Profile of Michael Grunstein

In 2001, half a decade after researchers announced the arrival of Dolly, the first mammal cloned from an adult somatic stem cell, scientists in the private sector decided to clone a pet cat. A couple of years later the enterprise went commercial, and eager pet owners lined up for the service. However, disappointment inevitably ensued: although the clones were genetically identical to the original pets, cloned cats often looked and acted nothing like their predecessors. Michael Grunstein, were he so inclined, might have said, "I told you so." Grunstein, a distinguished professor of biological chemistry at the University of California, Los Angeles (UCLA), has devoted a lifetime of research to exploring how identical genes can be expressed differently to create unique individuals. Elected to the National Academy of Sciences in 2008, Grunstein's findings have unraveled the secrets behind the subtle interplay among genes, proteins, enzymes, and chemical markers that determines how genes and gene components are read, expressed, transferred, and copied. These secrets were discovered not by working with people—or even cats—but with yeast.

A long strand of DNA must undergo several stages of dense packing to fit within the nucleus of a microscopic cell. In the first stage, a section of the DNA molecule coils around a set of eight proteins called histones, akin to DNA "thread" winding around a histone "spool." However, histones provide more than structural support. Thanks in part to Grunstein's work with yeast, researchers now know that histones help regulate gene expression. Grunstein's research concerns the function of acetyl groups—small molecules composed of carbon, hydrogen, and oxygen, that bind to chemical outcroppings on specific histones, or histone "tails". Using mutations in the lysine amino acids, at which acetyl groups are added, Grunstein found that the sites were necessary for gene activity. Acetylation is now thought to uncoil DNA in the chromosome. Once uncoiled, the genetic information within the DNA molecule becomes accessible so that it can be read, or transcribed. Removal of the acetyl groups, or deacetylation, causes the DNA to wind back, repressing transcription.

These concepts are not restricted to yeast, however. Grunstein's work had—and continues to have—implications for humans as well. Knowledge of the developmental regulation that occurs in histones is easily transferred to human genetics. In 2005 Grunstein's postdoctoral fellow, Siavash Kurdistani, and several coauthors described how to use patterns of histone modifications in cells to predict clinical outcomes of prostate cancer, the second leading cause of cancer death in American men (1). Histone modification patterns identified in that article predicted tumor recurrence independently of known clinical and pathological factors and better than any other biomarkers known at the time. As Grunstein says, quoting the French molecular biologist Jacques Monod, "What is true for *E. coli* is true for an elephant."

Michael Grunstein (center) shared the 2011 Lewis S. Rosenstiel Award for Distinguished Work in Basic Medical Science with C. David Allis (far left). Pictured (from left): Allis, Rockefeller University; James E. Haber, Director, Rosenstiel Basic Medical Sciences Research Center, Brandeis University; Grunstein; Frederick M. Lawrence, President, Brandeis University; and Michael Rosbash, Brandeis University.

A Teacher's Influence

Grunstein was born in 1946 in Romania, the only surviving child of two Holocaust survivors. After the war the family moved to Montreal, Canada, where Grunstein attended McGill University. Although his parents were intelligent, motivated, and careful to instill in him the value of hard work, Grunstein recalls that they were at times wary of his determination to make a living as a scientist. "Like many recent immigrants, they were self taught. They would have been much happier if I had gone to medical school. Still, they always supported and respected my decision.'"

Grunstein's first taste of science came from a summer job between university terms. Bored with his usual after-school jobs at gas stations, Grunstein secured a job performing gas chromatography at an industrial laboratory. The draw, says Grunstein, was the impressive equipment. He explains, "When you're separating the gasses from liquid air, you're separating them on an enormous column, which is several stories high. Here I was, 18 years old, working with these things."

Grunstein credits an instructor at McGill University for sparking his interest in genetics. "Probably the most important influence in college was a professor named John Southin," Grunstein recalls. "He taught genetics in a way that made it extremely interesting. Instead of teaching the facts of genetics, he would describe papers in which there was an error in the work. Finding the error was a challenge. It was fantastic!"

Tools of the Trade

Grunstein decided to pursue genetics in graduate school. At first, he assumed he should continue his education in Canada, where he lived. However, a faculty member at McGill, who had just returned from a sabbatical in Scotland, suggested that Grunstein expand his horizons by studying abroad. So Grunstein went to graduate school at the University of Edinburgh in Scotland. There he began working with a professor named Max Birnstiel. Under Birnstiel's tutelage, Grunstein helped characterize the first chemically isolated genes of an eukaryote—those for the rRNAs of the ribosome, where cellular protein synthesis takes place. Grunstein calls this period, "a breakthrough in my scientific life." At this point, the field of molecular biology was just picking up

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steam, and Grunstein sought out solutions to the practical aspects of genetic manipulation. "How do you deal with a gene in a test tube?" he asked. "Which portion of the gene is transcribed in the RNA? Which portion is not transcribed? How do you separate genes from each other?"

To answer these questions, Grunstein needed more time and more training. After graduating from the University of Edinburgh, Grunstein returned to North America to pursue postdoctoral research at Stanford University. His work with ribosomal RNAs in graduate school piqued his interest in messenger RNAs. Under the guidance of Larry Kedes, Grunstein began to examine the mRNAs and genes for the histone proteins. However, because scientists had not yet figured out an easy way to purify genes, he lacked the necessary tools to complete his studies. "At that time, you couldn't easily separate the genes for one messenger RNA from the genes for another. And separation is everything. If you couldn't separate them from each other you couldn't study them," he said.

Fortunately, Grunstein happened to be in the right place at the right time. By a stroke of luck, researchers at Stanford were developing the exact tools that Grunstein required, particularly a then-enterprising technique for studying genes called cloning. Grunstein knew that he had found what he needed, and so, he recalls, "I pestered my way into Dave Hogness's lab even though every lab bench was taken. I bothered him until he accepted me." Cloning would soon transform genetics research, but the technology was far from perfect. It remained unclear how to distinguish between distinct genes from different bacterial colonies. In Hogness's laboratory, Grunstein developed an approach called "colony hybridization" (2), which allowed researchers to use a DNA or RNA probe to isolate a single gene encoding an individual messenger RNA. For the field of genetics, this was a breakthrough. Finally, Grunstein had a tool that would make it possible to clone individual genes.

Falling in Love. . .with Yeast

Grunstein's technique soon opened up new avenues of genetics research. Beyond the tool's original intended use, researchers soon adapted the principle behind colony hybridization to clone genes in bacterial viruses, yeast, and even human cells. Eager to ride the momentum, Grunstein left Stanford in 1975 after three years as a postdoctoral scientist to set up a laboratory of his own at the UCLA. As a newly minted professor, Grunstein set out to identify genes for histones that switch on and off between one subtype and another during cellular development. His model organism of choice was the sea urchin, whose DNA contains many repetitive copies of histone genes. The sea urchins suited his work, at first. But bad weather complicated matters. "Sea urchins were hard to get, and their gametes were completely gone in the storms," Grunstein recalls. So he began looking for a different model organism.

In a fortuitous turn of events, Grunstein attended a scientific lecture on yeast genetics and "fell in love." With yeast he could perform experiments that were not possible in the sea urchin.

Burrowing into Histones

In a 1988 publication (3), Grunstein described how the nucleosome repressed yeast transcription in vivo. By blocking histone synthesis in replicating cells, Grunstein and his graduate student depleted the chromosomes of nucleosomes. Without histones, the pair found, every normally repressed gene they examined was activated. Before that work, researchers had reported in vitro experiments, but the field was far from convinced that the process worked the same way in living cells.

Some of Grunstein's earliest findings were also the most unexpected. Researchers had learned of numerous histone subtypes in flies and humans and sea urchins, but it remained unclear whether these subtypes performed essential functions. Grunstein's laboratory carried out the first cause-and-effect histone gene mutation experiments. They showed that when a subtype is removed from a cell, the cell survives quite well, suggesting that some histone subtypes performed redundant functions. "The field had generally thought that since histones are so conserved in evolution, anything you did to the histone would kill the cell. But here, we found that subtypes were not essential," he said.

Burrowing deeper, Grunstein began clipping away at the amino termini of histones and found that this entire region of each protein was not required for cell viability. Eventually Grunstein and his students developed a histone that lacked 25% of its sequence—and still, the cell lived. If the sequence was not required for viability, Grunstein wondered, why was the sequence there? Finally in 1988, a persistent graduate student studying histone H4 found that, although deletion after deletion in the amino terminus had no effect on viability, removal of the sequence surrounding lysine 16 affected a specific gene related to cell mating. Why would such a general protein affect a very specific function? His team soon found that lysine 16 interacted genetically with the SIR3 heterochromatin protein (4). "This was really special because it suggested a whole different type of regulation, where regulatory proteins interacted with histones to exert their function. That is now a common occurrence in the field called 'epigenetics'."

Day at a Time

Grunstein says the greatest joy of his career is not the awards he has received or the fields of study inspired by his work: it is the extraordinary graduate students and post-docs he has known during his years at UCLA. "I've been extremely fortunate," he says. "I've had some graduate students and post-docs over the years that I didn't consider to be my students so much as my colleagues—colleagues at an earlier stage in their development." Although he claims not to have any long-term goals beyond surviving to see the next five years, there are some questions that interest Grunstein. How does epigenetic switching take place? How do certain unusual histone lysines help regulate mammalian cell replication and differentiation? What are the heterochromatin-specific functions of the tail in a specific type of yeast histone known as H3?

In his Inaugural Article (5), Grunstein addresses this last question. In the article, Grunstein and his graduate student compiled genome-wide maps of histone binding within heterochromatin—a region of tightly packed DNA and histones located near the center and ends of a chromosome. The study finds that, whereas a related histone tail, the H4 N terminus, recruits proteins that promote deacetylation and gene silencing, the H3 N terminus neither recruits nor spreads these proteins. Instead, deacetylation at the H3 tail promotes restructuring of the packed DNA once the silencing proteins have already spread. Before this work, Grunstein recalls, "It wasn't clear why the H3 amino terminus was necessary for the repressive function of yeast heterochromatin." It was great to learn that it had a function distinct from that of histone H4.

Despite years of groundbreaking work, Grunstein still gets excited by new discoveries. Reflecting on his election into the National Academy of Sciences, he recalls, "I was thrilled. It's something that I always thought happened to other people. But when it happens to yourself, it's such a wonderful honor."

Jenny Ruth Morber, *Freelance Science Writer*

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