From One Experiment to the Next

The start was 1983, the first year anyone made a viral vector. My lab had been studying tumor viruses, and we got an idea: could we remove a gene that causes transformation and replace it with another unrelated gene?

We tried the experiment, in collaboration with Ted Friedmann at the University of California, San Diego, using a cDNA corresponding to the human gene for hypoxanthine phosphoribosyltransferase (HPRT), an enzyme that is missing in patients’ with Lesch-Nyhan syndrome. When we placed the cDNA in a mouse retroviral vector, the virus was capable of turning mouse and human cells lacking HPRT into normal HPRT-producing cells. The next thing I knew, the story was on the front page of the New York Times. I could not believe they had picked it up!

We will probably see the first successful use of gene therapy for treating cancer within the next 10 years. About 500 gene therapy clinical trials are being conducted, mostly in cancer patients, and about half-a-dozen are phase III trials. Thus, I believe I am being pragmatic. Although I will admit I am an optimist by nature. I always think my flight will be on time.

Seeing the clinical application of viral vectors is very rewarding, but that is only part of the story. Almost every study published today has used a gene vector for one of the experiments. Viral vectors have had fabulous utility in biology.

Most of the work we do is deductive. I am not the kind of scientist who sits and thinks, “How did life begin?” My work builds from one experiment to the next. For example, around 1995, it became clear that the viral vectors we were using had serious limitations. To overcome the problem, we looked for a vector that could integrate in the host cell DNA without causing toxicity and infect both dividing and nondividing cells. We turned to lentiviruses, the family of viruses that the human immunodeficiency virus (HIV) belongs to. Of course everyone thought we were nuts.

One by one, we eliminated each HIV gene, until 91% of the genome was gone. What was left was sufficient to carry a DNA sequence into a cell. Once we had shown that a lentivirus vector could infect nondividing cells, we asked: could it also infect a fertilized egg? We tried the experiment, and it worked. The resulting technology extended the capability of producing transgenic animals beyond the mouse.

A paper or a talk will often provide an idea for the next experiment. I have always worked like this. When I was a postdoctoral fellow in David Baltimore’s lab at the Massachusetts Institute of Technology (MIT), I heard a seminar by Georgii P. Georgiev, then the head of the Engelhardt Institute of Molecular Biology in Moscow. He had found that messenger RNA (mRNA) had a string of adenine nucleotides, which he speculated were either at the 3’ or 5’ end.

It was 1972, about 2 years after Baltimore’s discovery of reverse transcriptase and before anyone knew about poly(A) tails. It occurred to me that if the string of adenines were at 5’ prime end of mRNA, I would need only thymidine nucleotides to prime the synthesis of cDNA using reverse transcriptase. If it were at the 3’ end, I would need a mixture of all four nucleotides. I tried the experiment, and when I examined the purified products, the counts were so high I thought someone had contaminated the scintillation counter! After that experiment, I became very popular with my colleagues at MIT because many people wanted to make cDNAs.

David Baltimore was a wonderful mentor. He allowed me to follow my interests and intuition. I had been trained very differently in India. There the system was feudal, and students were not encouraged to think for themselves. That was part of the reason why I left when I was barely 19 to pursue a Ph.D. at the Weizman Institute in Israel. I then went to MIT in 1971.

I visit India two or three times a year for business and to see my father who turned 92 this year. Since 1983, I have been involved in helping to improve the country’s basic science research infrastructure. More recently, I have turned my attention toward cancer research in the country.

I came to the Salk Institute when I was 27. Baltimore had told me “Go to the Salk for few years and then get a real job.” But 31 years later, I am still here. As an immigrant, I was eager to put down roots. Also, many people started at the Salk at the same time I did, and we quickly became like a family. We were all young scientists and took great delight in each others’ successes.

I still come to work every day as excited as I was the first day I stepped through the door in 1974. Part of it has to do with the building itself—there is almost a mystical quality to it. But I also have a great job. It feels more like a hobby or like playing. Of course there are days—for example, when I get a letter of rejection from a journal—that I don’t feel as good. But these moments pass and don’t reduce my enthusiasm.

As told to Laura Bonetta, a science writer based in Bethesda, MD.