Biography of Nancy Hopkins

Geneticist Nancy Hopkins, professor of biology at the Massachusetts Institute of Technology (MIT; Cambridge, MA), has achieved unprecedented success in cloning vertebrate developmental genes by exploiting zebrafish as an ideal model organism. By using insertional mutagenesis, a technique pioneered in invertebrate animals such as *Drosophila* but long considered impossible to use in vertebrates, Hopkins's laboratory has cloned hundreds of genes that play a role in creating a viable fish embryo. This research has earned her several accolades, including 1998 election to the American Academy of Arts and Sciences and 1999 election to the Institute of Medicine. Hopkins has gained additional recognition for her revolutionary work on gender equity issues in science, including many awards and more than 400 requests to speak on the topic.

In 2004, Hopkins became a member of the National Academy of Sciences. In her Inaugural Article (1), published in this issue of PNAS, she and her colleagues describe 315 zebrafish genes essential for early development. Hopkins and members of her laboratory cloned these genes by using insertional mutagenesis, and they estimate that they have identified approximately 25% of the genes essential for early zebrafish development. The genes Hopkins's team has identified not only reflect an extraordinary level of evolutionary conservation from lower organisms, such as yeast and *Caenorhabditis elegans*, but also they may play a vital part in identifying the genetic basis for many human diseases.

Nancy Hopkins

Masterful Mentors

Hopkins was born in 1943 in New York, NY, into a family with several engineers and scientists; an uncle and a great uncle were both chemists, and another uncle was an engineer. However, she did not seriously consider becoming a scientist until her undergraduate years at Radcliffe College (Cambridge, MA), the formerly all-women annex of Harvard University. (Radcliffe College, now coed, is currently known as Harvard's Radcliffe Institute for Advanced Study.) Fond of art, math, and science, Hopkins briefly considered architecture and medicine as possible careers. Then, in her junior year, she attended a lecture given by the famous geneticist James Watson. The topic of the day was DNA. "After 1 hour, I said, 'That's it; that's going to answer every question I've ever had about life,'" said Hopkins. "I also thought it was ultimately going to lead to a cure for most human diseases."

Hopkins quickly switched plans from using applied science in medical school to performing basic biological research. After the lecture, she raced to Watson's laboratory at Harvard to inquire about working there. For the next year and a half, Hopkins finished her course work at Radcliffe while performing research in Watson's laboratory on bacteriophage, a model organism considered to have the most accessible genes. Her work exposed her to many distinguished scientists who passed through the laboratory, such as Francis Crick and Sydney Brenner. Although women of Hopkins's generation were rarely encouraged to pursue science as a career, Watson nurtured Hopkins's interests and supported her strong scientific leanings. "He told me, 'You should be a scientist. You have a one-track mind, just like me,'" she recalled.

After she graduated from Radcliffe in 1964, Watson encouraged Hopkins to continue her education in graduate school. However, she was reluctant to leave her friends and mentors in Watson's laboratory and the research projects with which she had become deeply involved. At the time, her strongest interest lay in a project to isolate the lambda phage repressor, a protein that controls the expression of other lambda genes. The project was led by geneticist Mark Ptashne, a former teaching assistant for one of Hopkins's classes at Harvard.

"I couldn't relate to the whole process of just going to get a Ph.D. so you could have some career. I was relating only to solving particular research problems in science," she said. Regretful about leaving Harvard, but heeding Watson's advice, Hopkins began a doctoral program at nearby Yale University in New Haven, CT. She soon found that no one at Yale was interested in isolating the repressor, which was then considered a daunting task. Consequently, after a year and a half, Hopkins left her program without a degree to join Ptashne at Harvard and work as his technician. Ptashne successfully isolated the isotopically labeled repressor about 6 months later (2).

Upon completion of her work with Ptashne, Hopkins received a friendly lecture from Watson. "Jim said, 'Okay, you've had your fun, now you have to go back to graduate school.' The next day, I was enrolled in Harvard," she said. For the next several years, Hopkins continued to study the repressor, characterizing its properties and interactions with operators. For her thesis, she used ge-

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 12792.

netics to define the operators that the repressor binds to, and then she isolated DNA from operator mutants. Her results showed that various mutations affected the repressor protein's ability to bind to DNA (3, 4). Hopkins completed her Ph.D. in 1971.

A Dynamic Career

The accessibility of genes in lambda phage originally drew Hopkins to genetics. However, as her studies progressed she became interested in applying her knowledge of genetics to a larger problem. Inspired by the fear she had felt when her mother contracted a mild form of skin cancer when Hopkins was a child, she decided that her next step would be to research the genetics of animal tumor viruses. Because of recent successes in studying bacteriophage genes, a small group of scientists was confident that cancer genes could eventually be just as accessible.

By the time Hopkins completed her Ph.D. at Harvard, Watson had established the Cold Spring Harbor Laboratory in Cold Spring Harbor, NY, particularly for studying tumor viruses. "I don't think I ever talked to him about my interest in tumor viruses. We were just independently on the same track," said Hopkins. However, when she decided to begin tumor virus research at Cold Spring Harbor, many colleagues in the phage field advised her against it. "A number of people who were still working on bacterial viruses said, 'Goodbye, we'll never hear from you again,' because they thought cancer research is the end of people's careers, it isn't ready to be tackled. They told me that people who go into cancer research are never heard from again," she said. "Fortunately, they were wrong."

Hopkins commuted to Cold Spring Harbor from Boston over the next 2 years for her postdoctoral studies, spending part of each week analyzing and characterizing DNA tumor viruses before taking a 10-hour train ride home to her husband. In 1973, she received a call inviting her to become a faculty member at the newly constructed Center for Cancer Research at MIT. Thrilled at the opportunity, Hopkins accepted. "The timing was just perfect to provide the right facility in which to work on cancer, the thing that I had wanted to work on all my scientific life," she said.

After setting up her laboratory in the summer of 1973, Hopkins reveled in the new research facility, built with an entire floor devoted to the study of tumor viruses. Much like her experience at Watson's laboratory at Harvard, she was surrounded by the leaders in biology; she was recruited by Salvador Luria and David Baltimore, and she shared an office with the newly recruited Phil Sharp. All three of these scientists were Nobel Prize winners, the latter two for work done at MIT. However, instead of continuing her research on DNA tumor viruses, Hopkins changed her focus to RNA tumor viruses, considered then to be a likely cause of many human cancers. Such a career shift would be difficult for a junior faculty member to make today; switching tracks could alienate familiar funding sources or lengthen the time to achieve tenure. "But back then, funding was easier to obtain, I suspect, and, to me, the two viruses seemed very similar and similar even to phage lambda," she said. "Intellectually, a virus is a virus."

Hopkins and her students set out to determine what features influence the host range for mouse RNA leukemia viruses, an important factor in understanding why viruses cause cancer. Her early studies in this area were some of the first genetic research on mouse RNA tumor viruses. In a pivotal article (5), published in the *Journal of Virology* in 1977, Hopkins and her colleagues obtained evidence that the viral capsid protein p30 was the determinant of the B- or NB-tropism of murine leukemia viruses, determining whether the viruses could grow well just on type B mouse cells or on both type N and type B cells. The result was surprising, because host range was commonly thought to involve proteins located on the surface of a virus; the p30 protein was known to reside deep inside the virus particle. Although Hopkins found this result fascinating, she sensed that its mechanism in deciding host range would be difficult to elucidate, and she did not pursue this line of research. Her intuition was right; researchers are still working to solve this problem. Interestingly, the capsid protein was recently determined to confer host range in the HIV and SIV viruses, determining whether they infect monkey or human cells (6).

Fishing for a Model

For the next 15 years, members of Hopkins's laboratory continued to study the mechanisms of host range and leukemogenesis by RNA tumor viruses, publishing more than 40 articles. Importantly, Hopkins identified transcriptional signals in RNA tumor viruses as a determinant of the type of leukemia a virus induces (7), a finding made without prior knowledge of the tissue specificity of enhancers, which was discovered concurrently in other laboratories. However, over time, Hopkins gradually felt that she again wanted to change fields. Although many of the mechanisms of cancer had been elucidated based on RNA tumor virus research, findings by Harold Varmus, Mike Bishop, and others had shown that oncogenes, and not viruses, were the major cause of human cancers. "The field of the molecular biology of cancer was clearly in good hands, and I wanted to make a change," said Hopkins.

After months of consideration, she decided that her new focus would be the genetics of vertebrate behavior, a field that had long interested her but was little studied at the time. "From the day I first heard about DNA in that classroom at Harvard, the three problems I wanted to work on were control of gene expression, cancer, and behavior," she said. "I just hadn't known when the latter two would become accessible. So I decided to go and see if the genetics of behavior might have become accessible by now." To begin her studies, Hopkins searched for a suitable vertebrate model. In a stroke of luck, she heard at a cocktail party at Cold Spring Harbor that German researcher Christiane Nusslein-Volhard had begun genetics studies on zebrafish.

Hopkins had been awed when she read about Nusslein-Volhard's previous discoveries on genes necessary for early development in the fruit fly, work that eventually earned her the Nobel Prize in Physiology or Medicine (8). Sensing that Nusslein-Volhard's nascent zebrafish research held the key to performing vertebrate genetics, Hopkins went to Germany in the late 1980s to plan behavioral genetics studies on the new model. However, when she arrived, Hopkins found that zebrafish genetics was not nearly as advanced as she had previously assumed. "I discovered that it was just so primitive," she said. She quickly abandoned the idea of doing behavioral genetics, deciding instead to focus on studying developmental genetics in zebrafish. In a short time, Hopkins discovered that the fish held several advantages over previous vertebrate genetics models: unlike mouse embryos, which are virtually inaccessible until birth, zebrafish mature outside the mother, remain transparent for a number of days, and become free-swimming, feeding larvae in just 5 days. Thus, any mutations that arise in the developing fish are easily visible.

However, the fish held some disadvantages as well. With conventional chemical or γ-ray mutagenesis as the only methods to produce mutations within the germ line, cloning zebrafish genes was tedious and slow, taking up to several years per gene. "I looked at this little animal and thought, 'Oh, if only

we could get the genes for early development from this guy.' But first, we had to develop a method of mutagenesis that would allow us to clone the genes very quickly," she said. Remembering the *P* element insertional mutagenesis technique commonly used in *Drosophila*, Hopkins wondered whether a similar method could be developed in zebrafish. Ironically, the method Hopkins's laboratory ultimately developed involved infecting germ-line cells with mouse retroviruses, the type of virus Hopkins had previously studied in her cancer research. By inserting a retrovirus in the germ line, insertional mutagenesis not only creates a genetic mutation but also concurrently marks the mutation with the virus's own genes as a tag.

The possibility of using mouse retroviruses to infect the fish germ line required using viruses with extended host range, called pseudotyped viruses. However, since their discovery some 20 years earlier, these viruses had never been grown to high enough titers to be useful for such a purpose. Through another piece of good fortune, Hopkins learned that Ted Freedman of the University of California at San Diego, motivated by his desire to use pseudotyped retroviruses for human gene therapy, had succeeded in growing these viruses to high titers. "I said, 'Oh my gosh,' and I ran to the telephone, called the lab, and said, 'Stop everything,'" she said. Hopkins quickly called Freedman and requested samples of the virus. When Hopkins's postdoctoral fellow, Shuo Lin, injected the virus into 3-hour-old fish embryos, the virus was incorporated into germ-line cells, although at low frequencies (9). Hopkins's graduate student, Nick Gaiano, was able to further increase virus titers about 100-fold, finally producing the first reliable infection rates (10).

Two questions remained before the method could be used for genetics studies: did retroviral infection cause mutations in the zebrafish germ line? And would the overall rate of mutagenesis be high enough to make a large screen feasible? Subsequent studies published in 1996 and 1999, along with technological innovations by her student and then postdoctoral fellow Adam Amsterdam, showed that the answers were a definitive yes (11, 12). For the first time, Hopkins's laboratories had succeeded in making insertional mutagenesis work in a vertebrate model.

Over the past several years, Hopkins and her colleagues have used the technique to perform a large-scale screen for all genes genetically essential for zebrafish development. It is still a laborious process: Hopkins's team finds important developmental genes by infecting the zebrafish germ-line cells with retroviruses, breeding the fish up to three generations, then examining developing embryos under a microscope. Hopkins notes that only the gene cloning happens quickly: "We had hundreds of thousands of fish pass through the lab in order to produce this work. It's a monumental undertaking." The final results of these efforts, some 315 developmental genes cloned, are revealed in her Inaugural Article (1).

According to Hopkins, many of the genes in her collection are the same genes that have proved genetically essential to development in *C. elegans* and to viability in yeast, reflecting a profound level of genetic conservancy and organization throughout evolution. Additionally, as was found by other laboratories, many defects that were lethal to developing embryos are much like those that plague humans, suggesting that similar genetic flaws are active in both species. For example, Hopkins suspects that mutations shown to cause cystic kidneys in zebrafish lie in the same pathway as the those involved in human cystic kidney disease (13).

With this research published, Hopkins says the next step will be to complete numerous shelf screens of the mutants, targeted searches to determine how each mutation contributes to a specific embryonic defect. Because this work requires understanding the development of many different organ systems in depth, Hopkins has readily agreed to collaborate with many additional laboratories. "Our goal is to give these mutants away to as many people as possible, because the more people who study them, the faster the research will happen," she said. "Almost every mutant has a fascinating story to tell, once it falls into the right hands."

Dual Interests

Besides her zebrafish studies, Hopkins has been captivated in recent years by another academic interest: gender equity in scientific research. During the mid-1990s, she and other tenured female faculty members in science at MIT conducted a wide-ranging study on potential gender biases at the school. The findings were startling: among them, the fact that the School of Science had only 15 tenured women in 1994, compared with 197 tenured men. Women often also held significantly less laboratory space and often earned less pay than their male counterparts did. A summary of the study published in 1999 (14) has been pivotal for inspiring change at MIT and many other institutions. Hopkins has since taken a part-time position with the school's higher administration to work on achieving gender equity and greater faculty diversity within MIT.

In the future, Hopkins would like to take more time outside her zebrafish work to explore again the feasibility of genetics research on behavior, including even gender inequalities in science and other forms of discrimination. However, before she embarks on this new career path she must choose an appropriate model: "I'm wondering whether or not it's possible to study that problem with the fish, or whether you have to study it in humans," she joked.

Christen Brownlee, *Science Writer*

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