

OCTOBER 08, 2007

The Making of a Scientist

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Mario Capecchi received a Kyoto Prize from the Inamori Foundation in 1996. The lecture he delivered when he accepted the prize in Japan in November 1996 tells the story of his remarkable life. The text of the lecture has had to be edited for length.

I hope that the title of this lecture, "The Making of a Scientist," is not misleading. I have no formula for generating creative scientists. To the contrary, my thesis will be that such a formula may not exist. My skepticism comes from a deep-rooted prejudice that creativity in science, or in any other discipline, may require the abrasive juxtaposition of unique sets of life experiences that are too complex to preorchestrate. It is in this spirit that I will share with you my own experiences as a tribute to such stochastic, chaotic influences. The only general interest of this story is that it exemplifies the antithesis of a nurturing environment, which all of us deeply want to believe is a conducive prerequisite for fostering thoughtful, creative human beings.

I was born in Verona, Italy on October 6, 1937. Fascism, Naziism and Communism were raging through the country. My mother was a poet, my father an officer in the Italian air force. This was a time of extremes and the juxtaposition of opposites. They had a passionate love affair and my mother wisely chose not to marry him. Considering the times, this took a great deal of courage on her part.

My mother was a beautiful woman with a passion for language and a flair for the dramatic. She grew up in a villa in Florence, Italy—magnificent gardens, a nanny, gardeners, cooks, house cleaners and private tutors for languages, literature and sciences. She was fluent in half a dozen languages. Her father was an archeologist born and trained in Germany. Her mother was a painter born and raised in Oregon, USA...

My mother's love 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 Naziism. In 1937 she moved to [a chalet in] the Tyrol, the Italian Alps...In the spring of 1941 the Gestapo and SS officers came to our home and arrested my mother. This is one of my earliest

memories. Although I was only 3-1/2, I sensed that I would not see her for many years. She was incarcerated as a political prisoner in Dachau, Germany...

My mother anticipated her arrest by the Gestapo. Prior to their arrival, she 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. In the late fall, the grapes were harvested by hand and put into enormous vats. The children, including me, stripped, jumped into the vats and became squealing masses of purple energy. I still remember the pungent odor and taste of the fresh grapes.

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.

In the spring of 1945, Dachau was liberated by the American troops. My mother survived the concentration camp and set out to find me...As a reminder of her flair for the dramatic, she found me on my ninth birthday. I am sure 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 reason: malnutrition. The prospects for most of those children 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 approximately one 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. We lay naked on those stripped beds. 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.

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

My mother and I lived with my Uncle Edward and his wife Sarah. Edward Ramberg, my mother's younger brother, was a brilliant physicist. Among his

many contributions was his discovery of how to focus electrons, knowledge which he used to build the first electron microscope. His books on electron optics have been published in many languages, including Japanese. Another achievement of which he was less proud was being a principal contributor to the making of both black and white, and color televisions. While I grew up in his home, television was not allowed...

My aunt and uncle were Quakers, and could not support violence anywhere in the world. During World War II, Edward did alternative service. He worked in mental institutions, cleared swamps in the South and was a guinea pig for the development of vaccines against tropical diseases. After the war, he lived on a commune in Pennsylvania, which he helped found...

Sarah and Edward took on the challenge of converting me into a productive human. This was a formidable task. I had received no formal education or training for living as a social being. Quakers do not believe in frills but rather in a life of service. My aunt and uncle taught by example. I was given few material goods but every opportunity to develop my mind and soul. What I made of myself was entirely up to me. The day after I arrived in America, I went to school. I started in third grade. I was a good, but not serious student in grade school and high school. I attended an excellent high school, a Quaker school north of Philadelphia, George School. The teachers were superb, caring, challenging and enthusiastic.

Sports were very important to me in high school. I played football, soccer, baseball and I wrestled. I was particularly proficient at wrestling: I enjoyed both the psychological and physical challenges of the game. At Antioch College, I became a serious student, converting all of my energy previously devoted to sports to academics. I first majored in political science in the hope of using this knowledge as a basis for the betterment of humankind. However, I soon became disillusioned with this field and switched to the physical sciences. I found great pleasure in the simplicity and elegance of mathematics and classical physics... Fortunately, Antioch had a work-study program and every three months we packed up our bags and set off for a new city and a new work experience. So off I went to Boston and M.I.T.

There, I encountered molecular biology. This was a new breed of science and scientist. Everything was new. There were no limitations. Enthusiasm permeated this field. Devotees from physics, chemistry and biology joined its ranks. The common premise was that the most complex biological phenomena could, with persistence, be understood in molecular terms and that biological phenomena observed in simple systems such as viruses and bacteria were mirrored in more complex organisms. An implicit corollary to this premise was that whatever we learned in one organism was directly relevant to all others, and that similar approaches could be used to study biological phenomena in all organisms. Genetics, along with molecular biology, became the principal means for dissecting complex biological phenomena into workable subunits. Initially, the focus of molecular biologists was bacteria and bacteria viruses. Soon all organisms came under its scrutiny.

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

Professor James D. Watson had a profound influence on my career. He personified molecular biology and his students were its eager practitioners. His bravado encouraged self-confidence in those around him. His stark honesty made our quest for 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...

Doing science in Jim's laboratory was a blast. As a graduate student, I was provided with what appeared to be limitless resources. I could not be kept out of the lab. Ninety-hour weeks were common. We were cracking the genetic code, determining how proteins were synthesized and isolating the enzymatic machinery required for transcription. At this time, Walter Gilbert also was working in Jim's laboratory. He was a member of the physics department, but was bitten by the molecular biology bug. Jim and Wally complemented each other brilliantly, because they approached science from very different perspectives. Jim was intuitive, Wally quantitative. As students, we received the benefits of both.

From Jim's laboratory, I joined the faculty, in the Department of Biochemistry at Harvard Medical School. I stayed there for four years, then decided to go to Utah. This was an unexpected move, but I enjoyed the maverick reputation acquired by moving west. Utah provided wide open space and an entirely new canvas upon which I could create a new career...

My entry into what was going to become the field of gene targeting started in 1977. I was experimenting with the use of extremely small glass needles to inject DNA directly into the nuclei of living cells. The needles were controlled by hydraulically driven micromanipulators and were directed into nuclei with the aid of a microscope. Using this experimental paradigm, I asked whether I could introduce a functional gene into cells by injecting the DNA directly into their nuclei. This procedure turned out to be extremely efficient. One in three cells received the DNA in functional form and went on to divide and stably pass the gene on to its daughter cells. The high efficiency of microinjection meant that it was now practical to use this technology to generate transgenic mice by the injection of DNA into one-cell zygotes which were then allowed to come to term as embryos in foster mothers...

An observation that I personally found fascinating from these early microinjection experiments was that when multiple copies of a DNA segment were injected into a cell, although they were integrated into a random location within the host chromosome, they were always present in head-to-tail concatemers. Such highly ordered concatemers could be generated in two ways: (1) By replication, for example by a rolling circle type mechanism; or (2) by homologous recombination. We were able to prove that

the concatemers were generated by homologous recombination. The significance of this observation was its demonstration that mammalian cells contain an efficient machinery for mediating homologous recombination. At the time, this was a startling discovery, because it was always assumed that the function of homologous recombination in all organisms was to ensure broad dissemination of the parental genetic traits to their offspring by shuffling these traits in the germ cells. Finding evidence for this activity in mouse fibroblast cells implied that all cells, somatic as well as germ cells, were capable of mediating homologous recombination. The machinery in somatic cells appeared to be very efficient, since I could inject over one hundred copies of a DNA sequence into a cell nucleus and they were all neatly incorporated into a single ordered head-to-tail concatemer. I realized immediately that, if I could harness this machinery to carry out homologous recombination between a newly introduced DNA molecule of our choice and the same DNA sequence in the cell's chromosome, I would have the ability to mutate at will any specific gene in the living cell...

We spent the next few years in my laboratory becoming familiar with the machinery that mediates homologous recombination in mammalian cells in order to determine its likes and dislikes so that we could exploit this machinery for our purpose of gene targeting. In 1980, I submitted an NIH grant application outlining experiments intended to test the feasibility of gene targeting in mammalian cells. This part of the grant was soundly rejected. In the opinion of the reviewers, the probability that the newly introduced DNA would ever find its matching sequence within the host genome was vanishingly small and, therefore, the experiments were not worthy of pursuit. Despite this rejection, I decided to forge ahead with these experiments using a paradigm that was capable of detecting gene targeting events at a very low frequency. Once we observed a gene targeting event, we could optimize the conditions to improve its efficiency. By 1984, we were confident that it was feasible to do gene targeting in cultured mammalian cells and I presented our work at a memorable symposium on homologous recombination held at the Cold Spring Harbor Laboratory. We resubmitted our grant application to the NIH. This time, the grant proposal was received with enthusiasm, and the new critique started with the words, "We are glad that you didn't follow our advice."

The next question we pursued was whether gene targeting could be extended to a whole animal, i.e., the mouse. Because of the low frequency of targeting events in mammalian cells, it was clear that doing the experiments directly in mouse zygotes would not be practical. Rather, targeting events had to be identified first in cultured cells to allow purification of a clonal cell line containing the desired gene disruption; these cells in turn could be used to generate mice capable of transmitting the mutation in their germline. I was familiar with the frustrations associated with previous attempts to obtain germline chimeras using embryonal carcinoma (EC) cells. However, in the summer of 1984, I heard at a Gordon Conference a discussion of EK cells that appeared to be much more promising in their potential for contributing to the germline. EK cells, which were developed in Martin Evans' laboratory in Cambridge, England, differed from EC cells in that they were obtained from

the early mouse embryo, rather than from mouse tumors.

By early 1986, our total efforts were focused on EK cells, now known as ES cells... The gene that we chose to disrupt in ES cells was *hprt*, because it provided us with the luxury of being able to select directly for cells containing the desired disrupted gene... [Our] experiments showed that ES cells were indeed able to mediate homologous recombination, and that the selection protocols required to identify cell lines containing the targeted disruption did not alter their pluripotent state in culture. This system also provided a good experimental paradigm for exploring the parameters that affect the efficiency of gene targeting. I believe that this study played a pivotal role in the development of the field by encouraging other investigators to begin using gene targeting as a means of determining the function of genes in mammals.

Although mammalian cells have the machinery to direct the newly introduced DNA to its endogenous target, we found that the targeting vector was also inserted into random sites within the host genome by nonhomologous recombination. The ratio of homologous to nonhomologous recombination events is approximately one to one thousand. Since disruption of most genes is not expected to produce a phenotype selectable at the cellular level, an investigator seeking a specific disruption must either screen through many colonies of cells in order to identify the rare colony that contains the desired targeting event, or use selections that enrich for cells containing the targeting event. Late in 1986, I conceived of a general strategy to enrich for cells in which a targeting event had occurred. It was based on key observations made during our studies of recombination involving exogenous DNA in mammalian cells. Incorporation of DNA segments at random nonhomologous sites involves insertions of a linearized vector through its ends, whereas recombination at a homologous target site involves crossover events occurring only through homologous sequences in the vector. Our strategy based on these observations, and known as positive-negative selection (PNS), uses two components. One component is a "positive selectable" gene, *neor*, used as a marker to select for cells that have incorporated the targeting vector anywhere in the recipient cell genome (i.e., at the target site via homologous recombination or at random sites via non-homologous recombination). The second component is a "negative selectable" gene, located at one end of the linearized targeting vector, used to select against cells that contain random insertions of the targeting vector. The net effect is to enrich 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 and succeeds whether or not the gene is expressed in the recipient ES cells. PNS is now the most frequently used procedure for enrichment of cells containing gene targeting events. The origin of the idea for positive-negative selection was not very romantic and involved purely deductive reasoning. Enrichment for cells containing the targeting event could be achieved either by direct selection for cells containing the targeting event or by elimination of cells that contained random insertions of the targeting vector. This fact, coupled with an appreciation of how information is transferred between an exogenous and an endogenous DNA sequence via homologous and nonhomologous

recombination, naturally led to the concept of positive-negative selection...

The use of gene targeting to evaluate the functions of genes in the living mouse is now a routine procedure. It is very gratifying to be able to pick up almost any major journal in the biological sciences and find the description of yet another gene "knockout" mouse. In the past five years, the in vivo functions of over two hundred fifty genes have been determined with this approach. It is relatively easy to project where gene targeting technology will go in the near future. It will continue to serve as the way to determine the roles of individual genes in mammalian biology. This will be accomplished by the generation of null mutations knocking out the genes of interest. Those investigators who desire deeper insights will generate an allelic series of mutations in a chosen gene to evaluate the effects of partial loss-of-function as well as gain-of-function mutations. To permit evaluation of potential multiple roles of a gene in different tissues, gene targeting will be used to engineer tissue-specific gene disruptions using the cre/loxP system.

Further, the technology soon should become available that will allow the investigator to turn chosen genes on or off in the adult or during any phase of mouse development. Finally, since most biological processes are mediated by interactions among a number of genes, such phenomena will be studied by combining multiple targeted mutations in a single mouse. There is no question that the mouse is a very complex organism. However, the broad range of genetic manipulations that are now available through gene targeting should provide a means for us to begin deciphering even the most complex of biological processes including development and learning.

I have taken you on a brief journey through my experiences. Looking back, I marvel at the resilience of the child. As I lay naked on that stripped bed so many years ago, my constant preoccupation was fixed on plotting an escape. In the absence of any apparent hope, the will to survive persists.

It is not clear whether those early childhood experiences contributed to whatever successes I have enjoyed or whether those achievements were attained in spite of those experiences. When dealing with human life, we cannot do the appropriate controls. Could such experiences have contributed to psychological factors such as self-reliance, self-confidence or ingenuity? I have always considered as a personal strength the ability to concentrate for long periods of time on a chosen topic at the exclusion of everything else that is going on around me. Is the ability to participate in such mental exercises learned from experiences of prolonged preoccupation?

What I have learned from my own experiences is that the genetic and environmental factors that contribute to such talents as creativity are too complex for us to currently predict. In the absence of such wisdom, our only course is to provide all of our children with ample opportunity to pursue their passions and their dreams. Our level of understanding of human development is too meager to allow us to foresee which of the children in our midst will be the next Beethoven, Modigliani or Martin Luther King.