruption of most genes in ES cells.

The experiments described above showed that mouse ES cells were a good recipient host for gene targeting. In addition the drug-selection protocols required to identify ES cell lines containing the desired gene-targeting event did not appear to alter their pluripotent potential.[16] I believe that this paper was pivotal in the development of the field, encouraging other investigators to begin to use gene targeting in mice as a means

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for determining the function of chosen mammalian genes in the living animal.

The ratio of homologous, that is, targeted insertions, to random insertions at nonhomologous sites in ES cells is approximately 1 to 1000.[16] Because the disruption of most genes does not produce a cellular phenotype that is selectable in cell culture, investigators seeking to disrupt a gene of choice would need to undertake tedious DNA screens through many cell colonies to identify the rare ones containing the desired targeting event.

To address this problem we reported in 1988 a general strategy to enrich cells in which a homologous targeting event has occurred.[41] This enrichment procedure, known as positive–negative selection, was derived from an observation in experiments done in our laboratory, namely that linear DNA molecules, when inserted at random sites in the recipient cell's genome, most frequently retain their ends, while sequences inserted at the target site, by homologous recombination, lose nonhomologous ends from the original vector (see Figure 17). Further, contrary to expectations from studies of homologous recombinations in yeast, we showed that even blocking both ends of the homology arms of a targeting vector with nonhomologous DNA sequences does not reduce the targeting frequency in mammalian cells.[41] This approach correspondingly has two components. One component is a positive selectable gene, *neo*^{r}, used, as described above, to select for recipient cells that have incorporated the targeting vector anywhere in their genomes (that is, at the target site by homologous recombination or at a random site by nonhomologous recombination). The second component is a negative selectable gene, *HSV-tk*, located at the end of the linearized targeting vector and used to select against cells containing random insertions of the target vector (medium containing the drugs, *gangcyclovir* or FIAU, kills cells expressing the *HSV-tk* gene but not cells expressing the endogenous mammalian thymidine kinase gene). Thus the positive selection enriches for recipient cells that have incorporated the targeting vector somewhere in their genome, whereas the negative selection eliminates those that have incorporated it at nonhomologous sites. The net effect is enrichment for cells in which the desired homologous targeting event has occurred. The strength of this enrichment procedure is that it is independent of the function of the gene that is being disrupted and succeeds whether or not the gene is expressed in ES cells. The validity of the procedure was shown by using it to enrich for ES cells containing targeted mutations in the *int2* gene, now known as *Fgf3*.[41] These experiments were carried out by Suzi Mansour, a talented postdoctoral fellow in our laboratory (Figure 18) and Kirk Thomas

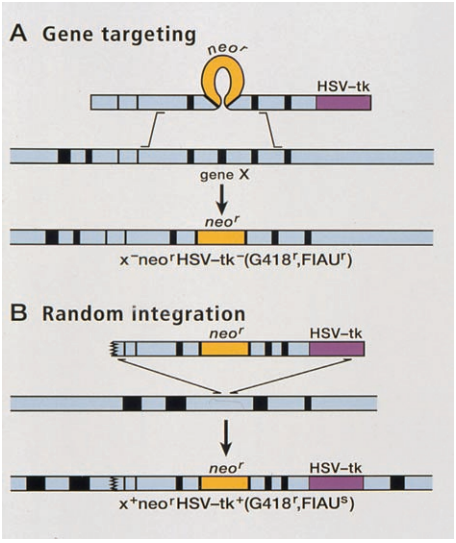

Figure 17. A) The positive–negative selection procedure used to enrich for ES cells containing a targeted disruption of gene *X*. The linear replacement-type targeting vector contains an insertion of *neo*^{r} in an exon of gene *X* and a linked *HSV-tk* gene at one end. It is shown pairing with a chromosomal copy of gene *X*. Homologous recombination between the targeting vector and the cognate chromosomal gene results in the disruption of one genomic copy of gene *X* and the loss of the vector's *HSV-tk* gene. Cells in which this event has occurred will be *X*$^{+/-}$, *neo*^{r}, *HSV-tk*$^-$ and will grow in medium containing G418 and F1AU. The former requires the presence of a functional *neo*^{r} gene and the latter kills cells containing a functional *HSV-tk* gene. B) Integration of the targeting vector at a random site of the ES cell genome by nonhomologous recombination. Because nonhomologous insertion of exogenous DNA into the chromosome occurs through the ends of the linearized DNA, *HSV-tk* will remain linked to *neo*^{r}. Cells derived from this type of recombination event will be *X*$^{++}$, *neo*$^{r+}$, and *HSV-tk*$^+$ and therefore resistant to growth in G418 but killed by presence of F1AU. Cells that have not received a targeting vector, will be *X*$^{++}$, *neo*$^{r-}$, and *HSV-tk*$^-$ and will be killed by the presence of G418. As a consequence this procedure specifically enriches for cells in which a gene targeting event has occurred.

Figure 18. A photograph of Suzanne Mansour. She worked in my laboratory as a postdoctoral fellow from 1987–1992.

(Figure 12). Positive–negative selection has become the most frequently used procedure to enrich for cells containing gene-targeting events. Using positive–negative selection we have found that the targeting frequency varies from gene to gene. With genes that exhibit a high targeting frequency, a high percentage of clones obtained after positive–negative selection contain the targeting event. The worst cases have been ones in which one in a hundred selected clones contains the desired targeting event. If the targeted gene is one expressed in ES

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cells, then the targeting frequency at that locus is likely to be high.

Extensions and more recent developments

The use of gene targeting to evaluate the functions of genes in the mouse is now routine. It is being used in hundreds of laboratories all over the world. Well over 11 000 genes have been disrupted in the mouse by using the described procedures. This is quite surprising considering that these disruptions have been done in individual laboratories in the absence of coordinated programs. Now, however, there are a number of funded national and international efforts to disrupt every gene in the mouse by gene targeting.[42] In addition, hundreds of human diseases have been modeled in the mouse by the use of gene targeting. These models allow study of the pathology of the diseases in much more detail than is possible in humans. In addition, the models provide a vehicle for subsequent development and evaluation of new therapeutic modalities including drugs.

To date, gene targeting has been used primarily to disrupt genes, producing so-called "knockout mice." However gene targeting can be used to alter the sequences of a chosen genetic locus in the mouse in any conceivable manner, thus providing a very general means for "editing" the mouse genome. It can be used to generate gain-of-function mutations or partial loss-of-function mutations. Gene targeting can also be used to restrict the loss of function of a chosen gene to particular tissues, yielding so-called conditional mutations. This is most commonly achieved by combining exogenous (nonmammalian) site-specific recombination systems, such as those derived from bacteriophages or yeast (that is, *Cre*/*loxP* or *Flp*/*FRT*, respectively), with gene targeting to mediate excision of a gene only where the appropriate recombinase is produced.[43–46] By control of where Cre- or Flp-recombinase is expressed, for example in the liver, a gene, flanked by *loxP* or *FRT* recognition sequences, respectively, can be excised in the desired tissue (for example, liver). Temporal control of gene function has also been achieved by making the production of the functional recombinase dependent upon the administration of small molecules or even on physical stimuli, such as light.[47–50] Such conditional mutagenesis has been very effective for more accurate modeling of human cancers, which are often restricted to particular tissues and even to specific cells within those tissues, as well as being initiated post birth.[51–54] In human cancers, the interactions between the host tissues and the malignant cells are often critical to its initiation and progression.[55,56] Thus, inclusion of these interactions in the mouse model also becomes critical if the mouse model is to accurately recapitulate the human malignancy.

Gene targeting is an evolving technology and we can anticipate further extensions to its repertoire. To date it has been used primarily to perturb the function of one gene at a time. We can anticipate development of efficient multiplexing systems that will allow simultaneous conditional or unconditional modulation of multiple genes. We can also anticipate improvements in exogenous reporter genes with parallel improvements in their detection, particularly with respect to capture times, resolution and sensitivity. Such improvements will undoubtedly be necessary if this technology is to make significant inroads in addressing truly complex biological questions, such as the molecular mechanisms underlying higher cognitive functions in mammals.

I have tried to take the reader through a brief, personal journey of my life, my development as a scientist, and our laboratory's development of gene targeting. In the process, I have tried to give credit to those who have helped me along the way to reach our goals. What I have failed to communicate is the enormity of the scientific community and how many scientists actually have helped in untold, countless ways. That list would be in the hundreds and thousands. As a scientist I have been fortunate to have visited many, many laboratories all over the world and to have talked with other scientists about their work and aspirations. It is through these conversations that one's vision broadens and an appreciation of the complexity and beauty of the biological world is reinforced. However the people that have been most influential are the members of my immediate family, Laurie Fraser and Misha Capecchi, my wife and daughter, respectively. Their support has kept me going, their sage advice has kept me from falling down too frequently, their love has made it all worthwhile.

The Nobel Prize has greatly rewarded a major segment of my life and, as a kind of demarcation invites some reflection. I hope that our contributions, among other developments, will be used by many to reduce suffering, improve our health and extend the productivity and fulfillment of our lives. Equally important, I hope that the new biological insights will yield a better understanding of ourselves as human beings and of our relationship to our environment, so that we may become better stewards of a fragile Earth. We live in a closed system and we have to gain the knowledge that will enable us to live in harmony with it. Neither we nor our planet can any longer afford the ravages of wars. Nor can the planet survive needless consumption. We must learn to distribute our resources more equitably among all peoples. As a scientist, I naturally find myself thinking about the future. As a people, we must learn to become more responsible for the consequences of our activities over much longer periods of time so that future generations may also enjoy this splendid world. It is my hope that science can combine with ethics to permit this.

Keywords: DNA · embryonic stem cells · gene targeting · homologous recombination · Nobel lecture

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[7] The reference here is to my first wife, Nancy McReynolds. She worked with me at Harvard and then helped me set up my laboratory in Utah. In addition to being my companion, Nancy was a critical contributor to my work before gene targeting. She is now an outstanding geriatric nurse and we are still good friends.

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