

# TURNING PAGES

Nobel Lecture, December 7, 2007

by

OLIVER SMITHIES

Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599-7525, USA.

I am fortunate in having been a bench scientist for almost 60 years, and perhaps somewhat prescient in having kept all my notebooks (of which there are more than 130 since I first began). Together they are a record of my happy life as a scientist. They are also a more or less complete record of the progression and logic of the work that brings me to Stockholm today, and of what I expect to continue when I return to North Carolina. My hope is that in the next 40 minutes or so I can share this progression with you by TURNING PAGES in these notebooks. And I want to talk to a large degree to the people up in the balconies – the students.

The first group of pages documents my CHANCE invention of molecular sieving electrophoresis. My first job was in Toronto, Canada, and I was looking for a precursor for insulin (which I never found!). In the course of this work, I was having trouble in studying insulin with filter paper electrophoresis, as my January 1<sup>st</sup>, New Year's Day, 1954 page illustrates. [“*Students, note the day!*”]. Insulin stuck to the paper and unrolled like a carpet. – the more protein that I used, the further the carpet unrolled. (Left panel, Figure 1).

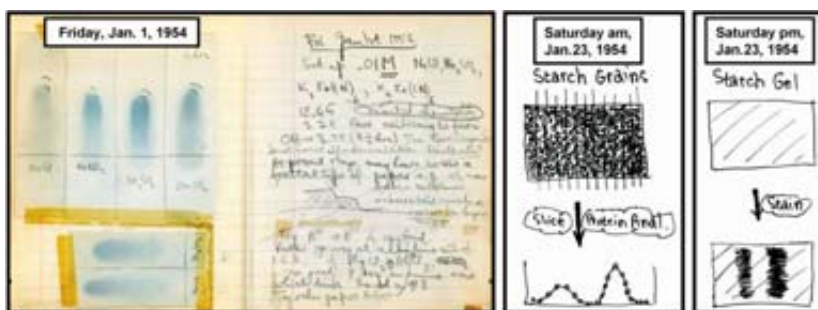


Figure 1.

Then, on January 23<sup>rd</sup>, 1954 (Middle panel, Figure 1) [“*Notice, students, Saturday morning!*”], I learned of a new method of electrophoresis that used a bed of moist starch *grains* (which do not adsorb proteins) for the electrophoretic medium, instead of moist filter paper (Kunkel and Slater, 1952). But, in order to find the separated proteins when using this method, it was necessary

to carry out a protein assay on each of about 40 slices taken from the moist starch bed. I had no technical help, not even a dishwasher, and I couldn't afford the time to do multiple protein assays for each electrophoresis experiment. Happily, however, when I was a boy I sometimes helped my mother with the laundry, and remembered that the boiled starch she used for my father's shirts set into a jelly when it was cold. This memory suggested to me that I could cook the starch grains, make a gel, carry out the electrophoresis, and then just *stain* the gel to find the proteins. (Right panel, Figure 1). As a consequence of raids on them when no one else was around, I knew the whereabouts of the best stockrooms in the Connaught Laboratory where I worked, and so I was able to find some starch and test the gel idea that afternoon. ["*Saturday, still*"] The starch gelled only when its concentration was high, but the result with insulin was, as I recorded in my notebook, "very promising!" I later found out that a high concentration of starch impeded the migration of large proteins more than small proteins. This need to use a high concentration of starch was the chance element in my invention of *molecular sieving* gel electrophoresis (Smithies, 1955). ["*Molecular sieving occurs, students, when you use polyacrylamide gels with proteins and agarose gels with DNA.*"]

Three months later, I tried electrophoresing serum – "just for a rough test" – and next day found a total of 11 components. At that time serum was thought to contain only 5 components (albumin, alpha 1, alpha 2, beta and gamma globulins), so I knew I was onto something likely to be important. I stopped looking for the insulin precursor, and began to study serum proteins.

Over the next 7 months I worked the bugs out of the starch gel electrophoresis method using serum from myself and from two of my graduate student friends at the University of Toronto, Gordon H. Dixon and George E. Connell, whom I co-opted to give blood. (Left panel, Figure 2) By the end of October, 1954, I was about ready to publish, when for the first time I ran a sample from a female, Beth Wade (B.W., right panel, Figure 2).

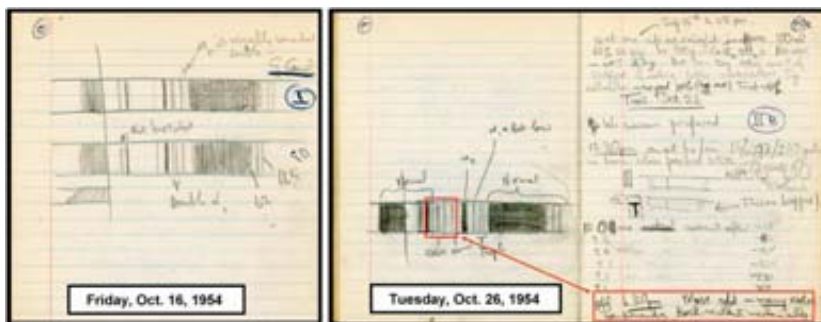


Figure 2.

My notebook entry on that day ("Most odd – *many* extra components") fails to record that I *thought* I'd found a new way of telling males from females! Indeed I called one type M, and the other type F, and found this designation

to be correct for several male-female comparisons over the next week or so. But, after a hilarious day when one pair of individuals had the M versus F electrophoretic patterns reversed, the gender distinction proved to be incorrect. In its place, I thought it likely that the differences had a genetic basis. So, I contacted Norma Ford Walker, at the Hospital for Sick Children in Toronto. She was a remarkable lady, “one of the founding members of the institutions of human and medical genetics in North America” (Miller, 2002). And together we showed that the differences in the electrophoretic patterns of individuals were determined by common and completely harmless variations in the gene (*Hp*) controlling haptoglobin – the chief hemoglobin binding protein in plasma (Smithies and Walker, 1955; 1956).

We identified three common phenotypes (and genotypes): Hp1-1, (*Hp<sup>1</sup>/Hp<sup>1</sup>*), Hp2-1 (*Hp<sup>2</sup>/Hp<sup>1</sup>*) and Hp2-2 (*Hp<sup>2</sup>/Hp<sup>2</sup>*). (Left panel, Figure 3).

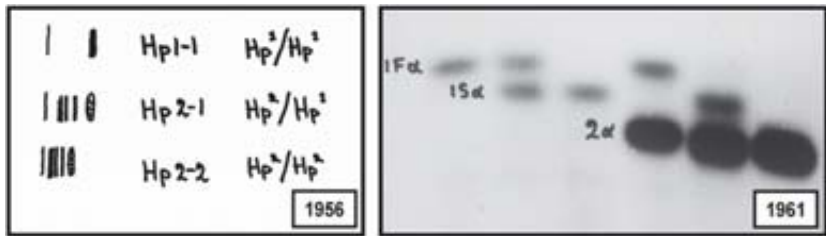


Figure 3.

This finding opened the next chapter in the book of my scientific life – an OPPORTUNITY to study the genetic differences in proteins, starting with the haptoglobins. This I undertook in collaboration with my ex-graduate student friends, Gordon Dixon and George Connell, who had by then come back to the University of Toronto as junior faculty members.

For many years I have advocated and practiced “Saturday morning” experiments, of which you have already had a sample. These experiments have the advantage of not needing to be completely rational, and can be carried out without weighing chemicals, and so forth. [“*But, students, not without proper lab-book notes.*”] And I carried out many of these in trying to simplify the complex electrophoretic patterns associated with the products of the *Hp<sup>2</sup>* gene. One of them included the use of phenol. This was short-lived because phenol dissolved my apparatus! Reducing the protein with beta mercaptoethanol ( $\beta$ ME) in the presence of urea, following a suggestion from Gordon, proved to be the key. But not without another hilarious incident that followed my accidental breakage of a bottle of  $\beta$ ME over my shoes. I put them on the windowsill for a while. But I didn’t have many pairs of shoes, and so I soon began to wear them again. Several days later, during a visit for other reasons to the local police station, I heard two old ladies whispering together. One asked the other, “Do *you* smell it?” Her friend responded, “Yes. Do you think it’s a *body*?” My shoes went outside on the windowsill for a while longer.

After learning how to separate haptoglobin into its subunits (alpha and beta), we found that its genetics were more complicated than Norma Ford

Walker and I had thought. Thus when George began purifying haptoglobin from single bottles of donated plasma we found (Right panel, Figure 3) that there are *three* common haptoglobin alleles ( $Hp^{1F}$ ,  $Hp^{1S}$  and  $Hp^2$ ), not two (published later in Connell *et al.*, 1962). We also noted that the  $Hp^2$  gene, the one which is associated with the complex protein patterns, appeared to produce twice as much alpha subunit as the other two genes ( $Hp^{1F}$  and  $Hp^{1S}$ ). And there were other findings that made us think that the  $Hp^2$  gene was more complicated than the  $Hp^{1F}$  and  $Hp^{1S}$  genes. For example, when Gordon compared the peptide maps of the  $hp1F\alpha$ ,  $hp1S\alpha$  and  $hp2\alpha$  haptoglobin subunits, the results were very puzzling, and we had great difficulty in believing them —  $hp2\alpha$  appeared to contain all the peptides present in  $hp1F\alpha$  and  $hp1S\alpha$ , plus an extra one. Then, during a get together in Toronto in 1961, I remember saying to Gordon and George, “Let’s *believe* our own data.” And I suddenly realized that the  $Hp^2$  gene was probably the product of some sort of recombinational event between the  $Hp^{1F}$  and  $Hp^{1S}$  genes that had generated a partially duplicated fusion gene. The  $Hp^2$  gene would consequently produce a larger protein having the same peptides as a mixture of  $hp1F\alpha$  and  $hp1S\alpha$  together with a novel junction peptide, “J”, not present in either  $hp1F\alpha$  or  $hp1S\alpha$ . (Left panel, Figure 4). We had become the first people to detect *non-homologous* recombination at the level of a gene! We called it “non-homologous”, because the recombination between the  $Hp^{1F}$  and  $Hp^{1S}$  genes was within regions that are unrelated in sequence.

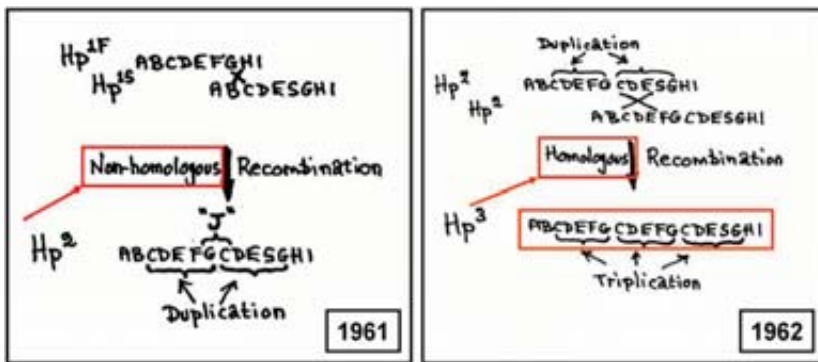


Figure 4.

We decided to present our data and our partial gene duplication hypothesis at the 1961 Second International Conference of Human Genetics in Rome. We also designed an experimental test that George was going to do before we each gave our part of the story at the conference. He would use the ultracentrifuge to determine the sedimentation coefficients of the alpha subunits with the expectation that the  $hp2\alpha$  subunit, which our hypothesis said was larger than  $hp1F\alpha$  and  $hp1S\alpha$ , would sediment more rapidly. We met in Rome on the evening before our talks to review George’s results, and he broke the bad news – the sedimentation coefficients of the three  $hp\alpha$  subunits did *not* differ. What to do? Well, we decided, despite this result, to go ahead with our

planned talks, with the understanding that in my part of the presentation I would describe our hypothesis and the experimental test of it that we had carried out. Then I would say “*We don’t believe the result, and I’ll go home and invent a new method for determining molecular sizes.*” The next two pages in my notebooks (Figure 5) show the implementation of that plan (Smithies, 1962). [“*Notice, students, that you shouldn’t always believe your results!*”]

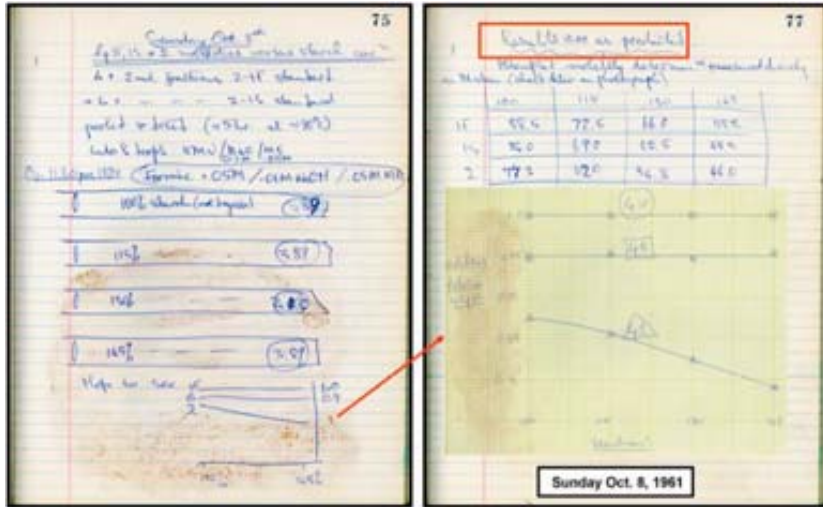


Figure 5.

The new method showed that  $hp2\alpha$  was bigger than  $hpIF\alpha$  and  $hpIS\alpha$ . (Later, when George got rid of aggregation by adding urea, the ultracentrifuge gave the same result.) Together we published our conclusion that the  $Hp^2$  gene was a partial gene duplication resulting from a non-homologous crossing-over event between the  $Hp^{IF}$  and  $Hp^{IS}$  genes in a heterozygous individual,  $Hp^{IF} / Hp^{IS}$ . (Smithies *et al.*, 1962).

The next part of this chapter in my science concerns the clear distinction between the randomness of *non-homologous* recombination and the predictability of *homologous* recombination. When I told Professor James H. (“Jim”) Crow, Chairman of Genetics at the University of Wisconsin, about our results, he referred me to some beautiful classical work involving the genes controlling the development of the eye of the fruit fly, *Drosophila*. In succession over a period of over 20 years, Tice (1914), Zeleny (1919), Sturtevant (1925) and Bridges (1936) provided evidence that a *unique*, non-homologous recombinational event, which occurred only once, had generated a duplication on the X chromosome of the fruit fly that changed the shape of the eye. They also showed that this duplication enabled unequal but homologous recombinational events that occasionally gave rise to a triplication or to a return to the unduplicated chromosome. We extrapolated this result to the haptoglobin genes, and expected that the same type of event would occur with them – namely that unequal but homologous recombination within the duplicated

region of the already larger  $Hp^2$  gene would likewise lead repeatedly to a still larger triplicated gene (Right panel, Figure 4). And we found this larger gene as an uncommon variant ( $Hp^3$ , but historically called  $Hp^{2l}$ ) that had arisen independently in all parts of the world where the  $Hp^2$  gene was already in the population. This was my first real understanding of the fundamental difference between the unpredictable nature of non-homologous recombination and the predictability of homologous recombination.

Later, in the late 1970s, I spent a sabbatical period in Fred Blattner's laboratory in the same building as my own laboratory, and learned how to work with DNA and with bacterial and bacteriophage mutants (and, as a concurrent sabbatical activity, learned to fly!). Then, when Fred's Charon bacteriophages were judged to be safe enough for use in cloning human genes, our groups collaborated in isolating and characterizing the two closely related genes that code for the human fetal globins,  $G\gamma$  and  $A\gamma$  (Blattner *et al.*, 1978; Smithies *et al.*, 1978). Subsequently, when we sequenced these two genes, we found clear evidence that DNA had been exchanged between them as a result of another type of homologous recombination, "gene conversion". (Slightom *et al.*, 1980). So, homologous recombination was very much a part of my scientific gestalt. And, not surprisingly, having worked with globin genes, I kept thinking that it ought to be possible to use DNA coding for the *normal* human  $\beta$  globin gene, which was now readily available, to correct the *mutant* human  $\beta$  globin gene that leads to sickle cell anemia, the most frequent disease caused by a single gene in people of African descent. But no one had demonstrated that such an event (now called "gene targeting") was possible with a genome as large as that of humans and other mammals, although it was known to occur in yeast (Hinnen *et al.*, 1978; Szostak and Wu, 1979) with a genome of less than one hundredth the size.

Then in 1982, while teaching a graduate course in molecular genetics at the University of Wisconsin, I came across a beautiful paper that catalyzed me to start writing the next chapter in my book of science – "PLANNING" to use homologous recombination to correct a mutant gene in the human genome. The catalytic paper was published in *Nature* on the first of April, 1982 (Goldfarb *et al.*, 1982). In this paper, the investigators described an elegant gene-rescue procedure to isolate a transforming gene from human T24 bladder carcinoma cells. This gene-rescue procedure depended on using mutant lambda bacteriophages that had a lethal amber chain-termination mutation which could be suppressed if the bacteriophages picked up a copy of *supF* (a mutant tRNA gene able to suppress amber chain-termination mutations). The amber mutant bacteriophages would not grow otherwise. The procedure was complicated, and I had to study the paper carefully in order to use it in teaching. This effort had, however, an unanticipated benefit. During the next 3 weeks I realized that I could use a modified form of Goldfarb's gene-rescue procedure in an assay to determine whether it was possible to place "corrective DNA *in the right place*" in the human genome.



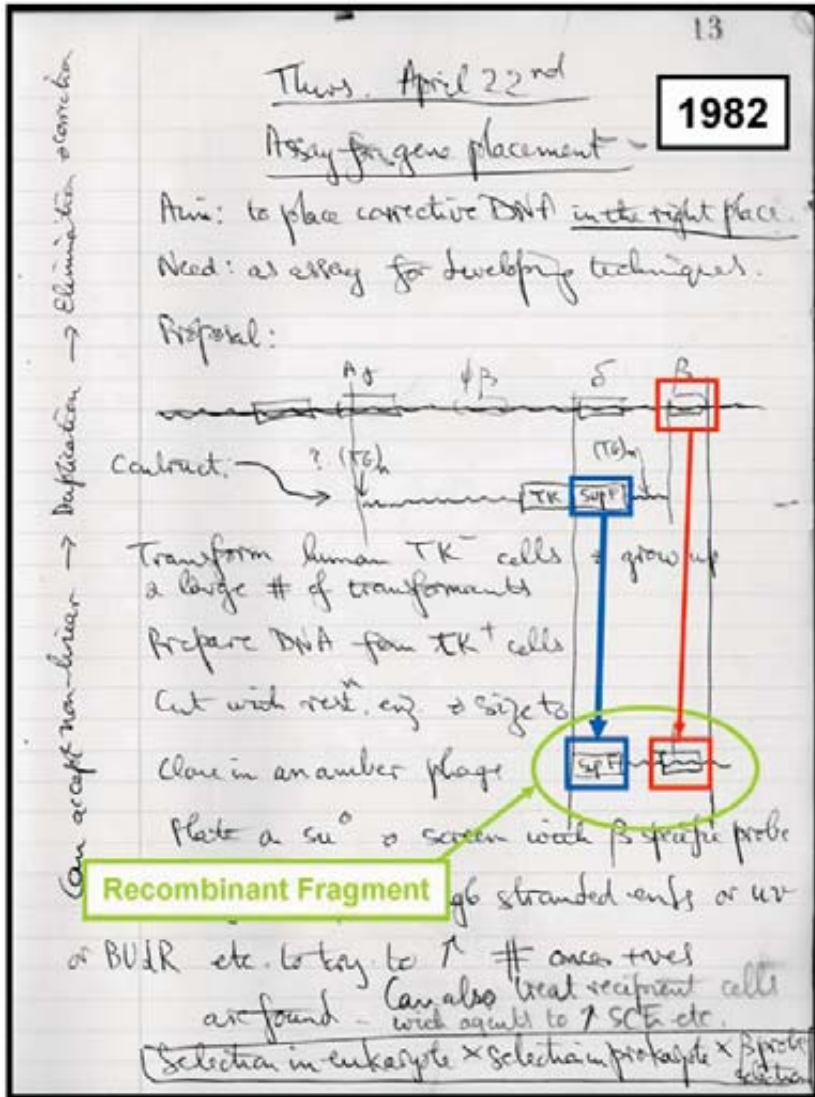


Figure 6.

On April 22<sup>nd</sup>, 1982, on page 13 of my  $\gamma$  notebook (Figure 6.), I summarized my idea under the heading “Assay for gene placement” (now called “gene targeting”). I proposed to make a DNA construct that included a large fragment of DNA covering the human beta-type globin genes, together with the *supF* gene and the thymidine kinase gene, *TK*. I would then introduce this DNA into human cells that were *TK*<sup>-</sup>, select for transformants that had become *TK*<sup>+</sup>, and then use gene rescue to look for a *recombinant fragment* in which the *supF* gene was now next to the  $\beta$  globin gene. This would prove that the incoming DNA had been inserted into the correct place. I was confident that I could detect gene targeting, even if it was *extremely* rare, because I had three levels of selection: selection in the eukaryotic *TK*<sup>-</sup> human cells

of transformants that had picked up the *TK* gene and so could grow in a HAT- containing medium; selection in the prokaryotic *E.coli* cells of mutant bacteriophages that could grow because they had picked up DNA fragments containing the *supF* gene; and selection by autoradiography of bacteriophages that also had  $\beta$  globin sequences. Only homologous recombination could generate the diagnostic recombinant fragment containing *both* the *supF* gene from the incoming DNA *and* the  $\beta$  globin gene from the target locus.

At that time DNA sequencers and DNA synthesizers were not yet available, so making the large targeting construct was difficult, and I had to clone it as a cosmid, which I called Cosos 17. Making this cosmid took me 7 months. Some idea of the complexity of this task is apparent from the next notebook pages that I show but will not attempt to explain (Figure 7.).

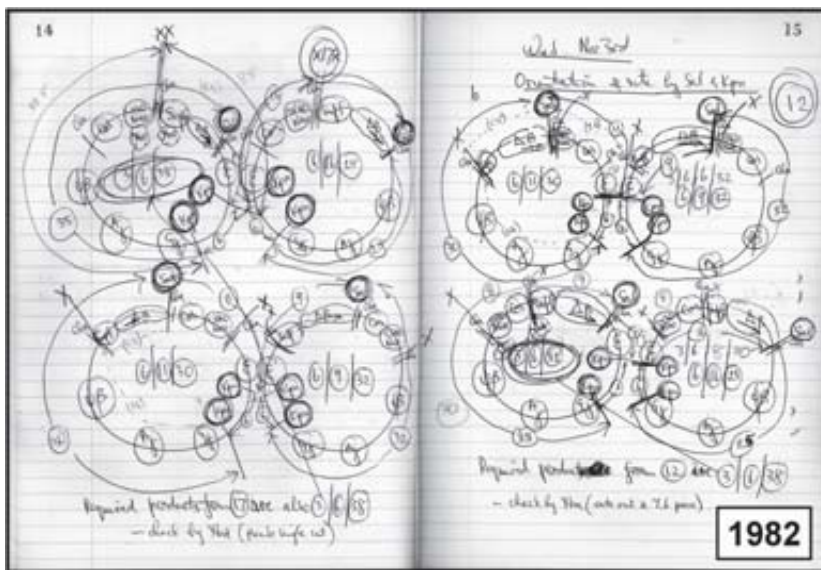


Figure 7.

By the end of 1982, I had sent Cosos 17 to my collaborator Raju Kucherlapati at the University of Illinois. He was to make a calcium phosphate precipitate with this DNA for transfection into another human bladder carcinoma cell line, EJ. Meanwhile, I began work on what turned out to be a scientifically dangerous experiment: I carried out a plasmid by plasmid recombination experiment to test whether the gene-rescue assay would work. The tester plasmid was  $\Delta\beta 17$ , a small precursor of Cosos 17. The mock target contained the human  $\beta$  globin gene. The good news was that both the recombination and the bacteriophage gene-rescue assay worked (Smithies *et al.*, 1984). The unforeseen bad news was that bacteriophages containing the diagnostic recombinant fragment were now present in the lab.

In May of 1983, Raju sent back to us the first DNA sample, RK41, from a gene-targeting experiment with Cosos 17 and the human EJ bladder carcinoma cells. On June 23<sup>rd</sup> (my 58<sup>th</sup> birthday), I started the bacteriophage assay



phase of this first real test of the overall scheme. 288 bacteriophages grew; 104 (34%) contained some  $\beta$  globin sequences; but, birthday or not, *none* hybridized to the critical  $\beta$  globin IVS2 probe! (Figure 8). So this first real experiment failed to provide any evidence that homologous recombination had occurred.

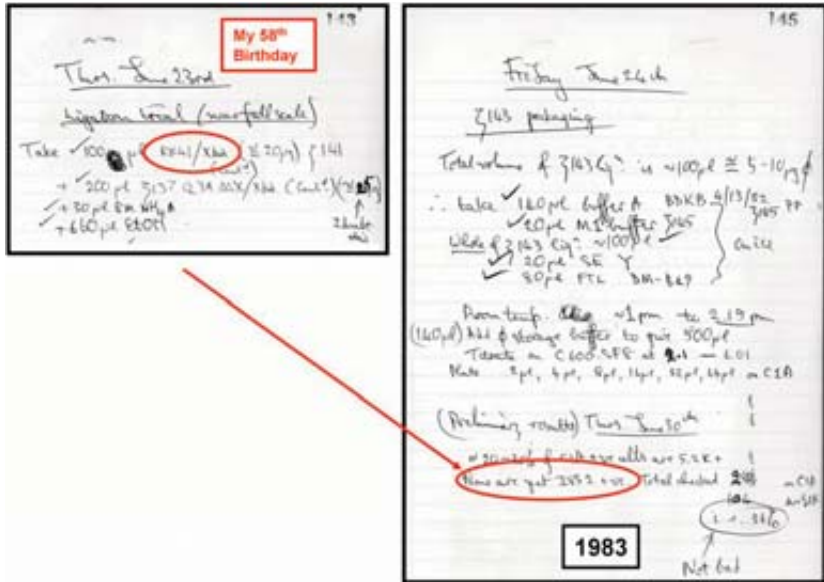


Figure 8.

Over a period of almost a year, my lab and Raju's lab continued experiments with the EJ cells, but without success. These negative results led my graduate student Karen Lyons to suggest that the failure might be because the drug-resistance gene, *Neo<sup>R</sup>*, which we were now using instead of *TK*, might not be transcribed when incorporated into the  $\beta$  globin locus of a bladder-related cell that does not express  $\beta$  globin. [“Students, you should keep going when things don't work; but you should also think carefully about what might be wrong.”] Two alternatives were available. We could retain the drug selection, but use cells that *expressed human  $\beta$  globin*; or we could continue to use the EJ bladder carcinoma cells but *without* using drug selection. One of our earlier collaborators, Art Skoultschi, gave us a cell line which he had made that was suitable for the first type of experiment. It was a mouse-human hybrid erythroleukemia cell line (which we called Hu11) that carried a human chromosome 11 and expressed human  $\beta$  globin (Zavodny *et al.*, 1983). Unfortunately the erythroleukemia cells grew in suspension, and could only be transformed by a newly devised procedure – electroporation (Potter *et al.*, 1984) – and no electroporator was then commercially available. So I spent the next few months designing and testing a homemade apparatus, which was constructed inside a baby bathtub from part of a plastic test tube rack and electronic parts from the local Radio Shack store. The final version of the apparatus, illustrated in schematic and real form in Figure 9, does not look impressive – but it worked, and was subsequently used for all the definitive experiments.

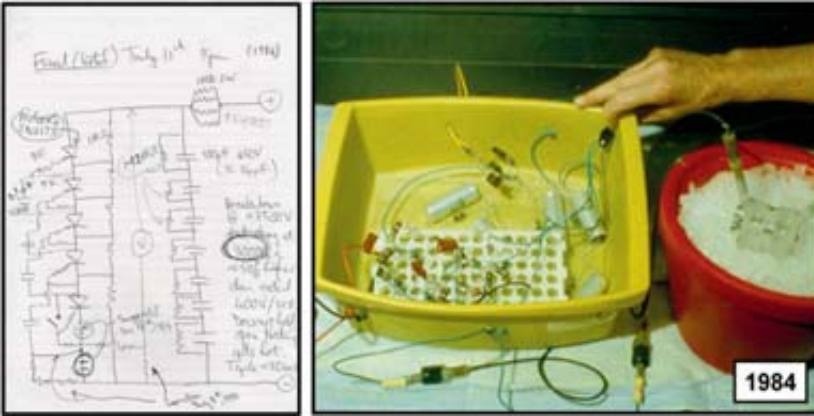


Figure 9.

[“Students: never make a complex piece of apparatus that can be bought in order to save money; but by all means make it to save the time that you will have to wait before some manufacturer makes it.”]

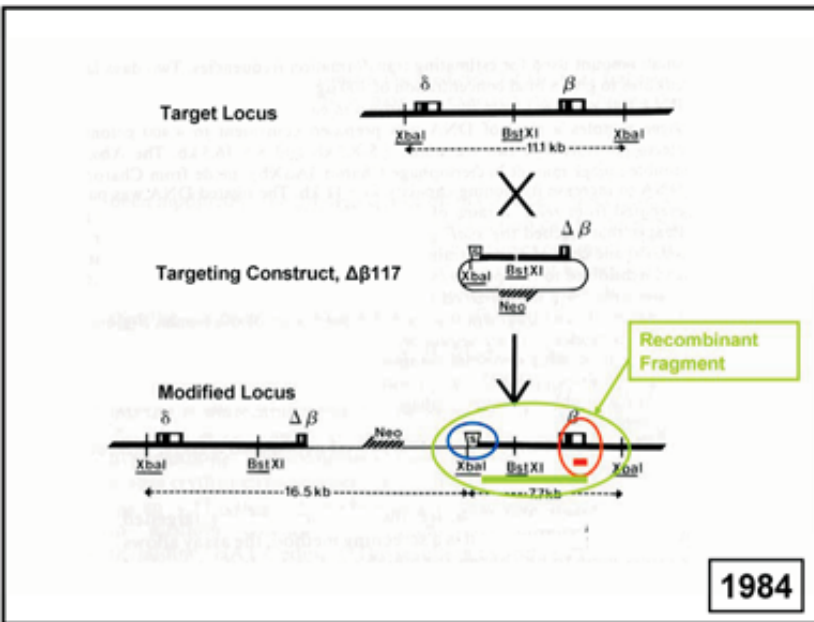


Figure 10.

Meanwhile Raju did an experiment of the second type, using the EJ bladder carcinoma cells without drug selection. This experiment also used a different targeting construct,  $\Delta\beta 117$ , illustrated in Figure 10. (Smithies *et al.*, 1984).  $\Delta\beta 117$  was the recombination tester plasmid  $\Delta\beta 17$  which I had modified so that it could be cut (with *Bst X I*) in the region of homology. This type of cut, we had already shown, increases the frequency of homologous recombination in mammalian cells, as it does in yeast (Kucherlapati *et al.*, 1984). Raju

treated the bladder carcinoma cells with *Bst*X I-digested  $\Delta\beta 117$ , grew them up without any drug selection, and then sent us DNA from the cells. My technician, Mike Koralewski tested this DNA with the bacteriophage assay in late August, 1984. He found one IVS2-positive bacteriophage, which I purified and showed had the hoped-for recombinant DNA fragment with *supF* next to  $\beta$  globin IVS2. This was good news.

But we began to have worries. One worry was that this single bacteriophage could have been a contaminant from our recombination tester experiment. (We had had a contamination problem in some earlier gene cloning experiments.) An even more serious worry was that the recombinant fragment present in the bacteriophage might have been formed by recombination in the *bacterial* cells used in the gene rescue assay, rather than in the *mammalian* cells used for the transformation. We were discouraged!

Fortunately, however, I had recently bought an airplane, and had flown it to Florida for a short sailing vacation with my pilot friends. This vacation re-energized me sufficiently that I could face starting the  $\Delta\beta 117$  experiments again – with two important changes. First, my postdoctoral fellow, Ron Gregg, who had been trying unsuccessfully to inactivate the *Hprt* gene in human fibroblasts, would electroporate *Bst*X I-digested  $\Delta\beta 117$  into the Hu11 cells that express the human  $\beta$  globin gene. Second, after Ron had isolated DNA from drug resistant transfectants, I would digest it with *Xba*I and size separate the restriction enzyme products into two fractions. One fraction would cover the size range 5.5 – 8.5 kb, and another would cover the range 8.5 – 16.5 kb. This fractionation had two purposes. It would reduce the amount of DNA to be packaged into bacteriophages; and, more importantly, it would separate *Xba*I fragments that were 7.7 kb long (the size of the *Xba*I recombinant fragment) from any fragments that were 11 kb long (the size of the *Xba*I fragment from the unaltered target locus). If the recombinant fragment was already present in the DNA from the Hu11 cells *before* the DNA had been exposed to bacteria, the 5.5 – 8.5 kb DNA fraction would give IVS-2 positive bacteriophages. If the recombinant fragment was the result of a recombinational event occurring in the bacteria, the 8.5 – 16.5 kb DNA fraction would give IVS-2 positive bacteriophages. In early 1985, this fractionation experiment was completed using size fractionated DNA from a flask containing ~ 1000 drug-resistant colonies. *Two* IVS-2 positive phages were obtained with the 5.5 – 8.5 kb fraction. (Upper panel Figure 11) Now we believed our results.

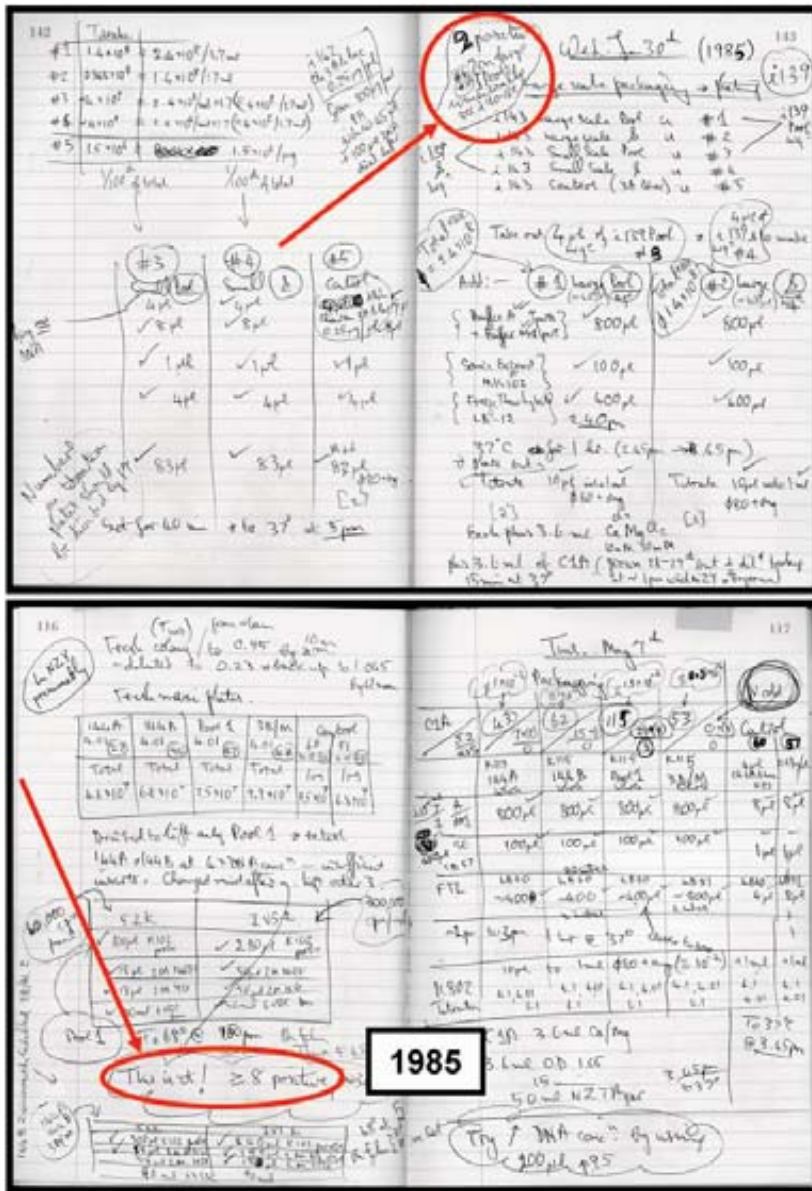


Figure 11.

It took three more months for me to iron out various problems with the gene rescue assay, and for Ron Gregg to generate pools of *individually cloned* Hu11 transformants. But by April we had identified a pool of about 300 cloned Hu11 transformants that gave *three* IVS-2 positive phages. And, in May, DNA from 30 sub-cloned Hu11 transformants from the 300 pool gave us *eight* IVS-2 positive phages (Lower panel Figure 11.). This meant that at least one of the 30 subclones was correctly targeted, and we could now use a *direct* test for recombination (a Southern blot of DNA from each colony) in place of the indirect bacteriophage assay.

On May 18<sup>th</sup>, 1985 [“Saturday, yet again!”], I Southern-blotted Ron’s electrophoresis gel of DNA from 11 of these 30 colonies (Figure 12). On May 20<sup>th</sup>, I noted on page 134 of my  $\kappa$  notebook that subclone “#20 is it!” – 3 years and 1 month and 7 notebooks after the original idea. In September of 1985, the paper (Smithies *et al.*, 1985) which I imagine the Nobel Committee considered my most important was published – after I was 60!

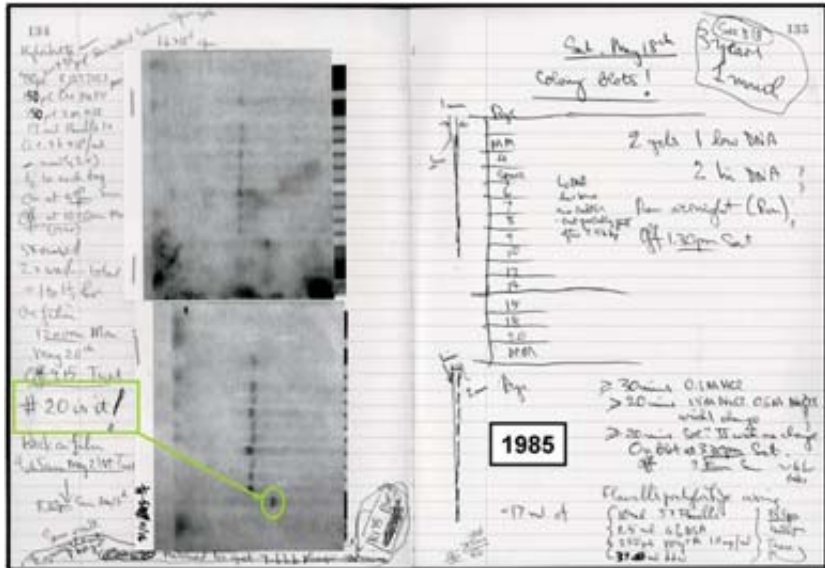


Figure 12.

I have already referred to all who contributed to this paper except one – Sallie Boggs. She was a visiting professor from the University of Pittsburgh. She chose, as her part in the work, to ensure that we had a “back-up” to the bacteriophage assay, in case it did not succeed. To implement this, she carried out Southern blots of DNA from 243 *individual* Hu11 transformants without ever using the phage gene-rescue assay. Although the phage assay, in the end, led to a correctly targeted colony before Sallie found a positive transformant, her work established that the electroporator we had made could introduce single copies of DNA into the cell genome without any other detectable changes in about 80% of transformants (Boggs *et al.*, 1986).

At this point, it was clear that gene targeting was impractical for any near-term use in the gene therapy that I had initially hoped. The frequency of targeting was *too low*. The bacteriophage assay we had used to detect targeting was *desperate* (indeed nobody, including me, ever used the assay again). But these experiments had told us that gene targeting was *possible*. We now knew that we could introduce DNA into a chosen site and alter a target gene in a pre-planned way. So, what to do? Well the first thing was to find a simpler system in which to improve the procedure. And towards this end several investigators in the field independently began experiments with genes that had a directly observable phenotype. Ron Gregg in our group chose the *Hprt* gene, which



makes cells resistant to HAT selection when it is normal, and makes them resistant to 6-thioguanine when it is disabled; Mario Capecchi also chose the *Hprt* gene; Raju Kucherlapati chose the *TK* gene. But success was slow in coming.

Then I heard about Martin Evans' work in isolating what we now call embryonic stem (ES) cells and using them to generate mice, and I immediately began to think about using gene targeting in these cells to modify genes in the mouse. Since ES cells grow rapidly and can be cloned from single cells, a low frequency of gene targeting would not be an issue. We could therefore modify a gene in the ES cells, and use the targeted cells to make animal models of human genetic diseases for study and for testing therapeutic procedures. As a step towards this end, in November 1985, Martin personally brought some of his cells to our lab (Figure 13). [“Students: Don’t be shy about asking other scientists for reagents or help!”]

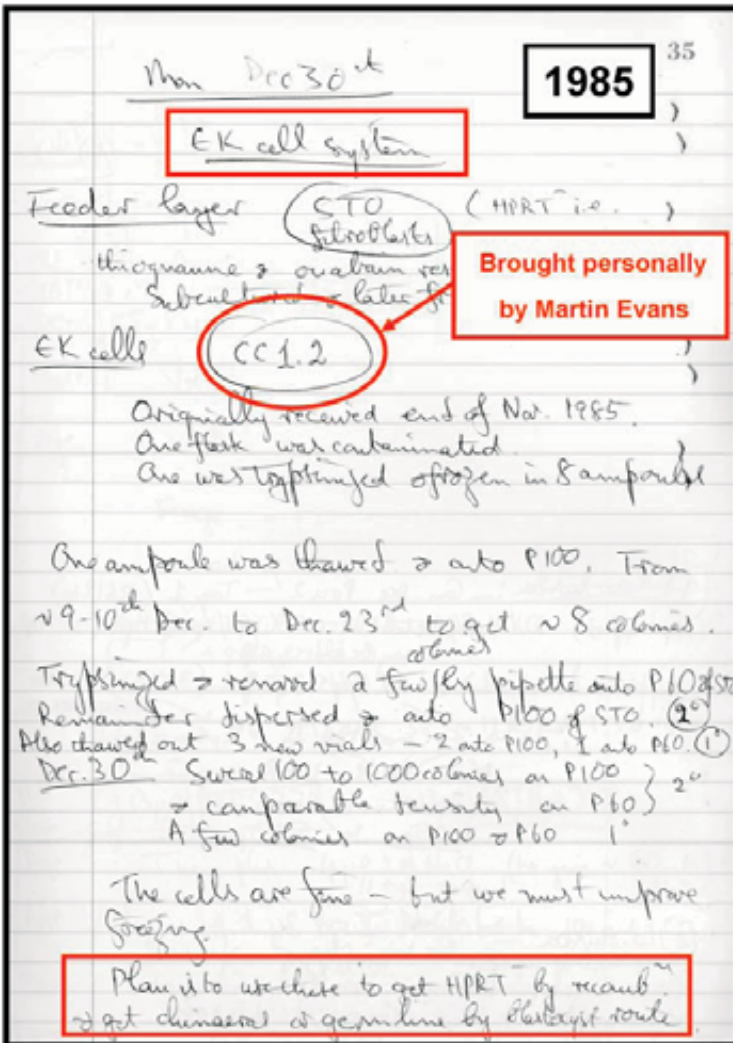


Figure 13.

Martin also put us in touch with Tom Doetschman who had experience with ES cells, which need to be handled correctly if they are to be capable of generating mice. In December of 1987, we published our first use of gene targeting in ES cells – to *correct* a mutation in the *Hprt* gene of E14TG2a ES cells that had been isolated by Hooper *et al.* (1987). The DNA construct, made by Nobuyo Maeda, worked the first time that Tom used it! The big colonies resulting from gene-corrected cells were easy to distinguish from the tiny residues left from cells in which the mutant gene had not been corrected. (Figure 14).

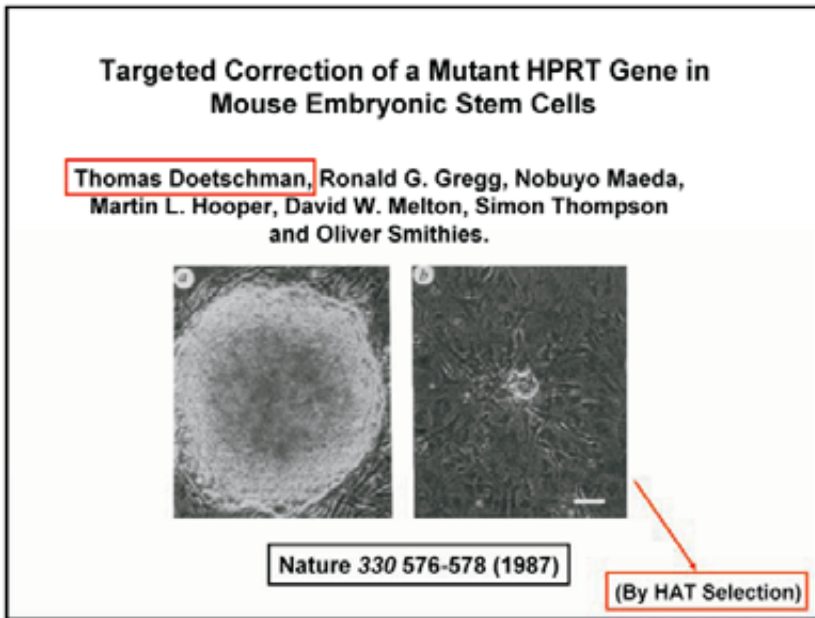


Figure 14.

Mario Capecchi independently contacted Martin Evans for help with ES cells within weeks of our contacting him. And his group's paper, describing a *knock out* of the normal *Hprt* gene in ES cells (Thomas and Capecchi, 1987), and ours describing *correction* of a mutant form of the gene (Doetschman *et al.*, 1987), were also within weeks of each other. Both had used drug-selection procedures to isolate the targeted cells, based on the enzymatic activity of HPRT.

However, a procedure was needed for targeting genes that did not have a directly selectable product. A big help would be to have a simplified *recombinant-fragment* assay. The polymerase chain reaction (PCR) described by Kary Mullis at Cold Spring Harbor in 1986 (Mullis *et al.*, 1986) looked to be eminently suitable for this purpose (Left panel, Figure 15), and I began to work on this idea a few months after hearing Kary talk. Again, no suitable apparatus was commercially available. So Hyung-Suk Kim and I made our own PCR machine, which I still use (Right panel Figure 15).

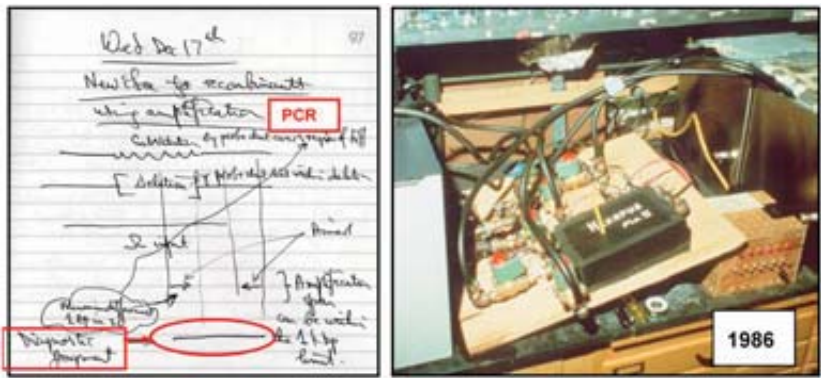


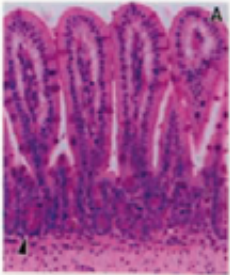
Figure 15.

Time does not permit me to describe many of the animal models that we have since made using gene targeting in ES cells, with the help of our PCR method of detecting the diagnostic recombinant fragment (Kim and Smithies, 1988), together with the powerful positive-negative selection method devised by Mario's group in 1988, as a "general approach for producing mice of any desired genotype" (Mansour *et al.*, 1988). But I can highlight some of them.

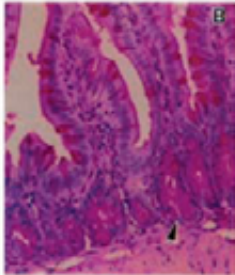
Bev Koller, as a post doctoral fellow in my laboratory, was the first to make a mouse model of cystic fibrosis, the most common single gene defect in Caucasians. (Figure 16) (Koller *et al.*, 1991; Snouwaert *et al.*, 1992).

**An Animal Model for Cystic Fibrosis  
Made by Gene Targeting**

John N. Snouwaert, Kristen K. Brigman, Anne M. Latour, Nadia  
N. Malouf, Richard C. Boucher, Oliver Smithies  
and **Beverly H. Koller**



A



B

Science 257 1083-6 (1992)

(By PCR)

Figure 16.

Nobuyo Maeda and her colleagues made a mouse model of atherosclerosis (Zhang *et al.*, 1992) that became a best-seller at Jackson Laboratories; it is an inspiring model of this genetically complex human disease that causes around 30% of deaths in advanced societies. (Figure 17).

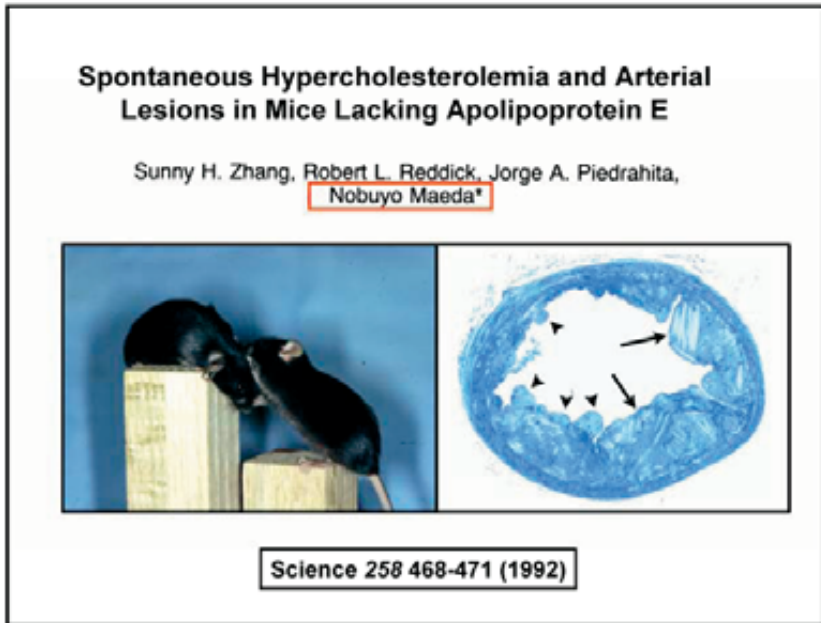


Figure 17.

John Krege led me into a very productive investigation of genetic factors important in another very common disease – high blood pressure (Krege *et al.*, 1997; Smithies, 2005). For this work we used a computerized blood pressure measuring apparatus made by John Rogers, who was at that time one of my glider pilot students (Krege *et al.*, 1995). I chose him to make the new machine (Figure 18) because he had told me about a computerized device that he had designed and built to detect the stones left in pitted cherries, which cause lost teeth in the eaters and lawsuits against the suppliers!

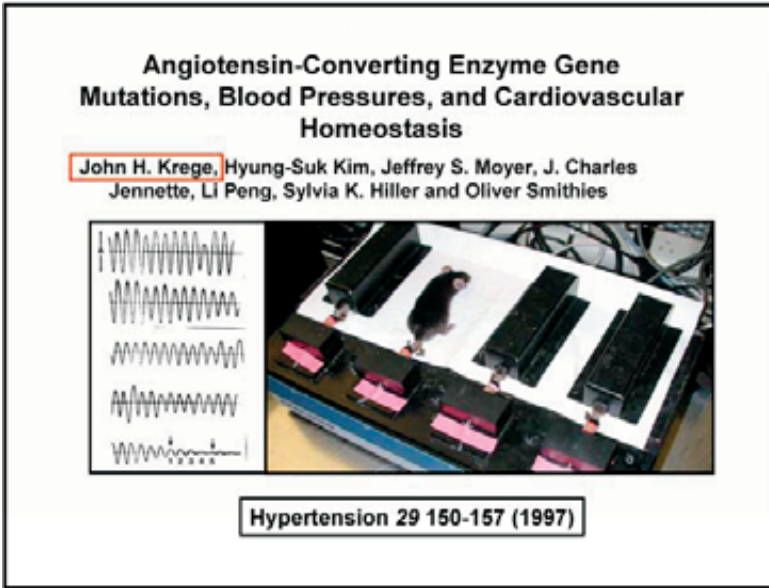


Figure 18.

Marshall Edgell helped me to use a different sort of mouse in computer simulations that explored how genetic factors influence blood pressure (Figure 19) (Smithies *et al.*, 2000).

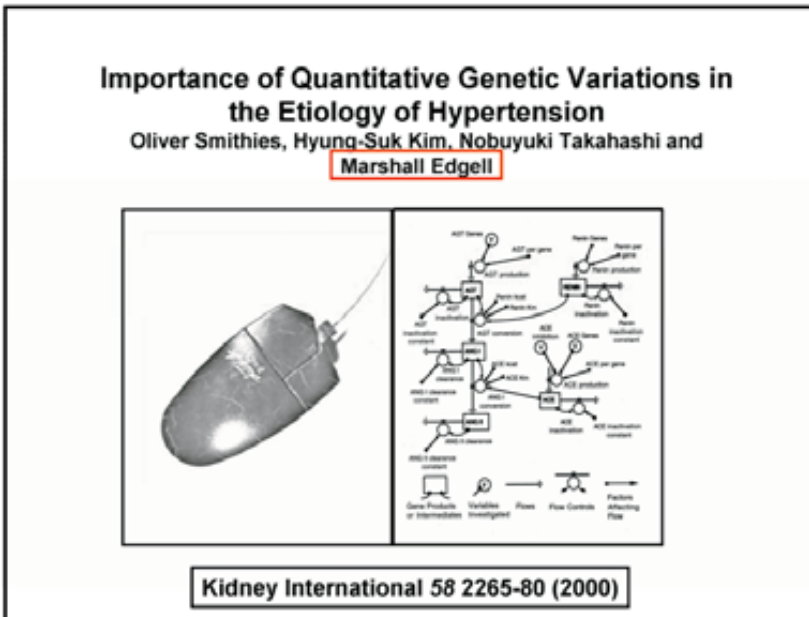


Figure 19.

Devising these and other simulations has helped me to uncover unexpected relationships and has stimulated ideas that I might not have had without



this work. In saying this, I stress that the greatest value of these relatively simple computer simulations does not stem from their ability to replicate experimental data, or even make predictions; rather it comes from forcing one to clarify which elements in a complex system are most critical, and how these elements integrate into a logically consistent whole. [“Students, try a simulation yourself; suitable generic programs for model building are available (for example Stella®) that you can use without being a computer expert”.]

Before closing, I want to mention a previous visit to the Karolinska Institutet on September 6<sup>th</sup>, 2002. During that visit, I heard Dr. Karl Tryggvason, who is here today, give a fascinating talk on how the kidney separates large molecules from small molecules. But I didn’t quite agree with him. And so afterwards, in the corridor, I asked him “Why doesn’t it clog?” His response was “That’s a good question!” which is the one most of us give when we don’t have an answer. Suddenly I thought that I already knew the answer, as a result of having recently written a scientific memoir of my undergraduate tutor, thesis advisor, and lifelong friend, A. G. (“Sandy”) Ogston (Smithies, 1999). In one of his papers, Sandy had derived an elegantly simple equation [ $f = e^{-\pi(R+r)^2n}$ ] that very accurately describes the behavior in gels of molecules of different sizes (Ogston, 1958). So, on my return to North Carolina, I wrote a brief communication on the topic and sent it to *Nature* (Figure 20).

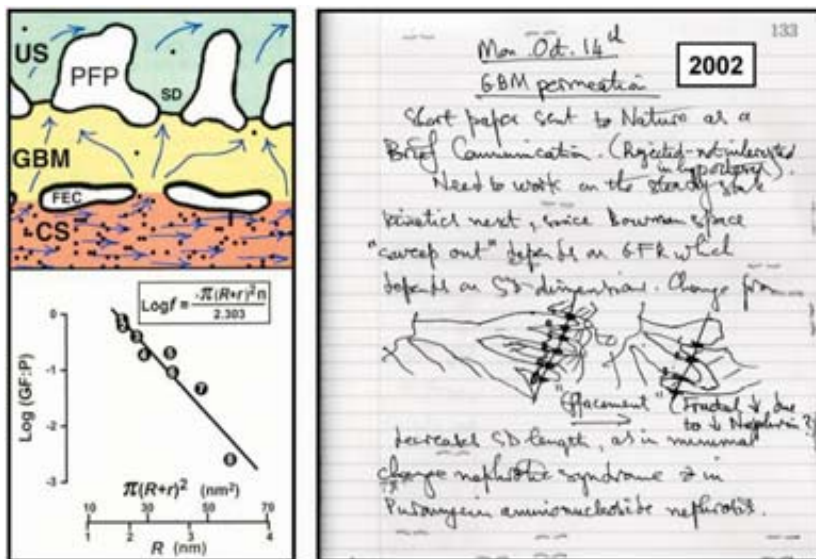


Figure 20.

It was rejected, I’m glad to say, because this caused me to write a better paper that described not only my hypothesis, but also a computer simulation of this aspect of kidney function [“Another simulation, students!”], and some testable predictions based on these ideas (Smithies, 2003). My personal scientific efforts are currently directed towards testing the predictions. And the last pages that I turn for you (Figure 21) illustrate the sequencing of a DNA construct made to implement this work.

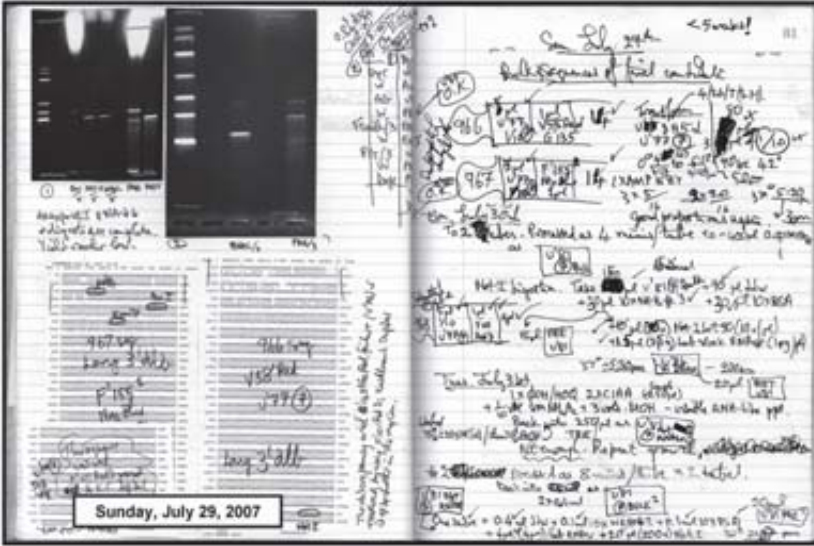


Figure 21.

[“At 82 it is still possible to work at the weekends!”]

What’s on the *next* page?

I don’t know!!

But that’s what makes science exciting!!!

Finally, in closing, I emphasize the importance of choosing a branch of science that makes your everyday work enjoyable, as mine has been. [“*Students: when it was not, I changed it!*”] I also emphasize the importance for a scientist to have other interests for diversion (mine is still flying) when science is being fickle. A happy relationship (mine is with my wife Nobuyo Maeda) can also be a source of comfort at such times – and can provide a captive audience with whom to share science’s much less frequent times of bliss. Scientific happiness is in sharing ideas and the daily excitement of new results with students, colleagues and other scientists. My adviser, Sandy Ogston, had it right when he summarized his view of our discipline. His words are the theme of my visit to Sweden. They capture better than I can what it means to spend a life doing science.

“For science is more than the search for truth, more than a challenging game, more than a profession. It is a life that a diversity of people lead together, in the closest proximity, a school for social living. We are members one of another.”

A. G. Ogston  
 Australian Biochemical Society Annual Lecture  
 August 1970, *Search*, Vol. 1, No. 2, 60-63.

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Portrait photo of Olivier Smithies by photographer Ulla Montan.