Gene pioneers: Donald Brown and Thomas Maniatis win the 2012 Lasker~Koshland Special Achievement Award in Medical Science

The 2012 Lasker~Koshland Special Achievement Award in Medical Science recognizes Donald Brown (Carnegie Institute of Washington) and Thomas Maniatis (Columbia University) (Figure 1), two scientists whose career-long contributions were seminal to our understanding of what genes are and our ability to study and manipulate them, and whose commitment to mentorship have had tremendous impact on a generation of scientists.

What is a gene?

In the nineteenth century, an Austrian monk began a set of experiments in a small garden plot. Gregor Mendel's detailed study of garden peas led him to understand that visible traits, such as the height or color of a plant, were determined by the combined inheritance of two physical particles from the two parent plants. Decades later, Theodor Boveri and Walter Sutton, analyzing meiotic cell divisions in grasshopper testes with the help of a microscope, hypothesized that Mendel's hereditary factors — genes — could be carried on chromosomes. The groundwork was thus laid for a basic understanding of inheritance, but the question remained: what is a gene, exactly? Further understanding these entities — both their molecular makeup and their regulation — would require the dedication of innumerable scientific careers, as well as technical innovations that allowed the isolation and manipulation their sequences.

Development

As a young man, Don Brown elected to follow in his father's footsteps and study medicine, and enrolled at the University of Chicago. At the time, he had not considered pursuing a research career, but the curriculum was research intensive, and it caught his interest. Further cementing his direction, Brown heard a journal club presentation of James Watson and Francis Crick's 1953 description of the double helix (1). "That really excited me," he said in a recent interview with the *JCI*, "I got more interested in biochemistry and research than I was in medical school. I did research every free moment."

Following his medical internship, Brown pursued those research interests at the NIH, where he joined a group working to uncover the molecular basis for schizophrenia, and later at the Pasteur Institute in Paris, where he worked with Jacques Monod on the control of gene expression in bacteria. Throughout these diverse experiences, though, he knew that his deepest interest lay in understanding development and embryology. He remembered, "I felt like it was a huge medically related problem, and not many people were working on it." Upon returning from Paris in 1960, he joined the embryology department at the Carnegie Institute of Washington, a place where his biochemistry background and exposure to the emerging science of molecular biology set him apart. "In those days, people talked a lot about 'a change in gene activity,' and what they meant was a change in enzymatic activity. But it seemed to me that if one was going to study gene activity, it would be reasonable to try to be closer to the gene and study RNA changes rather than protein changes."

Thus, Brown embarked on his independent research using frog oocytes and embryos as a model system. He became interested in ribosomes, and specifically the production of ribosomal RNA, during development. "While I was at the Pasteur Institute, two groups realized that transfer RNAs, ribosomal RNAs, and structural RNAs were in fact gene products just like messenger RNA." A mutant strain of the frog *Xenopus laevis* had been described by a group at Oxford that could produce embryos lacking a structure within the nucleus called the nucleolus, and Brown began a collaborating with John Gurdon to study them. These mutants died in the middle of embryogenesis — the same time, Brown knew, that cells normally accumulate large amounts of ribosomal RNA. Working together, Brown and Gurdon discovered that the anucleolar embryos were failing to make ribosomal RNA, establishing a critical role for the nucleolus in this process (2).

Later, Brown became aware of another interesting anomaly of the frog oocyte. The cells contain a huge number of ribosomes before fertilization (by comparison, the cells contain as many ribosomes as might be found in a million liver cells), and the number of nucleoli in those oocytes was also amplified (Figure 2). As he explained, "*Xenopus* cells have one nucleolus per haploid complement; oocytes are tetraploid, so they should have four nucleoli, but they don't: they have thousands." His group, and coincidently Joseph Gall's laboratory

Figure 1
Donald Brown (left) and Thomas Maniatis (right) won the 2012 Lasker~Koshland Special Achievement Award in Medical Science.

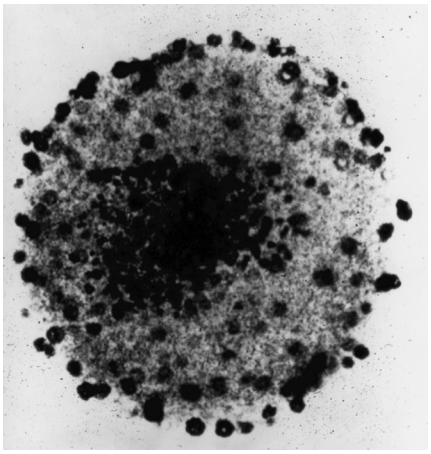

Figure 2
A mature oocyte from *Xenopus laevis*. Cresyl violet stain accentuates the thousands of nucleoli. Reproduced with permission from *Science* (3).

at Yale University, found that this was due to a dramatic amplification of ribosomal RNA gene number (3, 4).

These amplified genes, then, were a good source from which to attempt gene sequence isolation. A few years later, in 1971, Brown and his group were able to isolate the 5s ribosomal genes (5). Just the second gene sequences to be isolated, the 5s ribosomal genes were the basis for many of the studies that illuminated the nature of eukaryotic gene structure and regulation. This work also led to insight into the evolution of genes. Recalled Brown, "We purified the ribosomal and 5s genes from two different species of *Xenopus*. These species are probably 150 million years apart, but the genes are extremely similar, though the spacer regions had diverged almost completely. It was a perfect example that DNA regions that are used are carefully maintained during evolution, whereas regions that are not used diverge. And to make it more interesting, the spacers within a species are all pretty much the same, so they diverge from other species, but they all diverge together — something we call horizontal evolution."

Expanding the toolkit

Further investigation of genes and their regulation would require the development of new tools — specifically, tools that would enable scientists to identify, isolate, and control the expression of genes of interest. As Brown and colleagues were learning the nature of ribosomal genes in the mid-1960s, Tom Maniatis was beginning his graduate studies at Vanderbilt University, under the tutelage of Leonard Lerman. There he studied the structure of compacted DNA by small-angle X-ray scattering and through his reading developed a strong interest in gene regulation. Eventually this interest led him to a postdoctoral position in the lab of Mark Ptashne at Harvard University, where he worked on bacteriophage lambda repressor interactions with DNA and discovered multiple binding sites within the operator (6). Maniatis's postdoctoral work was an important impetus to his later discoveries; as he recently told the *JCI*, "It was through that study that I really developed a desire to do similar things in eukaryotic cells; but at the time that wasn't possible, because we didn't have the technology."

A chance meeting with a young physician, Argiris Efstratiadis, then a student in Fotis Kafatos's lab, would quickly change that. Maniatis and Efstratiadis began collaborating on a project to clone cDNAs — DNA copies of transcription products. They succeeded in isolating full-length DNA copies of β-globin gene mRNA, a key step toward establishing a system in which they could study gene regulation (7, 8). Standing between them and the next step, however, was a surprising hurdle: the City Council of Cambridge, Massachusetts.

In the mid-1970s, the Cambridge City Council instituted a moratorium on recombinant DNA research, citing possible safety concerns. Thus, Maniatis, who at the time was transitioning from a postdoctoral to a faculty position at Harvard, could not begin the process of inserting isolated β-globin cDNA into a plasmid in his own lab. Looking back on this era, Maniatis sees a parallel with the current controversies over stem cell research; "There are different sources of concern, because the stem cell controversy is tied to deeply held political and religious beliefs, but the impact in slowing research progress was very similar." Fortunately for Maniatis, Jim Watson offered him the space to do the experiments at the Cold Spring Harbor Laboratory. There, Maniatis and his colleagues were able to insert the cDNA into a plasmid and propagate the cDNA sequence in bacteria (Figure 3 and ref. 9). This provided a powerful tool for isolating genes from total genomic DNA and for producing proteins in large amounts for structural and functional studies.

Molecular cloning — a field guide

Following this success, and facing an extended recombinant DNA research ban in Cambridge, Maniatis moved his research program to Caltech. While there, he continued to develop new methods for the isolation and manipulation of gene products. This included the generation of genomic DNA libraries — collections of recombinant bacteriophage that as a set contained all of the DNA sequence present in the genome — which made it possible for researchers to easily identify and isolate any gene. As this work was opening new opportunities to biologists interested in genes and gene regulation, Jim Watson invited Maniatis back to Cold Spring Harbor to teach a summer course on molecular cloning. With his postdoctoral fellow Edward Fritsch, Maniatis assembled experimental protocols to create the course manual. Later, Fritsch and Maniatis worked with Joseph Sambrook to adapt the course manual into a book, *Molecular Cloning: A Laboratory Manual*, which has since become an invaluable resource in molecular biology labs worldwide (10).

Remarkably, Maniatis recalls not anticipating the impact this manual would have. "The only person who really appreciated that was Jim Watson," he said. "We were all so busy with our own research and with the job of writing the manual. It required an enormous amount of work, and wasn't simply putting together a bunch of protocols, because at the time, there were no kits out there, no easy ways to do things. We had to provide a conceptual background for every chapter so the reader had some ability to troubleshoot if things didn't work." The product of these efforts allowed scientists from diverse backgrounds, and those who might not be able to enroll in courses like the one at Cold Spring Harbor, to employ these techniques and make successful forays into a new and exciting field.

New tools and new directions

With the development of recombinant DNA technology, Brown's work on ribosomal genes advanced rapidly, and his work contributed to our understanding of transcriptional control with the discovery of an internal control region within the 5s RNA gene (11, 12). This helped lay the groundwork for Robert Roeder's efforts in isolating the first gene-specific transcription factor (13).

Later, Brown's interests turned in a different direction, and his lab became a leader in the field of metamorphosis. He explained, "I wanted to work on a complex biological problem of gene expression changes, and a perfect one was amphibian metamorphosis. Even by 1990, no relevant gene associated

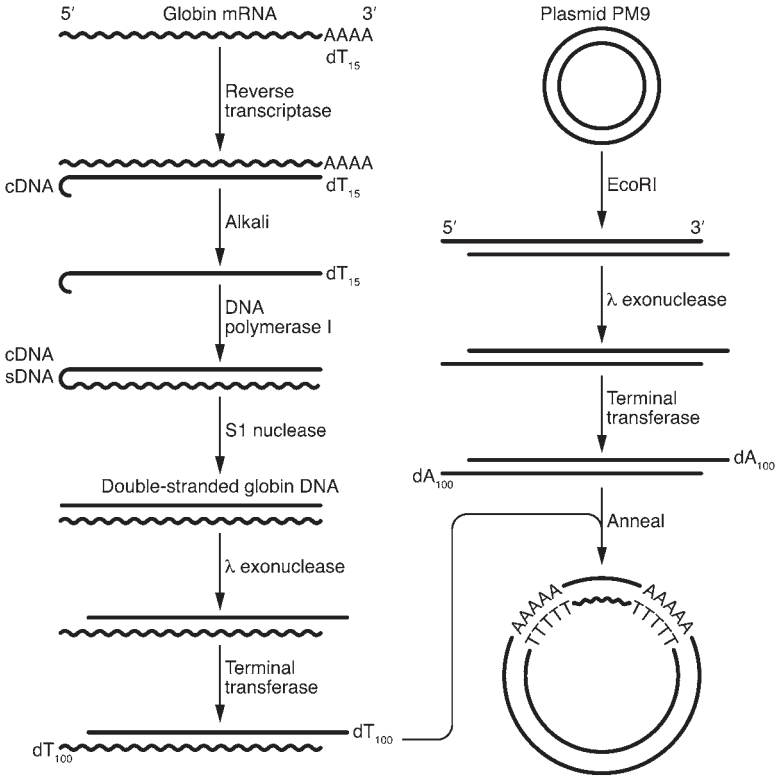

Figure 3
The synthesis and cloning of double-stranded DNA from purified rabbit globin mRNA using the sequential activities of reverse transcriptase and DNA polymerase I. Reverse transcriptase generates a short hairpin that functions as a primer for second-strand synthesis. The hairpin was cleaved by S1 nuclease, the ends were prepared for the terminal transferase reaction by λ-exonuclease treatment, and oligo-dT was added to the ends. The plasmid PM9 vector was cleaved by EcoRI and subjected to oligo-dA addition. The cDNA was annealed to the plasmid DNA through dA:dT base pairing and the hybrid DNA molecule introduced into *E. coli* bacterium. Reproduced with permission from *Cell* (9).

with it had been cloned — it lagged so far behind. Here was this wonderful biological problem that was still being studied by anatomists and endocrinologists — molecular biologists hadn't joined in at all." Brown's group stayed on the cutting edge of molecular technology: "When transgenesis in *Xenopus* became available in 1996, we could do a lot of things that we could never do before, and that was tremendous fun."

After his pioneering work in recombinant DNA technology, Maniatis set out to use the tool to understand the molecular mechanisms of transcription and splicing. As he says, "We followed our nose," and his group made important contributions to the understanding of the fundamental mechanisms of gene expression and signal transduction. Recently, this interest led them to discover a family of genes called the protocadherins, the alternative splicing of which generates cell surface diversity and is important in the development of the nervous system (14). In the last decade, his group has become increasingly interested in the study of molecular neuroscience, motivated in part by a personal loss. Recalled Maniatis, "My sister had been diagnosed with ALS, and while she was ill, I was approached by the ALS Association to chair a committee for non-neurologists: for molecular biologists and cell biologists to look into new approaches to the disease. I chaired that committee for several years, but I wanted to find a way to contribute directly in my lab." In the years since, his group has made important observations about the cell-autonomous and non-cell-autonomous nature of the disease and continues to probe the mechanisms of neurodegeneration.

Lasting legacies

The Lasker~Koshland award recognizes, in addition to their remarkable research achievements, the dedication Brown and Maniatis have demonstrated to mentorship. The first and second editions of the manual Maniatis wrote sold well over 100,000 copies and is still the go-to resource for molecular biologists attempting complex experiments. He also takes his role as mentor at the personal level seriously and has tremendous pride in his trainees; "In my lab, what I try to do is achieve a balance between offering independence and advice. And I think it's working; if you look at my Web page, you'll notice all of the former students and postdocs in my lab — they're really an impressive group."

In 1980, Brown founded the Life Sciences Research Foundation (LSRF), which awards postdoctoral fellowships to promising young scientists. The foundation partnered with the US government and with industry to fund the fellowships; as he explained, "So many people that I knew were going to companies, starting companies . . . It occurred to me that they would have a lot of memory of the great advances that had taken place in academic institutions, that they would remember where their roots were, and want to set up partnerships." The fellowships are unique in that they fund oft-ignored, but important, science. Brown explained, "We really wanted to support things that didn't get a lot of support, like plant biology and environmental research." In the last 30 years, LSRF has funded 450 of these fellowships. In addition, while the director of the embryology department at the Carnegie Institute from 1984 to 1996, Brown established a position for young investigators, designed to give newly minted PhD recipients a tremendous amount of research independence, which, he believes, is a prototype for how research should be done.

What's next?

After four decades of dedication to research, Brown retired in 2010. "I've given all my frogs away," he said, "and my days are a lot different than they used to be." Brown remains active with the LSRF and is currently helping to plan an alumni reunion in

celebration of their 30th year. After nearly 30 years at Harvard, Maniatis recently moved to the Columbia University Medical Center, where he is Chair of the Department of Biochemistry and Molecular Biophysics. Regarding his future, he noted, "We're finally in a position to understand what the protocadherin genes do, both in development and in disease processes. Also, the advances in human genetics and whole genome sequencing are beginning to reveal the very complex interactions between motor neurons and other cell types that are the basis of ALS . . . I guess the short answer is that I'm very excited about molecular neuroscience, and hope to continue to contribute research advances."

Kathryn Claiborn

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