The first transgenic mice: an interview with Mario Capecchi

Mario Capecchi recently won the Nobel Prize for contributions to genetics that have catapulted the mouse to the status of the most valuable of all animal models. He has a personal story that is as rich and interesting as his science. Here, he discusses the journey that led him to gene targeting and his vision for the future.

The ability to remove or mutate genes to assess their function has forever changed the fields of biology and medicine. A pioneer of this technology, Mario Capecchi was shaped by a unique childhood that taught him persistence and self-reliance. He was born in Italy during World War II. When he was only 4 years old, his American mother was imprisoned in a German concentration camp and soon after he found himself homeless and fending for himself on the streets of Italy. After 4 years alone, he was reunited with his mother and they moved to the USA. He quickly developed a passion for physics and his early work with James Watson further influenced his scientific mind. His discoveries leading to gene targeting are rapidly unlocking the mysteries of the mammalian genome.

In 2007 you won the Nobel Prize for your role in developing the process of gene targeting, in which homologous recombination is used to replace an endogenous gene with one that has been modified. What inspired you to approach such a monumental challenge?

There was a paper by Wigler and Axel [in *Cell*, 1977] in which they formed a precipitate of DNA and calcium phosphate on top of cells and found that the cells took up the DNA. These cells started out lacking a particular gene that was necessary for survival in a chosen medium, but if the cells stably incorporated the gene into their genome then they survived; the efficiency was roughly one cell in every million. Further, they showed that the added DNA was randomly incorporated into the host cell genome.

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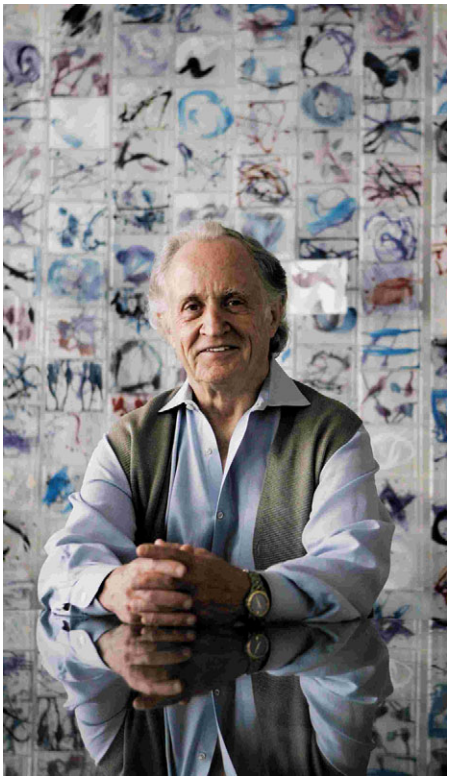
My thought was that the cells were using endocytosis, where cell membranes engulf exogenous material, to internalize the DNA. Cells usually do this with the intent of shuttling the internalized material to lysosomes to degrade it, and then use whatever is useful to them. So, my thinking was that most of the DNA in those early experiments ended up being degraded and that very little of it was delivered to the nucleus where it could function. I thought that if I made very small hypodermic needles and stuffed the DNA directly into the nucleus itself maybe the efficiency would be much higher. I did it, and it turned out that efficiency was much higher. Now one in every three cells, rather than one in a million cells, incorporated the gene into its genome in a functional form. That was interesting and important, but didn't answer the question of how the DNA was inserted into the genome.

Wigler and Axel were using a lot of DNA, and what they did was mix plasmid DNA containing their gene of interest with salmon sperm carrier DNA, which was cheap to buy, to get the DNA concentration high enough to form the precipitate. Because I was injecting individual molecules, I could choose to put in 1, 10 or 100 molecules. When I put the needle into the nucleus, the solution that I was injecting had a distinct refractive index so I could watch the solution spread out in the nucleus. I used that information to measure the volume I was adding to the cells. So, I could control how much I was injecting and then I could also control the concentration of DNA that I was injecting and, consequently, how many molecules were being injected. I found that the DNA was randomly incorporated into the genome, but if I put in 10 copies or 100 copies, or even 1000 copies, of the same DNA sequence into the nucleus, all of the molecules were



inserted into the genome in the same orientation. DNA has an orientation, just like reading a text. If I started by putting all of the separate DNA copies into the nucleus and they ended up incorporated into the genome at a single locus in the same orientation, then this process couldn't happen randomly.

One possibility is that the cell picks up a piece of DNA and uses it as a template, like a sausage machine, to synthesize more copies of the DNA in a process that would end up producing DNA copies all in the same orientation. This would be a synthetic way of generating the observed head-to-tail concatamers. The other possibility was that the DNA copies are put together by homologous recombination, a naturally oc-

curring process in which similar DNA sequences on chromosomes can exchange information with each other. Since homologous recombination always maintains sequence orientation, the DNA that has been newly inserted by homologous recombination would also be in the same orientation. I could readily distinguish between these two potential models and showed that head-to-tail concatamers were generated by homologous recombination.

At that time, people knew about the process of homologous recombination but they thought it was primarily restricted to myosis (i.e. during the formation of sex cells, sperm and eggs). When sperm and egg come together you have chromosomes derived from your mother and father, but these chromosomes are not intact chromosomes derived from one maternal or paternal grandparent. Rather, the chromosomes that you received from your mother or father are extensive mixtures derived from both of your maternal or paternal grandparents, respectively. The extensive exchanges between the pairs of chromosomes derived from your grandparents are mediated by homologous recombination. This process generates much, much greater variation in the DNA content that is present in each of your children than if your sperm or egg contained intact copies of chromosomes obtained from one grandparent or the other. This shuffling insures that each of your progeny gets an extensive and unique mixture of DNA derived from each set of grandparents.

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As I mentioned before, people knew that homologous recombination occurred during the formation of sperm and eggs, but I was working with somatic cells derived from skin fibroblasts. Scientists were not aware that extensive homologous recombination also takes place in somatic cells. I showed that the homologous recombination machine was present in somatic cells and that it was actually very efficient, because even if I put in as many as 1000 copies of the same DNA molecule into a cell, they were all incorporated into a single head-to-tail concatamer, all lined up one after the other in the same orientation.

The reason that somatic cells use homologous recombination is that every day each of your cells receives about 10,000 insults to its DNA. The insults arise from oxygen radicals produced in the cells, or from sunlight, or from everything else that's happening to your poor cells. Often, a DNA strand gets broken. When it breaks, you not only lose the gene at the breakage point, but upon cell division you lose all the genes that are no longer associated with a centromere. The first thing a cell wants to do is stick those two pieces of DNA back together so that, rather than losing a thousand genes, it just loses one gene. Once that is done, the damaged DNA at the junction is tagged and the cell can use the copy of the gene from the other homologous chromosome to repair it. For example, if the maternal copy is broken, then the paternal copy can be used to repair the damaged gene. Homologous recombination is the machinery that mediates this repair. Every cell in your body has to have very efficient homologous DNA repair machinery or it could not survive.

Since the machinery is there in every cell I asked, 'how can we fool Mother Nature to use this machinery for our advantage?' What if I could convince the homologous recombination machinery that I'm presenting the 'good copy' of the gene to the cell, and that the cell needs to replace its own copy with my copy? Initially, at least, I wanted to present the cell with a 'bad' or defective copy of a chosen gene, to knock out that gene. This thought process led directly to gene targeting.

As soon as I saw all of those pieces of newly added DNA lining up in the genome, and I could prove that this process was mediated by homologous recombination, I knew what I wanted to do and I even knew that I wanted to do it in mice. Unfortunately, mouse embryonic stem (ES) cells didn't exist at that time and they would be necessary to create mice with the designed modifications in their germline. Fortunately, about 4 years after we started all of these studies, Martin Evans' group were the first to report the isolation of ES cells. (Sir Evans was a co-recipient of the 2007 Nobel Prize.) I asked if I could come to his lab and learn how to work with ES cells right then and there. He was very generous to let my wife, Laurie, and I visit his lab. When I arrived he said to me, "Nobody seems to be interested in my cells" and I told him, "They will be, just wait!"

After you developed this new technology there were limitless genetic unknowns available for study. Why did you choose to focus on the *HOX* gene family?

It was timing. At that time, Walter Gehring in Switzerland had just characterized *HOX* genes in Drosophila. As Ed Lewis had shown, these genes are responsible for specifying segmental identity in Drosophila. Walter Gehring and others showed that these genes contained DNA-binding domains called homeo boxes. Amazingly, they also showed that *HOX* genes not only existed in Drosophila, but also in frogs, mice and humans. This story was breaking at the same time as we were thinking about which gene to tackle with our technology for modifying mouse genomes.

This was all happening in the mid-1980s. All of a sudden, these *HOX* genes were being characterized at the DNA level and identified in many species. Up until then, people knew that, for example, the citric acid cycle was going to be the same in all species from plants to humans, so they thought of metabolism as being conserved, but they didn't think that the genetic circuitry responsible for development would be the same among disparate species. The conservation of *HOX* genes among many unrelated species was the first insight that allowed people studying Drosophila to recognize that their work in Drosophila development also had something to do with our own development, and that was very exciting.

I knew I could carry out gene targeting in mice and I thought, 'What should I do with it?' The smart thing, I knew at the time, would be to work in immunology, because you can isolate B cells and you can characterize them in vitro and in vivo. At that time, many genes involved in immunology were being identified but an assay did not exist to determine what they were doing. Also, you can wipe out the immune system without killing the animal. From a pragmatic point of view, immunology would have been a perfect system to attack, but I didn't enjoy the study of immunology so I

wasn't interested in pursuing that avenue. I was much more interested in mammalian development. However, if I chose to disrupt a developmental gene that functions very early, it may kill the mouse and I would not have much to look at. But, I thought that *HOX* genes were going to be involved later in development because, based on Lewis's work with Drosophila, they were likely to be involved with forming the body plan.

So, I thought that *HOX* genes were working at the right time to provide an interesting phenotype to analyze. My feeling was that the many *HOX* genes were going to have some functional overlap, called redundancy, allowing us to look at that redundancy by combing separate *HOX* gene mutations by breeding. At the same time, they functioned late enough in development for the embryos to have probably progressed sufficiently for us to decipher what had happened. Even then, after sampling opinions from different investigators one lab would tell me, 'nothing will happen because these genes are so redundant that any effect from losing one *HOX* gene will be covered by the presence of another', and another lab would say, 'it's going to result in a puddle and you are not going to be able to decipher it. These genes are so important that you are just going to get a mess.'

Fortunately, the story was in between. It doesn't give you a puddle and there is some redundancy, but there was always a unique aspect to the function of each gene. For a while, gene targeting, particularly in knockout mice, received a bad rap in the sense that people would say, 'I've done this experiment and I don't see any phenotype, my gene doesn't do anything.' My retort would be to simply say, 'you have to look in the right place because every gene has to have a function.' After a few generations, if a gene doesn't have a function, it is lost by mutation. Every gene has to have a function if it is maintained.

How did you approach something as different, at this point in your career, as mouse phenotyping? Did you consult a pathologist?

No, I'm a great believer that the lab should do everything internally. The reason is that when you do it all and watch the story develop, you are much more involved. Further, a pathologist looks at a lot of slides and doesn't have the same investment or motivation to look at each slide with care. But, if it's your PhD thesis on the line, you are going to look at those slides very carefully and really see if there is a difference resulting from the targeted mutation.

This self-reliance also gives you flexibility. You control the pace. The greater your personal involvement, the greater your commitment to solve the problem properly.

Does that philosophy influence the way you structure your lab?

Yes, I seek diversity. I have people joining our laboratory from medicine, molecular biology, neurobiology and developmental biology. I always look to see who is missing. There is a constant flux of people coming and leaving, so I always bring in people from different disciplines.

The other advantage of this internal diversity is that everybody looks at problems from their own perspective, so if you have a group of people from different backgrounds they will look at problems from very different vantage points, and I think that enriches the whole lab.

What areas do you think will be most influenced by gene targeting technology in the future?

All biological phenomena is mediated or influenced by genes. Therefore, gene targeting will influence the study of all areas of biology. Neurobiology, for example, was surprisingly one of the last disciplines to adapt gene targeting. I think this reflects the complexity of the nervous system. Complex systems often require the use of conditional mutagenesis and that wasn't available in the beginning. The problem is that if you knock out a gene required for liver development, for example, the mouse is going to die, but that same gene may also have a function in the brain that cannot be evaluated owing to lethality. You have to have a way of separating these two functions in the animal and that requires conditional mutagenesis.

There are still problems to overcome in gene targeting. With current conditional mutagenesis protocols, we can readily perform processes that occur in a day, but we cannot operate within minutes, seconds or milliseconds. Yet, our thought processes operate by millisecond scales. As we are talking, our thoughts are taking shape at tremendous speeds so, if we are to thoroughly understand these processes, we need switches that can operate at such speeds, but this technology is not currently available. A recent development is the ability to activate genes with light, which at least allows the switches to be turned on and off very quickly. All of these speed-related advancements are spurred on by computers that allow rapid processing of large quantities of information. In addition to rapid switches, the capture time for signals (i.e. reporter genes) will also have to be increased enormously. Technology has always been important for the advancement of science, but my guess is that in the next 20 years we are going to witness remarkable advancements as our ability to process information increases dramatically.

I am a very gene-centric guy. Not because genes do everything, but because genes are the easiest place to be able to dissect complex biological phenomena with great precision. By controlling genes you can control function and then see what happens. You can't do this any other way. If we could control genes at very high speed, then we could discover exactly how and when the functions for each gene were required, even for complex processes such as laying down memories. This would allow us to approach more and more complex problems, particularly the processing of information in the brain.

I think the mouse is the best model organism to address such problems. However, our brain is much more complicated than the mouse brain. It would be nice to be able to perform molecular genetics on something like ourselves or a monkey, but for ethical and pragmatic reasons we cannot do that.

Do you mean that we need to achieve greater humanization or primatization of the mouse?

In a sense yes, in an organ- or subsystem-specific way. It sounds crazy but there are reasons to think it may not be as crazy as it sounds. An example for this is that there are two kinds of bats, big ones (mega bats) and small ones (micro bats). People initially thought, as late as the 1980s, that mega bats were derived from primates and micro bats were derived from rodents. The basis for that conclusion came from looking at the histology of the brain. Mega bats have a brain that looks histologically like a primate brain and micro bats have brains that look like a rodent brain. But, DNA analysis tells us that all bats were derived from rodents, suggesting co-evolution of the mega bat

brain in its resemblance to the primate brain. Because this co-evolution occurred in fewer than 70 million years, the major histological differences between our brain and the mouse brain could not have involved changes in more than a few genes, not even 10 genes.

Do you think we'll be able to create a primate-like brain in a mouse?
I think it is not impossible at the subsystem level, perhaps not today or tomorrow, but eventually, subsystem by subsystem. To me, such a scenario is more likely than carrying out molecular genetics in a primate, which would require an enormous investment in time and cost. Further, personally, for ethical reasons I would have great difficulty working on a primate. When I look at a primate, it looks just like me. The ethical issues are enormous.

At one point in your career, you made a big change from physics to biology. What led you to make such a career shift?
I went to Antioch College, which had a work/study program where you study for a quarter and then work for a quarter. At any time, half of the student body was working and the other half was studying on campus. The jobs were all over the country and determined by your academic interests. If you were a lawyer, you would obtain clerking jobs and if you were a scientist you got lab jobs. So, I was going all over working in different labs just at the time when molecular biology was being born. I eventually ended up at the Massachusetts Institute of Technology (MIT). Physics is an elegant discipline; the only discipline that is more beautiful than physics is mathematics. But experimental physics, particularly particle physics, was involving the use of bigger and bigger machines, and larger and larger groups of scientists. I wanted to study a science where the individual scientist was a more integral participant in the experiments and, at that time, molecular biology was a real draw. I think one reason why there was such a boom in molecular biology at that time, was that people from separate disciplines: chemists, biologists and physicists, were turning their expertise to biology. It was naive but there was a feeling that we could solve any problem, no matter how complex.

You did much of your genetics training in James Watson's [who won the 1962 Nobel Prize for his contribution to understanding the structure of DNA] lab. It seems like a pretty exciting time to be working in someone's lab who was a central figure in genetics.
I had applied to three places: MIT, Cal Tech and Harvard. I went to Jim's lab and asked him where he thought I should go. He looked at me and said, "Here! You would be crazy to go anywhere else". That's how I ended up in his lab at Harvard and it was a terrific choice.

You have unique ideas and have made uncommon contributions to science. Certainly we are all influenced by our childhood, and yours is one of the most unusual that I know. Do you think that growing up alone on the streets from 4 to 9 years of age influenced you in ways that have affected your career?
Certain aspects may have, for example self-reliance. If you are on the streets you have to be able to rely on yourself and gather everything that is required for survival. You have to get your own food, find your own clothes and shelter, and so on. So you are dependent on yourself and nobody else. I think that is the way that I like to look at our lab. If we need new expertise, it is better to develop it internally than to farm it out. That is where I see the most direct influence.

Another aspect may be intense concentration. On the streets you have to concentrate. Particularly in wartime when all resources were short, nobody wanted to give up their food. To survive you have to steal food, but people with the resources are quite aware that there are many people out there who may want to have a share of their resources. They are watching their food and you have to outfox them. You do that with patience and observation. It forces you, at an early stage, to be observant and patient. Science is a mixture of talents, at one end of the spectrum you are thinking about new things and new ideas – flights of imagination. You think about what does not exist and try to make it so. At the other end of the spectrum, science depends on paying attention to details. There is a lot of repetition to science; for example, an experiment may have 10 different steps and each step needs to be done with precision. To be successful in experimental science you need to have talents at both ends of these extreme processes. It is good to have great ideas but if you can't put your ideas into practice then they are useless. Lots of people have great ideas, but you have to convert the ideas into practice.

My experience also shows that children are extremely resilient. If they are put into a situation, they will work out a solution. You may look at your own child who is 4.5 years old and wonder, 'how could my child exist alone out in the world?' But put into that situation many children can do it and do do it. I should also point out that there is a selection process; you would not be talking to the failures. The only ones that you are going to be able to talk to are the ones that have survived.

What qualities do you think promote good and creative science?
I think of the process of science as a series of concentric circles where the small circles in the centre are where most people are working. As you move further and further out to the edge of the larger circles you approach science fiction. What you have to do is find the circle in which you are comfortable. I like to work near the edge of the largest circle and hope that I don't step over that edge, because then I would be wasting my time. I like to go out there and work on things that require not only thinking about the problem, but often developing the technology needed to solve the problem, which is why the problem is way out there. The further out you go, the longer a project is likely to take you. If it took less time someone would have already done it. Such problems require long-term commitments to the project and an environment that will support such long-term commitments.

When a new student comes into my laboratory I spend several days just talking with the student to find out what their interests are. If they are interested and committed to a project then they will do a good job and if not, then no matter how hard they try, they are not going to do a good job. It is important to find out what is of interest to you and what projects are you willing to commit a lot of your time and energy to. Science isn't easy. It requires enormous commitment, and a lot of work, time and thinking. But the rewards are tremendous. You are generating new knowledge that can make a difference to the welfare of our planet.

I build pictures of what I want to do. Once I formulate the picture, I find out

what is missing and needed in order to do it, then ask where might those missing pieces exist. This approach requires me to look way beyond what I am doing and see what other people are working on, even in completely different fields such as engineering, to find out whether they are doing something relevant to my work. I have to draw some analogies to get an idea going. There is a mixture of almost fanciful ideas, which are close to the realm of being science fiction, mixed with the practicality of the details and seeing where the pieces will come from and how I might put them together to make something new. The most valuable information concerning biological problems comes from nature. I see if nature has done it before and, if so, how has she done it? Using this question, I may gain insight on how to do it in our experimental setting. If nature has done it, at least I know that whatever we want to do is doable, even if I don't yet know how to do it myself. If nature hasn't done it, I may be in trouble because she has had a long time to get it done.

An example is the involvement of *HOX* genes in regulating finger length. There are people with big hands and people with small hands. How does that happen? We can alter *HOX* genes in mice and change the lengths of the phalangal bones by about 10%. When you go out and look in nature, you find that there are organisms where these small bones are longer than their body length. That is hundreds-fold longer. How has nature accomplished this?

In terms of molecular genetic analysis, almost everything we know has been acquired from the analysis of bacteria, yeast, *C. elegans*, Drosophila, mouse, zebrafish, chick and Xenopus. That is, our representation of nature has been restricted to just those eight model organisms out of the entire biosphere of thousands upon thousands of species. In the past, we have also emphasized what is common among these organisms. There may be just as much to be learned by finding out what makes these organisms all different from each other. This area is infinite and could occupy scientists for hundreds of years. It is an area that is currently peaking our interest. Genomes from many different species are very quickly becoming available but, to date, comparisons between these genomes are restricted to in silico comparisons. What would really be fun is to be able to access this wealth of information experimentally. So, we need to develop techniques that would allow much greater experimental access to this enormous repertoire of biological information.

Interviewer comment

At the conclusion of this interview, I asked Dr Capecchi why he chose, in his early career, to leave his position at a coastal Ivy League institute and mecca of scientific discovery for the more isolated environment of the University of Utah, where he has been since 1973. He made two points that I frequently hear echoed by scientists at other more isolated institutions that, despite their location, are renowned for their creative contributions to science. First, although being a member of the Harvard faculty, he was continuously asked about his progress: 'What is new?' The desire, or need, to give continuous updates can have the effect of channeling investigators to work on short-term projects, at the expense of long-term, high-risk projects. Dr Capecchi wanted to work on more long-term projects like the development of gene targeting in mice, which took 10 years to develop. Second, he wanted to work in an environment that was diverse, collegial and fun; a place that took its commitment to including new, young faculty and their ideas very seriously. Often, many of the best-known scientific institutions are predominantly filled with senior, established faculty. In a newly formed institution, there can be a unique opportunity to develop an academic environment that benefits from the youthful vigor of a mixture of junior and senior scientists with unique perspectives. He indicates that these qualities make the University of Utah an exciting place to extend his career.

DMM is excited to be able to present Dr Capecchi's personal story here. We are grateful to him for discussing his experience in pioneering the technology that introduced gene targeting to scientific research. We also appreciate his candor in addressing some of the additional special qualities and experiences that make him so unique.

Mario Capecchi was interviewed by Kristin Kain, Associate Reviews Editor for DMM.

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