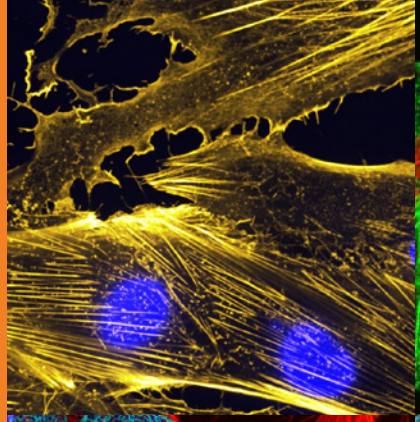
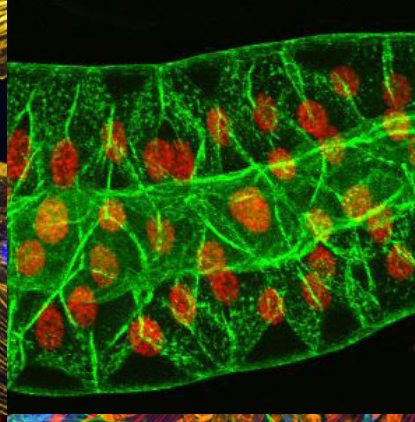
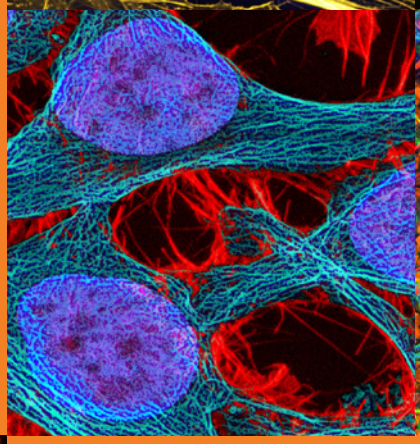
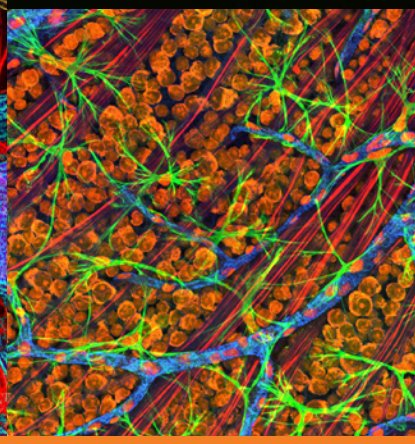
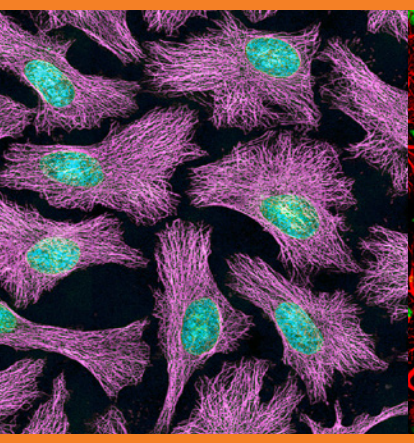
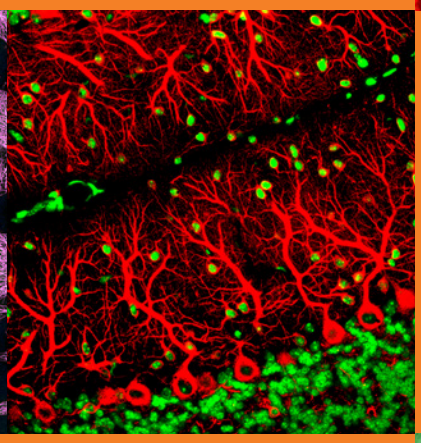
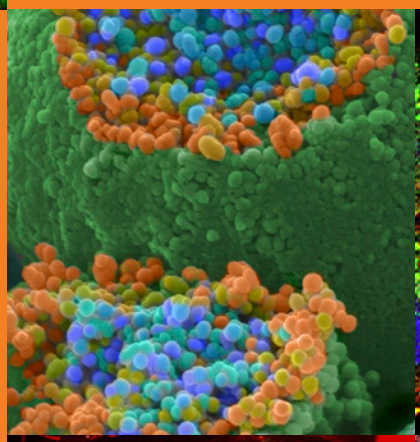
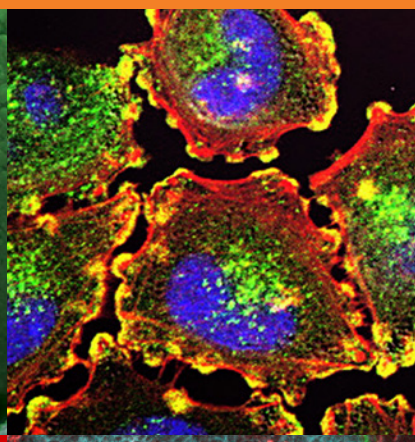
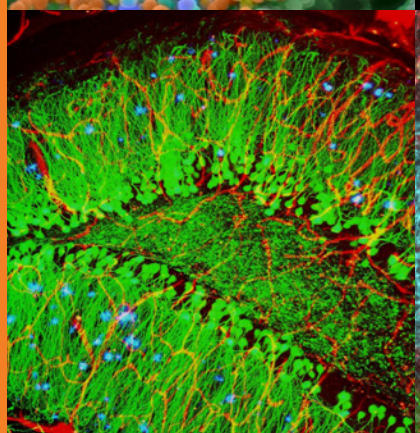
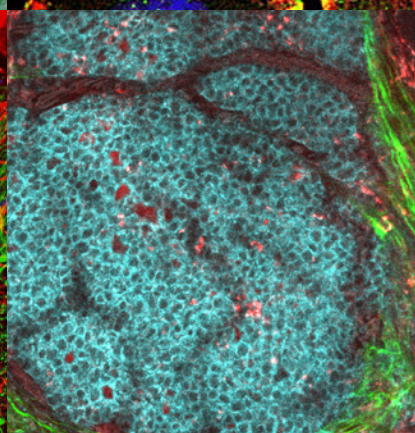







CELEBRATING 40 YEARS of RITA ALLEN FOUNDATION SCHOLARS

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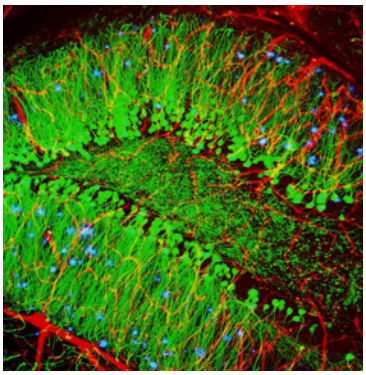


ORIGINS

"The results of these ventures are never certain but, in the long term, their collective impact is extraordinary."
– Elizabeth G. Christopherson, President and Chief Executive Officer, Rita Allen Foundation

FROM THE PRESIDENT

In 1976, Robert Weinberg was in his third year as an assistant professor at the Massachusetts Institute of Technology. He was already demonstrating great potential as a cancer researcher, according to the nomination letter MIT President Jerome Wiesner wrote to the Rita Allen Foundation in March of that year: "Dr. Weinberg is one of the most promising cancer virologists of his generation."

The Foundation's Scientific Advisory Committee also recognized Dr. Weinberg's abilities, and named him one of the first Rita Allen Foundation Scholars. He went on to identify the first human cancer-causing gene and the first tumor suppressor gene, earning numerous awards for his discoveries.

On the pages that follow, we tell the stories of Dr. Weinberg and other former Scholars—early-career scientists pursuing high-risk, fundamental research on which the Rita Allen Foundation's leaders made farsighted bets. In the process, we reveal the importance of our commitment to investing in creative minds and open-ended endeavors in basic research. "You can't really predict what you haven't found yet," says Dr. Weinberg, who used the Foundation's funding to conduct a number of experiments he had never proposed. The results of these ventures are never certain but, in the long term, their collective impact is extraordinary.

The delight we take in these achievements represents the kind of pride we have in all of our Scholars' work. We are continually inspired by the diversity and creativity of their research, and by their passion for exploring big, consequential questions at the forefront of their fields. It is a privilege to engage in supporting this group of innovative thinkers.

The profiles in these pages offer a marvelous glimpse into the lives of scientists. These began as an effort to capture the discoveries and perspectives of our Scholars 10 years after they received the Rita Allen Foundation award—thus, the preponderance of midcareer Scholars from the classes of 2003 and 2004. As we prepared to mark the 40th anniversary of the program, we expanded this project to include a broader range of Scholars, spanning the classes of 1976 to 2010 and reflecting the variety of research our awards now support in cancer, neuroscience, immunology and pain. It has been energizing and illuminating to learn about what sparked these Scholars' interest in science, the unexpected and fascinating turns in their careers, and what questions are shaping the future of their research.

Even as we celebrate 40 years of exploration and discovery, we know that this anniversary is, in many ways, about new beginnings. We treasure the opportunity to select each new class of Scholars—this year from nominations by 61 institutions—and with great admiration we follow their progress in tackling vital scientific questions. We look forward to fostering greater connections among our remarkable community of Scholars, and to sharing the stories of more Scholars in the coming years.

Warm regards,

Elizabeth G. Christopherson
President and Chief Executive Officer
Rita Allen Foundation

EXPLORATION AND DISCOVERY
40 Years of the Rita Allen Foundation Scholars Program

The Rita Allen Foundation Scholars program selected its first class of Scholars in 1976 as one of the first philanthropic fellowship programs of its kind for early-career biomedical scholars. The program grew out of a deep interest in improving human health, guided by input from leaders in biomedical research who pointed to the long-term promise of supporting scientists early in their careers—a time when they were exploring vital but still unproven ideas and struggling to find resources.

With the Scholars program, the Foundation began what would become its defining approach to philanthropy: investing in the earliest stages of big ideas that have the power to be transformative. New resources allowed the Foundation to hire its first chief executive officer and open its first office in 2009. With new Board members and a honed strategic outlook, the Foundation continued to build on the Scholars program while expanding its venture philanthropy work to invest in innovative solutions to strengthen our democracy. Today, the intersection of science and democratic engagement is a promising new horizon—science requires robust public support to thrive, and it is in turn an essential element of solutions across society.

The pioneering work of Rita Allen Foundation Scholars has inspired the Foundation's Guiding Principles. The Foundation seeks to advance new ideas and discoveries that address the root causes of challenging problems, cultivating curiosity, creativity, learning and collaboration. It is willing to take smart risks in order to explore the unknown. While discovery is by nature unpredictable, the Scholars program demonstrates the value over time of investing in curiosity-driven inquiry. Rita Allen Foundation Scholars have made transformative contributions to their fields of study, and former Scholars have won recognition including the Nobel Prize in Physiology or Medicine, the National Medal of Science, the Wolf Prize in Medicine, the Lasker~Koshland Award for Special Achievement in Medical Science, and the Breakthrough Prize in Life Sciences.

Each year, the Rita Allen Foundation's Scientific Advisory Committee of leading scientists and clinicians selects a new class of early-career scientists in the fields of cancer, immunology and neuroscience, nominated by research institutions across the United States. Since 2009, Scholars have also been selected for the Rita Allen Foundation Award in Pain in partnership with the American Pain Society.

The program is marking its 40th anniversary by extending an invitation to all current and former Scholars to gather to share their research and perspectives on the future of their fields. With this anniversary program, the Foundation is creating new opportunities for all Scholars to add to their strengths as leaders and expand their networks of knowledge and collaboration.

More than ever, the complex, interdisciplinary nature of today's biomedical frontiers requires an ability to form connections across boundaries of specialty. Since 1976, Rita Allen Foundation Scholars have been distinguished by their bold approaches to basic scientific questions that address enduring problems, as well as their potential for learning, leadership and collaboration.

The Rita Allen Foundation was made possible by the generosity of members of the Allen and Cassel families, including Charles Allen, Jr., Rita Allen Cassel, Milton Cassel and Lucette Cassel. We also express deep gratitude to Dr. Howard Hiatt and Margaret E. Mahoney, whose vision shaped the Rita Allen Foundation Scholars program, as well as to all who have subsequently contributed to building this enduring resource for pioneering research by early-career biomedical scientists.

UNEXPECTED CONNECTIONS: ON CANCER BIOLOGY, ORIGINAL IDEAS AND INTELLECTUAL LINEAGE

A conversation with Arnold Levine, Honorary Chair of the Rita Allen Foundation Scholars 40th Anniversary Meeting and former Chair of the Scientific Advisory Committee

CLIFF MOORE

"You look for ideas that are original, because original ideas are always at a premium. It's always wonderful when you see one and you hadn't thought about it. You get smarter when you read it."

Arnold Levine got his start in science as a microbiologist, applying new tools from the nascent field of molecular biology to examine how viruses co-opt cellular machinery. Through his investigations of a cancer-causing virus, he became one of the first scientists to characterize the cell cycle regulator p53, and went on to demonstrate its critical role in human cancers. More recently, Levine has taken original approaches to integrating physics and mathematics with biological research.

Levine is Professor Emeritus in the School of Natural Sciences at the Institute for Advanced Study, where he established the Simons Center for Systems Biology in 2004. He served on the Rita Allen Foundation's Scientific Advisory Committee from 2000 to 2009, and chaired the committee for several of those years. Levine has also chaired the National Institutes of Health Commission on AIDS Research and the National Academies Cancer Policy Board. He currently serves as a scientific advisor to the American Association for Cancer Research and Stand Up To Cancer.

Here, Levine illuminates the history of his research on p53 and reflects on celebrating four decades of high-risk, high-reward research by young scientists.

How did the discovery of the p53 protein come about?

I came in 1968 to Princeton [as an assistant professor of biochemistry]. By 1979, we were focused very heavily on the question: How does this [SV40] virus cause cancer? We knew the viral protein that caused cancer—it was called T antigen, or tumor antigen. We were constantly finding it associated with another protein, which was a cellular protein. That protein had a 53,000 [Dalton] molecular weight, and we named it p53.

At the time, everybody agreed this was a good target for understanding the cancer, but no one thought it had anything to do with human cancer. It caused cancer in hamsters. It was a peculiar side effect, but I fell in love with it and wouldn't give it up. I tried to convince every new postdoctoral fellow or graduate student who came into my lab to work on this project.

By '81 we had a portion of the gene cloned, and by '83 we had the [full] gene. Then a big fight ensued. The gene we got was the normal copy of the gene, but there were two other labs that tied us in the cloning—everything was a race at that time. They thought it was an oncogene. They thought that when you put the DNA back into cells, it would cause cancer. We said, "No, it's not causing cancer."

Then a wonderful graduate student named Phil Hinds, who's now at Tufts University School of Medicine, and a postdoctoral fellow named Cathy Finley took our copy of the DNA and [another lab's] copy of the DNA—theirs would cause cancer and ours would not. They mixed the two and put them in cells, and the cells never got cancer. So this was, for the first time, an indication that p53 was a tumor suppressor gene. By '89 we had published that it was really a tumor suppressor gene. At that moment in time, it all fell in place. Several other labs started to find the same thing.

When mutated forms of the p53 gene were uncovered in human cancers, what pleased me the most was that we had done something that was going to be important in human cancer. Here I was, at Princeton University, not at a medical school. We worked with mice. We didn't work with humans, but I knew that we had discovered something that was going to be important in human cancer.

How would you describe the process of selecting Rita Allen Foundation Scholars?

You look for ideas that are original, because original ideas are always at a premium. It's always wonderful when you see one and you hadn't thought about it. You get smarter when you read it. [At first] I wasn't sure what you'd get from an interview. But it turns out that once young people start talking about their science, they forget that they're afraid or intimidated by the group around the table, or that this is an important thing for them. They just go into a zone of science.

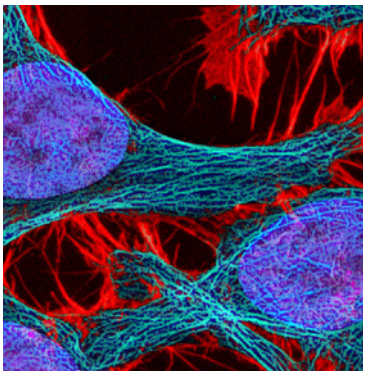
Suddenly you can see how this person reasons at blackboard. It's a whole set of new questions. You make an objection, and they tell you why it's not correct. Or you make an objection, and they say, "Yes, that's true, but..." It just opens up the logic trains in their head, the depth at which they understand their subject and the excitement they're feeling about moving ahead. Those are things that you don't get from a paper.

In your view, what is most significant about bringing together all the Rita Allen Foundation Scholars for a 40th anniversary meeting?

One of the wonderful things about academia is that it's just like families. You run a lab, you have your graduate students and postdocs, and they go out and they have their graduate students and postdocs, and they have theirs, and they have theirs, and it's like a lineage. It's not a genetic lineage. It's an intellectual lineage. Rita Allen has an intellectual lineage, and [at the meeting, the Scholars will] meet each other for the first time.

The second thing is, there are many meetings. These meetings tend to be very large. They tend to be very impersonal. There tend to be too many subjects, and they tend to be very technical. The Rita Allen meeting is planned to be much more diverse, and to tackle subjects like the future of science. Not only the future of our science, but the future of physics, or the future of all science in China. These aren't subject matters [Scholars would] ever hear at [other] meetings, but they're subject matters that are going to be important to them as they go through the rest of their lives. So I think [the meeting is] an educational opportunity that will enrich their abilities to make decisions about the future and make them better scientists.

Visit http://www.ritaallenfoundation.org/raf-news/arnold-levine.htm for an extended version of this conversation.



SCIENTIFIC ADVISORY COMMITTEE

"We want to invest in individuals who have proposed great ideas to study and clearly have been successful. But we are not investing in a specific idea per se, because if their proposal doesn't work, we want to know that they can move on and do something equally creative."

—Kathleen Foley (1978), Rita Allen Foundation Medical Advisor

PIONEERING PAIN RESEARCHER INVESTS IN NEXT GENERATION OF SCHOLARS

A conversation with Kathleen Foley, Rita Allen Foundation Medical Advisor and 1978 Rita Allen Foundation Scholar

MEMORIAL SLOAN KETTERING CANCER CENTER

"The idea of the interview is...to give [candidates] the opportunity to present their research plan, to answer questions and to see how they respond in that setting. It is very much about the science, their ideas, their innovative and creative approaches and their risk-taking."

Kathleen Foley was just beginning to delve into the emerging field of pain assessment and treatment when she was selected as a Rita Allen Foundation Scholar in 1978. As a member of the second class of Scholars, she used her Foundation grant to study pain in patients suffering from shingles.

Today, Foley is recognized as a leader in developing global standards for pain management and palliative care. She is Attending Neurologist Emeritus in the Pain and Palliative Care Service at Memorial Sloan Kettering Cancer Center. In 1981 Foley helped to establish this first-of-its kind, team-based service, which ensures that pain management and palliative care are integrated into cancer treatment for all patients. From 1994 to 2003, she directed the Project on Death in America, which focused on changing attitudes and policies toward end-of-life care.

Foley joined the Rita Allen Foundation's Scientific Advisory Committee in 1997, and became the Medical Advisor in 2009, leading the committee in selecting each class of Scholars. She introduced the idea of a partnership between the Rita Allen Foundation and the American Pain Society to identify and support promising young investigators working to decipher the myriad mechanisms of pain signaling, with the potential for clinical applications. Each year since 2009, two Rita Allen Foundation Scholars have been designated as recipients of the Award in Pain.

Here, Foley recounts her unexpected introduction to clinical pain research, and reflects on the courage and ingenuity she seeks in new generations of Rita Allen Foundation Scholars.

How did you first get involved in pain research?

At the end of my neurology residency program at New York Hospital [now New York–Presbyterian], my mentor Jerry Posner, who was one of the cochairs of our department, said, "We need someone to come and be a clinician-researcher at Memorial Sloan Kettering Cancer Center. We need someone to study pain."

I said, "Well, I don't know anything about pain." And he said, "That's fine. Not too many people do."

I had the opportunity to work with some great researchers. I spent the first year as a fellow in what is called neuro-oncology, a new field that studied tumors of the brain and metastatic disease in the rest of the neurological system. We made the decision that pain was a neurologic complication of cancer—that's how we characterized it in the department of neurology—and we did analgesic [pain relief] studies on cancer patients.

The analgesic study group at Memorial was well established in the field of clinical analgesic studies, but I had no experience with using analgesic medicines clinically. I had been through an internship and three years of a superb neurology program and been a chief resident, but I had probably never written an opioid prescription for a patient, and had never thought much about how to treat patients with pain. It was humbling in the first couple of years, because I needed to learn about pain, and at the same time I needed to treat cancer patients.

As the Rita Allen Foundation's Medical Advisor, how do you go about interviewing and selecting Rita Allen Foundation Scholars?

There is a two-stage process. All the applications are reviewed and ranked by all the members of the Scientific Advisory Committee, and then the top eight or 10 candidates come for an interview. The idea of the interview is not to be intimidating to the investigators, but to give them the opportunity to present their research plan, to answer questions and to see how they respond in that setting. It is very much about the science, their ideas, their innovative and creative approaches and their risk-taking.

We want to invest in individuals who have proposed great ideas to study and clearly have been successful. But we are not investing in a specific idea per se, because if their proposal doesn't work, we want to know that they can move on and do something equally creative. There is also a lot of emphasis placed on their ability to bring together molecular biology with physics and mathematics, and with innovative techniques. Some are tool developers, and some are asking very basic biological questions. Others are more translational.

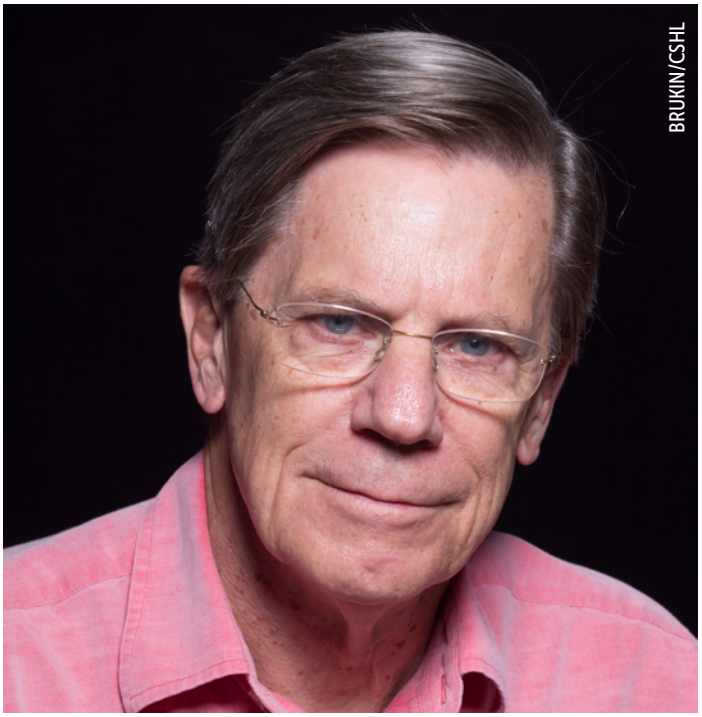
Not all of them come with this idea about how they're going to change the world because they discover a new fact, but rather that this is such an important basic scientific question that they need to answer it. We do have a list of topics—cancer, immunology and neuroscience—but we're expansive in how we view those categories. It is more important that an idea is well developed and challenging, and that the individual has a track record of doing good work.

As we look ahead to our 40th anniversary plenary meeting, why is it important that we bring together all of our Scholars?

The Rita Allen Foundation award has had an enormous influence on people's careers, and I think we have not captured that enough. So we'd like to hear more about those experiences. Also, they're part of a tribe because they received this award. They've all uniquely gotten to their current positions through various paths, and they have a host of experiences that may help the next generation. So I think we want to say, "We're glad you had a Rita Allen award. How can you mentor and support the next generation of Scholars?"

Visit http://www.ritaallenfoundation.org/raf-news/kathleen-foley2.htm for an extended version of this conversation.

DOUGLAS FEARON
Attacking Disease with Insights



"What I'm doing now is more exciting to me than anything I've ever done. I always felt that I was preparing myself to finally attack a really important, big problem."

Early on in his medical training, Douglas Fearon relished the "scientific problem solving" required to find effective treatments for patients. But as he progressed through his medical degree, internship and residency at Johns Hopkins University, Fearon also grew frustrated by the inability to truly cure diseases.

"We were controlling the physiology of the disease, but we weren't reversing the basic process of the disease," he says. "I really wanted to cure people, and I started to get interested in research, with the faith that if we understood more about disease, we could then come up with cures."

He decided to focus on understanding autoimmune disease, "because it was fascinating in kind of a literary sense: the body's immune system attacking the body," says Fearon, whose undergraduate English literature major at Williams College still provides a frame of reference. "It's paradoxical that a host system that we need to protect ourselves against microbial infection somehow makes a mistake and starts destroying cell tissue."

On the advice of Mary Betty Stevens, one of his mentors at Johns Hopkins, Fearon pursued a clinical research fellowship with immunologist Frank Austen at Harvard Medical School. Fearon characterized some of the key molecules and regulatory steps involved in the "complement" innate immune system, a collection of more than 30 different proteins that complements the pathogen-fighting powers of antibodies, macrophages and other immune components.

Fearon later returned to Johns Hopkins as a professor of medicine, and in 1993 joined the faculty of the University of Cambridge in the U.K. His research group explored the biology of B and T cells, the white blood cells of the adaptive immune system that produce antibodies and kill off pathogens. Notably, Fearon and his colleagues discovered that C3, a central complement protein that binds to foreign molecules on invading microbes, helps B cells recognize pathogens, connecting the innate and adaptive immune systems.[1]

Fearon has also investigated the ties between cancer and the immune system. In recent years he has collaborated with 2004 Rita Allen Foundation Scholar David Tuveson, who trained

in Fearon's laboratory as an M.D.-Ph.D. student. A research team led by Fearon and Tuveson used mouse models of lung and pancreatic cancer to show that small populations of cells in the connective tissues of tumors produce a protein that protects cancer cells from attack by the immune system.[2]

Further work revealed that this effect is mediated by CXCL12, a protein that coats cancer cells and prevents the intrusion of T cells into tumors. However, a drug that specifically inhibits the CXCL12 receptor on T cells can reverse the effect, allowing T cells to dismantle tumors.[3] The drug, known as plerixafor, is now in phase 1 clinical trials for pancreatic cancer at both the Cancer Research UK Cambridge Research Institute and Weill Cornell Medical Center.

Fearon joined the Rita Allen Foundation's Scientific Advisory Committee in 2002, and has held a joint appointment at Cold Spring Harbor Laboratory and at Weill Cornell Medical College since 2014. Here, he reflects on his decision to devote his career to research, the complications of translating laboratory results into clinical therapies, and the similarities between sports and science.

What made you decide to focus more on research than on clinical work?

Early on, I observed that people in academic medicine always touted how you had to be a triple threat: You had to be a great teacher, you had to be a great clinician and you had to be a great researcher. And on top of that, you probably had to be a great administrator. But when I looked at older academic physicians who had had remarkable early research careers, they often had kind of petered out, because they were continuing to do the same thing that they had been doing when they were younger.

I thought the reason was that they simply didn't have enough time to explore new ideas, which actually takes a lot of time. Often you don't know what's relevant to your research, so you need to learn a lot more than what you actually end up using. And the medical profession demands that you do so many things, that you don't have time to spend thinking about new ideas. I was at Hopkins in the mid-'80s when I realized this. I was a professor of medicine and head of rheumatology, and I was running a graduate program in immunology. I got very worried, because I wanted to go into research to make a discovery, not to be a prominent academic physician.

So I decided to move to Cambridge, where I would have an opportunity to focus on thinking about my research. I also had a sense, which became stronger over the years, that at an institution like the University of Cambridge, you are encouraged to think about long-term problems. You don't have to say every day that you did something great. Now that I spend half of my time at Cold Spring Harbor, it's a little bit like Cambridge—a quiet environment. Weill Cornell [Medical College] is also a great institution, but being a medical school and clinical center, it is more hectic and active. But the advantage there is that I can establish clinical connections, which I must do at this point in my research.

What are some of the challenges you're facing as you work to apply the results of your laboratory research to a clinical trial?

The clinical trial we're doing is testing a very fundamental idea we developed by studying mouse pancreatic cancer. In the mouse system we can more or less control all the variables and get a clean readout of whether or not we're hitting our therapeutic target. Now, there are enough examples of studies in mice that do not translate into humans. The biology somehow differs, but the other thing that happens is that you can't control variables as well when you start to do the experiment with humans. I have an intense fear of us not doing the study properly and getting a false negative. If we get a false negative, I know the pharmaceutical industry will walk away from our work, and the concept will not advance to human therapy.

We're only treating these patients for one week, and there's not going to be an effect on cancer growth, or I don't expect it. So we're developing assays that can tell us that we turn on the immune reaction in the tumor. There's no standard way to show you turned on the immune reaction in a tumor, other than showing that the tumor gets smaller—in trials where patients continue using the drug for weeks. The company that's providing us with the drug is only

allowing us to treat the patients for one week. In a mouse, I know we can show an effect in one week. I just don't know that yet in humans.

What did you learn from your experiences with college football? Do you see any parallels between playing sports and doing research?

I had always enjoyed sports, and enjoyed it very much at Williams. My junior year we went six [wins] and two [losses]. We were very intense about our commitment to football, and we were expecting to have a very, very good season our senior year. But senior year, we went two and six.

The resiliency that football taught us was that if you lost a game, you had next week to redeem yourself. I think that's been good for my research: If the experiment doesn't work, keep on thinking, and keep on working. What I'm doing now is more exciting to me than anything I've ever done. I always felt that I was preparing myself to finally attack a really important, big problem. It's just like football: When you're young, you play on a freshman team, and then your next year, you might be second-string, and you're gradually getting better until you get to be first-string.

References

1. Dempsey, PW, Allison, ME, Akkaraju, S, Goodnow, CC, and Fearon, DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science* 271 (1996), 348-350.
2. Kraman, M, Bambrough, PJ, Arnold, JN, Roberts, EW, Magiera, L, Jones, JO, Gopinathan, A, Tuveson, DA, and Fearon, DT. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science* 330 (2010), 827-830.
3. Feig, C, Jones, JO, Kraman, M, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci USA* 110 (2013), 20212-20217.

JEFFREY MACKLIS (1991)
Making and Mending the Brain's Machinery


ALEXANDROS POULOPOULOS

"What I saw was the brain as a machine that also thought. That connected [my interests in] literature, philosophy, intellectual history and biophysics, and all of a sudden my whole world came together and I said, 'I want to do that.'"

Early in his research career, Jeffrey Macklis set out to unite two seemingly disparate fields of neuroscience: neural development and brain repair. At the time, this was an unorthodox idea, and "being a brain repair guy was a little shady," he says. Still, he thought, "if we figured out how the brain was built, then maybe we could figure out how to rebuild it or fix it, and we might also be able to figure out something about why it breaks."

Macklis has made strides in understanding the cells and pathways of the brain's cerebral cortex, the outer layers of tissue critical for voluntary movement, sensation, thinking, memory, language and consciousness. His research has shown that it is possible to rebuild cortical circuits by coaxing new neurons integrated in the adult brain into specific developmental tracks. And Macklis continues to explore the origins of nerve cell diversity, providing insights into autism, neurodegenerative diseases and spinal cord injuries.

Macklis earned bachelor's degrees in both bioelectrical engineering and literature from the Massachusetts Institute of Technology. During his junior year, his research mentor, mechanical engineering professor Ernie Cravalho, encouraged him to apply for early admission to the Harvard/MIT Health Sciences and Technology (HST) program, which integrates science and engineering with medical training. In an HST neuroscience course, Macklis arrived at an unexpected realization during a neurophysiology lecture by "this curly-haired Swedish guy by the name of Torsten Wiesel" (a 1981 Nobel Laureate and an emeritus member of the Rita Allen Foundation's Scientific Advisory Committee).

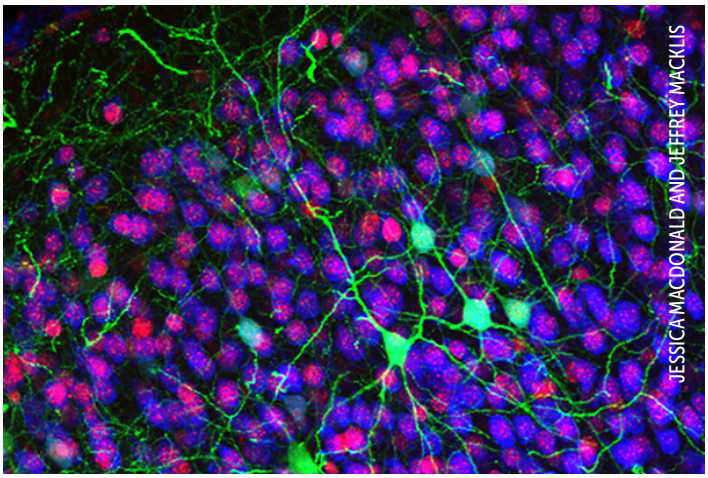
"He showed us amazing experiments on vision in cats, using electrical recordings," Macklis recalls. "What I saw was the brain as a machine that also thought. That connected [my interests in] literature, philosophy, intellectual history and biophysics, and all of a sudden my whole world came together and I said, 'I want to do that.'"

Wiesel graciously invited Macklis to talk neuroscience over tea and cookies, and, based on Macklis' desire to investigate how the brain is built, suggested he reach out to Harvard's Richard Sidman. Macklis describes Sidman as "at that time among the world's leading developmental neurobiologists"

who had "pioneered the ideas of genetic underpinnings of neuron- and circuit-specific development and degeneration before molecular manipulation of the nervous system existed." Macklis conducted both graduate and postdoctoral research in Sidman's laboratory, where he focused on myelinating glia and their mutations, biophysical approaches for targeted activation of long-distance circuits, and cell type-specific neuronal degeneration to investigate integration of new neurons.

Macklis began his own research program at Harvard Medical School, and in 1991 he was selected as a Rita Allen Foundation Scholar. He recalls that the Foundation's Scientific Advisory Committee (including Wiesel) was surprisingly receptive to his idea of combining studies of cortical development with neuronal repopulation and regeneration. The award "gave me a mandate and provided some resources to just go after these venturesome combinations of fields," he says. "That really enriched the lab, and let us take some risks in directions that I otherwise would have been a bit hesitant to go in."

Using mice as a model for brain development and neuronal repopulation from progenitors, Macklis induced selective degeneration of subtype-specific neurons in the neocortex. He showed that under the right conditions, transplanted embryonic neurons could migrate to new positions, acquire correct neuron subtype identity and begin to restore functional connectivity to long-distance targets. His group's subsequent work examined the mechanisms behind this regeneration, which contributed to the seminal finding that progenitors already existing within the adult brain are capable of being manipulated to generate new long-distance cortical "projection" neurons, partially repairing degeneration of circuitry in the neocortex.[1]



Jeffrey Macklis studies the development of the cerebral cortex, the most complex, outer layers of the brain critical for voluntary movement, sensation, thinking, memory, language and consciousness. Shown here are callosal projection neurons (green), which connect the two hemispheres of the cerebral cortex and are known to develop abnormally in some cognitive disorders.

Macklis joined the Foundation's Scientific Advisory Committee in 2007. Today, Macklis and his team have broadened their investigations of the cerebral cortex, working to more deeply understand molecular controls over development and diversity of neuronal subtypes, the subcellular mechanisms guiding the formation of axons and synapses, and how these processes are perturbed in developmental and neurodegenerative diseases.

Here, Macklis recalls his early impressions of science and engineering, and reflects on some of the highlights from his research career.

How did you first get interested in science, and what was your first research experience?

My father was an aerospace engineer who worked on the space program. I was surrounded by telescopes, gyroscopes and early computers; I traveled to launches at the Kennedy Space Center and watched satellites and manned spacecraft being built. But I was also observing grasshoppers and frogs and crayfish from local creeks, and trying to figure out how muscles moved limbs, and how frogs caught flies. I think I was always interested in some combination of biology and machinery.

In high school, I thought maybe I would be a physicist who studies people, but I didn't know what that meant. In a National Science Foundation-sponsored program for high school seniors at the University of Pennsylvania, we had Tuesdays and Thursdays for independent pursuits, and I wanted to use that time to see what academic research was all about. I got involved in a wonderful lab [led by] Professor Fred Ketterer. He enabled me to basically be his undergraduate thesis student, and then, because I did well with building instruments and inquiring about cellular membranes in a quantitative way, he let me have more freedom. Fred was my earliest research mentor, and he was very generous with me.

How has your work changed our understanding of development and repair in the cerebral cortex?

When I started my lab there was very little knowledge about integrating new neurons into existing circuitry to repair that original circuitry in the brain, and in fact the dogma was that it was ludicrous to have goals in that direction. It was largely thought that mammalian brain circuitry was complete, developed, hardwired. Cerebral cortex circuitry in particular was considered by many to be much too complex for regeneration. My lab produced some of the first evidence that under the right cellular conditions, with open synaptic space, we could manipulate precisely correct immature developmental neurons to rebuild cortical and other brain circuitry that connected across long distances and regenerated functional circuitry.

At that point, others had identified that there were progenitors—what some in the field now would call stem cells—resident in the brain in a couple of locations, making new neurons in the hippocampal dentate gyrus and the olfactory bulb, which deal with memory and smell, respectively. So my lab started asking whether there might be small numbers of such progenitors in the adult brain, perhaps residual from development, that were already poised to make new neurons in the cerebral cortex—this most elegant, highest-level part of the brain that makes us think and talk and move and feel and integrate.

People thought that was a little wacky, but in the early 2000s we had a series of papers that startled the field. We were the first to show that we could make the same manipulations as before, but instead of transplanting immature neurons to rebuild circuitry, we could recruit small but real numbers of new, subtype-specific long-distance "projection" neurons from a subset of these progenitors right within the brain itself. Then we began to figure out the genes and molecules and the logical organization that build the hundreds and thousands of distinct subtypes of neurons in the cerebral cortex, which connect and integrate motor and sensory and associative, cognitive information, and provide output to the spinal cord and the outside world.

Where are you going next in your research?

We'd like to contribute to understanding how neural wiring and circuitry get implemented at the subcellular level, and how they might go wrong or "break" later on. As genomics has gotten more and more powerful, the genes that come up in human genomic studies for neuropsychiatric diseases—from bipolar disorder and schizophrenia to motor disorders, intellectual disabilities and autism spectrum disorders—are enriched with genes that have to do with the functioning and maintenance of synapses. Synapses start their development as so-called "growth cones," so we've gone directly after the molecular biology of subtype-specific growth cones.

Going all the way back to my first conversation with Torsten Wiesel in his office, I've been centrally interested in the notion that the way a neuron and its circuitry function is derived from how they were built. So after several years of developing new approaches and technologies, I think we're finally close to figuring out how growth cone-guided circuits and synaptic machines are built in diverse, subtype-specific ways, and what might go subtly wrong with building those circuits and machines in a whole range of neuropsychiatric diseases.

Reference

1. Magavi, SS, Leavitt, BR, and Macklis, JD. Induction of neurogenesis in the neocortex of adult mice. *Nature* 405 (2000), 951-955.

GREGORY HANNON (2000)
Tools for Tough Questions

CRUK PIONEER AWARD

"As scientists, we produce science—we produce results, we produce insights. But I think it's much more important that we produce scientists."

Gregory Hannon grew up in western Pennsylvania, where his father worked as a quality inspector at a factory. Hannon had professional aspirations, and assumed he was "destined" to become a physician. "Growing up, I didn't even know that the job I do now existed," he explains. As an undergraduate at Case Western Reserve University, Hannon took a job as a lab assistant, and this taste of the research life changed his plans.

Working with structural biologist Joyce Jentoft, Hannon learned to use nuclear magnetic resonance (NMR) spectroscopy to determine the structures of proteins, and wrote one of the first software programs for visualizing protein structures on a personal computer.[1] Hannon stayed at Case Western for his Ph.D., delving into the mysteries of RNA processing in the laboratory of Timothy Nilsen. His research was fueled by an unexpected turn of events: another student in the lab got scooped on the discovery of trans-splicing—a phenomenon in which RNA molecules transcribed from different genes are joined together and translated into a kind of fusion protein.

Although others had demonstrated the existence of trans-spliced RNAs, the production mechanism of these oddball molecules remained unknown. So Hannon and his colleagues examined trans-splicing in *Ascaris lumbricoides*, a giant parasitic roundworm with eggs that could be easily collected and induced to develop synchronously—helpful properties for gathering sufficient RNA material to visualize and study.

By investigating trans-splicing in cell-free extracts from developing embryos, Hannon was able to decipher the details of the reactions that append a specific "spliced leader" sequence of nucleotides to an RNA molecule, thus marking it for trans-splicing.[2] Further research revealed the identities of the molecular labels that enable two strands of disparate RNA to associate and recruit proteins that splice them together.[3] This work "added new twists to our understanding of RNA metabolism," Hannon says. Today, researchers are investigating the potential of trans-splicing to correct disease-causing mutations.

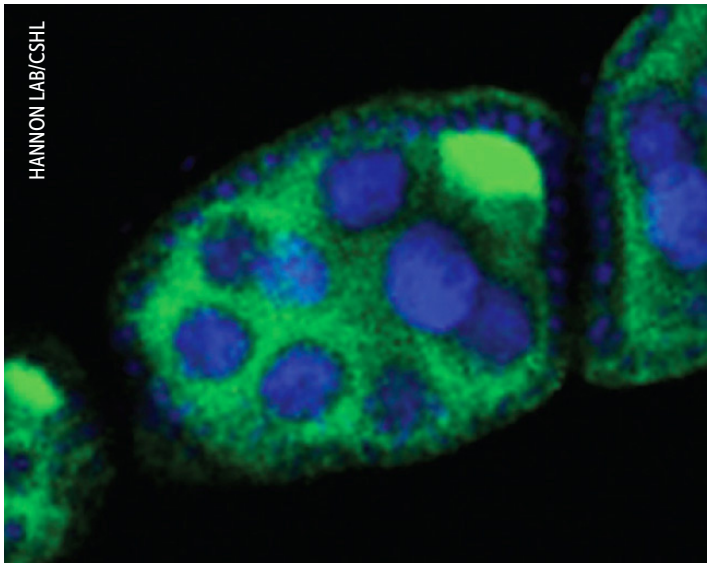
Hannon switched gears as a postdoc, moving to Cold Spring Harbor Laboratory and working with yeast geneticist David Beach to study the activities of cyclin-dependent kinases,

key cell cycle regulators whose mutation can lead to cancer. Hannon and Beach discovered a tumor suppressor gene, p15, whose product inhibits cyclin-dependent kinases.[4] When it functions properly, p15 can respond to external signals by halting cells in an early phase of the cell cycle, preventing further growth and cell division. Hannon contributed to the discovery of two related cyclin-dependent kinase inhibitors, p16 and p21. Together these represented a new class of cell cycle regulatory proteins, which have turned out to play major roles as tumor suppressors.

After joining the faculty of Cold Spring Harbor Laboratory in 1994, Hannon continued to explore the underpinnings of cell cycle control. By the time he was named a Rita Allen Foundation Scholar in 2000, Hannon had shifted his focus to developing genetic tools, driven by a desire to go deeper into biological questions than biochemistry had allowed him to. "If there's one theme, it's that we don't really have a single field," he says of his team's work.

Hannon studied the mechanisms of RNA interference—a process by which a double-stranded RNA molecule can block a complementary messenger RNA from being translated into a protein, effectively "silencing" the corresponding gene and allowing researchers to deduce the normal function of the silenced gene. Hannon's group successfully targeted specific transcripts for silencing in fruit fly cells, demonstrating that the silenced transcripts were enzymatically degraded.[5]

Later, they characterized the enzymes Dicer and Argonaute, central players in the silencing process,[6,7] and developed a method to stably knock down gene expression in mammalian cells.[8] By 2002, so many scientists were exploring various aspects and applications of RNA interference that *Science Magazine* declared small RNAs "Breakthrough of the Year." (Andrew Fire, a 1989 Rita Allen Foundation Scholar, shared the 2006 Nobel Prize in Physiology or Medicine for the discovery of RNA interference.)



A developing fruit fly egg chamber, showing "nurse cells" that support the egg (blue) and a transposon (bright green), which will lead to sterility in the fly that develops from this egg. Gregory Hannon studies the Piwi-interacting RNA pathway, which protects the genomes of gamete-producing cells from disruptive transposons.

Hannon went on to make key advances in understanding the Piwi-interacting RNA pathway, which is vital for fertility in all animals, as it protects the genomes of gamete-producing cells from disruptive transposons. In 2014, Hannon moved his lab to the Cancer Research UK Cambridge Institute.

Hannon has served on the Rita Allen Foundation's Scientific Advisory Committee since 2009. Here, he describes his renewed focus on tumor biology, his drive to develop new research technologies, and the joys of training and supporting young scientists.

Where are you going next in your research?

In our cancer research, we're trying to understand tumors in 3-D, or better yet, in 4-D. We want to characterize a lesion as a whole: Which cells are there, what are the cells doing, and how are they communicating with each other? How does heterogeneity within tumors impact both disease initiation and progression, and especially treatment response? Now that we've moved to a place that is very cancer-focused, we hope to use what we've learned to have a direct impact on patients.

In terms of basic biology, we want to better understand the mechanistic details of the Piwi-interacting RNA pathway—a deeply conserved process that is absolutely critical for defending germ cell genomes. It's a very complicated small RNA-based system that operates in every animal and is essential for fertility.

We want to understand how the system is put together, down to the level of protein structures and interactions. We've had lots of surprises so

far, including RNA acting as a means of epigenetic inheritance.[9] I think there will be a lot more surprises as we learn more about the mechanistic details, and I think those insights will flow into other related fields—less specialized than these very specific germ cell phenomena.

What are the greatest challenges in your work?

The big challenge is that almost everything in science is technology-related. If the technology is available to ask an important question, it gets asked. We tend to do things that aren't quite possible with toolkits that are out there, so we're always running up against technical barriers to answering questions that are deeply biological.

In any thread of work in my lab over the last couple of decades, there are always key points where we've either made or adopted some cutting-edge approach that transformed what we could do. There are half a dozen of these projects in the lab right now. For instance, we're trying to work with very small bits of clinical sample to determine how we can predict whether early breast cancer will progress or not.

There are challenges around trying to understand the biology of very rare dormant cell populations, where you've got to pick through a whole tissue to find one cell and figure out what it's doing—which neighbors it's talking to and how. These kinds of problems are at the edge of what is currently possible. So we spend most of our intellectual effort thinking about solutions to these technical issues.

What do you see as the most significant impacts of your work?

We've tried to develop really good tools, get them into people's hands, and hope that, by having them deployed broadly, we can have an impact on our basic understanding of science, but also have a clinical impact that is beyond what we could do as an individual lab.

The other way to magnify your impact is by training people. As scientists, we produce science—we produce results, we produce insights. But I think it's much more important that we produce scientists. When I look at the people who have been trainees in the lab, and at where they've ended up in their careers, it amazes me constantly. It's great to hear from them when they get their first job, have their first child or publish their first paper from their own lab. They still want advice on things, and it reminds me that I'm building this science family—it's like having children. One of the most important things I've learned is that we are teachers before anything else.

References

1. Hannon, GJ, and Jentoft, JE. MOLECULAR DESIGNER: an interactive program for the display of protein structure on the IBM-PC. *Comput Appl Biosci* 1 (1985), 177-181.
2. Hannon, GJ, Maroney, PA, Denker, JA, and Nilsen, TW. Trans splicing of nematode pre-messenger RNA in vitro. *Cell* 61 (1990), 1247-1255.
3. Hannon, GJ, Maroney, PA, Yu, YT, Hannon, GE, and Nilsen, TW. Interaction of U6 snRNA with a sequence required for function of the nematode SL RNA in trans-splicing. *Science* 258 (1992), 1775-1780.
4. Hannon, GJ, and Beach, D. $p15^{INK4B}$ is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* 371 (1994), 257-261.
5. Hammond, SM, Bernstein, E, Beach, D, and Hannon, GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 404 (2000), 293-296.
6. Bernstein, E, Caudy, AA, Hammond, SM, and Hannon, GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409 (2001), 363-366.
7. Liu, J, Carmell, MA, Rivas, FV, Marsden, CG, Thomson, JM, Song, JJ, Hammond, SM, Joshua-Tor, L, and Hannon, GJ. Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 305 (2004), 1437-1441.
8. Paddison, PJ, Caudy, AA, and Hannon, GJ. Stable suppression of gene expression by RNAi in mammalian cells. *Proc Natl Acad Sci USA* 99 (2002), 1443-1448.
9. Brennecke, J, Malone, CD, Aravin, AA, Sachidanandam, R, Stark, A, and Hannon, GJ. An epigenetic role for maternally inherited piRNAs in transposon silencing. *Science* 322 (2008), 1387-1392.

These Q&As with three members of the Scientific Advisory Committee are edited versions of those originally published in 2011 and 2012.

JOAN STEITZ

Sterling Professor of Molecular Biophysics and Biochemistry, Yale University School of Medicine

In 1963, Joan Steitz became the sole woman in a class of 10 to begin graduate studies in biochemistry and molecular biology at Harvard University. She earned her Ph.D., completed post-doctoral work in Cambridge, England, and joined the faculty at Yale University. Today, she is best known for discovering and defining the function of small nuclear ribonucleoproteins (snRNPs), which occur only in higher cells and organisms. The Sterling Professor of Molecular Biophysics and Biochemistry at Yale, Steitz is a member of the National Academy of Sciences and the recipient of the 2015 Connecticut Medal of Science.

"It is clear that these young researchers stand out, and I am attracted to those in science who are doing something a little bit different."

What have been some of the highlights of your career?

Coming from my background and having to encounter some barriers as a woman scientist, at the beginning I was not entirely sure male grad students would want to work in a lab headed by a woman. But Yale had men who were both eager and very good who joined the lab. We continued working on aspects of my postdoc project, and I received my first recognition prize in 1975—the Passano Foundation Young Scientist Award.

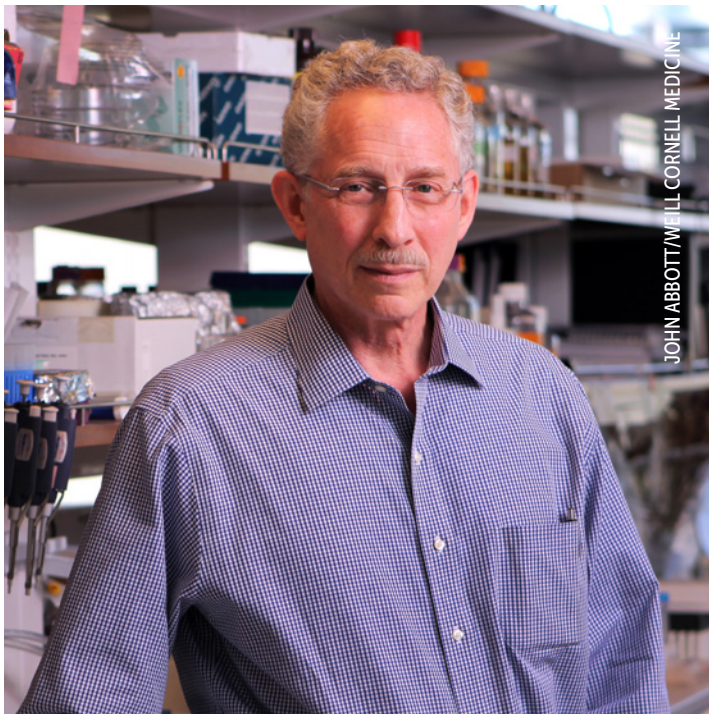
Subsequently, we began a new line of research that is related to lupus, an autoimmune disease that develops when patients make antibodies against their own DNA, snRNPs or ribosomes, the body's protein-making factories. My colleagues and I identified snRNPs as the building blocks of the RNA splicing machinery, which is essential for making functional messenger RNAs in mammalian cells. We have also studied other snRNPs involved in excising a rare, divergent class of introns, and still other snRNPs involved in pre-ribosomal RNA processing. Today our

work continues to focus on noncoding RNAs and the roles they play in the regulation of gene expression.

Would you share your thoughts about the selection process for Rita Allen Foundation Scholars?

I find that the interview process is a truly positive and unique aspect of the program's selection process. Most other awards do not include interviews, and you can only get so much from the written submissions. Meeting the applicants and finding out that they are not what you necessarily expected is much more valuable than relying on written documents. I have served on a number of selection committees, and getting to know the Scholars one-on-one is a major strength of the Rita Allen Foundation program.

I also like that not everyone is doing the same type of research. There is a wide selection of projects to choose from and support. It is clear that these young researchers stand out, and I am attracted to those in science who are doing something a little bit different.



"Major game-changing findings result when we entrust researchers with the freedom to explore."

CARL NATHAN

R.A. Rees Pritchett Professor and Chair, Department of Microbiology and Immunology, Weill Cornell Medical College

Co-Chair, Program in Immunology and Microbial Pathogenesis, Weill Graduate School of Medical Sciences

1984 Rita Allen Foundation Scholar

A graduate of Harvard College and Harvard Medical School, Carl Nathan trained in internal medicine and oncology at Massachusetts General Hospital, the National Cancer Institute and Yale before joining the faculty of The Rockefeller University and Weill Cornell Medical College. He is a member of the National Academy of Sciences, the Institute of Medicine and the American Academy of Arts and Sciences. He is the 2009 recipient of the Robert Koch Award for his research on mechanisms of defense against bacterial pathogens and the 2013 recipient of the Anthony Cerami Award in Translational Medicine.

What influenced your decision to pursue a career in science?

The directions we take in life are very much influenced by the people we meet. My father introduced me to one of his college friends, the late physician-scientist Lester Grant, who turned me on to science when he hired me as an assistant animal handler at New York University Medical Center the summer after my freshman year in high school. I

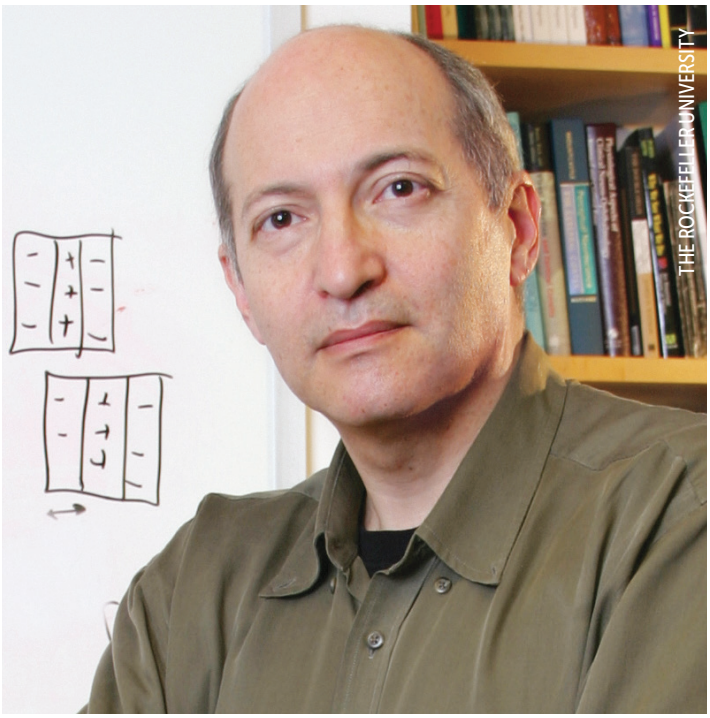
worked for Lester for five summers. Washing rabbit cages let me learn research from the bottom up. Lester loved students and excelled at communicating his enthusiasm for learning and for science. He introduced me to the NYU greats, including Lewis Thomas, and ignited my interest in medicine.

After leaving NYU, during my oncology fellowship at Yale, I realized that despite my love for both clinical medicine and research, I was in the wrong specialty to combine them, given that I needed quiet moments to think every once in a while. That is when I decided to go all research.

How is the research environment different for today's Rita Allen Foundation Scholars than it was when you received your award?

Let me instead emphasize something that remains the same. The special value of Rita Allen Foundation support was, and still is, flexibility. With National Institutes of Health grants it is difficult to deviate from original outlines, as grants are increasingly treated as contracts with set timelines and deliverables. Major game-changing findings result when we entrust researchers with the freedom to explore.

The Rita Allen funding permitted me the latitude to investigate leprosy. That work ultimately led to discoveries that advanced our understanding of tuberculosis. Along the way we identified specific regulatory and biochemical mechanisms of cell killing and resistance that are broadly applicable, including to cancer. The cytokines and enzymes whose properties we discovered underlie a great deal of cell signaling relevant not only to infectious disease and cancer, but to much of medical physiology. I could not have launched this decades-long line of inquiry without the flexibility of the Rita Allen award.



CHARLES GILBERT

Arthur and Janet Ross Professor, Laboratory of Neurobiology, The Rockefeller University

1986 Rita Allen Foundation Scholar

Charles Gilbert received his M.D. and Ph.D. from Harvard Medical School, where he held an academic appointment until he joined The Rockefeller University in 1983 as an assistant professor; he is now head of the Laboratory of Neurobiology at Rockefeller. In 2004 he was named the Arthur and Janet Ross Professor at Rockefeller. A member of the National Academy of Sciences and the American Academy of Arts and Sciences, he has received numerous awards, including the W. Alden Spencer Award from the Columbia University College of Physicians and Surgeons and the Edward M. Scolnick Prize in Neuroscience from the McGovern Institute for Brain Research at MIT.

What was your pathway to a career in science?

As a boy I was inspired by books about scientific research, such as *Arrowsmith* by Sinclair Lewis, where the fictional character worked as a physician-researcher at an institute modeled on The Rockefeller University. I had forgotten the connection when I first began working at Rockefeller, and it is ironic that it had such an influence and I ended up working here.

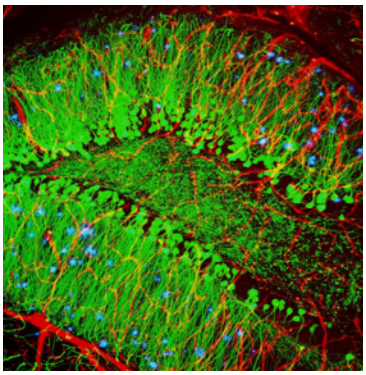
I had my first research experiences during college, where I worked at Cold Spring Harbor Laboratory. The work I did there was at the molecular level, with bacteriophage, but after a time my interests migrated toward doing something more systems-oriented. I was fortunately able to tailor my own program to pursue my degrees at Harvard, where I worked with Torsten Wiesel [an emeritus member of the Foundation's Scientific Advisory Committee] and David Hubel, who did seminal work on neural systems research for which they received the Nobel Prize. From that time to the present, I have had a continuing interest in studying the circuitry underlying the processing of sensory information by the cerebral cortex.

What questions are you exploring in your research?

Today, my team and I study the visual cortex, with a focus on the mechanisms of perceptual grouping, object recognition and perceptual learning. The job of the visual cortex is to take signals coming from the retina, to group features of visual scenes belonging to objects, and to identify them. We investigate the mechanism, at the level of cortical circuitry, by which this occurs.

Perceptual learning, the way visual experience shapes the strategy by which the cortex analyzes sensory information, is another major interest of ours. We examine the contributions of different cortical areas along the visual pathway that facilitate this learning and are characterizing the functional changes occurring at the level of individual neurons.

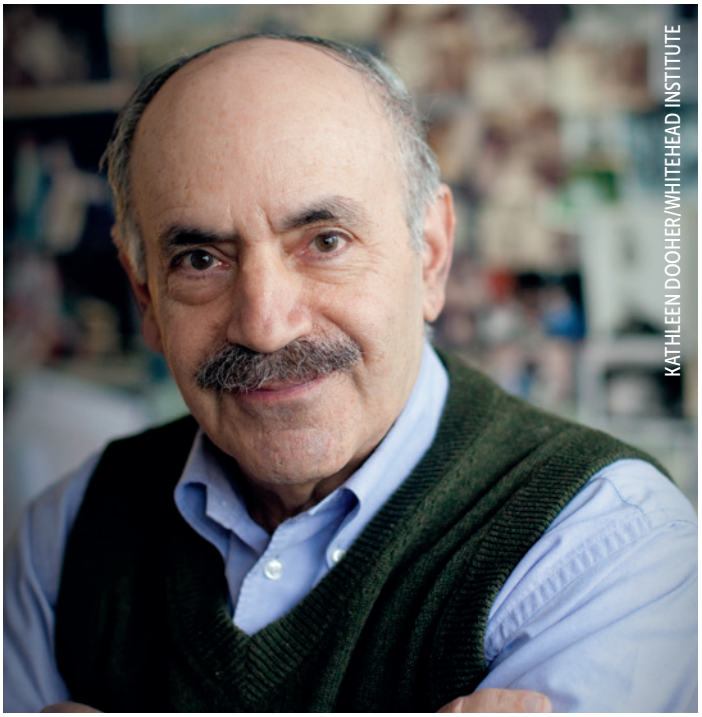
An important outgrowth of this research is the role of top-down influences in visual perception: how the brain's internal representations of the world, acquired through experience, shape the way we analyze the ongoing stream of visual input. We have discovered that neurons are adaptive processors, changing the kind of information they carry under different task conditions, and that cortical connectivity is dynamic, whereby neurons select the inputs that enable them to take on different functional roles. We are now exploring the possibility that behavioral disorders such as autism involve deficits in this process.



KEYNOTE SPEAKERS

"What propels me every day is... the joy and pleasure of being in the lab and interacting with the people of my laboratory group, discussing science. That's what makes it all worthwhile."
–Robert Weinberg (1976)

ROBERT WEINBERG (1976)
The Genesis of Cancer Genetics


KATHLEEN DOOHER/WHITEHEAD INSTITUTE

"It represented the first experimental proof that inside cancer cells, in the absence of any cancer viruses, there actually lay mutant cellular genes that are responsible for the malignant behavior of these cells. That work was just picking up then, and having the Rita Allen award was an enormous support."

Robert Weinberg began his studies at the Massachusetts Institute of Technology with the intention of preparing for medical school, but before long he was dissuaded from this path. "I heard that doctors had to stay up all night," he says, "so I switched to becoming a biologist."

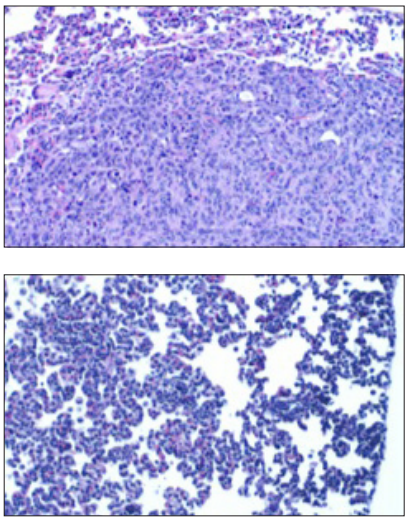
Weinberg soon found that he relished the research life—he thrived on the continuous inquiry and tinkering of the scientific process. He went on to make a number of pivotal discoveries that have transformed our understanding of the genetic origins and development of cancer. He is known for identifying the first human oncogene, *ras*[1] (an abbreviation for rat sarcoma virus, in which the gene's homolog was first characterized); he and his colleagues also isolated the first known tumor suppressor gene, *Rb*[2] (for retinoblastoma, a type of eye cancer).

Weinberg first tried laboratory research as an undergraduate at MIT, where he got to know David Baltimore (then a postdoc in James Darnell's lab), who showed him the high standard of intellectual rigor required to perform first-class science. Weinberg stayed on at MIT for his Ph.D., working in Sheldon Penman's laboratory and using human cell cultures to investigate the formation of ribosomal RNA and small nuclear RNA.[3] After postdoc stints at Israel's Weizmann Institute of Science and at the Salk Institute near San Diego, he returned to MIT to assume a faculty position, working first as a research associate with Baltimore until the MIT Cancer Center first opened in 1974. By then Baltimore had established his own laboratory studying the genetics, replication and expression mechanisms of RNA viruses—with a growing interest in mammalian tumor viruses as a means of understanding cancer. (Baltimore shared the 1975 Nobel Prize in Physiology or Medicine for his findings on the ability of RNA tumor viruses to reverse transcribe their RNA genomes into DNA.)

In 1973, Weinberg became an assistant professor in the MIT Department of Biology, and was selected as a member of the inaugural class of Rita Allen Foundation Scholars in 1976. During this time his research group was transitioning from its focus on cancer-causing retroviruses to studying how chemical carcinogens, whose ability to cause cancer was unrelated to tumor virus infections, were successful in transforming normal

cells into cancer cells. "In 1979 we found that introducing DNA prepared from chemically transformed cancer cells into normal cells altered the behavior of the latter,[4] converting them from normal cells into cancer cells," he explains.

"That was a fundamentally important discovery," he adds, in that it enabled the subsequent identification of oncogenes—cancer-causing genes—present in the DNA of these chemically transformed cells and then in a variety of human cancer cells. Weinberg and his team later demonstrated that converting fully normal cells into tumor cells actually required the introduction into these cells of at least two oncogenes.[5] Still other work led to isolation of the first known tumor suppressor gene, termed *Rb*. These two classes of genes—oncogenes and tumor suppressor genes—are often likened to the accelerators and brake pedals of cells.



JING YANG

Robert Weinberg and his research team have shown that a transcription factor called Twist1, known for regulating embryonic development, also plays a key role in breast cancer metastasis. The image on the top shows a metastatic nodule in the lung of a mouse with a breast tumor. Knocking down the expression of Twist1 in breast tumor cells prevents metastasis—the image on the bottom shows normal lung tissue.

Many of these studies were performed in mouse or rat cell cultures. "In the following years," Weinberg says, "we became interested in how to convert normal human cells into cancer cells, a task that turned out to be much more challenging." In 1999, his research group succeeded at this task, which required the expression of two specific oncogenes in combination with a gene encoding the catalytic subunit of the telomerase enzyme.[6] Expression of this enzyme allowed cells to continue lengthening their chromosome ends, or telomeres, and proliferate continuously for unlimited periods of time. (Telomere regulation has been extensively investigated by Titia de Lange, a 1995 Rita Allen Foundation Scholar).

Beginning in 2004 Weinberg turned his attention to the process of metastasis, in which cancer cells disseminate from primary tumors to distant sites in the body, where they often form secondary tumors. His group showed that a transcription factor known for regulating embryonic development also plays a key role in breast cancer metastasis,[7] and that metastases arise from cancer cells that possess stem cell properties. Since then, "we have focused strongly on how metastasis occurs in human carcinomas and how the metastatic colonies are founded in distant tissues," Weinberg says, "which is a critically important problem, because 90 percent of cancer-associated mortality is caused by metastases, not by primary tumors."

He has received an impressive set of accolades for his pioneering discoveries, including the National Medal of Science (1997), the Wolf Prize in Medicine (2004) and an inaugural Breakthrough Prize in Life Sciences (2013; Titia de Lange was among the 10 other winners). However, he says, "what propels me every day is not the recognition, but sincerely the joy and pleasure of being in the lab and interacting with the people of my laboratory group, discussing science. That's what makes it all worthwhile."

Here, Weinberg recalls the significance of becoming a Rita Allen Foundation Scholar, and shares his thoughts on the state of cancer research and the training of young scientists.

What was the role of the Rita Allen Foundation award in your research career?

I was a junior assistant professor at MIT at the time. My work was moving ahead, but at a rather slow pace. My future was not so certain. I can say that being honored by receiving a Rita Allen award was a great boost for my morale. We had just begun to do some experiments that turned out to be the most important in my career, demonstrating that if you looked inside chemically transformed cells—that is, cells that have been transformed to a cancerous state through exposure to chemical carcinogens—that those cells actually can carry mutated genes, known as oncogenes, that are responsible for the aberrant behavior of those cells.

These days it sounds rather humdrum, but at the time it represented the first experimental proof that inside cancer cells, in the absence of any cancer viruses, there actually lay mutant cellular genes that are responsible for the malignant behavior of these cells. That work was just picking up then, and having the Rita Allen award was an enormous support.

What are some of the major challenges in cancer research?

People have not yet confronted the complexity of cancer in a realistic fashion. Although there are many "moonshots" proposed and many attempts at personalized medicine, the reality is that we still don't really understand what makes cancer cells tick and how to effectively kill them. Overall, the clinical applications of basic cancer research have come at a remarkably slow pace, with the one exception of immunotherapy of cancer, which has exploded over the last five years and has proven to be very useful in effectively treating a subset of human cancers. Moreover, those promoting many kinds of molecularly targeted anticancer therapies and cancer genome sequencing have made promises that, to my mind, are unrealistic and unrealizable. We still confront some fundamental problems when trying to understand the complexity of how individual cancer cells operate and what makes them resistant to various kinds of therapy.

What skills do you think are most important for young scientists?

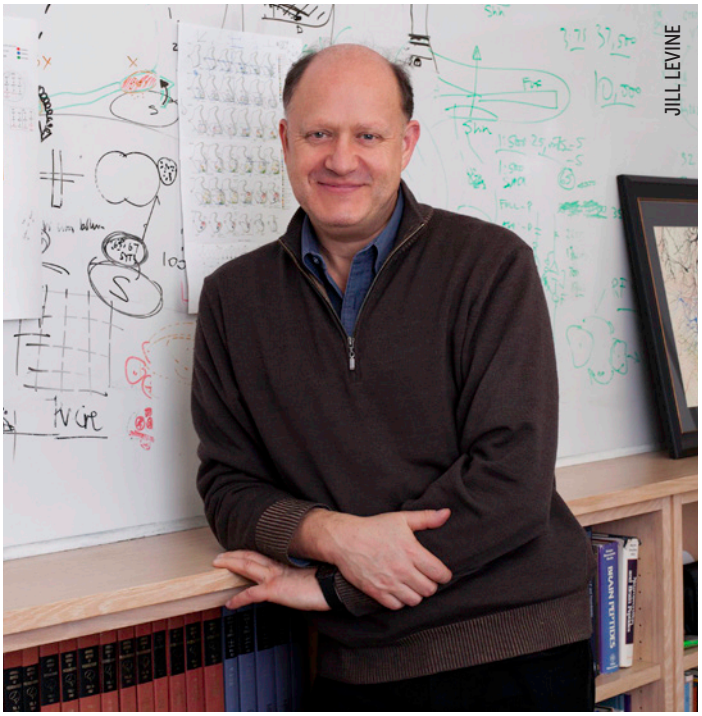
To my mind, the most difficult thing to master is not the experiment that can be done or the data that can be obtained. The most difficult thing is the prioritization of which experiments one does and which ones one doesn't do. The number of possible experiments one can think up is more than the stars in the universe, while the number of truly interesting and important experiments is far more limited. The ability to acquire a taste for what's important and what's trivial is really the most challenging task for most graduate students, and beyond that, for postdocs.

I often confront this problem when I am running my own research group meetings, when one of my trainees gets up and tells us that they want to undertake this or that experiment. The experiment they propose is bound to yield data, maybe unequivocally interpretable, rigorously supported conclusions. But then I will pose the question, "Why do you actually want to do that particular experiment?" This forces them to begin to think on their own about precisely why they want to prioritize doing certain experiments and defer from doing others. Being able to distinguish the wheat from the chaff is a critically important taste in science and, to my mind, one that's not easy to develop.

References

1. Parada, LF, Tabin, CJ, Shih, C, and Weinberg, RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus *ras* gene. *Nature* 297 (1982), 474-478.
2. Friend, SH, Bernards, R, Rogelj, S, Weinberg, RA, Rapaport, JM, Albert, DM, and Dryja, TP. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 323 (1986), 643-646.
3. Weinberg, RA, and Penman, S. Processing of 45 s nucleolar RNA. *J Mol Biol* 47 (1970), 169-178.
4. Shih, C, Shilo, BZ, Goldfarb, MP, Dannenberg, A, and Weinberg, RA. Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proc Natl Acad Sci USA* 76 (1979), 5714-5718.
5. Land, H, Parada, LF, and Weinberg, RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* 304 (1983), 596-602.
6. Hahn, WC, Counter, CM, Lundberg, AS, Beijersbergen, RL, Brooks, MW, and Weinberg, RA. Creation of human tumour cells with defined genetic elements. *Nature* 400 (1999), 464-468.
7. Yang, J, Mani, SA, Donaher, JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117 (2004), 927-939.

THOMAS JESSELL (1984)
Linking Molecules to Perception and Motion



"It is rewarding to be able to return to the same general problem and do it in a new, more incisive way. That's part of what science is about—sticking with a problem and letting new technologies reveal new aspects of that physiology."

Growing up in London, Thomas Jessell was immersed in both art and science from an early age. His mother worked as a paintings conservator, while his grandfather was an organic chemist who introduced him to the beauty of molecular structures. "I was torn between whether to train as a biochemist or whether to become an art historian," Jessell recalls.

Ultimately, he enrolled in the Department of Pharmacology at the University of London's Chelsea College. "Molecular biology as a sophisticated discipline didn't exist in Britain in the early 1970s, so pharmacology was the way that you dissected complicated aspects of neural function," he says. "I got intrigued by the power of neuropharmacology to dissect circuits and link them to behavior—this is why I became hooked."

After a year's training at The London Hospital, Jessell pursued a Ph.D. in neuropharmacology at the University of Cambridge with Leslie Iversen. "It was a time when the function of peptides in the nervous system was first being appreciated," he says. "I spent most of my graduate career working on one of these peptides, called substance P, which had a long and illustrious history" and was thought to transmit pain and other sensory signals. Jessell and Iversen investigated the actions of substance P in the trigeminal nucleus, a portion of the spinal cord involved in sensing touch, pain and temperature. Their work led to the finding that opiate drugs inhibit the release of substance P from the terminals of primary sensory nerve cells that mediate nociception [the sensing of potentially harmful stimuli].[1]

This type of "presynaptic inhibition," in which a neuron prevents the excitation of connected neurons, has proved to be essential for the functioning of neural circuits in the spinal cord and other parts of the nervous system. Nearly 40 years after completing his doctoral work, Jessell continues to explore aspects of presynaptic inhibition, albeit in the context of proprioception, which connects sensory information and movement.

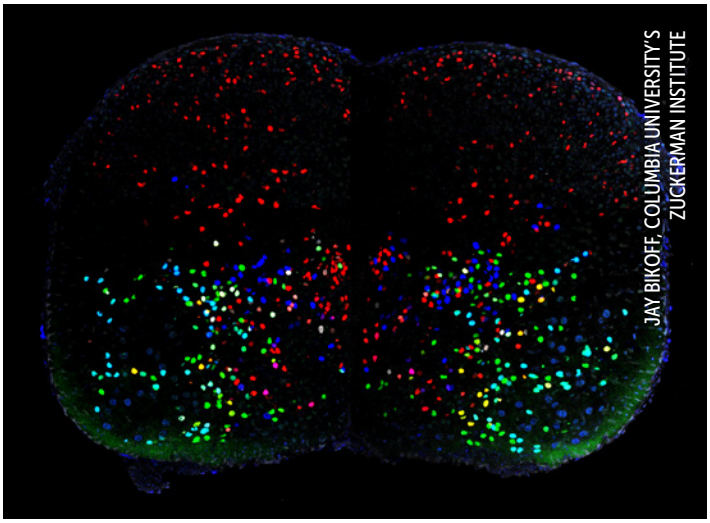
"It is rewarding to be able to return to the same general problem and do it in a new, more incisive way," he says. "That's part of what science is about—sticking with a problem and letting

new technologies reveal new aspects of that physiology." For instance, Jessell's research team recently used a combination of molecular genetic approaches and statistical analyses to investigate the diversity of inhibitory neurons in the mouse spinal cord, revealing neural "microcircuits" that control sensory input, as well as hip, ankle and foot movement.[2]

Jessell's career has paralleled the rise of molecular and cell biology and their applications to neuroscience. He entered the developing field as a postdoc at Harvard Medical School with Gerald Fischbach (now the Scientific Director of The Simons Foundation). "Up till the point that I walked in his lab, I had no concept of the immeasurable interest in cell biology, and Gerry—in one way or another—was a physiologist with cell biological affinity," Jessell recalls. "I learned at Harvard what cell biology was all about, and I also learned something about the primacy of neuroscience as a new and emerging discipline."

He joined the Harvard Department of Neurobiology as an assistant professor in 1981, and became a Rita Allen Foundation Scholar in 1984. At the time, Jessell says, "We were just exploring the cell biology of sensory motor transmission. [The award] allowed me to do things with a confidence and with a resolution that would not have happened had I not received that endorsement." His group examined the localization of specific protein and carbohydrate molecules in spinal cord sensory neurons during embryonic development, providing insights into the maturation of the neurons and the interactions between them.

After moving to Columbia University in 1985, Jessell broadened his studies of signaling and cell patterning in the nervous system. He teamed up with David Julius in Richard Axel's lab to clone and characterize two subtypes of the serotonin receptor that mediate the effects of this key neurotransmitter, and with his own group began to decipher the chemical cues that guide axons—the long projections of nerve cells—to their targets in the developing central nervous system. Many of their analyses focused on the activities of the notochord and the floor plate, crucial organizing structures for neural development in all vertebrate animals. Jessell and his group later applied their growing knowledge of spinal motor neuron differentiation to demonstrate that mouse embryonic stem cells could be induced to form functional motor neurons.[3]



Thomas Jessell and his colleagues recently developed a novel method for identifying distinct classes of neurons based on the expression patterns of 19 transcription factors. Here, various classes of V1 interneurons in the mouse lumbar spinal cord are labeled with different colors according to their transcription factor "fingerprints" and corresponding roles in motor control.

Jessell served on the Rita Allen Foundation's Scientific Advisory Committee from 1999 to 2007. Today, he is a Howard Hughes Medical Institute Investigator, and a Professor of Biochemistry and Molecular Biophysics and Neuroscience at Columbia, where he is codirecting the new Mortimer B. Zuckerman Mind Brain Behavior Institute. His laboratory continues to apply emerging molecular, physiological and behavioral tools to understand how nerve cells in the spinal cord control movement.

Here, Jessell considers the potential of new techniques to answer old questions, reflects on the joy of discovery, and shares one of his sources of inspiration.

What is most exciting about your current research?

The brain only does three things: it extracts information from the sensory world around it, it stores and retrieves that information when useful, and it converts it into behavior through action and movement. Half the brain is designed to activate behaviors through movement in very precise ways. And the minute you stop to think about that, it poses all sorts of questions and challenges that we are trying to answer, in small ways, through mouse genetics. I know that in my lifetime I'm never going to achieve a satisfying answer, because these are big cognitive problems...you have to realize the impact that you are going to make on a field is limited by the enormity of the problem. You have to set your goals on small, incremental discoveries that drive a field forward.

I am still studying presynaptic inhibition in the spinal cord—through an interest and an access to this one small set of inhibitory interneurons. Suddenly we can approach things that we were puzzled by in the past, and now there are reagents and methodologies that have never been dreamed of before: gene targeting to manipulate individual neuronal populations in mice, the ability to manipulate proteins through expression of toxins that kill neurons, or the ability to activate neurons with precision. Now we are in a position to address some of the major challenges in the field, to resolve why presynaptic inhibition is so important. We've answered that at one level, but of course every answer is associated with dozens more questions.

What do you think young scientists need to be successful?

The first advice I give to young scientists is to do what you're passionate about, because your ability to make interesting observations will depend on that. And what is in vogue today will be not necessarily the stuff of the moment tomorrow. Who can judge what is relevant in science?

I also tell my graduate students that during the course of their Ph.D. career they will discover something that is new and has never been realized in the history of humankind. It may not be a big thing, but it's going to be something, and you have to revel in that little element of discovery. I think that's what makes an interesting scientist: the curiosity, the persistence and the gratification when you discover something, no matter how small, about something you care about.

When you aren't in your lab or helping to direct the Mind Brain Behavior Institute, where might we find you?

You might find me in Chelsea art galleries looking at various productions by artists, which are very similar to the scientific pursuit. In both science and art, you've got to be comfortable doing things at the very edge of rationality, and trying to change things and trying to express your intuition and passion. When I'm deeply troubled about something that's gone awry in the lab, it's amazing the beneficial influence of immersing myself in the world of art—shaking me out of myself and inspiring me in a different way. So I like doing that, and I like walking up and down Broadway, observing anonymously the rich world that is New York City.

References

1. Jessell, TM, and Iversen, LL. Opiate analgesics inhibit substance P release from rat trigeminal nucleus. *Nature* 268 (1977), 549-551.
2. Bikoff, JB, Gabitto, MI, Rivard, AF, et al. Spinal inhibitory interneuron diversity delineates variant motor microcircuits. *Cell* 165 (2016), 207-219.
3. Wichterle, H, Lieberam, I, Porter, JA, and Jessell, TM. Directed differentiation of embryonic stem cells into motor neurons. *Cell* 110 (2002), 385-397.

TITIA DE LANGE (1995)
The Complex Puzzle of Chromosome Ends

JOHN ABBOTT/THE ROCKEFELLER UNIVERSITY

"The more I thought about it, the more I saw that there was a real problem with chromosome ends, and it was beginning to look like an impossible issue for nature to deal with. The problem is that we have linear chromosomes, they have ends, and those ends look like broken DNA, but the cell should not treat them like broken DNA."

When asked to describe her research, Titia de Lange says she has been "working on the same simple question for 25 years." That's a modest account of a dogged dedication that has revealed information critical to understanding both genome maintenance and cancer development.

De Lange studies how cells protect the ends of their chromosomes, or telomeres, from getting chewed up by DNA repair machinery. She has examined this erosion in early-stage cancer, explaining how the loss of telomere function can generate genome instability and drive cancer progression.

While de Lange's efforts have yielded striking advances, she would never have expected these achievements when she began her "meandering path" toward becoming a research scientist.

After finishing high school in the Netherlands, de Lange had to choose a career track before starting her university studies. "I decided I wanted to study chemistry, because that was the only thing I enjoyed," she says. "But there were no women in chemistry—not just professors, but no students either. So I decided to go to biology because they offered a biochemistry track, and I thought that would be close enough."

The "descriptive" nature of her biology training and the endless memorization of anatomical details left de Lange uninspired, until she met molecular biologist Richard Flavell (now at the Yale School of Medicine) at the University of Amsterdam. In Flavell's laboratory, then at the National Institute for Medical Research in Mill Hill near London, de Lange completed the equivalent of a master's thesis, helping to identify a genetic translocation implicated in a rare form of thalassemia, a disorder marked by abnormal production of hemoglobin.[1] "That was where I first saw how science is really done," de Lange recalls. "It was a very vibrant, competitive, international lab. It was a lot of fun, so that made me stay in science."

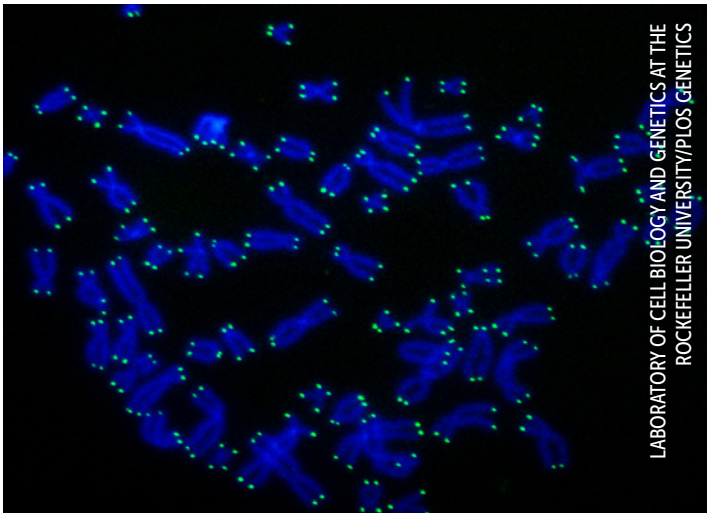
She earned a Ph.D. at the Netherlands Cancer Institute, where she worked with Piet Borst to study the genetic underpinnings of variant surface glycoproteins in the parasite *Trypanosoma brucei*, which causes African sleeping sickness.[2] These investigations piqued de Lange's interest in telomeres, as the

glycoprotein genes' positions near the ends of chromosomes appeared to facilitate the duplications and transpositions that help the parasite dodge the immune system.

This line of research led de Lange to a postdoctoral fellowship at the University of California, San Francisco, where she examined the structure of human telomeres in the laboratory of Harold Varmus.[3] (Varmus shared the 1989 Nobel Prize in Physiology or Medicine with J. Michael Bishop for pioneering work on cancer-causing oncogenes, and later directed the National Institutes of Health and the National Cancer Institute.) De Lange discovered that sperm cells have longer telomeres than somatic cells, and that tumor cells have notably short, unstable telomeres—findings that continue to guide telomere research.

After establishing her own laboratory at The Rockefeller University in 1990, de Lange began to focus on identifying telomere-associated proteins and assessing their roles in shielding telomeres from DNA repair processes. She was a Rita Allen Foundation Scholar from 1995 to 1998, a fruitful period for her research group. De Lange and postdoctoral fellow Bas van Steensel (now a research group leader at the Netherlands Cancer Institute) demonstrated that the human telomeric-repeat binding factor protein TRF1 regulates the length of telomeres.[4] They also discovered that a related protein, TRF2, prevents telomeres from fusing with one another.[5] (Agata Smogorzewska, who conducted her Ph.D. research with de Lange and became a Rita Allen Foundation Scholar in 2010, also coauthored this study and was the lead author on later work integrating the roles of TRF1 and TRF2.[6])

Around the same time, de Lange and her team identified an enzyme called tankyrase that interacts with TRF1[7] and has since been shown to release TRF1 from telomeres, allowing



Titia de Lange studies how cells protect their chromosome ends, or telomeres, from damage by DNA repair mechanisms. This microscopic image shows human chromosomes labeled with a telomere-specific probe (green).

access by the telomere-lengthening enzyme telomerase. And a collaboration with Jack Griffith, an electron microscopy specialist at the University of North Carolina at Chapel Hill, revealed that TRF2 functions to remodel telomeres into a "loop" structure, tucking in overhanging single strands of DNA and protecting the chromosome end.[8]

By 2005, de Lange's work had led to the realization that a dynamic complex of six proteins, which she dubbed shelterin, function together to regulate the length of telomeres and "shelter" them from DNA damage response and repair pathways.[9] "For the last 15 years, we've been doing experiments to figure out how these proteins do this, and we're not done yet," she says. Here, de Lange recalls the origins and progress of her investigations into telomere protection, and details emerging evidence on links between telomeres and tumors.

How did you come to focus on telomere biology?

I can't say I had a brilliant eureka insight, but the more I thought about it, the more I saw that there was a real problem with chromosome ends, and it was beginning to look like an impossible issue for nature to deal with. The problem is that we have linear chromosomes, they have ends, and those ends look like broken DNA, but the cell should not treat them like broken DNA.

As we were learning about how cells respond to broken DNA, the problem of how telomeres could prevent all this became more complex and mysterious. My model was that there would be proteins [associated] with telomeres that would fulfill this function. How, I didn't know, but I thought, "There have to be some proteins there that do this."

What has your research revealed about how cells maintain their telomeres?

At first, this project was so risky that nobody in my lab wanted to work on it, so the students and postdocs worked on other things, and I worked with a technician on finding a protein [TRF1] that bound to telomeric DNA.[10] It took five years, and it was a terrible project—once we identified it, purifying this

protein took the whole supply budget of my single NIH grant. But once we got it, in the next five to seven years we identified a number of other proteins. Other people started working on this as well, and by 2005 I proposed that all these proteins formed a single complex that I called shelterin, for the sheltering of chromosome ends.

Now we know which protein has which task. It's a beautiful little complex. It only has six members, but it is able to repress six very different pathways that cells can activate at DNA ends. One issue is that when the telomere is linear, it has DNA ends that cells can respond to. So the complex folds back the telomere; it tucks the DNA end away so that cells can't see it. This is an elegant architectural solution. I would say that the identification of the shelterin complex and the understanding of how it solves this end protection problem—that's partly due to my contribution.

What questions do you want to answer going forward?

With regard to the end protection problem, we are not there yet. We understand how two of the six pathways are repressed, and we're making progress toward the others, but there's a ways to go to understand mechanistically how this really works.

In addition, I think the field will more and more turn its attention to what happens to telomeres during tumorigenesis. We know that telomeres shorten in normal, even somatic [non-gamete] cells, because telomerase is switched off. That shortening provides our tissues with a replication barrier. A cell can only divide until it runs out of telomeric DNA, and then it has to stop—that's a good way to prevent cancer from arising. It works very well, but cancer always finds ways around tumor-suppressor pathways. When cells continue to divide even though their telomeres are too short, you get genome instability, which can actually promote tumorigenesis—this is referred to as telomere crisis.

We now realize that telomere crisis is very frequent in human cancers at an early stage, and that some of the massive genome rearrangements that we observe in full-blown tumors can be ascribed to telomere dysfunction. So my lab is increasingly focused on understanding the actual consequences of telomere dysfunction in the early stages of tumorigenesis. I would like to better understand the exact genomic signatures of telomere crisis so that we can identify which tumors have lived through that misery in their proliferative past, and perhaps this can help us with prognosis, diagnosis and so on.

References

1. Kioussis, D, Vanin, E, de Lange, T, Flavell, RA, and Grosveld, FG. Beta-globin gene inactivation by DNA translocation in gamma beta-thalassaemia. *Nature* 306 (1983), 662-666.
2. De Lange, T, and Borst, P. Genomic environment of the expression-linked extra copies of genes for surface antigens of *Trypanosoma brucei* resembles the end of a chromosome. *Nature* 299 (1982), 451-453.
3. De Lange, T, Shiue, L, Myers, RM, Cox, DR, Naylor, SL, Killery, AM, and Varmus, HE. Structure and variability of human chromosome ends. *Mol Cell Biol* 10 (1990), 518-527.
4. Van Steensel, B, and de Lange, T. Control of telomere length by the human telomeric protein TRF1. *Nature* 385 (1997), 740-743.
5. Van Steensel, B, Smogorzewska, A, and de Lange, T. TRF2 protects human telomeres from end-to-end fusions. *Cell* 92 (1998), 401-413.
6. Smogorzewska, A, van Steensel, B, Bianchi, A, Oelmann, S, Schaefer, MR, Schnapp, G, and de Lange, T. Control of human telomere length by TRF1 and TRF2. *Mol Cell Biol* 20 (2000), 1659-1668.
7. Smith, S, Giriat, I, Schmitt, A, and de Lange, T. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. *Science* 282 (1998), 1484-1487.
8. Griffith, JD, Comeau, L, Rosenfield, S, Stansel, RM, Bianchi, A, Moss, H, and de Lange, T. Mammalian telomeres end in a large duplex loop. *Cell* 97 (1999), 503-514.
9. De Lange, T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes & Dev* 19 (2005), 2100-2110.
10. Chong, L, van Steensel, B, Broccoli, D, Erdjument-Bromage, H, Hanish, J, Tempst, P, and de Lange, T. A human telomeric protein. *Science* 270 (1995), 1663-1667.

ANDREW FIRE (1989)
The Resonance of Gene Silencing


LINDA A. CICERO/STANFORD NEWS SERVICE

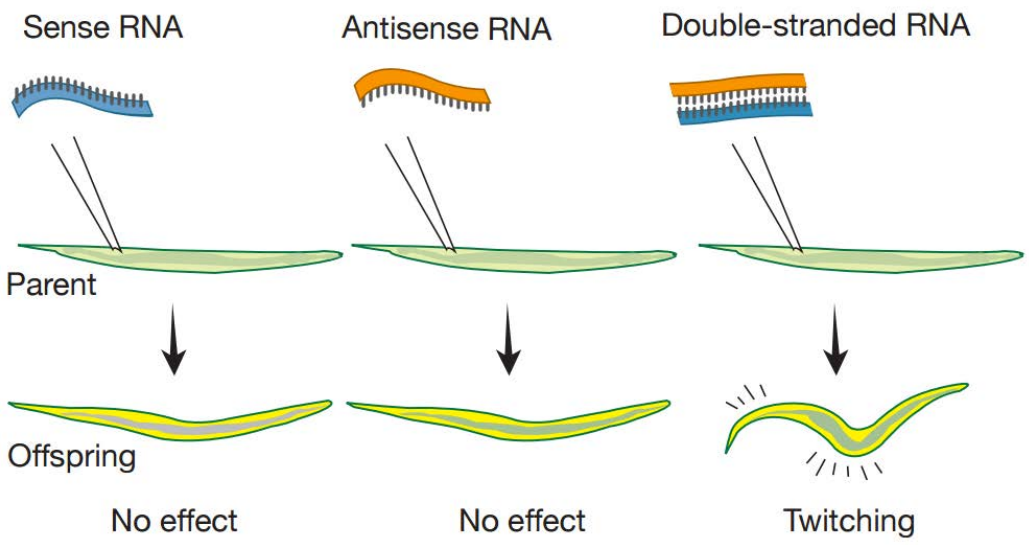
Andrew Fire helped to revolutionize genetic research when he and his colleagues discovered the phenomenon of gene silencing by RNA interference in the nematode roundworm *Caenorhabditis elegans*.[1]

Now a professor at the Stanford University School of Medicine, he and his research group continue to use *C. elegans* to examine molecules that pattern gene expression. They also collaborate on studies of various other systems in which knowledge of molecular repertoires may inform disease surveillance and intervention strategies.

Fire studied mathematics as an undergraduate at the University of California, Berkeley, and then earned a Ph.D. with molecular biologist Phillip Sharp at the Massachusetts Institute of Technology. Fire investigated the details of transcription (by which the instructions in DNA are transcribed into messenger RNA) in adenovirus, a cause of the common cold and an important genetic model.[2] *C. elegans* was also emerging as a powerful model system for cell differentiation and development. As a postdoctoral fellow in Cambridge, England, Fire worked with the *C. elegans* research group at the Medical Research Council Laboratory of Molecular Biology to advance the application of molecular biology approaches in the millimeter-long roundworm.

In 1986, Fire started his own laboratory at the Carnegie Institution of Washington in Baltimore, where he set out to explore gene regulation during early worm development. He was a Rita Allen Foundation Scholar from 1989 to 1993, when his group continued to develop new tools for introducing DNA into *C. elegans*, and began to study the roles of genes controlling muscle diversification in the worm.[3]

During this time, Fire's research revealed glimpses of a phenomenon that would lead to the landmark discovery, in 1998, of RNA interference—a mechanism that detects double-stranded RNA and leads to the degradation of (single-stranded) messenger RNA molecules with matching sequences, thus interfering with the expression of specific genes. In 2006, Fire was awarded the Nobel Prize in Physiology or Medicine along with Craig Mello for their research on this "gene silencing" process.



Andrew Fire and his colleagues demonstrated the phenomenon of RNA interference in the nematode worm *Caenorhabditis elegans*. Injecting worms with double-stranded RNA containing the code for a muscle protein caused the worms to twitch—an effect similar to that seen in worms that do not produce functional muscle protein due to genetic mutations.

"This day is a wonderful chance to acknowledge that science is a group effort," Fire said in a Stanford University news story about the prize. "The advances cited in the Nobel award grew from original scientific inquiry from numerous research groups throughout the world."[4]

RNA interference quickly became a boon to biological research, and has been widely employed as a method to study gene functions in plants and animals. Research has revealed that RNA interference is critical for regulating gene expression during organismal development, defending plants and some animals against viruses, and silencing potentially disruptive elements in the genome. Fire and other investigators (including Gregory Hannon, a 2000 Rita Allen Foundation Scholar and member of the Foundation's Scientific Advisory Committee) have also worked to decipher the molecular events involved in RNA interference.

References

1. Fire, A, Xu, S, Montgomery, MK, Kostas, SA, Driver, SE, and Mello, CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391 (1998), 806-811.
2. Fire, A, Baker, CC, Manley, JL, Ziff, EB, and Sharp, PA. In vitro transcription of adenovirus. *J Virol* 40 (1981), 703-719.
3. Okkema, PG, Harrison, SW, Plunger, V, Aryana, A, and Fire, A. Sequence requirements for myosin gene expression and regulation in *Caenorhabditis elegans*. *Genetics* 135 (1993), 385-404.
4. Conger, K. Andrew Fire shares Nobel Prize for discovering how RNA can switch off genes. *Stanford Report*, October 4, 2006, accessed May 5, 2016, http://news.stanford.edu/news/2006/october4/nobel-100206.html.

YIGONG SHI (1999)
Illuminating the Cell's Critical Systems


TSINGHUA UNIVERSITY

Yigong Shi received his early training in biology and biotechnology at Tsinghua University in Beijing, China. After nearly two decades building his research career in the United States, he returned to Tsinghua, where he is helping to advance the university's life science faculty while continuing his work to elucidate the mechanisms of fundamental cellular events.

Shi is best known for piecing together the fine-scale molecular interactions that control programmed cell death, known as apoptosis. This tightly regulated cellular suicide is vital for organismal development and for the maintenance of healthy tissues: When apoptosis goes awry, it can lead to cancer and other diseases. Shi's research team recently resolved the atomic structure of the "apoptosome," a wheel-like ring containing seven subunits of the protein Apaf-1 that assembles under cell death-promoting conditions to activate central apoptotic proteins known as caspases.[1]

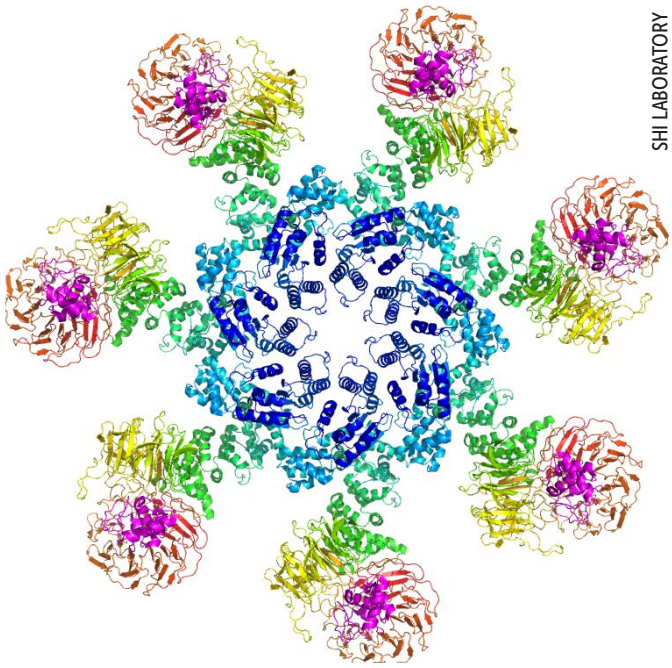
Shi earned a Ph.D. in molecular biophysics at Johns Hopkins University. He and his advisor, Jeremy Berg, examined the interactions of zinc finger proteins with both DNA and RNA—essential activities for the proteins' roles in regulating gene expression.[2] During a postdoctoral fellowship in Nikola Pavletich's lab at Memorial Sloan Kettering Cancer Center, Shi delved more deeply into structural biology, providing insights into how cancer-causing mutations disrupt the functions of tumor suppressor proteins known as SMADs.[3,4]

In 1998, Shi joined the faculty of Princeton University, and the following year he became a Rita Allen Foundation Scholar. During Shi's first few years at Princeton, he and his team began to decipher the machinery of apoptosis. They made several key discoveries, solving the structure of Smac, a caspase-activating protein, and revealing the molecular interactions that underlie the protein's activity.[5,6] Their results helped to explain the developmental defects observed in certain fruit fly mutants, and prompted Shi to cofound TetraLogic Pharmaceuticals, which works to develop anticancer drugs informed by Smac's apoptosis-promoting properties.

In 2008 he returned to Tsinghua, where he is now Vice President of the university and Director of the Institute of Biomedicine. Shi's laboratory research remains focused on using

structural and biochemical methods to understand critical cellular activities, including apoptosis, RNA splicing, and protein changes relevant to Alzheimer's disease. Since 2013, Shi and his team members have elucidated the atomic structures of γ-secretase, which is responsible for the generation of β-amyloid peptide, and the spliceosome, which executes RNA splicing.

In a 2014 profile for the *Proceedings of the National Academy of Sciences*, Shi said he is often asked why he returned to China. "I usually reply by asking, 'why not?,'" he said. "I have helped create a strong biomedical research community and been witness to changes."[7]

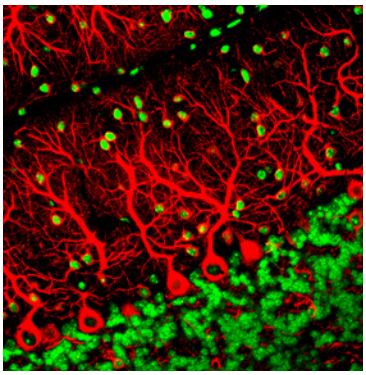


SHI LABORATORY

Yigong Shi's research team recently resolved the atomic structure of the "apoptosome," a wheel-like ring containing seven subunits of the protein Apaf-1 that activates a programmed cell death process known as apoptosis.

References

1. Zhou, M, Li, Y, Hu, Q, Bai, XC, Huang, W, Yan, C, Scheres, SH, and Shi, Y. Atomic structure of the apoptosome: mechanism of cytochrome c- and dATP-mediated activation of Apaf-1. *Genes Dev* 29 (2015), 2349-2361.
2. Shi, Y, and Berg, JM. Specific DNA-RNA hybrid binding by zinc finger proteins. *Science* 268 (1995), 282-284.
3. Shi, Y, Hata, A, Lo, RS, Massagué, J, and Pavletich, NP. A structural basis for mutational inactivation of the tumour suppressor Smad4. *Nature* 388 (1997), 87-93.
4. Shi, Y, Wang, YF, Jayaraman, L, Yang, H, Massagué, J, and Pavletich, NP. Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-beta signaling. *Cell* 94 (1998), 585-594.
5. Chai, J, Du, C, Wu, JW, Kyin, S, Wang, X, and Shi, Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. *Nature* 406 (2000), 855-862.
6. Wu, G, Chai, J, Suber, TL, Wu, JW, Du, C, Wang, X, and Shi, Y. Structural basis of IAP recognition by Smac/DIABLO. *Nature* 408 (2000), 1008-1012.
7. Davis, TH. Profile of Yigong Shi. *Proc Natl Acad Sci USA* 111 (2014), 16234-16235.



SCHOLAR PROFILES

"I fell in love with research: the pursuit of fundamental questions about how our cells, organs and bodies work; the aesthetic of the data; the heady experience of being the first—really the first—to observe something."
–Susan Dymecki (1999)

TOM MANIATIS (1978)
Mastering Methods and Exploring Molecular Mechanisms


COLUMBIA UNIVERSITY MEDICAL CENTER

"When we developed cDNA and genomic cloning methods, we clearly recognized the potential impact of these methods on eukaryotic molecular biology. Less obvious were the enormous impact that the molecular cloning manual would have on the propagation of the technology worldwide, and its impact on biomedical science."

Tom Maniatis launched his scientific career by experimenting with early ultraviolet lasers to scrutinize the development of chicken embryos. This undergraduate research experience, at the University of Colorado, Boulder, "taught me the importance of bringing technological developments to important biological problems," Maniatis recalls.

Devising and applying new technologies have since become hallmarks of Maniatis' work, from pioneering protocols for identifying, isolating and cloning genes in the late 1970s, to more recent investigations of the nervous system using mouse models and stem cells.

As a Ph.D. student with Leonard Lerman at Vanderbilt University, Maniatis established new methods for studying the structure of compact DNA using X-ray scattering,[1] with relevance for understanding DNA dynamics in both chromosomes and viruses. These investigations "required designing and building instruments, and using some of the earliest computer algorithms to interpret the diffraction data," Maniatis notes.

Fascinated by the emerging capabilities of molecular biology to reveal the intricate forms and functions of genetic material, Maniatis moved to Harvard University for a postdoctoral fellowship. He worked with Mark Ptashne (now at Memorial Sloan Kettering Cancer Center) to characterize gene regulation in bacteriophage lambda,[2] a virus that infects *E. coli* bacteria and has become both a model system for understanding genetics and a key tool for genetic engineering. Maniatis and Ptashne traveled to Fred Sanger's laboratory in Cambridge, England, to make use of Sanger's still-in-progress DNA sequencing techniques, for which Sanger later received a Nobel Prize in Chemistry. The characterization of the protein-DNA interaction in phage lambda set the stage for understanding the "genetic switch" in the life cycle of the phage—between dormancy, or lysogeny, and lysis, in which the phage kills the host cell and releases newly assembled viral particles.

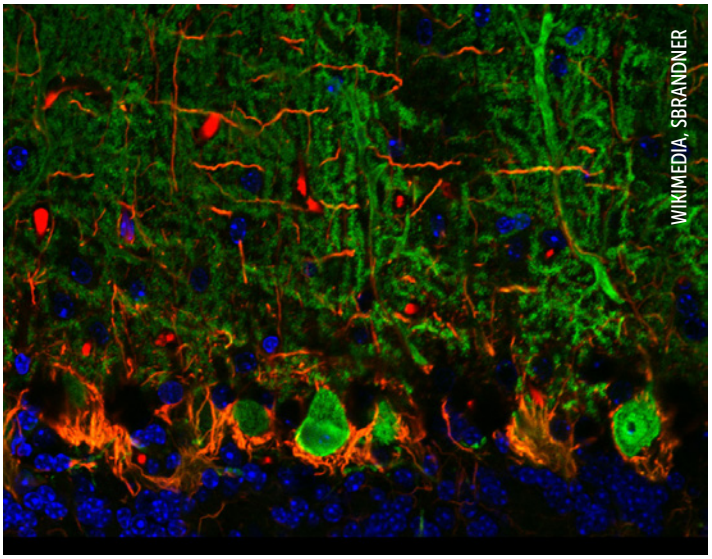
Maniatis and his colleagues then began forging a new approach for studying genes in eukaryotic organisms (plants, animals and fungi)—a process for cloning complementary DNAs (cDNAs, the functional versions of genes produced from messenger RNA precursors) and using these cloned cDNAs to isolate the corresponding genes from genomic

DNA libraries. Maniatis was unable to continue this work at Harvard, however, due a moratorium on recombinant DNA research in the city of Cambridge, Massachusetts.

Fortunately, Maniatis was offered faculty positions at Cold Spring Harbor Laboratory and then at Caltech, where he was a Rita Allen Foundation Scholar from 1978 to 1979. This was a fruitful period for his research, as he completed the development of the first full-length cDNA clone (beta-globin) with his collaborators Fotis Kafatos and Argiris Efstratiadis at Cold Spring Harbor. Subsequently, Maniatis and his team at Caltech developed the first complete genomic DNA libraries—collections of cloned copies of all the genomic DNA in *Drosophila* (fruit fly), rabbit and human cells. "The *Drosophila* and human libraries were widely used by the *Drosophila* developmental biology and human genomics communities for many years to isolate and study individual genes," Maniatis says.

Using the human genomic DNA library, the Maniatis group was the first to clone human genes—delta-globin and beta-globin,[3] which encode two subunits of hemoglobin, the vital oxygen-carrying protein found in red blood cells. Maniatis' group went on to characterize the full structure of the human globin gene cluster[4] and map genetic mutations in the globin genes[5] responsible for forms of an inherited disease called thalassemia.

The city of Cambridge eventually lifted its ban on recombinant DNA and established new research regulations. In 1980, Maniatis returned to Harvard, where he helped to shape the University's nascent Department of Molecular and Cell Biology. Around the same time, he also embarked on a project that would lead to perhaps his most enduring contribution to science: Maniatis coauthored *Molecular Cloning: A Laboratory Manual*, which was first published in 1982 and has since become ubiquitous in laboratories throughout the world—a trove of protocols and tips for investigating genetics and cell biology. In 2012 Maniatis received the Lasker~Koshland Award for Special Achievement in Medical Science; the award announcement cited the transformative impact of the manual and praised Maniatis for "fundamental discoveries concerning the nature of genes."



Tom Maniatis studies molecular neuroscience, including the disease mechanisms of amyotrophic lateral sclerosis (ALS). ALS involves degeneration of Purkinje neurons, which are among the largest brain neurons and form abundant dendritic spines. Purkinje neurons (shown here in green) function in motor coordination in the brain's cerebellum.

Maniatis has continued to make use of ever-advancing molecular tools to study the regulation of immune responses. His lab has also made fundamental contributions to understanding the mechanisms of pre-mRNA splicing.

He moved to Columbia University in 2009, and during the past two decades he has branched out into neuroscience, pursuing the function of protocadherins—a large family of cell surface proteins—in brain wiring. In addition, prompted by his sister's death from amyotrophic lateral sclerosis, or ALS, he now investigates the underpinnings of this neurodegenerative disease.

Here, Maniatis shares his thoughts on the far-ranging influence of molecular cloning methods and reflects on the next frontiers of his research in neuroscience.

When you helped to develop methods and write the *Molecular Cloning* manual, what did you think the impact of this work would be? Has anything surprised you about the development of molecular biology and recombinant DNA technology?

When we developed cDNA and genomic cloning methods, we clearly recognized the potential impact of these methods on eukaryotic molecular biology. Less obvious were the enormous impact that the molecular cloning manual would have on the propagation of the technology worldwide, and its impact on biomedical science.

I was a Rita Allen Scholar at Caltech when I was asked by Jim Watson [best known for helping to discover the structure of DNA; also an emeritus member of the Rita Allen Foundation's Scientific Advisory Committee]

to teach the first "Molecular Cloning" course at Cold Spring Harbor. Ed Fritsch, then a postdoc in my lab, and I assembled all of the cloning and analysis protocols from my lab in a loose-leaf notebook and prepared reagents for the course.

The cloning course was a great success, and at the end of the course Jim asked me to turn the course notebook into what became the *Molecular Cloning* manual. He also convinced Joe Sambrook, at that time the scientific director at Cold Spring Harbor, to join the effort, and I convinced Ed Fritsch to join. The hardest part was integrating the necessary biological background with the protocols in such a way that novices could learn to troubleshoot. Others have told us that this was, in fact, the basis for the success of the manual. It allowed naive investigators who did not have access to nearby experts to successfully apply the then-new technology to their biological problems. This, in conjunction with the timing of publication (early in the application of the technology), led to an enormous impact, internationally, in nearly every aspect of biomedical research. I believe that only Jim Watson fully understood the potential impact of the manual.

When you think about the future of your research and your fields of study, what possibilities are most exciting to you?

The development of recombinant DNA methods in the late 1970s was just the beginning of rapid and dramatic technological advances, ranging from the development of sophisticated tools for genome manipulation (including the recent development of CRISPR technology), to optogenetics and high-throughput DNA and RNA sequencing, to single-cell and single-molecule technologies. These technologies are profoundly advancing our understanding of the brain.

My lab is currently focused on two projects in the area of molecular neuroscience. The first is directed toward understanding the expression and function of the clustered protocadherin genes, which we discovered more than 15 years ago. New technologies have made it possible to probe deeply into the molecular mechanisms involved in the generation and function of single-cell diversity in the brain, the structure and function of protocadherin proteins, and, most recently, the function of protocadherins in the development of brain circuitry in vivo. Massive whole-exome [expressed genes] or genome sequencing studies of individuals with autism have led to the identification of DNA sequence variants in the protocadherin gene cluster, and these sequence changes occur in sequences which we know to be essential for protocadherin function. Thus we are poised to understand not only the function of protocadherin genes in brain wiring, but also their possible role in neurological diseases.

Our work in ALS has been similarly impacted, with the identification of close to 20 new ALS genes during the past decade based on next-generation sequencing technology, and progress in understanding ALS disease mechanisms. These important advances in genetics have identified new pathways and potential targets for drug development. Our particular focus is currently on ALS genes that function at the interface between innate immunity, inflammation and autophagy [the destruction of cells]. I think it is fair to say that more has been learned about ALS genetics and disease mechanisms in the past 10 years than in the preceding 100 years.

References

1. Maniatis, T, Venable, JH, and Lerman, LS. The structure of psi DNA. *J Mol Biol* 84 (1974), 37-64.
2. Maniatis, T, and Ptashne, M. Structure of the lambda operators. *Nature* 246 (1973), 133-136.
3. Lawn, RM, Fritsch, EF, Parker, RC, Blake, G, and Maniatis, T. The isolation and characterization of linked delta- and beta-globin genes from a cloned library of human DNA. *Cell* 15 (1978), 1157-1174.
4. Proudfoot, NJ, Shander, MH, Manley, JL, Gefter, ML, and Maniatis, T. Structure and in vitro transcription of human globin genes. *Science* 209 (1980), 1329-1336.
5. Fritsch, EF, Lawn, RM, and Maniatis, T. Characterisation of deletions which affect the expression of fetal globin genes in man. *Nature* 279 (1979), 598-603.

BRUCE STILLMAN (1983)
The Foundations of DNA Replication

"[The Rita Allen Foundation award] was the first independent money that I had, and it enabled me to think about exactly what I wanted to do in the longer term."

Bruce Stillman counts himself lucky to have been born in 1953, the year James Watson and Francis Crick announced their discovery of the double helix structure of DNA, and to have embarked on his research career during the early days of recombinant DNA technology. "It was almost perfect timing…in terms of being involved in the field of molecular biology, which has revolutionized many areas of science," he says.

As an undergraduate at the University of Sydney, Stillman conducted thesis research on bacterial genetics in Keith Brown's lab, where he worked to dissect the regulation of amino acid synthesis in *E. coli*. During that year Brown was on a sabbatical at Stanford University, and wrote effusive letters to Stillman about the exciting possibilities of recombinant DNA research and the study of DNA tumor viruses.

"I looked around Australia for any labs that worked on DNA tumor viruses," Stillman recalls. "There was only one, in Canberra, and they worked on adenovirus." So that's where he began his Ph.D. work with Alan Bellett at Australian National University's John Curtin School of Medical Research. Stillman investigated DNA replication of adenovirus, which is not known to cause cancer in humans, but served as an important model system, and is capable of interfering with cell cycle regulation in a manner similar to cancer-causing viruses. He and Bellett studied an unusual virus-encoded protein and its role in initiating DNA replication.

At that time, Stillman says, he was eager to shift his focus to research that would advance understanding of cancer. Both adenovirus and simian virus 40 (SV40), the main models for DNA tumor viruses, were known to infect cells and stimulate cellular DNA synthesis, a process driven by the cancer-causing proteins that the viruses produced. "Nothing was really known about cellular DNA synthesis in the late '70s, so I decided I was going to try to figure it out," he says.

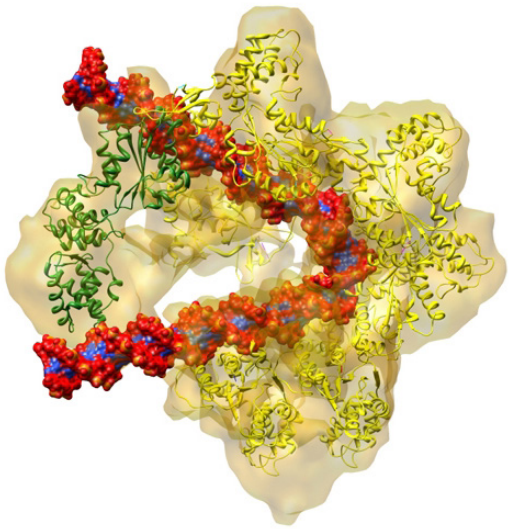
He knew that Cold Spring Harbor Laboratory was one of the top places for tumor virus research, and in 1979 he began a postdoctoral fellowship there, working in a laboratory headed by Michael Mathews (now at Rutgers New Jersey Medical School). But unusually, he was allowed to continue to focus

on adenovirus DNA replication, and in 1982 published work identifying a rather unique DNA polymerase that initiates virus DNA replication using a protein as a primer.[1]

Stillman soon became a staff investigator at Cold Spring Harbor, continuing his own research program on DNA replication, and in 1983 he was selected as a Rita Allen Foundation Scholar. The award enabled him to take his research in new directions. He chose to begin working with SV40, whose replication was more similar to that of cellular chromosomes, making it a more useful model than adenovirus for identifying cellular proteins that participate in DNA replication. Following up on work from Thomas Kelly's lab, Stillman and his team started isolating these cellular proteins using in vitro techniques. He also developed a system to examine the deposition onto the replicating DNA of histones[2]—the proteins that form the "spools" around which strands of DNA are wound in eukaryotic chromosomes. Knowledge of the histone deposition process has had "an impact on [understanding] the inheritance of epigenetic states of gene expression," he says.

Around the same time, Stillman's group also set out to study DNA replication in yeast. These investigations culminated in the identification, in 1992, of the multiprotein complex that binds to origins of DNA replication, the sequences on chromosomal DNA where DNA duplication begins.[3] "It's an ATP machine that starts the entire process of chromosome replication," Stillman says. (The lead author on this work was a postdoc, Stephen Bell, a 1995 Rita Allen Foundation Scholar who is now a professor of biology at MIT.)

Stillman's lab went on to reconstitute both SV40[4] and the initiation of yeast chromosome replication in vitro.[5] "The combined investigations of these two systems led to the discovery of lots of cellular DNA replication proteins," he says. "Now we know in great detail the mechanisms of how the proteins involved in DNA replication are controlled by the cell division cycle regulatory machinery, a machinery identified by others. The integration of cell cycle regulation and DNA replication is giving us a profound understanding of how these processes are controlled in normal cells and how they go wrong in cancer cells, which was an original goal."



BROOKHAVEN NATIONAL LABORATORY

Bruce Stillman has characterized the origin recognition complex, a group of six proteins (shown in yellow in this illustration) that binds to origin of replication sequences on chromosomal DNA (red and blue). The recruitment of the Cdc6 protein (green) also prepares the DNA for unwinding and subsequent replication—an essential process for cell division.

Along with making fundamental contributions to understanding DNA replication, Stillman has served in various leadership positions at Cold Spring Harbor for more than 25 years; he has been President of the Laboratory since 2003. Here, Stillman reflects on the significance of the Rita Allen Foundation award, and discusses Cold Spring Harbor's changing roles in translational research and science education.

How did becoming a Rita Allen Foundation Scholar influence your research?

It was the first independent money that I had, and it enabled me to think about exactly what I wanted to do in the longer term. I started to look around at what was going to be important, and two things came to the fore. One was the SV40 virus, because that virus replicates like the cell chromosome. It was a great system to figure out how the DNA replication fork proteins work, because all of the proteins that replicate SV40, except one, are cell proteins. We identified and purified all of those cell proteins and figured out how they worked. Almost all of them were previously unidentified proteins, and turned out to be very important proteins also involved in DNA repair and DNA recombination, in addition to cell DNA replication.

I also used the award to think about working on cell chromosome replication itself, and was able to recruit additional people to the laboratory. One of them

was John Diffley, who originally came to work on adenovirus, but he and I discussed the possibility of working on yeast. That had an enormous impact on the entire field of replication, because John started doing this in my lab. He's now a major player in the field, as are many other former postdocs. We were working on SV40 and yeast in parallel, and that turned out to be incredibly powerful. Now we work on yeast and human cells in parallel to do comparative chromosomal replication.

As the leader of a basic research institution, how do you think about balancing support for basic versus translational research?

Our faculty members are coming to me all the time, saying, "Look, I've been working on this discovery, and if I had some extra money, we could do added value research, which would potentially be applicable in health care and the development of therapeutics." I see the mission of Cold Spring Harbor as performing important basic research, discovering new processes and developing new ideas, but translational research is not something that we should ignore. It is a fine balance of allocation of resources.

One of the biggest new drugs approved for breast cancer [treatment] is from Pfizer—the cyclin-dependent kinase 4 (CDK4) inhibitor palbociclib. The target for this drug was discovered at Cold Spring Harbor by David Beach, and also by Chuck Sherr at St. Jude Children's Research Hospital, in 1994, but this important drug just got approved in 2015. That timeline is way too long, and I think institutions have a responsibility to accelerate the rate of discovery.

But we also have to understand that we can't all become pharmaceutical companies; there has to be a very good working relationship between the two, and also with health systems. Last year we signed an agreement with one of the largest health systems in the United States, Northwell Health, which has transformed our ability to integrate the pinnacle of science with clinical cancer research, without taking resources away from basic science.

How does Cold Spring Harbor make an impact on science beyond the research of its faculty?

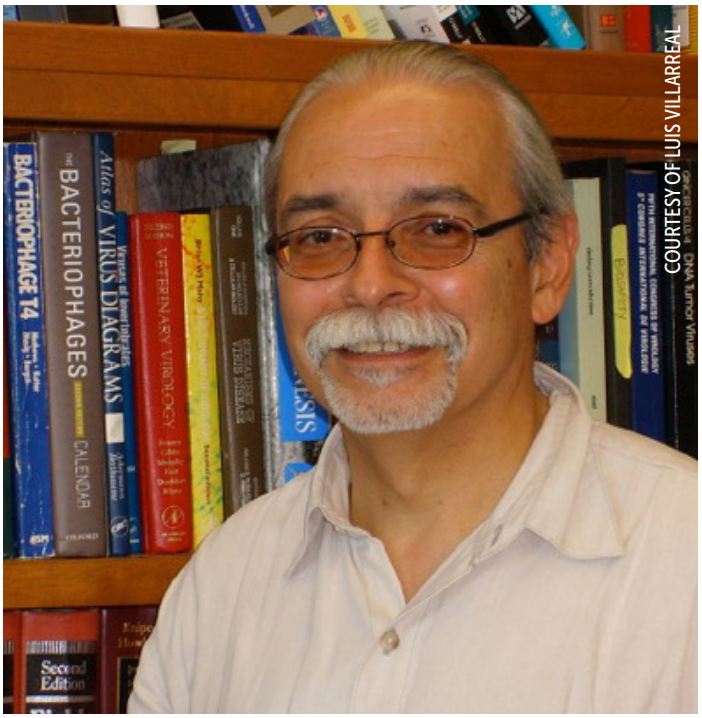
We have about 9,000 people a year come here for scientific meetings and courses. That's one of the reasons why I've stayed at Cold Spring Harbor—the world's scientists come here, so you get to meet a lot of people—that networking is very important.

We also have a DNA Learning Center that I'm very proud of. This program teaches laboratory-based science to more than 30,000 middle and high school students a year just on Long Island, either in our center or at their schools. We have also helped start DNA Learning Centers throughout the United States and around the world—we have just opened a very large center in China. We think about such teaching as a generational thing: if you start engaging kids in scientific methods early on, then they'll grow up thinking like that.

References

1. Stillman, BW, Tamanoi, F, and Mathews, MB. Purification of an adenovirus-coded DNA polymerase that is required for initiation of DNA replication. *Cell* 31 (1982), 613-623.
2. Stillman, B. Chromatin assembly during SV40 DNA replication in vitro. *Cell* 45 (1986), 555-565.
3. Bell, SP, and Stillman, B. ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. *Nature* 357 (1992), 128-134.
4. Waga, S, and Stillman, B. Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication in vitro. *Nature* 369 (1994), 207-212.
5. Evrin, C, Clarke, P, Zech, J, Lurz, R, Sun, J, Uhle, S, Li, H, Stillman, B, and Speck, C. A double-hexameric MCM2-7 complex is loaded onto origin DNA during licensing of eukaryotic DNA replication. *Proc Natl Acad Sci USA* 106 (2009), 20240-20245.

LUIS VILLARREAL (1983)
A Life in Viruses



COURTESY OF LUIS VILLARREAL

"Viruses are the unending front of evolution...they continue to shape the trajectory of life on the planet, including that of humans."

With epidemics and outbreaks of Ebola virus, Zika virus, West Nile virus and others posing serious threats, many virologists are scrambling to learn how to better fight these human pathogens. Luis Villarreal has studied viruses for nearly 50 years, and considers them much more than infectious agents. For him, viruses are at the leading edge of biology, and a vital driver of the evolution of all living things—humans included.

"What interests me is, what does it take for a virus to develop a persistent relationship with its host?" Villarreal says. "We have persistent, stable relationships that are passed from generation to generation, and you seldom, if ever, see an acute outcome with these species. But in other related or non-related hosts there can be disease consequences. So we know that in some circumstances these very same viruses can be quite harmless, while in others they are capable of causing severe and lethal infections."

As an example, Villarreal cites avian influenza that emerged in China and has been traced to a particular lake where the virus mutated and resulted in the deaths of millions of domestic birds. Yet avian influenza is found in a range of wild bird species throughout the world, and for the most part these birds are not harmed by its stable persistence in their genomes. "There are these completely different relationships of viruses to hosts, and that is what fascinates me—what are the evolutionary consequence of these relationships?" he asks. "The assertion I'm making is that viruses have provided the raw material to influence the complexity of all living things."

Throughout his career, Villarreal has developed tools to study viruses and has deciphered some of the fundamental processes of viral replication and transcription. Recently he has been consumed by concepts and theories of evolution as they relate to what he calls the "virosphere"—the large network of viruses that inhabits our planet. "My work really comes together in my recent thinking on the role viruses have in life in general," he says.

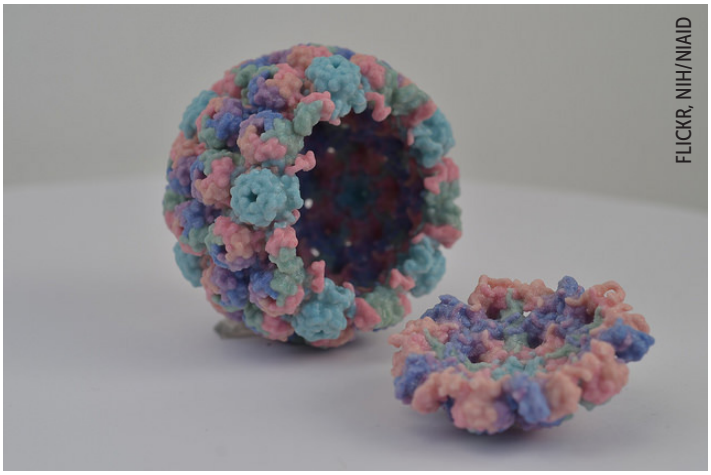
Villarreal grew up as an "urban migrant" in East Los Angeles. His family moved often because his father bought, fixed up and sold houses to supplement the family's income. "I had a

very fragmented educational experience, but at the same time, that made me more resilient and adaptable," he says. Villarreal was persistent in his curiosity: he experimented with chemical reactions and took things apart (including motorcycles) to understand how they worked.

His interest in virology was seeded as an undergraduate at California State University, Los Angeles, when he saw an electron micrograph of an arbovirus inside the cytoplasm of a cell. "It was clearly the interface of chemistry and life," he recalls. "It was this crystalline structure that had clear biological consequences. I was looking at it from the perspective of a chemist. I wanted to understand what the biology was."

After receiving his bachelor's degree in biochemistry, Villarreal entered a Ph.D. program in the Division of Biological Sciences at the University of California, San Diego, working in John Holland's laboratory on negative-strand RNA viruses. Villarreal characterized the behavior of vesicular stomatitis virus (VSV) and the related rabies virus, showing that defective particles made from mutant VSV virions, when mixed with virulent, wild-type virions, resulted in a persistent infection but did not kill the host.[1] "This idea of defective [virions] that can interfere with replication of the wild-type virus, modifying the trajectory of an acute infection—that's a topic that still interests me today. I think it has big consequences for the outcome of the relationship between a virus and host," he says.

Villarreal's experience in the Holland lab solidified his commitment to research, but he felt that he needed to broaden his scope beyond negative-strand RNA viruses. In 1976, he began a postdoctoral fellowship at Stanford University in the laboratory of Paul Berg, who is credited with constructing the first recombinant DNA molecule (Gilbert Chu, a 1988 Rita Allen Foundation Scholar, also conducted postdoctoral research with Berg). Villarreal developed the now widespread technique of in situ plaque hybridization, demonstrating its utility to identify simian virus 40 (SV40) by its DNA sequence rather than its biological properties.[2] "For me, it was the first step in manipulating a genome and starting to understand transcription," he says.



Luis Villarreal has studied how viruses such as polyomaviruses establish and maintain persistent relationships with their mammalian hosts. Shown here is a 3-D printed model of a polyomavirus capsid.

In 1980, Villarreal started his own laboratory at the University of Colorado School of Medicine, and developed the first direct transfection of polyomavirus DNA into the tissue of newborn and adult mice.[3] Villarreal became a Rita Allen Foundation Scholar in 1983, and in 1985 he moved to the University of California, Irvine, where he directed the Center for Virus Research from 2000 to 2010 and is now a professor emeritus in the Department of Molecular Biology and Biochemistry.

Villarreal has continued to study the mechanisms of tissue-specific replication and transcription of SV40 and other mammalian polyomaviruses. "These viruses make their living in their host as unapparent, persistent lifelong infections," he says. According to Villarreal, this concept of persistence is fundamental to how viruses work and is the common thread that runs through his research.

Here, Villarreal describes his unwavering perspective on virology, how the scientific community's view of viruses has changed, and the complexities of assigning meaning in biology.

Some biologists view viruses as a tool to understand biological systems. Did you initially see viruses this way?

Many researchers do see them as a simple model that can be manipulated to understand how genes or [DNA] replication work. In fact, scientists have been highly productive in elucidating the mechanisms of those processes using viruses. I suppose I never really thought of them that way, but rather as fundamental—as units capable of significant adaptation and modification of the survival dynamics of their hosts.

How have scientific perspectives on viruses evolved since you first began to study them?

I first started teaching virology to medical students in the late 1970s, when we had eradicated smallpox from the planet, and many other viral infections, including measles and polio, seemed to be on the wane because of vaccines. At that time, it was declared by various famous virologists and directors of the National Institutes of Health that virology was going to be a thing of the past—something that medical students would study in their textbooks, but nothing that was going to affect the future of medicine, because we had figured it all out!

Now, with millions of people still affected by emerging viruses, that view couldn't have been more wrong. It's striking that every year we have some new viral emergence. Viruses are the unending front of evolution. We need to give them that descriptor officially, as opposed to just treating viruses as some transient mutational phenomenon. They continue to shape the trajectory of life on the planet, including that of humans. Yet it's never admitted that this is the front of biological evolution and that it affects human survival.

What is prominently on your mind right now?

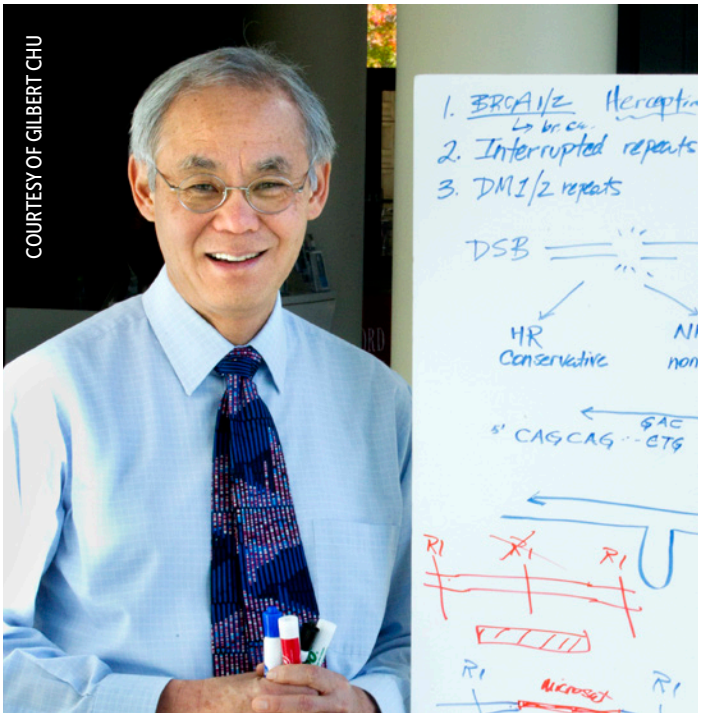
One of the most fundamental concepts to emerge for me in the last five years has come from talking to the philosopher Günther Witzany about the history of science and language. Assigning meaning to information is part of a social enterprise. We have codes that specify meaning, including the biological code of DNA, and the history and context of these codes matter in how we apply them.

For example, a herpes virus can sit inside a peripheral neuron as a strand of DNA in a silent state for a lifetime. That same DNA strand inside a brain neuron can cause fatal encephalitis. The same host, code and virus, but a different history and context result in a vastly different outcome: persistence or acute death. A code can't have meaning unless there is a population using it, both in society and in biology. This line of thinking leads to the need for a deeper evaluation of where we as a society stand in terms of understanding biology.

References

1. Holland, JJ, and Villarreal, LP. Persistent noncytocidal vesicular stomatitis virus infections mediated by defective T particles that suppress virion transcriptase. *Proc Natl Acad Sci USA* 71 (1974), 2956-2960.
2. Villarreal, LP, and Berg, P. Hybridization in situ of SV40 plaques: detection of recombinant SV40 virus carrying specific sequences of nonviral DNA. *Science* 196 (1977), 183-185.
3. Dubensky, TW, Campbell, BA, and Villarreal, LP. *Proc Natl Acad Sci USA* 81 (1984), 7529-7533.

GILBERT CHU (1988)
DNA Dreamer



Gilbert Chu (wearing a genome tie) with his portable classroom, giving office hours for medical students.

"Discovering that I can still help after all available treatments have failed was something that I didn't expect. I often think, 'Well, this person needs me. I have to figure out how to help in ways not covered by the medical guidelines.' I didn't realize that would be so satisfying."

Gilbert Chu's passion for biology stretches far back. By ninth grade, he was conducting brain surgery in rats and corresponding with researchers about implanting brain electrodes. But during his first week of college at Princeton, a run-in with a premed student who demanded to copy his lab report sent him into the arms of his other love, the beauty of math and physics. Chu focused on elementary particles, the basic building blocks of matter, and earned a Ph.D. in theoretical physics at the Massachusetts Institute of Technology. During his postdoc at the University of California, Berkeley, a professor of physics and radiology challenged him to devise a mathematical method for using one of those elementary particles, the positron, for medical imaging of tumors. The excitement of meeting this challenge led Chu to realize that biomedical research was his true calling.

After getting an M.D. from the joint Harvard/MIT Health Sciences and Technology program, and completing a medical residency at Massachusetts General Hospital, Chu went on to Stanford for a fellowship in medical oncology followed by a postdoc in the lab of biochemist Paul Berg, the father of recombinant DNA research. Chu set out to clone the genes responsible for xeroderma pigmentosum (XP), an inherited disease that often causes cancer and is characterized by the body's inability to repair sun-damaged DNA.

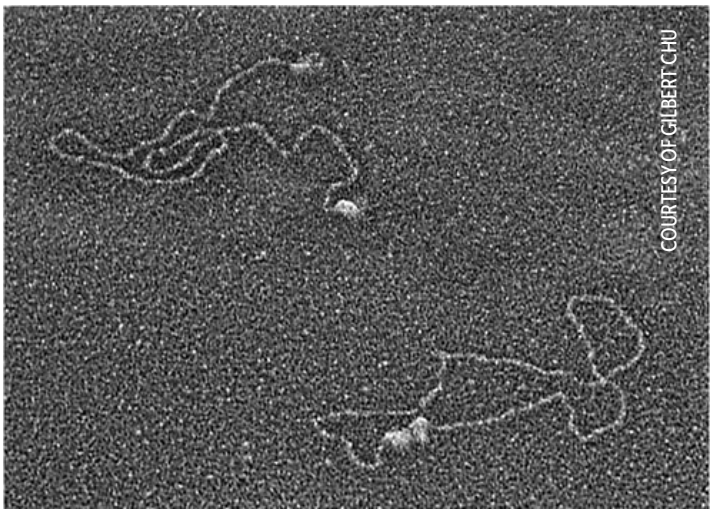
Those were early days for molecular biology, and techniques for working with DNA were spotty. Chu wanted to clone the XP genes by introducing DNA into XP cells with an electric field, a method called electroporation. One of Berg's former students had developed a method in which hand-held electrodes delivered pulses of unknown voltage, sending sparks flying across the solution. "It was just haphazard, and it sometimes sent the researcher flying as well," Chu recalls. So Chu built an optimized electroporation device[1] that was commercialized by the laboratory supply company Bio-Rad and for several years served as standard issue in molecular biology labs. "I'm very proud of that," he says.

In 1987, Chu launched his own lab at Stanford, continuing his work on XP with the aim of untangling the biochemical mechanisms underlying DNA damage and repair. The following year he became a Rita Allen Foundation Scholar. His lab identified two partner proteins that stick to damaged

DNA, one of which turns out to be missing in a subset of patients with XP.[2] They then worked backward to determine the protein's molecular role in targeting the ultraviolet radiation damage for repair.[3]

Later his lab discovered the role of another protein, Ku, in repairing DNA double-strand breaks.[4] Meanwhile, in the clinic, Chu kept encountering patients who were experiencing serious toxicities from cancer treatment—particularly ionizing radiation, which causes DNA double-strand breaks. He dove into genomics, using the emerging technology of DNA microarrays to gain a global picture of gene expression levels under different conditions. Chu compared gene expression patterns in patients who experienced radiation toxicity versus those who did not. But he couldn't separate the signal from the noise, largely because no established method for analyzing gene microarray data existed. Chu and his colleagues developed a new approach that was soon widely adopted as the standard in the field. "That was a very short paper[5]—five pages long, at least five years to produce—but it had an enormous impact on people's ability to analyze microarray data reliably," Chu says. "And my background in math and physics made it possible."

A few years later, Chu's group published results from a small clinical trial that showed the technique could potentially predict patients' radiation toxicity.[6] Although the work was never developed into a clinical test, Chu has found himself following his clinical nose in his research. "I want to use my physics and medical background to help people," he says. "So I try to make choices that I think are going to help the greatest number of people."



Early work in Gilbert Chu's lab explored the biochemical mechanisms underlying DNA damage and repair. This electron microscope image shows two proteins that his lab implicated in the process, DNA-PK and Ku, binding to DNA ends (upper left) and bringing the ends together (lower right)—the first steps in a pathway that repairs DNA double-strand breaks.

Here, Chu muses on his diverse interests, his latest passion, and the grace he finds in being a part of his patients' lives—all the way to the end.

What convinced you to change your course from theoretical physics back to biology?

I've always been interested in many things, which gave me the flexibility to maintain my love for biology, even as I kept doing my physics. I remember being on a bus with two other graduate students in theoretical physics, going between Harvard and MIT. It was in the 1970s; we were worried about our place in the world, and we thought things were going to hell in a handbasket. Martin Luther King had just been assassinated. The U.S. was bombing Vietnam and Cambodia. Riding the bus with us was a junior faculty member. He said, "You know, I don't know if we're really helping the world. If we really wanted to help we'd all go into medicine."

All three of us graduate students on that bus ride ended up going to medical school. I was last. When I was at Berkeley, I realized that if I kept waiting it would be too late. I'd never taken a biology class, but I did extremely well on the MCATs because I'd been reading biology voraciously. The Harvard/MIT M.D. program was very welcoming to people with my background. I ended up doing a summer project with the immunologist Herman Eisen, and then spending a year in the lab with Phil Sharp. And then, I was hooked!

What are you working on these days?

One of my patients became mentally dulled and then delirious from chemotherapy. I figured out that the chemotherapy caused a dramatic increase in her blood ammonia, which is a brain toxin. So I sequenced her exome [all the protein-coding genes] and did a small clinical trial to show that patients susceptible to this phenomenon aren't rare. We're

trying to get the work published, and I think it's going to explain a large number of cases of what cancer patients call "chemobrain."

In doing the clinical study I discovered how hard it is to measure blood ammonia levels. It must be done at a laboratory connected to a hospital, because the sample must be obtained intravenously and processed by a central lab within half an hour. So I started looking for a method to measure ammonia in blood from a finger-stick, and provide results in the home or at the bedside. Serendipitously, at a dinner fostering interdisciplinary collaborations, I met a chemist whose graduate student had made a device that might work for blood. So we've done a clinical trial showing that our device is just as accurate as the Stanford clinical lab. We want to start a company and get funding for FDA approval.

Looking back on the different strands of your career, what have you found the most surprising?

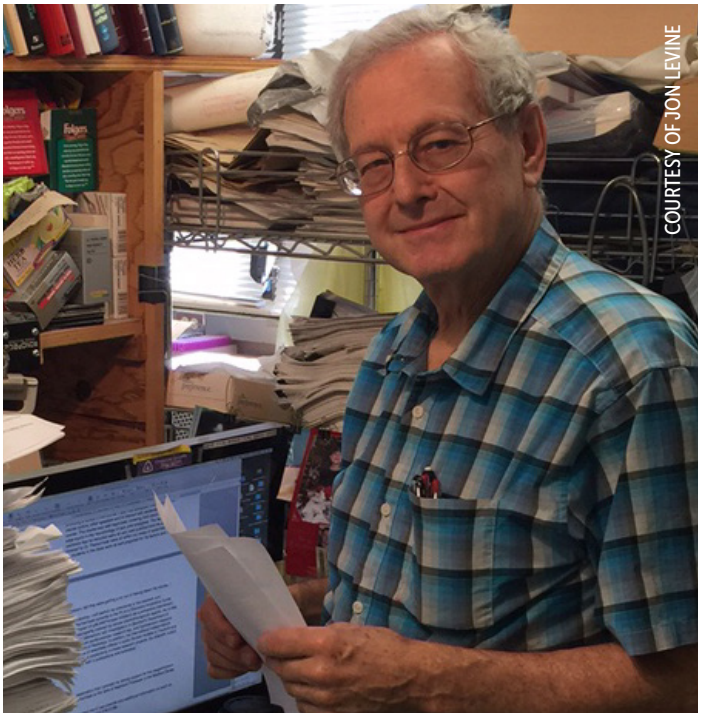
I'm not surprised at all that I love the science and the teaching. The thing that has surprised me the most is that I treasure my relationships with patients. My goal was to use research to help cancer patients. And I wanted a foot in the clinic so that it could inform my research. But because we're a tertiary care referral center, I often see patients who have run out of options. In working with them, I often take on the role of teacher, confessor and counselor. There's no formula; everybody is different.

Discovering that I can still help after all available treatments have failed was something that I didn't expect. I often think, "Well, this person needs me. I have to figure out how to help in ways not covered by the medical guidelines." I didn't realize that would be so satisfying.

References

1. Chu, G, Hayakawa, H, and Berg, P. Electroporation for the efficient transfection of mammalian cells with DNA. *Nucleic Acids Res* 15 (1987), 1311-1326.
2. Chu, G, and Chang, E. Xeroderma pigmentosum group E cells lack a nuclear factor that binds to damaged DNA. *Science* 242 (1988), 564-567.
3. Tang, JY, Hwang, BJ, Ford, JM, Hanawalt, PC, and Chu, G. Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. *Mol Cell* 5 (2000), 737-744.
4. Smider, V, Rathmell, WK, Lieber, MR, and Chu, G. Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. *Science* 266 (1994), 288-291.
5. Tusher, VG, Tibshirani, R, and Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98 (2001), 5116-5121.
6. Rieger, KE, Hong, WJ, Tusher, VG, Tang, J, Tibshirani, R, and Chu, G. Toxicity from radiation therapy associated with abnormal transcriptional responses to DNA damage. *Proc Natl Acad Sci USA* 101 (2004), 6635-6640.

JON LEVINE (1988)
A Passion for Deciphering Pain


COURTESY OF JON LEVINE

"We have made a lot of progress in treating acute pain, but the treatment of chronic pain has lagged far behind. One of the critical scientific questions is, 'What *is* chronic pain?'"

As an undergraduate studying biophysics, Jon Levine didn't see himself going into medicine. He trained as a neuroscientist, first earning a Ph.D. at Yale, and then beginning a postdoc at the University of California, Berkeley, on the genetics of flight in fruit flies.

It was 1972, and on his way to the lab he would pass people protesting the Vietnam War or rallying for other political causes; somehow, against that backdrop, his research on flies felt lacking in relevance, he recalls.

A mentor connected him with Howard Fields, a new hire at the University of California, San Francisco, who was starting a lab and clinical practice in pain. On Wednesday afternoons, Levine would accompany Fields to see patients, many of whom suffered from devastating pain syndromes. "We would walk back across the street and I would be in tears," Levine says. "One day, he just put his arm around me and said, 'Jon, if you can learn to deal with this, you can learn to deal with anything.'"

Levine soon enrolled in medical school at UCSF, continuing to work with Fields. In 1978, the duo published a landmark paper on the placebo effect, showing that it is mediated by endorphins—natural opioid compounds produced by the central nervous system.[1] This was the first study to identify a biological basis for the phenomenon. During a subsequent fellowship in rheumatology, also at UCSF, the chief of the division—the legendary physician and hospital administrator Jack Stobo—discouraged him from pursuing pain research, so Levine proposed investigating a broader question related to the nervous system's role in inflammatory disease: Why do people who have strokes not get arthritis on the affected side of their bodies?

Back then the field was still underdeveloped; most researchers explored pain's genesis in the central nervous system. Levine's work, however, prioritized the periphery—a focus he maintained when he launched his own lab in 1983. "One of the interesting aspects of studying the effect of stroke on pain and inflammation was seeing how injuries in the central nervous system can cause a downstream effect—a secondary injury in the peripheral nervous system," he says. His lab began to develop a program dissecting peripheral contributions to pain, including how processes such as inflammation can sensitize

the body's pain machinery. Five years into his academic post, Levine became a Rita Allen Foundation Scholar, setting out to define the role of specific molecules in this process and identify compounds that might modulate it.

In the decades since, Levine has made wide-ranging and fundamental contributions to pain research—seeking synergistic effects between different pain drugs;[2] identifying a dramatic difference in the way men versus women respond to opioids;[3] participating in cloning the heat-activated capsaicin receptor,[4] which is found in neurons that process pain; and characterizing the mechanisms that underlie chronic versus acute pain.[5] "There is no area of pain that I'm not interested in," he says.

Levine says researchers still have a tremendous amount to learn about pain, despite advancements in recent decades. "We have made a lot of progress in treating acute pain, but the treatment of chronic pain has lagged far behind," he says. "One of the critical scientific questions is, 'What *is* chronic pain?'"

Solving that and other conundrums will require clinicians, researchers and advocacy groups working closely together to define the most clinically relevant issues, Levine says. It will also require extensive mechanistic studies in humans—in addition to in animal models, which often show an unclear relevance to human pain syndromes.

Here, Levine discusses some surprising discoveries—both in the clinic and in the lab.

What experiences spurred your early commitment to pain research?

One morning during my junior surgery rotation in medical school, I saw a young woman who had been brought in by an ambulance; she was lying in the hallway screaming. A nurse said she had been shot through the window of her apartment, but the trauma surgeons had determined that the bullet wasn't near any vital organs, and that they'd get it out maybe tomorrow. I asked, "But what about her pain?" And the nurse said, "It's OK, it won't kill her."

So I went to talk to Howard [Fields], and he gave me a recent paper from the *Annals of Internal Medicine* about the undertreatment of pain.[6] It looked at the recommended drug dosages for people with a certain amount of pain, how much clinicians actually prescribe for these patients—which was less—and how much nurses actually administer—which was even less. By the time all was said and done, these patients were being vastly undertreated for very straightforward pain—not even chronic pain, just basic stuff. So I told the head of the trauma service that I would make it illegal to not treat people's pain properly. He invited me to give surgery grand rounds at San Francisco General Hospital about that, after which everybody said, "Oh, this is terrible! We'll do better!" I went back to my rotations, but several years later—it was before HIPAA, so I could look at patients' records—there was no change at all. There's no question that we need to fix this.

How did you discover the sex difference in how well opioids work?

The work with sexual dimorphism is something we're very passionate about. We were studying the interactions of different pain drugs that work on the endogenous pain control pathway through different neurotransmitter receptors. Because each drug has dose-related side effects, we thought we could maybe produce synergy between their analgesic effects, limiting the side effects. In one of our experiments, by chance, there were statistically more women in one group and more men in the other. When we wrote up the paper, we noted this, but said that we didn't think it affected the findings. It was a very short paper, and the reviewer said, "Well, you have the data; why not run the analysis?" I would love to kiss this person now. About a half hour after my colleague went to run it, he came back and said, "You're not going to believe this—everything we're looking at has to do with sex." This completely turned things around for us.

We were looking at a class of narcotics called kappa-opioids—nalbuphine, butorphanol, pentazocine—which are available clinically. When we went back for a closer look, we found that they're great analgesics for women—as good as morphine, but that in men, they can make pain worse. The OB-GYNs have known for years that these are great drugs, because they only treat women. Back then, Parke-Davis [a subsidiary of Pfizer] had been developing a kappa-

opioid, and somebody from their group was visiting just as we finished this work on sex differences but before we published. The company had done three studies. In one, they got very good analgesia with the kappa-opioid, and in the other two, they got terrible results. Those two were wisdom tooth studies, but they only used men because they didn't want to have to deal with the menstrual cycle. The one where it worked was in women, for post-episiotomy pain. I asked this guy, "Is there any chance this could be due to sex differences?" He said, "Absolutely not."

What exciting new areas of pain research are you pursuing now?

We've gotten very interested in the environment in which the cell lives—that is, the extracellular matrix and its role in pain. One of the most common new treatments for osteoarthritis pain is to inject a non-protein extracellular matrix molecule called hyaluronan into joints. It changes the pain phenotype as effectively as injecting corticosteroids. So we're studying how, in the setting of inflammation, hyaluronan is chewed up into little bits and pieces, and how those bits and pieces are toxic to the pain system. We're also looking at a population of pain-sensing neurons and their interaction with an extracellular matrix molecule called versican, which plays an incredibly important role in activating nociceptors [nerve cells that respond to pain]. If we could figure out what binds to this molecule, that compound could lead to a therapy that could silence cells important for certain pain syndromes, while leaving protective pain mechanisms intact.

References

1. Levine, JD, Gordon, NC, Jones, RT, and Fields, HL. The narcotic antagonist naloxone enhances clinical pain. *Nature* 272 (1978), 826-827.
2. Miaskowski, C, Taiwo, YO, and Levine, JD. Kappa- and delta-opioid agonists synergize to produce potent analgesia. *Brain Res* 509 (1990), 165-168.
3. Gear, RW, Miaskowski, C, Gordon, NC, Paul, SM, Heller, PH, and Levine, JD. Kappa-opioids produce significantly greater analgesia in women than in men. *Nat Med* 2 (1996), 1248-1250.
4. Caterina, MJ, Schumacher, MA, Tominaga, M, Rosen, TA, Levine, JD, and Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389 (1997), 816-824.
5. Joseph, EK, Reichling, DB, and Levine, JD. Shared mechanisms for opioid tolerance and a transition to chronic pain. *J Neurosci* 30 (2010), 4660-4666.
6. Marks, RM, and Sachar, EJ. Undertreatment of medical inpatients with narcotic analgesics. *Ann Intern Med* 78 (1973), 173-181.

SUSAN DYMECKI (1999)
Serotonin Circuit Master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"The more I delved into it, the more I became fascinated by the neurons that signal through serotonin...How does this neuronal system accomplish so many different tasks, affecting everything from mood and cognition to breathing, heart rate and temperature?"

Growing up, Susan Dymecki loved biology, math and engineering. But from the time she donned skates at age 13, she was consumed by an entirely nonacademic passion: ice dancing. She competed nationally throughout high school, and after enrolling at the University of Pennsylvania, took a leave of absence to train and compete internationally.

When her skating partner needed to retire early, Dymecki found herself at a crossroads. She returned to Penn to complete her bachelor's degree in biomedical engineering, staying on for a master's to conduct research on the effects of electrical stimulation on bone growth and repair. Next, she embarked on an M.D.-Ph.D. at Johns Hopkins University School of Medicine, working with Stephen Desiderio, who studies the underlying genetics of the immune system. "Steve was a tremendous mentor and scientist from whom I could learn and grow," Dymecki says. Under his tutelage and that of John Niederhuber, then a visiting professor at Hopkins who later headed the National Cancer Institute, Dymecki cloned a previously unknown gene—a member of the SRC proto-oncogene family—and surmised that it did its job specifically in antibody-producing B cells.[1] Discovering a new gene, she says, "was incredibly motivating."

Dymecki then moved on to new challenges. At the time, scientists were just beginning to adopt revolutionary techniques for inserting or deleting specific sequences in the DNA of mice, creating ways to probe the functions of gene and cellular networks with unprecedented precision. Dymecki became a staff fellow in the Carnegie Institution of Washington's Department of Embryology in Baltimore, where she established its first transgenic mouse facility and adapted a powerful gene manipulation tool from yeast called Flp-FRT for use in mammals. By applying this technique to switch the activity of genetically engineered reporter transgenes on or off in specific cells at specific times during in utero development, she was able to reveal the journey various cells take across space and time—and the genes involved—in the assembly of different brain areas.

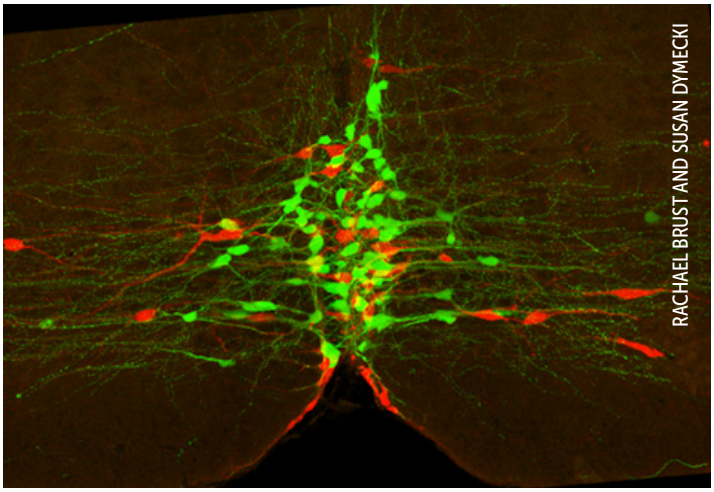
In 1998, Dymecki joined Harvard Medical School's Department of Genetics as an assistant professor, and the following year she became a Rita Allen Foundation Scholar. Starting with Flp-FRT, her lab expanded its set of genetic tools and

used them to map how different types of neurons in the brainstem—the region connecting the brain and the spinal cord—find their correct positions during development.

In the past decade, Dymecki has continued her work on neural patterning in brain development. One major focus of her current work is on so-called serotonergic neurons, which use the neurotransmitter serotonin to communicate. Serotonergic circuits in the brain modulate a dizzying range of functions, from breathing, body temperature and heart rate to dimensions of mood and cognition. Dymecki's lab uses transgenic tools and techniques to probe these neurons' functions—for example, turning certain genes on or off during distinct periods of development or in specific brain regions or types of cells.

For Dymecki, who also heads Harvard's Ph.D. Program in Biological and Biomedical Sciences, teaching and mentoring have taken a place beside research as central elements of her career. "One of the best parts of my job," she says, "is working with the young trainees who will be the next generation of scientists shaping our world."

Here, she reflects on her ice-dancing past, her love of serotonergic neurons, and the joys of collaboration.



Susan Dymecki studies the functions of serotonergic neurons, which use the neurotransmitter serotonin to communicate. Here, two subtypes of serotonergic neurons with different functions (one marked green and one marked red) intermingle in the brainstem.

What made you decide to give up ice dancing and resume your education, and how did your years of athletic training influence your career?

Returning to school was something I always knew I would do. What prompted the decision was that my dance partner needed to stop for personal reasons, and I was unable to find another partner with whom I felt I could progress. Another consideration was that my family was going through a challenging time. I was giving up a dream, but I had a strong sense that it was time to begin a new journey and ignite a new passion.

I expected to chart an M.D.-centric course, with Ph.D. training on the side. But I fell in love with research: the pursuit of fundamental questions about how our cells, organs and bodies work; the aesthetic of the data; the heady experience of being the first—really the first—to observe something. I also loved the single-minded devotion of it all, much like training in figure skating. That feeling, so similar to the intensity I felt on the ice, helped me recognize that my heart lay in the laboratory and not in clinical medicine.

What drew you to the serotonergic system? What's so interesting about this particular neurotransmitter?

In 2001, I learned from a colleague, Dr. Hannah Kinney, that dysfunction in this system seemed associated with sudden infant death syndrome (SIDS). I had just had my first child, and I couldn't imagine anything more tragic than the sudden unexpected loss of an infant. How does one ever recover? I felt that if using our tools to better understand this neuronal system could help understand and eradicate SIDS deaths, I had a responsibility to pursue it.

The more I delved into it, the more I became fascinated by the neurons that signal through serotonin, with their elaborate ramifications, innervating a wide range of brain regions to modulate specific physiological processes or behaviors. How

does this neuronal system accomplish so many different tasks, affecting everything from mood and cognition to breathing, heart rate and body temperature? What we are learning is that there is a surprising heterogeneity in the types of serotonin neurons that exist in the brainstem raphe—a set of midline regions in the brainstem from which these neurons project. Differences in gene transcription over the course of development help to shape the suite of neurobiological tasks that each neuron will engage in.

How have transgenic techniques changed neuroscience, and what do you predict they will continue to bring to the field?

Studies of neuron electrophysiology, behavior, neuroanatomy and circuitry have all been greatly advanced through genetic techniques. By using genes expressed in cell type-restricted patterns, we can gain genetic access to subsets of neurons in a living animal and drive the expression of a wide range of molecules that label those specific types of neurons or regulate their biological activity. Then we can visually follow neurons as they develop—really watch axons and dendrites extend to their targets—which is the first step in mapping a neural circuit. We can use such effector molecules to turn a neuron's activity up or down, and then study the behavioral or physiological consequences. We can also isolate types of neurons and discover the range of genes they express, as well as which of those genes enable unique functions. The possibilities are endless. My goal was to get in on the ground level and make a contribution that many could build upon.

Reference

1. Dymecki, SM, Niederhuber, JE, and Desiderio, SV. Specific expression of a tyrosine kinase gene, *blk*, in B lymphoid cells. *Science* 247 (1990), 332-336.

HAO WU (2002)
The Cellular Dimensions of Immunity

"We saw that these intracellular signaling molecules were forming very large higher-order structures...this kind of signaling complex has now established its role as the overarching principle for innate immune responses."

Hao Wu was a medical student in Beijing when she first saw the images that would change the course of her career. At an international biochemistry meeting, Michael Rossmann of Purdue University presented the intricate three-dimensional structures of viruses that his group had elucidated using X-ray crystallography.

"At the time, I actually didn't know what X-ray crystallography meant, but that really sparked my interest," Wu recalls. She learned that the technique involved illuminating protein crystals with beams of X-ray radiation, and then mathematically analyzing the beams' diffraction patterns to deduce the arrangements of atoms in the protein. "Instinctively, I thought this could be a really good fit for me, because of my interest in math and physics," she says.

Wu was so enamored of the idea that she left medical school and moved to the United States to pursue a Ph.D. with Rossmann, who had recently become known for producing the first atomic-resolution structure of an animal virus[1]—a type of rhinovirus that causes the common cold. Wu herself tackled the structure of canine parvovirus, which can lead to serious disease in puppies (although animals are now commonly protected by a vaccine).

Wu and Rossmann examined the architecture of the 60 individual coat proteins that form the canine parvovirus's icosahedral shape—a soccer ball-like structure with 20 flat surfaces.[2] Their work helped to illuminate how viral particles are put together within host cells, including how coat proteins interact with viral DNA as it is packaged inside an assembling protein shell.

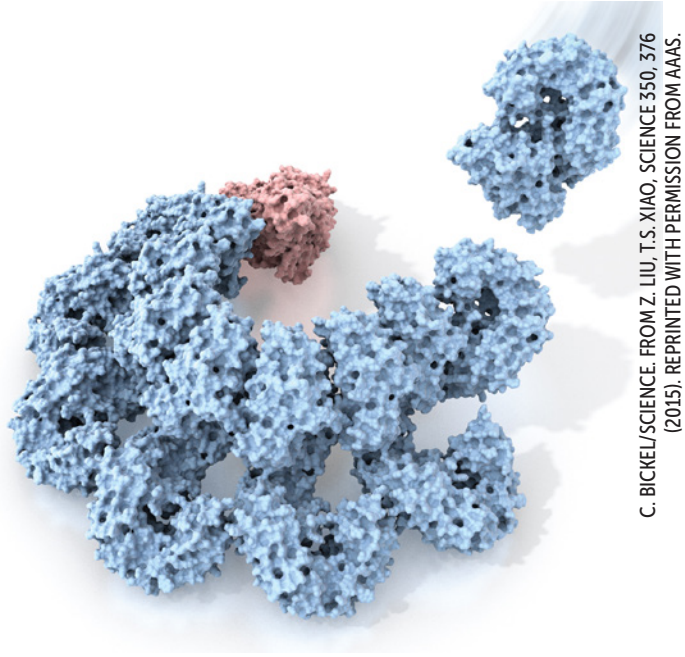
Fascinated by the power of structural studies to reveal new facets of biological activities, Wu continued to investigate protein architectures as a postdoc in the laboratory of Wayne Hendrickson at Columbia University (where 2003 Rita Allen Foundation Scholar Christopher Lima also conducted postdoctoral research). Wu and her colleagues mapped the structure of human chorionic gonadotropin (hCG), a hormone that triggers the production of progesterone during early pregnancy.[3] She also studied the anatomy of CD4, an immune receptor involved in HIV infection, and showed how

CD4 proteins may form pairs when mediating the recognition of foreign molecules by immune cells.[4]

After joining the faculty at Weill Cornell Medical College, Wu turned her attention to tumor necrosis factor-receptor-associated factors (TRAFs), key components of cellular signaling pathways that regulate immune responses and are inappropriately activated in autoimmune conditions such as rheumatoid arthritis and Crohn's disease. Wu's team identified unique attributes of TRAF6[5] and explored its potential as a therapeutic target for osteoporosis.

Wu became a Rita Allen Foundation Scholar in 2002, and began to investigate how a viral protein called p35 prevents cells from committing suicide (apoptosis)—a response meant to purge the body of infected cells.[6] Understanding the activities of p35 has helped researchers to examine the involvement of apoptosis in various disease processes. Wu's research group also uncovered an interaction between amyloid beta, the main protein found in the brain plaques of Alzheimer's patients, and a particular alcohol dehydrogenase enzyme.[7] Drugs that inhibit this interaction are now being developed as potential Alzheimer's therapies.

In 2012 Wu moved to Harvard Medical School, where her team has characterized the complex, higher-order structures of "inflammasomes"—multiprotein complexes that assemble in response to the recognition of, for example, proteins from bacterial pathogens.[8] Once assembled, inflammasomes initiate an array of responses that may lead to cell death, the production of antibacterial molecules and the influx of immune cells to the site of infection. To investigate these elaborate bundles of proteins, Wu's group has begun to use cryo-electron microscopy, a structural imaging technique that, in contrast to X-ray crystallography, does not require the laborious process of producing protein crystals.



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Hao Wu studies the assembly of inflammasomes—multiprotein immune signaling complexes. Here, the protein NAIP2 (red) recognizes a component of a bacterial pathogen, triggering changes in a complex of NLRC4 proteins (blue) that activate inflammatory responses.

In light of emerging knowledge about inflammasomes and other innate immune signaling complexes, Wu and her colleagues have challenged traditional views of cellular signaling mechanisms as linear pathways.[9] They argue that the assembly of multiprotein complexes may enable immune responses to reach a critical threshold, causing cells to unleash powerful inflammatory signals. Wu's team is now beginning to apply this framework to drug discovery studies, for which she received a 2015 Director's Pioneer Award from the National Institutes of Health.

Here, Wu reminisces about getting her start as a scientist, and explains shifting perceptions of cellular signaling.

What was your first research experience?

In medical school, I worked with an immunologist. Maybe that's why I'm still studying immunology—it got me very interested in all of these different immune diseases. At the time, I worked on lupus, a chronic autoimmune disease that causes inflammation in many of the body's tissues. We isolated blood samples from lupus patients and from normal donors—actually, from ourselves! Then we analyzed them using what I think was the very first flow cytometer in China—a machine that can sort cells based on defined molecular properties. We actually helped install the machine.

We were able to see a difference between immune cells with the CD4 surface protein in normal individuals versus lupus patients, and we tried to provide some insights into the disease, so to me that was all extremely interesting. Later I ended up continuing this interest by studying cellular immune responses.

How has your work led to new ideas about how cells propagate immune signals?

We and other researchers have discovered these so-called "higher-order structures" involved in mediating signal transduction. In a classical signaling pathway, you have a receptor protein, which gets activated by a ligand molecule, and that causes some kind of conformational change in the receptor. That change then propagates the signal to the inside of the cell, where another protein is activated, which triggers second messenger molecules that can set off broader responses. So that's what we were thinking when we started studying the receptors of the immune system, such as the TNF family of receptors and Toll-like receptors.

But as we were applying structural approaches to these studies, we realized that we did not see a similar motive signaling process. Instead, we saw that these intracellular signaling molecules were forming very large higher-order structures. And more recently we discovered that these are filamentous structures that polymerize into a helical symmetry, that there are multiple kinds of structures within a given signaling complex, and that together they form micron-sized spots or puncta inside the cell—which you can see even with a light microscope. That's what I call signaling machinery, or a "signalosome" that mediates the transmission as well as the amplification of the signal. Since our initial discovery, this kind of signaling complex has now established its role as the overarching principle for innate immune responses.

References

1. Rossmann, MG, Arnold, E, Erickson, JW, et al. Structure of a human common cold virus and functional relationship to other picornaviruses. *Nature* 317 (1985), 145-153.
2. Wu, H, and Rossmann, MG. The canine parvovirus empty capsid structure. *J Mol Biol* 233 (1993), 231-244.
3. Wu, H, Lustbader, JW, Liu, Y, Canfield, RE, and Hendrickson, WA. Structure of human chorionic gonadotropin at 2.6 A resolution from MAD analysis of the selenomethionyl protein. *Structure* 2 (1994), 545-558.
4. Wu, H, Kwong, PD, and Hendrickson, WA. Dimeric association and segmental variability in the structure of human CD4. *Nature* 387 (1997), 527-530.
5. Ye, H, Arron, JR, Lamothe, B, et al. Distinct molecular mechanism for initiating TRAF6 signalling. *Nature* 418 (2002), 443-447.
6. Xu, G, Rich, RL, Steegborn, C, Min, T, Huang, Y, Myszka, DG, and Wu, H. Mutational analyses of the p35-caspase interaction. A bowstring kinetic model of caspase inhibition by p35. *J Biol Chem* 278 (2003), 5455-5461.
7. Lustbader, JW, Cirilli, M, Lin, C, et al. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* 304 (2004), 448-452.
8. Zhang, L, Chen, S, Ruan, J, et al. Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. *Science* 350 (2015), 404-409.
9. Kagan, JC, Magupalli, VG, and Wu, H. SMOCs: supramolecular organizing centres that control innate immunity. *Nat Rev Immunol* 14 (2014), 821-826.

AJAY CHAWLA (2003)
Beyond Immunity

COURTESY OF AJAY CHAWLA

"[The award] provided me recognition, in the sense that an elite group of scientists have gone through this path before—and it puts you with them. And then the expectations are also pretty high that you're going to do something equivalent or something similar."

Ajay Chawla delighted in exploring the mechanics of everyday objects from an early age. At first, he recalls, "I spent most of my time taking things apart and not putting them together." This impulse served him well in school, where he was drawn to math and science, and considered a career in chemistry.

As an undergraduate at Johns Hopkins University, Chawla majored in bioengineering, attracted by the challenges and possibilities of this emerging discipline. He joined the laboratory of microbiologist Robert Maier (now at the University of Georgia), where he investigated the metabolism of *Azotobacter vinelandii*, a soil bacterium that converts its food into usable energy with remarkable speed. Chawla helped to isolate and study the genes encoding the bacterium's cytochrome *d* oxidase, an electron-transporting enzyme embedded in the cell membrane.[1]

This experience fueled Chawla's fascination with biology and led him to pursue interests in both research and medicine in an M.D.-Ph.D. program at the University of Pennsylvania. His graduate work focused on parsing the functions of nuclear receptors—transcription factors that were known to regulate metabolism and appeared to play pivotal roles in heart disease and diabetes.

Working with endocrinologist Mitchell Lazar, Chawla explored the activities of a nuclear receptor called peroxisome proliferator-activated receptor gamma (PPARG), finding high levels of PPARG expression in the fat cells (adipocytes) of mice. Chawla documented the striking rise in PPARG levels during the differentiation of adipocytes from their precursor cells.[2]

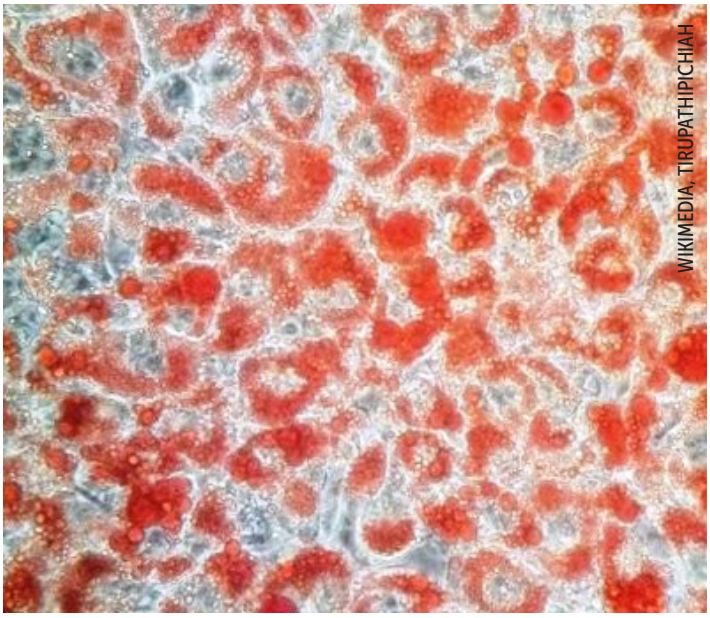
But directing the fate of fat cells was not the only purpose of PPARG: the receptor also participated in the formation of foam cells—lipid-packed macrophages (a type of white blood cell of the immune system) that are a hallmark of arterial plaques. And PPARG seemed to be involved in regulating insulin resistance, as a new class of antidiabetic drugs called TZDs had been shown to act through PPARG.

In parallel with his medical residency at the University of California, San Diego, Chawla pursued postdoctoral research

with Ronald Evans at the Salk Institute for Biological Studies. Continuing his investigations of PPARG, Chawla showed that its activation could lead foam cells to shed their accumulated cholesterol.[3] And in mice genetically prone to hardened arteries, introducing macrophages lacking PPARG caused more severe arterial lesions. These results helped to explain why TZDs tended to keep arteries clear, and provided genetic evidence that PPARG played a role in coronary artery disease.

Chawla became a Rita Allen Foundation Scholar in 2003, shortly after establishing his own research group at Stanford University. There, he discovered that in macrophages, PPARG directs a pathway of "alternative activation," distinct from classic bacteria-fighting activities, that reins in insulin resistance.[4]

Since joining the faculty of the University of California, San Francisco, in 2010, Chawla and his group have mapped even more intersections between metabolism and the immune system. Their work has provided evidence that macrophages infiltrate nearly every organ of the body,[5] and working together with other immune cells, they contribute to tissue regeneration, and even enable fat tissue to generate heat in response to cold temperatures.[6]



WIKIMEDIA, TIRUPATHIPICHIAH

A culture of differentiated fat cells (adipocytes). Fat molecules are stained with the dye Oil Red O. Ajay Chawla has studied the role of the nuclear receptor PPARG in adipocyte differentiation.

Here, Chawla discusses his career path, his approach to research, and his latest venture—deciphering the details of how mammals adapt to the cold.

After your initial interest in engineering, what made you decide to go to medical school, and then to pursue research full-time?

I'm one of those people who had to try different things. It wasn't clear to me what I wanted to do with my life—I wasn't preprogrammed. I went through college wanting to be a biomedical engineer, and that was after wanting to be a chemist. And then I wasn't sure if I wanted to be an engineer or a physician or a scientist.

So I went to medical school. But within a week, I realized I couldn't do it—it was very different from engineering. So I entered the M.D.-Ph.D. program. And even during my clinical training, I would get up in the morning, and I would always think about what was going on in the lab. That was a very clear internal guide for me.

I don't see patients anymore, but I still value every moment of my medical training, and it has provided me with a very interesting perspective on biological problems. My true love, though, is at the bench. There's nothing more exciting than discovering something new in the lab.

You've uncovered many unexpected roles for immune cells—in arterial disease, insulin sensitivity, adaptation to cold...how do you figure out what to study next?

I call it "following your nose." You get data in the lab that suggests something, and you just follow it. But I have a couple of filters that I use, which came from my mentors.

One is that the first observation, if you're going to follow it, has to be pretty robust. Because by the

time you drill down into the mechanism, if it's small, you'll lose this effect. Second, for every experiment you do, think about three steps ahead, and do the thought experiment. And at the end, ask yourself what you've discovered, and how important it is—be honest about it.

Over time, my interests have shifted into trying to understand how the immune system is not only controlling metabolism, but also participating in other programs—dealing with environmental changes, dealing with tissue repair mechanisms and other things. This was understudied, although people are starting to study it more now. It's exciting, because when something is understudied, you have more opportunities to discover new things.

What is one of the big questions you still want to answer?

I have a fairly good-sized effort right now trying to understand how organisms—in particular, mammals—adapt to changes in the environment. We're using the experimental paradigm of cold to understand the process.

Our ability to regulate our body temperature is necessary for survival. We understand how the neuroendocrine system controls the ability to sense and regulate body temperature. This works well when you simply have to sense and activate a program that is already established.

But when you require adaptation and acclimatization for longer periods, it turns out that the innate immune system participates. This provided us with an experimental paradigm to try to understand how immunity instructs acclimatization to environmental cold.

How our bodies adapt to the cold seems like such a fundamental question. Why is so much still unknown about this?

We have ignored cold in some ways, because we can regulate our environmental temperature. When was the last time you actually felt really cold? You change the thermostat in your home or office, or wear warmer clothing. So we don't pay attention to it much.

Using the mouse as a model, we want to understand the signals and mechanisms by which a mammal acclimatizes to colder temperatures, and then to try to figure out how that knowledge can be applied. If you know how to turn on a program that is very thermogenic and catabolic, it can perhaps also be used for tackling disease states like obesity.

References

1. Moshiri, F, Chawla, A, and Maier, RJ. Cloning, characterization, and expression in *Escherichia coli* of the genes encoding the cytochrome *d* oxidase complex from *Azotobacter vinelandii*. *J Bacteriol* 173 (1991), 6230-6241.
2. Chawla, A, Schwarz, EJ, Dimaculangan, DD, and Lazar, MA. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* 135 (1994), 798-800.
3. Chawla, A, Boisvert, WA, Lee, CH, et al. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol Cell* 7 (2001), 161-171.
4. Odegaard, JI, Ricardo-Gonzalez, RR, Goforth, MH, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* 447 (2007), 1116-1120.
5. Wynn, TA, Chawla, A, and Pollard, JW. Macrophage biology in development, homeostasis and disease. *Nature* 496 (2013), 445-455.
6. Nguyen, KD, Qiu, Y, Cui, X, et al. Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature* 480 (2011), 104-108.

CHRISTOPHER LIMA (2003)
Structure Meets Function

"This enabled me to do things that I couldn't necessarily do with more traditional funding sources. To get that boost when you're probably the least sure of what you should be doing to have a successful career...having that level of guidance was really a turning point."

As an undergraduate at The Ohio State University, Christopher Lima split his time between studying biochemistry and playing the trombone in a ska band.

When the time came to choose between these two pursuits, Lima opted for a career in science. He delved into structural biology, first as a graduate student with Alfonso Mondragón at Northwestern University, where he mapped the doughnut-like structure of a bacterial topoisomerase enzyme to shed light on how it breaks and reseals DNA.[1]

Lima then conducted postdoctoral research with Wayne Hendrickson at Columbia University, where he crystallized two divergent members of the cryptic histidine triad (HIT) protein family.[2] Emerging genomic sequences showed that the proteins were highly conserved among organisms from bacteria to humans, but their functions were not well understood.

The proteins' structures suggested that they might transfer or remove nucleotides from other molecules. Years later, this work "came back to haunt me in a very satisfying way," Lima says, as his colleagues found that a HIT protein called DcpS was responsible for removing the molecular "caps" of partially degraded messenger RNA molecules, thus preventing their interaction with the translation apparatus.[3] Lima and colleagues then went on to resolve the structure of a DcpS-cap complex.[4]

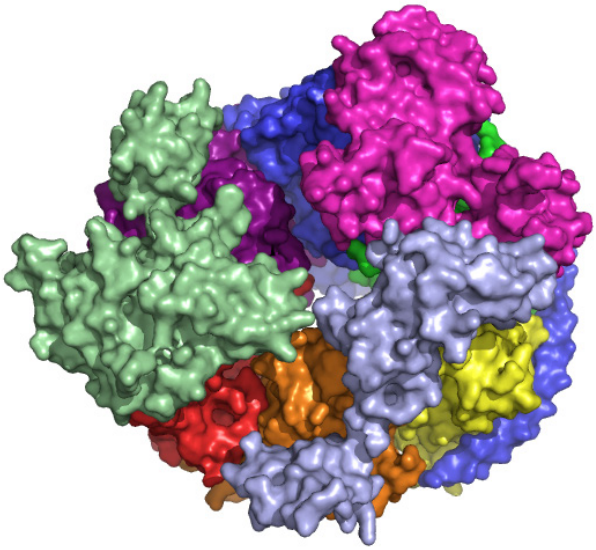
Mechanisms of RNA processing have since become a major area of focus for Lima, who established his own research group at Weill Cornell Medical College in 1998, and is now an Investigator of the Howard Hughes Medical Institute at Memorial Sloan Kettering Cancer Center. Lima and his team examine fundamental cellular processes that determine the fates of proteins and RNAs—with implications for the regulation of the cell cycle, gene expression and other vital activities.

Lima became a Rita Allen Foundation Scholar in 2003. At the time, small ubiquitin-like modifier (SUMO) proteins had recently been discovered. Researchers knew that these little tags could be attached to larger proteins, and that their presence was required for cells to properly grow and divide. But the specific targets of SUMOs, and even the mechanisms of their attachment, were unclear.

Structural studies by Lima and his group helped to illuminate the interactions between SUMOs and the enzymes that link them to other proteins—interactions critical for cell growth, stress responses and other functions.

Lima also had a longstanding interest in the molecular processes that generate and modify RNAs, which form diverse structures that mediate many aspects of the transformation from genetic information into actual cellular activities. This interest led Lima to explore the stunning complexity of the dozen or more proteins that make up the RNA exosome, which has the ability to either chew up RNA molecules or to convert them into mature, functional forms. How the exosome discriminates between these possibilities remains unclear.

Here, Lima reflects on the enigmas of the exosome, the future of structural biology and the intrinsic artistry of science.



CHRISTOPHER LIMA

A model representing the architecture of the human RNA exosome core, which consists of nine distinct protein subunits, as elucidated by Christopher Lima and his team.

Why was the exosome so difficult to decipher? How did you do it?

When we started working on the exosome, it wasn't even clear which proteins were in it, or how many proteins or what the stoichiometry was. It turned out that the complex is dynamic: it has nuclear forms, it has cytoplasmic forms, and it has all sorts of different cofactors and accessory factors.

We realized that it was almost going to be an intractable problem until we were able to reconstitute it in vitro from individual components—which we did, more than a decade ago now.[5] It was a landmark example of a brute force effort, in the sense that we tried basically every combination of every possible factor in order to get these complexes to form. But we succeeded.

We characterized the activities of our reconstituted samples, and showed that in many cases they were not as anticipated. We were then able to genetically modify individual components, rather than modifying the organism, to generate an intact complex with modified components. That was very critical, because most of the subunits are essential for viability. By looking in vitro, we were able to see what the individual subunits were actually doing.

Where are you going next with the exosome?

Now the big question is how it's regulated. In some ways, it's analogous to the proteasome, which everybody looked at as a trash can: you put ubiquitin on the protein, it finds the proteasome, and the proteasome just degrades—it has no other option. But it turns out there are multiple levels of regulation even at the site of degradation, and I suspect very strongly that that is also going to be true for the RNA decay system.

Several projects in my lab right now are focused on the question of how fate is decided once RNA reaches the exosome. When you make a particular structured RNA or structured piece of nucleic acid and you screw that up, how do you sense that it's screwed up? How is that molecule labeled as screwed up, and then how does it get to the exosome to be interrogated?

I think that that level of quality control is something we understand at the genetic level right now. But at the mechanistic level, we have no clue.

What's next for the field of structural biology?

The methodology defined the field for quite some time, and it still does in some cases. But now it's the biology that's driving the bus.

The big challenge is to become biologists who use structure, rather than structural biologists who explain other scientists' biology with structure. As our systems are growing in complexity, this is becoming the major source of effort—not so much figuring out which structure to solve, but figuring out how the system behaves as a whole.

There are tens of factors that have to interact with one another to decide the fate of a particular RNA or protein. And that's way more complicated than it was when I started in science. So it's really about understanding the biology deeply enough.

Looking back at your career choice between science and the arts, how do you feel about that decision now?

At the time, I wasn't quite aware of how creative science can be, if you're lucky enough to have the opportunity. When I realized that the level of creativity I was employing in these other parts of my life could be applied to my science, that's where it really took off. Now I spend most of my time trying to think of new ways to get at the problems we're investigating, and that still flexes those creative muscles.

I don't view science as a job—it's more of a lifestyle. If I couldn't come into my office, I'd be sitting at home thinking about the same stuff. It's really a privilege.

References

1. Lima, CD, Wang, JC, and Mondragón, A. Three-dimensional structure of the 67K N-terminal fragment of *E. coli* DNA topoisomerase I. *Nature* 367 (1994), 138-146.
2. Lima, CD, Klein, MG, and Hendrickson, WA. Structure-based analysis of catalysis and substrate definition in the HIT protein family. *Science* 278 (1997), 286-290.
3. Wang, Z, Jiao, X, Carr-Schmid, A, and Kiledjian, M. The hDcp2 protein is a mammalian mRNA decapping enzyme. *Proc Natl Acad Sci USA* 99 (2002), 12663-12668.
4. Gu, M, Fabrega, C, Liu, SW, Liu, H, Kiledjian, M, and Lima, CD. Insights into the structure, mechanism, and regulation of scavenger mRNA decapping activity. *Mol Cell* 14 (2004), 67-80.
5. Liu, Q, Greimann, JC, and Lima, CD. Reconstitution, activities, and structure of the eukaryotic RNA exosome. *Cell* 127 (2006), 1223-1237.

LAURA JOHNSTON (2004)
How Life Shapes Up

COURTESY OF LAURA JOHNSTON

"Whenever cells have to live together as a group, they have to become, essentially, social communities—to interact, cooperate and function together. Our hypothesis is that cell competition ensures this kind of cooperative behavior by recognizing and eliminating rogue cells before they do damage."

Laura Johnston worked as a chemist at an oil refinery, a city council staffer, and a landscape and portrait artist before shifting her focus to biomedical research.

Now, she seeks to understand how cells push and prod one another as an organism grows and develops. She has spent the past two decades exploring the intricate circuits that lead formless masses of fruit fly (*Drosophila*) cells to become eyes, legs and wings. In the process, Johnston has uncovered a key pathway involved in cell competition, a critical activity for the formation of healthy adult tissues.[1]

She has spent the better part of her scientific career teasing apart the functions of Myc, a powerful genetic regulator that is mutated in many human cancers. In *Drosophila*, alterations of the *myc* gene delay development and diminish the size of the adult fly.

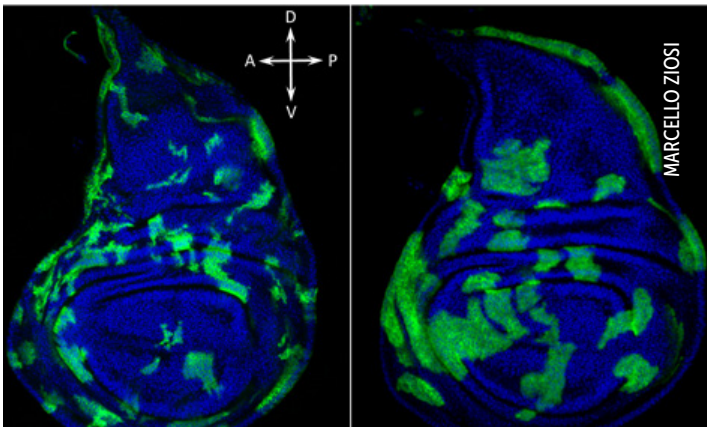
Johnston first investigated *myc* mutants as a postdoc working with Bruce Edgar (a 1995 Rita Allen Foundation Scholar) at the Fred Hutchinson Cancer Research Center in Seattle. She and her colleagues examined how Myc controls cellular growth in the larval precursors of adult fly wings, called imaginal discs.

They found that cells with more Myc protein grew faster and larger than cells with less Myc.[2] And at early stages of development, direct competition between *myc* mutant cells and wild-type cells could cause the mutant cells to be eliminated completely. Johnston and her coauthors hypothesized that the growth differences they observed might result from disparities in protein synthesis.

After establishing her own research group at Columbia University Medical Center in 2000, Johnston went on to show that Myc-mediated cell competition was required for the fruit fly wing to reach its proper size in the adult.[3] Another insight into the nature of this intercellular battle emerged from analyzing the interactions between cultured *Drosophila* cells with different levels of Myc. Johnston and a postdoc in her lab found that higher-Myc "winner" cells did not need to directly contact lower-Myc "losers" to induce the death of the weaker cells.[4]

The experiments suggested that both winner and loser cells were releasing molecular signals to affect one another's growth. While these discoveries pointed to Myc as a key modulator of cell competition during development, the precise mechanisms mediating the conflict remained elusive.

However, recently Johnston's lab identified a secreted cytokine that is required for the death of the "loser" cells. Strikingly, their results suggest that cell competition relies on some of the same molecules that allow animals to respond to invading bacteria and fungi. In 2014, her team published a study of cell competition in wing discs from flies with mutations in various components of two innate immune pathways, Toll and IMD. Some of these mutations, they found, compromised the ability of weaker loser cells to induce their own death in response to neighboring cells with higher Myc levels.[5] Johnston and her group are now following this lead as they continue to examine the underlying mechanisms of cell competition.



Drosophila wing discs containing non-competing neutral groups of cells (green, left) and competing groups of cells expressing extra Myc (green, right).

Here, Johnston reflects on the implications of cell competition for disease and evolution, her unusual career path, and the parallels between art and science.

Why is cell competition important for human health?

Information about fitness is transmitted between cells all the time, and we didn't realize that before. But it's becoming more and more clear that this is taken advantage of in diseases like cancer, where tumor cells can sort of cheat and pretend they're more fit, and trick the tissue into thinking that they shouldn't be gotten rid of, while normal cells that are relatively less fit than the cancer cells end up being kicked out.

We're trying to understand how this happens. Very recently we discovered that some of the same molecules that are used to sense bacterial and viral pathogens in our bodies—innate immune signaling components—are also used to distinguish cells within our growing tissues that are either less fit or more fit.

Now we're very excited about trying to understand, first of all, the various molecules that are involved, and how these molecules interact with each other. And then also, why does this happen?

What's your current thinking on why cell competition evolved?

We hypothesize that this is a mechanism that optimizes tissue health, and makes sure that cells that are not very functional don't contribute to the adult. We think this mechanism may have evolved from the onset of multicellular animals.

Whenever cells have to live together as a group, they have to become, essentially, social communities—to interact, cooperate and function together. Our hypothesis is that cell competition ensures this kind of cooperative behavior by recognizing and eliminating rogue cells before they do damage.

How did you arrive at a career in science again after your stints in politics and art?

I've always been interested in nature and in biology, and I've also always been fascinated with art—I've always drawn and painted. Those kinds of things went hand in hand for me for a long time.

When I went to college, I studied biology, and I loved it. But then I got involved in local politics and took a job doing that for a while. I ended up getting married and having a son, and at that point I dedicated myself to learning how to paint.

But eventually I decided I wanted to go back to school. First I got a job as a technician. I already had a lot of lab experience, and I just felt very, very comfortable in a lab.

Do you miss painting?

I did have to put painting aside when I went back to graduate school. But I realized that doing experiments and thinking about biology is so much like doing art. You have ideas in your head, and you have to somehow translate those ideas into something more concrete. As an artist, you draw and paint on a canvas or a piece of paper. But you're always sort of nudging it and changing it.

It's the same when you're doing experiments. You start with an idea, you try to develop that idea, and you do experiments to test various aspects of the idea. Sometimes they don't work out the way you think. So you rethink, and you change a little bit. It's so much like making a piece of art. Science has been very satisfying for me in that respect, even though I can't do art in the traditional sense.

References

1. Johnston, LA. Competitive interactions between cells: death, growth, and geography. *Science* 324 (2009), 1679-1682.
2. Johnston, LA, Prober, DA, Edgar, BA, Eisenman, RN, and Gallant, P. *Drosophila myc* regulates cellular growth during development. *Cell* 98 (1999), 779-790.
3. de la Cova, C, Abril, M, Bellosta, P, Gallant, P, and Johnston, LA. *Drosophila* Myc regulates organ size by inducing cell competition. *Cell* 117 (2004), 107-116.
4. Senoo-Matsuda, N, and Johnston, LA. Soluble factors mediate competitive and cooperative interactions between cells expressing different levels of *Drosophila* Myc. *Proc Natl Acad Sci USA* 104 (2007), 18543-18548.
5. Meyer, SN, Amoyel, M, Bergantiños, C, de la Cova, C, Schertel, C, Basler, K, and Johnston, LA. An ancient defense system eliminates unfit cells from developing tissues during cell competition. *Science* 346 (2014), 1258236.

SENTHIL MUTHUSWAMY (2004)
Tackling Cancer in Three Dimensions

"When I started my research program we were addressing something that was not commonly thought about as an important regulator of cancer biology, which is cell polarity. [With the Rita Allen Foundation award], the way in which we could address these questions without having to provide all the necessary initial evidence...was a big blessing for us."

In 1990, Senthil Muthuswamy was a young graduate student in biology at McMaster University in Hamilton, Ontario. He had just moved to Canada from his native India after completing a bachelor's degree in agricultural sciences at the Tamil Nadu Agricultural University and a master's degree in genetics at the Indian Agricultural Research Institute in New Delhi.

Although he had enjoyed plant genetics, Muthuswamy became more interested in mammalian biology and embarked on a major transition in his training—and in the process found a new research interest he has been pursuing ever since.

"I was completely clueless about animal physiology and molecular biology," he recalls. "So I took two pathology courses, and that completely changed my perspective." Learning to decipher the differences between healthy and cancerous tissues gave him a cell structure-centric view of cancer. "I realized that cancer is a disease of loss of tissue structure and organization," he says. "That is the cardinal feature used by pathologists to define a disease state, whether it is cancer or anything else."

At that time, most cancer researchers, using tumor cell lines, were focusing on signaling pathways that went awry in cancer, inducing unchecked proliferation. But Muthuswamy wanted to study how cancer caused the cellular changes he had seen on pathology slides of tumor samples. He joined the laboratory of William Muller, who was then establishing mouse models of breast cancer. Focusing on the role of the Src enzyme in initiating tumors, Muthuswamy and his colleagues showed that in transgenic mice, activation of Src is necessary to induce mammary tumors.[1] He then further teased out the signaling pathway of Src-activated mammary tumors, including the protein's interaction with the oncogene HER2 (also known as neu) during tumor formation in mice.[2]

Despite working on tumor mouse models, Muthuswamy was focusing on how genetic mutations perturb intracellular signaling, rather than on cell biology. He didn't forget the structural changes that tissues undergo as cancer develops and progresses. "What I learned in that pathology course still stayed with me," he says.

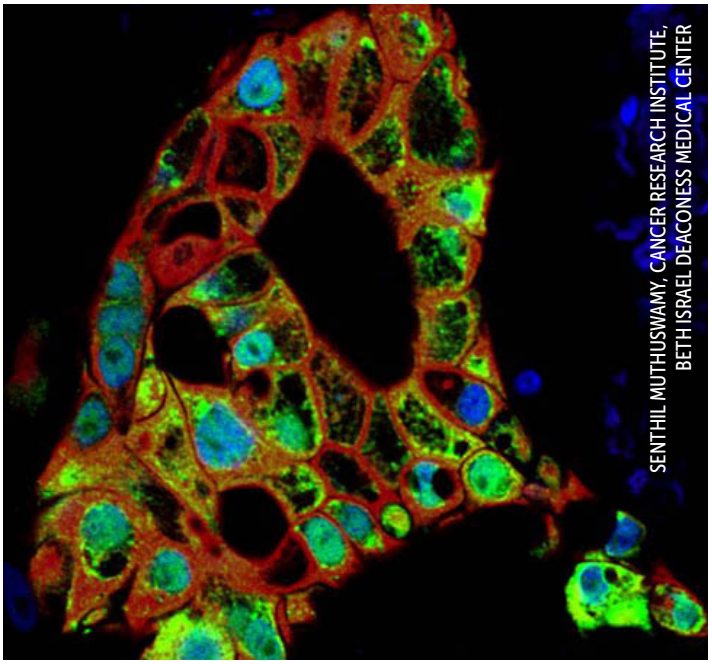
Muthuswamy pursued a postdoc with Joan Brugge, a cell biologist at Harvard Medical School. He recalls the conversation he had with Brugge when he first joined her lab: "We said, 'We need to think about growing epithelial cells in three-dimensional cultures, so that we can really understand the tissue structure and how oncogenes disrupt this organization,'" he recalls.

Mimicking epithelial tissue structure in the lab was not a concept being seriously considered by the cancer research community, Muthuswamy says. Starting from scratch, he set up a new system in Brugge's lab—clusters of mammary cells, called acini, cultured in three-dimensional basement membrane gels. He wanted to address how cell polarity is disrupted by oncogenes in epithelial tumors. "It was a slow process… there was a lot of self-doubt," he says.

Muthuswamy and Brugge teamed up with Mina Bissell of the Lawrence Berkeley National Laboratory in California, one of the few cancer researchers who were using three-dimensional culture systems to address how tumor cells develop in the context of surrounding tissues and organs. Using this culture system, Muthuswamy and his collaborators demonstrated that two receptors overexpressed in breast tumors, EGFR and HER2, have different capabilities to induce mammary tumorigenesis in polarized cell cultures,[3] while both receptors are equally important for transforming unpolarized fibroblasts into tumor cells.

"There was context-dependent biology that was unique for each receptor. We found evidence that HER2 could disrupt the apical base polarity of epithelial cells, while EGFR could not," says Muthuswamy. The work was a proof of concept that a three-dimensional model system can provide insights into how signaling drives cancer that cannot be revealed by standard methods of growing monolayers of tumor cells in the lab.

By the time Muthuswamy was looking to start his own laboratory, the cancer community "was welcoming to the idea of using three-dimensional epithelial cells to probe cancer biology, although this was not being done at most research institutions," he says. Muthuswamy became an assistant professor at Cold Spring Harbor Laboratory in 2001, probing how HER2 disrupts cell polarity. He became a Rita Allen Foundation Scholar in 2004.



Senthil Muthuswamy uses tumor "organoids" to study the roles of cell polarity in cancer.

His research group showed that activated HER2 in epithelial cells immediately causes tight junction mislocalization, which perturbs the distribution of apical polarity proteins in the cells. Then, in 2006, they found that HER2 interferes with cell polarity by physically disrupting a complex called Par.[4] "That was how we made the first connection between cell polarity proteins and oncogene signaling," says Muthuswamy. His lab continues to address how oncogenes affect cellular polarity pathways and cellular architecture. Recently, his group has expanded three-dimensional culture systems to grow pancreatic tumor cells derived from patients' surgical samples as three-dimensional tumor organoids.[5]

Here, Muthuswamy describes the origins of his interests and expands on the importance of cell polarity disturbances in cancer.

When you were younger, who influenced you to pursue a career in biology?

My dad was a soil chemist, an agriculture professor, and I used to do work in his soil chemistry lab after school when I was in high school. I would do small things, like comparing the volumes of different soil samples depending on their composition. He really liked motivating his students toward science. There's a long lineage of people who were trained under him at various levels. But my interest in biology came from my uncle, who is a pediatrician, and he continues to be a true inspiration for me. He instilled in me the idea that if you are really passionate about a

problem, you should dedicate your life to studying it. That concept has stuck with me.

Is disturbance of cell polarity now seen as a major consequence, or even a hallmark, of cancer?

It is beginning to gain a lot of attention now. When I started my lab, cell polarity was not commonly thought about as an important regulator in cancer biology. Cell polarity is a property by which cells create asymmetry within themselves, whether that's differential distribution of proteins or organelles or junction proteins. Cancer biologists accept and acknowledge that intracellular organization is lost in cancer cells, but the mechanisms by which this happens are not well understood. When I began my laboratory it was considered to be an extremely high-risk project, and the Rita Allen award was very helpful because it allowed me to ask these questions.

Now it is accepted that disruption of cell polarity is another arm of cancer biology that has not really been investigated well. What is really missing is whether this is a cause or a consequence of cancer. Data from our lab and others are beginning to suggest, in fact, that structural changes can be causal. Simple experimental systems, such as cells grown on a plastic dish, are poor models to study changes in cell polarity. In addition, it's not a topic that is being widely investigated, and that is why it has not actually made an impact in the minds of [prominent cancer researchers] that it's a hallmark of cancer.

It also depends on whom you ask. If you ask pathologists, they will tell you that cell polarity has an essential role in cancer—that if you do not see any changes in cell structure, then it's not a disease state. There are still big unanswered questions in the field about what exactly is the role of cell polarity pathways in the process of cancer initiation. But our lab and others are beginning to find answers.

What aspect of your research is most exciting to you right now?

Science in my lab is really bubbling right now, and I have a great group of colleagues to work with—it's fantastic. We're focusing on how a polarity protein called Scribble regulates intracellular organelle biology. What we see is that polarity proteins are regulating organelle physiology and organelle biology, and in doing so they are regulating differentiation and stress adaptation by cells. Another focus is coming up with ways in which we can keep primary patient tumor cells alive in culture to test responses to drug combinations and use the information for making clinical decisions. This is really interesting, because for the first time in my academic career, we can do something that can directly help patients, which has really resonated with me.

References

1. Guy, CT, Muthuswamy, SK, Cardiff, RD, Soriano, P, and Muller, WJ. Activation of the c-Src tyrosine kinase is required for the induction of mammary tumors in transgenic mice. *Genes Dev* 8 (1994), 23-32.
2. Muthuswamy, SK, and Muller, WJ. Direct and specific interaction of c-Src with Neu is involved in signaling by the epidermal growth factor receptor. *Oncogene* 11 (1995), 271-279.
3. Muthuswamy, SK, Li, D, Lelievre, S, Bissell, MJ, and Brugge, JS. ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nat Cell Biol* 3 (2001), 785-792.
4. Aranda, V, Haire, T, Nolan, ME, Calarco, JP, Rosenberg, AZ, Fawcett, JP, Pawson, T, and Muthuswamy, SK. Par6-aPKC uncouples ErbB2 induced disruption of polarized epithelial organization from proliferation control. *Nat Cell Biol* 8 (2006), 1235-1245.
5. Huang, L, Holtzinger, A, Jagan, I, et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat Med* 21 (2015), 1364-1371.

DAVID SABATINI (2004)
Fueling Cell Growth


CHRISTOPHER CHURCHILL/WHITEHEAD INSTITUTE

"Our lab does a lot of metabolism work, and researchers tend to study metabolism in just intact cells. I think we need to know about different compartments in the cell and what's inside them. Getting into the cell and measuring things within the cell is quite a challenge, and we're trying to address that."

When asked if he has any advice for young researchers, David Sabatini emphasizes the importance of approaching problems in creative ways. He speaks from experience: As a graduate student at Johns Hopkins University, he struck out on his own rather than undertaking one of the existing research questions in his advisor's lab.

At age 24, he led studies that uncovered a key regulator of cellular growth. More recently, he has emerged as an authority on applying the precision DNA-editing technique CRISPR/Cas9 in human cells.

Sabatini credits his mentors and parents for inspiring his love of research and his powers to think imaginatively. Raised by a cell biologist father and a pathologist mother, he worked as an undergraduate at Brown University with Albert Dahlberg, who studied bacterial ribosomal RNA.

Sabatini entered the M.D.-Ph.D. program at Johns Hopkins in 1990 and joined the laboratory of Solomon Snyder, a well-known psychiatrist, neuroscientist and pharmacologist. "One of the most valuable things I received from Solomon was tremendous freedom to follow my own scientific interests," says Sabatini.

On his own initiative, Sabatini decided to probe the functions of rapamycin, an antibiotic originally isolated from soil bacteria in the early 1970s that was used as a control drug by the Snyder laboratory and was already in development as a clinical drug for organ transplant patients (the drug was later approved by the Food and Drug Administration). In the early 1990s, the molecule's effects on cells and tissues were virtually unknown. "At the time, rapamycin was neither well studied nor famous," says Sabatini.

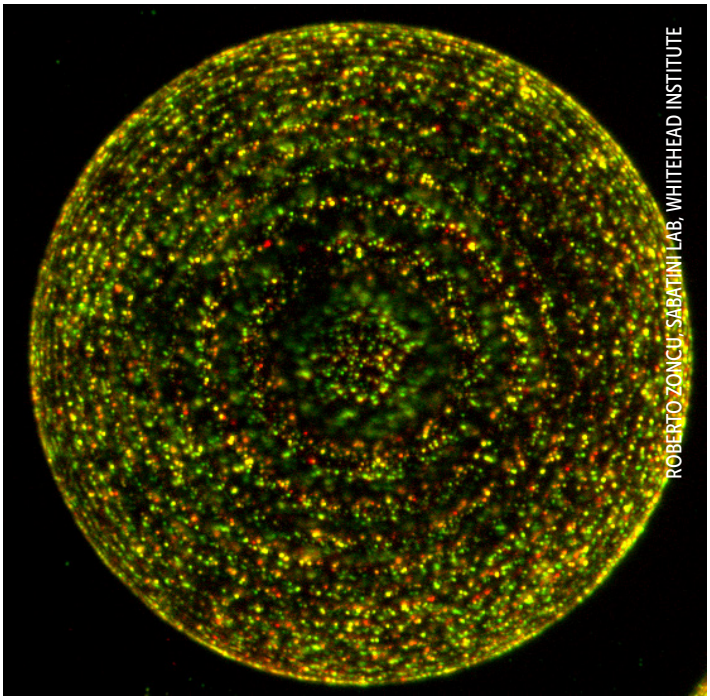
In Snyder's lab, Sabatini discovered the protein target of rapamycin in mammals, known as the mammalian target of rapamycin (mTOR—also called RAFT1).[1] It turned out that mTOR, a serine/threonine kinase, is at the center of numerous signaling pathways for communication both within and between cells, including those that regulate cell metabolism, proliferation, growth and survival. Because of its central role in these processes, mTOR has since been connected to many

diseases, including cancer and diabetes. By the time Sabatini returned to the medical school component of the M.D.-Ph.D. program, he was convinced that research, rather than clinical practice, would be his focus. "I ended up falling in love with the process of scientific pursuit," he says.

After completing his degrees, Sabatini became a Whitehead Fellow at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, which allows select young scientists to run their own labs without the teaching obligations of professors. He continued to study mTOR signaling, discovering that within cells, mTOR is part of two large multiprotein complexes called mTORC1 and mTORC2 that are part of distinct signaling pathways.[2] The mTORC1 pathway is a master growth regulator that couples nutritional and environmental signals to promote cell growth, while mTORC2 is thought to promote cell survival.

In 2002, Sabatini was appointed an assistant professor of biology at the Massachusetts Institute of Technology and a member of the Whitehead Institute for Biomedical Research. He was selected as a Rita Allen Foundation Scholar in 2004, having proposed to tease out the signals that regulate mammalian cell growth—the accumulation of cell mass and increases in size—through the mTOR pathway. Sabatini identified several key players, including Rictor (rapamycin-insensitive companion of mTOR), part of the mTORC2 complex that regulates insulin signaling.[3] Sabatini's team linked mTOR to critical pathways mutated in cancer: his team found that mTORC2 directly activates another signaling protein, Akt, that is deregulated in many types of cancer and that has roles in insulin signaling and glucose sensing. They also found one of the first factors that signal the level of amino acids to the mTORC1 pathway.[4]

In addition to mTOR signaling, Sabatini's lab studies how nutrient metabolism is different in cancer compared to normal tissue.[5] His group is working to characterize essential genes in the human genome,[6] and has helped to develop large-scale lab tools such as a genetic screen applying CRISPR/Cas9 genome editing in human cells.[7]



David Sabatini studies signaling pathways involving the mTOR kinase, a key regulator of cell metabolism, proliferation, growth and survival. Using in vitro techniques, Sabatini's lab has uncovered the details of mTOR pathways. In one experiment, the lab recapitulated the observation that inside cells, mTOR localizes to lysosomes—cell organelles involved in nutrient homeostasis and quality control—in the presence of amino acids: Here, lysosomes (red)—are pre-bound to the surface of agarose beads. The mTOR protein (green) is then added and, in the presence of amino acids, binds to the lysosomes, making them appear yellow.

Here, Sabatini shares advice for young researchers, and reflects on his career-spanning work on mTOR and the challenges of studying signal transduction.

What advice do you have for those just starting out in research?

What I try to emphasize to students is the importance of developing how you think about new problems. I think the hard part of biology is determining what to study, because once you pick a topic and begin to make inroads, you know what you're going after and you follow your results. But deciding what to work on and being creative with that, I think, is very hard, particularly if you're going to do things that are new and original.

I'm not sure what the secret is for making that choice. A little bit of it is gaining some experience in conducting research and being willing to just try things. That's what I learned from Solomon [Snyder]—that it's OK to just try things. I try to teach my students not to talk themselves out of doing something, but to at least try. I also have encouraged people not to do the next follow-on thing. When students come to me after reading a new paper and say, "Now we can do this next thing," I don't like that. I really prefer to do things that are original to our lab.

What has continued to capture your attention since your early scientific career?

Early on, even when I just started my lab and had been working on mTOR, what always fascinated me was the sensing of nutrients. I was interested in sensing in general—how we sense our environment. Part of that had come from classes at Johns Hopkins, where the neuroscience classes had sections on sensing—how pH and temperature are sensed.

At the time, we actually didn't know most of the molecular basis of these things. But I always thought that was kind of cool: how the stomach knows it's full, for example. The mTOR pathway was known to be sensitive to nutrients, and in particular to amino acids. I think in many ways the most significant work that we've done, and are doing, is figuring out the molecular basis of amino acid sensing.

How does that actually happen? We are now finding the actual sensors of amino acids and seeing the structures of these molecules. To me, that's the most fun work that I've done, because it dates back to my Ph.D. Maybe this is very noncreative, but I've continued that research and I think it's been quite productive to see this pathway unravel. My training, especially the big-picture training by Solomon, has really enabled this research. He taught me to do my own thing and not worry about risk. Of course, the risk has to be supported, which is where funding like the Rita Allen Foundation's and the generosity of the Whitehead Institute in general have been key for me.

Are you thinking about any research questions that can't be answered using current technology and will require new tools?

I think we're at this pretty amazing time in biology for measurements. We're able to measure quite a few things at sensitive levels. What I like about the state of technology now is that there are so many questions we can ask with what exists. We can measure the genome by sequencing it, we can sample the proteome and we can measure metabolites. We can also perturb the genome using CRISPR, which is highly enabling, even though dealing with redundancy in the human genome is still a challenge.

Our lab does a lot of metabolism work, and researchers tend to study metabolism in just intact cells. I think we need to know about different compartments in the cell and what's inside them. Getting into the cell and measuring things within the cell is quite a challenge, and we're trying to address that.

Another challenge for people like me, who have done a lot of research on signal transduction, is that much of it is in cells in culture. Now we need to understand how these pathways work in vivo in different tissues. Biology reuses pieces, so the big challenge will be to figure out how these standard pieces we've identified are modified in various tissues and in different disease states.

References

1. Sabatini, DM, Erdjument-Bromage, H, Lui, M, Tempst, P, and Snyder, SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* 78 (1994), 35-43.
2. Kim, DH, Sarbassov, DD, Ali, SM, King, JE, Latek, RR, Erdjument-Bromage, H, Tempst, P, and Sabatini, DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 110 (2002), 163-175.
3. Sarbassov, DD, Ali, SM, Kim, DH, Guertin, DA, Latek, RR, Erdjument-Bromage, H, Tempst, P, and Sabatini, DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* 14 (2004), 1296-1302.
4. Sancak, Y, Peterson, TR, Shaul, YD, Lindquist, RA, Thoreen, CC, Bar-Peled, L, and Sabatini, DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320 (2008), 1496-1501.
5. Birsoy, K, Possemato, R, Lorbeer, FK, et al. Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature* 508 (2014), 108-112.
6. Wang, T, Birsoy, K, Hughes, NW, Krupczak, KM, Post, Y, Wei, JJ, Lander, ES, and Sabatini, DM. Identification and characterization of essential genes in the human genome. *Science* 350 (2015), 1096-1101.
7. Wang, T, Wei, JJ, Sabatini, DM, and Lander, ES. Genetic screens in human cells using the CRISPR-Cas9 system. *Science* 343 (2014), 80-84.

DAVID TUVESON (2004)
Decoding a Cryptic Cancer

COURTESY OF DAVID TUVESON

"[The award] was very helpful in allowing me to work on several areas which were risky…it allowed me to venture into areas of therapeutic development that have led to the really exciting projects that we have going on right now in my lab."

David Tuveson was born a naturalist. Growing up in Michigan, he says, "I was always outside—looking under a rock, going out on the water to observe and catch things and let them go…I've loved science ever since I was conscious."

As an undergraduate at the Massachusetts Institute of Technology, Tuveson explored a range of disciplines, and was impressed by the rigorous quantitative approaches involved in scientific research. "I learned that one of my major weaknesses was chemistry," he says. "So I decided to study it, and I loved it." He was interested in treating disease in patients, but also wanted to contribute to research aimed at improving medicine. His professors encouraged him to pursue an M.D.-Ph.D., and he was accepted into the program at Johns Hopkins University.

Along with his medical training, Tuveson conducted preclinical research with immunologist Douglas Fearon (now at Cold Spring Harbor Laboratory and also a member of the Rita Allen Foundation's Scientific Advisory Committee). He examined the structures and activities of cell surface receptors that trigger the immune system's B cells to generate antibodies and enhance defenses against pathogens.

As a medical student in Baltimore, Tuveson saw patients suffering and dying of AIDS as HIV stripped away their immune systems. He resolved to apply his training to investigate treatments for the disease. But by the mid-1990s, when Tuveson was a medical resident at Brigham and Women's Hospital in Boston, the advent of triple therapy was beginning to transform AIDS from an early death sentence into a chronic condition.

Tuveson turned his attention to another devastating, untreatable disease. "I decided to study a cancer nobody was interested in, which was pancreatic cancer," he says. "The patients died quickly and miserably. There was no scientific understanding of it." Today, pancreatic cancer is the fourth leading cause of cancer deaths in the United States; only 6 percent of patients survive more than five years beyond their diagnosis.

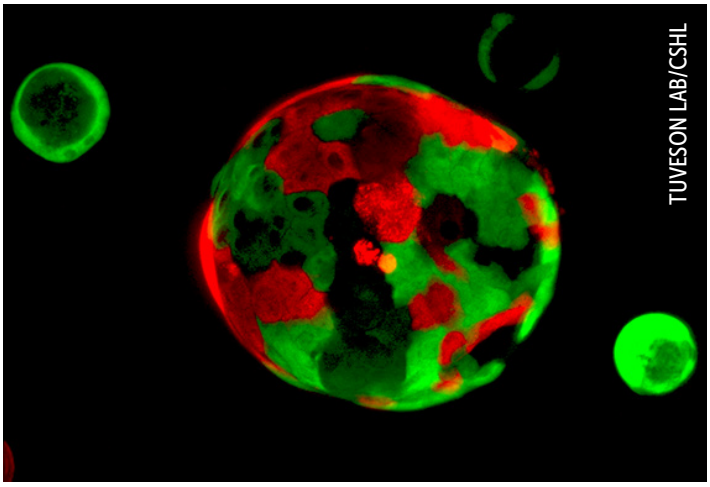
During his hematology and oncology fellowship at Dana-Farber/Partners CancerCare, Tuveson conducted postdoctoral research with MIT cancer biologist Tyler Jacks. He concen-

trated on developing improved models for studying human cancers in mice, with particular attention to the role of a gene called KRAS, which encodes a signaling protein that regulates growth and metabolism, and is mutated in many human tumors.

Tuveson, Jacks and their colleagues developed a model for studying the most common type of lung cancer, adenocarcinoma, in mice, through controlled activation of a mutant form of KRAS in the lungs.[1] Early experiments using this model revealed a previously unknown cell type as a potential culprit in tumor growth. Tuveson then began working to adapt this technique to model pancreatic cancer in mice.

In 2002 Tuveson became an assistant professor at the University of Pennsylvania, where he and his lab completed the development of the mouse model for studying pancreatic cancer.[2] After becoming a Rita Allen Foundation Scholar in 2004, Tuveson continued to investigate the underpinnings of pancreatic cancer and the cellular havoc caused by mutations in KRAS and other oncogenes, with the goal of devising therapies to counteract the aberrant signaling pathways that drive the progression of cancer.

Over the past decade, Tuveson's group (at the Cancer Research UK Cambridge Research Institute from 2006 to 2012, and then moving to Cold Spring Harbor Laboratory in 2012) has further developed techniques for understanding pancreatic cancer, revealing layers of immune cells and fibroblasts that shield tumor cells from chemotherapy, as well as testing new combinations of therapies to help overcome refractory tumors.[3,4] Recently, Tuveson and his team built a new tool that could transform approaches to both research and treatment: an "organoid" model of pancreatic cancer—a cell culture method that closely reproduces the three-dimensional architecture and tumor progression that occur within the human body.[5]



David Tuveson and his team have developed an "organoid" system for growing pancreatic tissue in three-dimensional culture. Organoids consisting of normal cells (red) and cancer cells (green) are cultured together to identify therapies that selectively target cancer cells.

Here, Tuveson describes the power of the organoid system, the unique challenges posed by pancreatic cancer, and emerging efforts to improve outcomes for the disease.

Pancreatic cancer remains one of the most difficult cancers to diagnose and treat. What was the state of the field when you entered it, and how were you able to make progress?

When I entered the field in the late '90s, there was nobody to talk to. The National Cancer Institute was not funding very many R01s [Research Project Grants] focused on pancreatic cancer because nobody was submitting applications. One of the main problems was that there was no model system for the disease, and patients would get sick and die so quickly that you couldn't really learn about the disease from patients.

I was in the right place at the right time, in that we really needed a model system for the disease, and Tyler Jacks was willing to support me in developing it, and that paid off. But there was no way of knowing that that was an intelligent idea. In the hospital I had mentors who asked me if I wanted to become a mouse doctor—they questioned how I was spending my time.

I've learned the hard way that you have to stick to your ideas and be very persistent—but also very patient. Most major breakthroughs don't happen because somebody has some thought, does a quick experiment and has a eureka moment. It always requires struggling and trying many different approaches.

Where is pancreatic cancer research headed in the next decade?

In pancreatic cancer we need a marriage between much better diagnostics, so we can follow patients while we treat them, and much better therapies, so that we have a pretty good shot at killing cancer cells. I think both of these areas are going to see some real advances over the next five to 10 years.

Cancer diagnostics come in several flavors—our current methods include pathology, like pap smears, colonoscopy and mammography; and blood tests, which we have for choriocarcinoma [embryonic cancer occurring during pregnancy] where you can pick up the same biomarker that you would use for pregnancy. We need better tissue- or blood-based diagnostics so we can identify patients when they're still healthy, and so we can monitor patients very carefully as we treat them.

We also need effective therapies that work to extinguish the cancer cells. I don't think that's a pipe dream. They will probably be combinations of therapies, as is the case for many viral or bacterial diseases.

How will the organoid system developed by your team help to improve the treatment of pancreatic cancer?

The organoid system allows you to grow both normal and cancerous human pancreas tissues indefinitely in three-dimensional culture. This method allows you to unambiguously determine the molecular changes in the cancer compared to the normal cells. You can sequence the DNA or proteins. You can look at metabolism and the immune system.

Organoids also offer a very attractive strategy for personalized medicine because they're alive, so you can determine which genes are mutated and which biochemical pathways are activated. Then you can test whether those changes matter for the growth and survival of cancer cells, because you have the perfect companion—the normal tissue from that patient. So you can ask why a certain drug kills cancer cells in a patient but doesn't harm the patient's normal cells. That's the step that's been missing in drug development.

Organoid production should become part of how we take care of cancer patients. So when you meet a patient with a new diagnosis, you grow the tissue and send it for testing. Then you have it ready, so that if the disease comes back after standard therapy, you make another organoid, and then you can compare the two to figure out why the disease came back. Organoids will allow us to more effectively follow and choose therapies for patients as we attempt to exterminate the cancer from their bodies.

References

1. Jackson, EL, Willis, N, Mercer, K, Bronson, RT, Crowley, D, Montoya, R, Jacks, T, and Tuveson, DA. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* 15 (2001), 3243-3248.
2. Hingorani, SR, Petricoin, EF, Maitra, A, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 4 (2003), 437-450.
3. Olive, KP, Jacobetz, MA, Davidson, CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 324 (2009), 1457-1461.
4. Neesse, A, Frese, KK, Bapiro, TE, et al. CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreas cancer. *Proc Natl Acad Sci USA* 110 (2013), 12325-12330.
5. Boj, SF, Hwang, CI, Baker, LA, et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell* 160 (2015), 324-338.

HILARY COLLER (2005)
When Cells Sleep


BRIAN WILSON

"He suggested that I consider applying microarray technology to a 'dead-end' cell cycle state of quiescence...I liked the idea of being a pioneer in studying a cell cycle state that was physiologically important but poorly understood."

Hilary Coller caught the biomedical research bug early—as a junior high school student she took part in research that resulted in a first-author scientific publication. Coauthored with her father Barry Coller, a blood and vascular biologist at The Rockefeller University, the paper explored a technical issue in making monoclonal antibodies, which are widely used as protein markers in biomedical research.[1]

As her interests matured, Coller was drawn toward the policy dimensions of research. After completing her undergraduate degree in biochemistry and molecular biology at Harvard, she embarked on a master's program in technology and policy at the Massachusetts Institute of Technology. She was interested in pursuing research that would be helpful to policymakers, so for her Ph.D. she joined the MIT laboratory of William Thilly, who was studying chemicals in the environment and their ability to cause cancer in humans. Her doctoral research explored the pattern of mutations such chemicals induced, focusing specifically on the DNA in the mitochondria, the power-supply organelles of the cell.[2]

Having explored the external causes of mutations so intensively in her graduate work, Coller decided to spend her postdoc understanding cancer from a more cell-intrinsic perspective. She stayed on in Cambridge for a one-year fellowship at the Whitehead/MIT Center for Genome Research, where she worked with pioneering genomicists Eric Lander and Todd Golub (as well as cancer transcriptional regulation expert Robert Eisenman of the Fred Hutchinson Cancer Research Center in Seattle) to explore the functional pathway of the transcription factor and cancer driver Myc in regulating the cell cycle.[3]

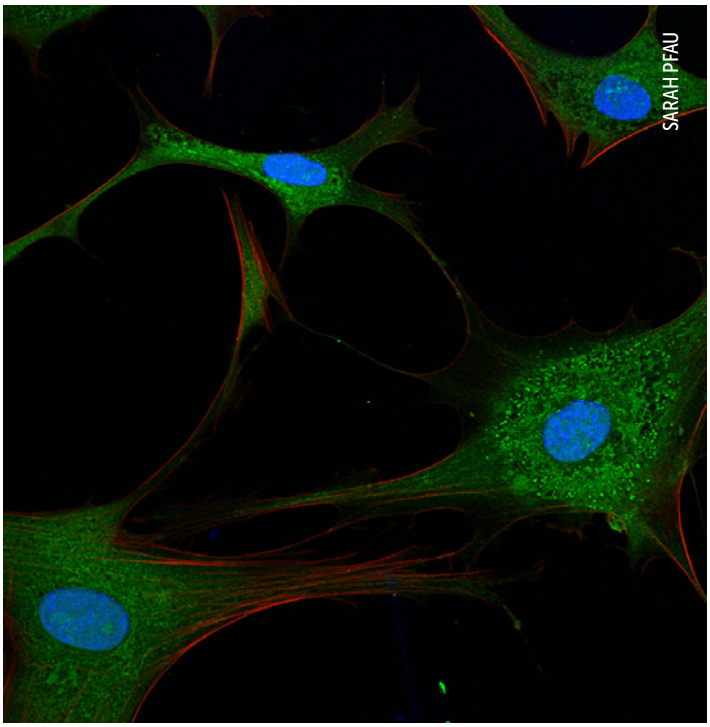
Next, she headed west for a second postdoc with Jim Roberts at the Fred Hutchinson Cancer Research Center, excited to apply the genome-wide analysis technologies she had learned to understanding the cell cycle and the causes of cancer. Cancer cells are characterized by rampant cell division and overgrowth—processes that are relatively well studied. Coller began to explore what happens when cells exit this proliferative cell cycle and become quiescent—not actively dividing, but maintaining their capacity to do so. This process is important in cancer, as its reversal may explain why tumors

that have lain dormant, sometimes for years, reawaken to cause a recurrence of a patient's cancer.

Coller's early work on these sleeper cells suggested that, rather than passively lying inert, quiescent cells may be engaged in certain as-yet-unknown tasks. Quiescence, she and her colleagues proposed in a publication that laid out a novel view of the phenomenon, "was not simply a downstream consequence of exit from the cell cycle," but an actively regulated process.[4]

In 2005 Coller accepted an assistant professorship at Princeton University. She spent her very first day on the job in hot pursuit of high-quality paper to print her Rita Allen Foundation Scholars Award application. She raced to the FedEx drop-off box to meet the submission deadline, and was selected as an RAF Scholar later that year. She and her colleagues set out to understand the attributes of quiescent cells and the molecular mechanisms that regulate them. In 2010 they reported that quiescent cells can be as metabolically active as proliferating cells, despite the long-held assumption that they are napping on the job.[5]

Interfering with the shift between quiescence and an active cell cycle could provide a therapeutic avenue for cancer treatment, Coller says. For example, it may be possible to prevent tumor resurgence by keeping the dormant cells in that state, or finding a way to target and kill them; another possibility is kicking them back into the cell cycle and targeting them with selective therapies.



Hilary Coller uses fibroblasts, cells that form connective tissue in animals, to study cell quiescence, in which cells reversibly exit the cell cycle. Shown here are primary human fibroblasts visualized with fluorescent stains.

Here, Coller recalls her early encounters with molecular biology and discusses the many remaining unknowns about cell quiescence.

What was your very first research experience?

In eighth grade, I remember learning about how a specific mutation in the gene encoding the sickle cell protein [a component of hemoglobin, which carries oxygen in the blood] results in sickle cell disease. I thought this was absolutely fascinating and got very excited about biomedical research. My first research experience was for a science fair project in ninth grade. My father was working with monoclonal antibodies and suggested I tackle a mathematical question about making monoclonal antibodies as a research project. I enjoyed working on the problem and presented it at the science fair. My father and I eventually published the work—it was my first scientific publication.

What drew you to studying quiescent cells, and what elements of the phenomenon has your lab focused on?

Early in my second postdoc, my advisor, Jim Roberts, pointed out to me that a lot of the important events in the cell cycle are post-translational modifications [at the protein level], and that these would be missed by microarrays, which only detect changes in gene expression. But he suggested that I consider applying microarray technology to a "dead-end" cell cycle

state of quiescence. He said that not very much was known about this state, but that the cells have RNA, and would be amenable to transcriptomic analysis. I liked the idea of being a pioneer in studying a cell cycle state that was physiologically important but poorly understood.

One aspect of quiescence that we have been investigating is how quiescent cells reorganize their metabolic pathways. We've found that quiescent cells don't necessarily get "sleepy" and shut down their metabolism, but can maintain high levels of metabolic activity. Further, while metabolic pathways are often considered unimportant or routine "housekeeping" functions of the cell, we've found that metabolic pathways can also have an important regulatory role. Understanding how and why quiescent cells reorganize their metabolic pathways has been a very interesting question for my laboratory.

What exciting directions is your lab currently pursuing?

A major focus for my laboratory for the past two years has been developing tools and methods for understanding quiescent versus proliferative cells in vivo using mouse models. This has been driven by the increasing availability of tools to visualize or monitor cell biological processes and manipulate specific pathways in intact organisms. We are also very focused on making connections between disease states and our basic research observations about quiescence in the laboratory. For example, we are establishing the infrastructure to ask whether the pathways that we found to be important for the transition between cellular proliferation and quiescence are relevant for patients who fail to mount a proper wound healing response, which is an important public health problem.

References

1. Coller, HA, and Coller, BS. Statistical analysis of repetitive subcloning by the limiting dilution technique with a view toward ensuring hybridoma monoclonality. *Hybridoma* 2 (1983), 91-96.
2. Coller, HA, Khrapko, K, Torres, A, Frampton, MW, Utell, MJ, and Thilly, WG. Mutational spectra of a 100-base pair mitochondrial DNA target sequence in bronchial epithelial cells: a comparison of smoking and nonsmoking twins. *Cancer Res* 58 (1998), 1268-1277.
3. Coller, HA, Grandori, C, Tamayo, P, Colbert, T, Lander, ES, Eisenman, RN, and Golub, TR. Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. *Proc Natl Acad Sci USA* 97 (2000), 3260-3265.
4. Coller, HA, Sang, L, and Roberts, JM. A new description of cellular quiescence. *PLoS Biol* 4 (2006), e83.
5. Lemons, JM, Feng, XJ, Bennett, BD, et al. Quiescent fibroblasts exhibit high metabolic activity. *PLoS Biol* 8 (2010), e1000514.

DIANA BAUTISTA (2010)
An Itch for Knowledge


MARK JOSEPH

"My goal is to define how these different systems...are interacting with one another to drive the pathophysiology of disease. I'm having a lot of fun finding collaborators in different fields who are excited about this, and finding ways to bring our different perspectives together to tackle it."

A chance introduction to the tricky issue of water pollution first attracted Diana Bautista to science. Growing up in Chicago, she was the first member of her family to finish high school, and she began college as a fine art major. But she floundered, and soon dropped out to figure out what she wanted to do.

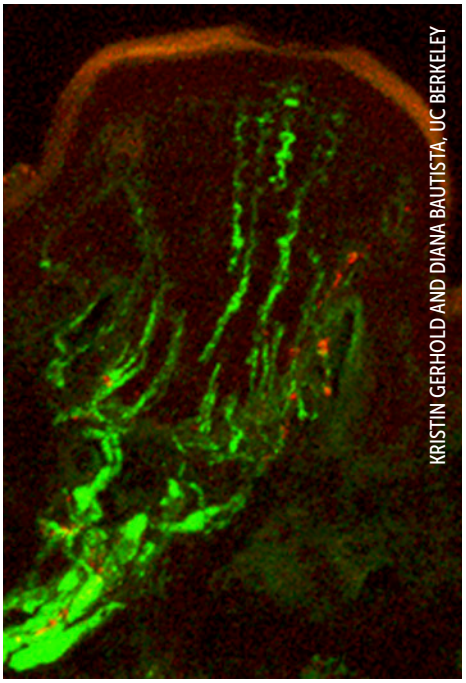
A temporary job for an environmental group sent her to a public hearing about dioxin contamination in the Great Lakes. "It really got me thinking about chemistry and epidemiology and human disease, and I started teaching myself some things to be able to understand scientific reports," Bautista says.

She set her sights back on school, choosing an environmental science program at the University of Oregon because it included chemistry as well as ecology. Once there, however, a neuroscience class captured her attention—as did a fateful work-study placement, in which she was tasked with making fly food for Peter O'Day's lab.

His team was investigating visual transduction in fruit flies, and O'Day fostered her interest by letting her do experiments and teaching her about cell signaling and the nervous system. Eventually, he asked Bautista if she had ever considered a career in research. "I was shocked when I found out that you could get paid to go to graduate school," she recalls.

Bautista earned a Ph.D. at Stanford in the laboratory of Richard Lewis, where she studied calcium channels and transporters and how they work together to control calcium dynamics. She was fascinated by the notion that a molecule as ubiquitous as calcium could have varied and specific effects in different cell types, and learned to probe these effects with calcium imaging and electrophysiology. For her postdoc, she returned to her first love, sensory neurobiology. Working with David Julius at the University of California, San Francisco, she set out to apply the biophysics and cellular techniques she had just mastered to the study of pain signaling.

In Julius' lab, she worked with mice engineered to lack specific pain receptors, exploring the functional roles of those receptors in detecting different types of pain stimuli. "I had a very specific skillset, but I went there to learn molecular biology, mouse genetics and behavior," she says. "In David's lab I also



(Top) Diana Bautista has studied the molecular basis of touch in the star-nosed mole, which has a collection of 22 unusual-looking mechanosensory appendages, or rays, surrounding its nose. This is the most sensitive tactile system in of any animal. (Right) The sensitivity of the star-nosed mole's tactile system is mediated by tens of thousands of specialized touch organs, called Eimer's organs, which stud the rays of its star. Shown here is a confocal micrograph of a single Eimer's organ, with a neural cytoskeletal protein stained green and substance P, a touch- and pain-associated neuropeptide, stained red.

learned how to take a basic science question and approach it using many different techniques."

In 2008 Bautista launched her own lab at UC Berkeley. Two years later Bautista became a Rita Allen Foundation Scholar, and her lab began to use knockout mice to investigate whether sensory signaling molecules involved in pain were also implicated in itch. The wasabi receptor, TRPA1, they found, seemed to specifically mediate chronic itch that is insensitive to antihistamine treatment.[1] Since then, Bautista's team has begun to explore how skin cells and immune cells collaborate with the nervous system to drive chronic itch.[2] They have also identified several subtypes of somatosensory neurons that appear to be dedicated to acute and chronic itch, separate from the pain pathway.

Currently a faculty member in the Department of Cell and Developmental Biology at UC Berkeley, Bautista sees her role as a scientist extending well beyond the confines of the lab. Part of her mission is to bring students from diverse and nontraditional backgrounds into the fold through teaching and outreach, and by mentoring students in her lab just as she herself was mentored. "As someone who didn't think about science until a later age, I feel I've been really lucky in getting to have this amazing career in research and education," she says. "I definitely want to pass it on to the next generation."

Here, Bautista muses on the mysteries of itch, explains her group's work with an unusual research animal, and reflects on the power of collaborative approaches in science.

What's so compelling about studying pain and itch?

When I started looking through the literature and reading about pain, I didn't see much published on the molecular mechanisms of pain signal transduction and hypersensitivity. I thought I must not be looking in the right journals, and so I asked my thesis committee where to look. And everyone said there wasn't a lot known about touch and pain from a molecular and cellular perspective. That seemed shocking to me. That's such a fundamental thing—how we experience tactile sensations, and how pain—whether it's cold pain or mechanical pain—is detected by the nervous system.

While people see the need for studying chronic pain, chronic itch is a little bit under the radar. In the past, that was true of pain: It was generally considered as a secondary aspect of other diseases, and not necessarily something that needed to be studied in and of itself. But these days, chronic pain is acknowledged as a highly debilitating condition that

affects millions of people. Meanwhile, I think most of us think about itch as just some irritating thing that goes away a couple days after you get that mosquito bite. We really have to change that. Yes, it does accompany many diseases, but it is a disease in and of itself that causes a decreased quality of life, on par with that of chronic pain.

What was it like working with the star-nosed mole?

It's a really interesting animal. We are interested in touch all along the spectrum, from how we experience pleasant or gentle touch, to painful, mechanical transection [a cut in the skin], to itch. We wanted to use the star-nosed mole as a model for understanding light touch because it has a specialized touch organ called the star organ, which is the most sensitive tactile system in the animal kingdom. The moles live in tunnels, and they compete for food with a lot of other animals. They have the ability, using this tactile organ, to detect small insects and larvae in these tunnels that other animals can't detect at all.

We collaborated with Ken Catania at Vanderbilt, who is the world's expert on star-nosed moles, to look at these animals' tactile system to try to figure out what makes them so sensitive. The idea was to use the mole as a model system to figure out what molecules are selectively mediating light touch versus pain. We identified a number of candidate genes that were enriched selectively in the neurons that innervate the touch organ, and we're looking at the role of these genes in the mouse and human sensory systems. We're also interested in exploring how it is that the mole tactile organ is so touch-sensitive and yet relatively insensitive to pain.

What research directions are you most excited about now?

I realized that to really understand chronic itch and chronic pain and inflammatory disease, we will need to think globally. I'm trained as a neuroscientist, and I came into this field thinking about how the neurons transduce information. But when you look at all of these inflammatory disorders, you see that it's not just the nervous system that's acting crazy and hyperactive. The epithelial cells, the immune system and the nervous system are all acting in abnormal ways. So my goal is to define how these different systems—these pathways and cell types—are interacting with one another to drive the pathophysiology of disease. I'm having a lot of fun finding collaborators in different fields who are excited about this, and finding ways to bring our different perspectives together to tackle it.

References

1. Wilson, SR, Gerhold, KA, Bifolck-Fisher, A, Liu, Q, Patel, KN, Dong, X, and Bautista, DM. TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nat Neurosci* 14 (2011), 595-602.
2. Schwendinger-Schreck, J, Wilson, SR, and Bautista, DM. Interactions between keratinocytes and somatosensory neurons in itch. *Handb Exp Pharmacol* 226 (2015), 177-190.

DAVID PROBER (2010)
Sleeping Like the Fishes

David Prober examines a zebrafish.

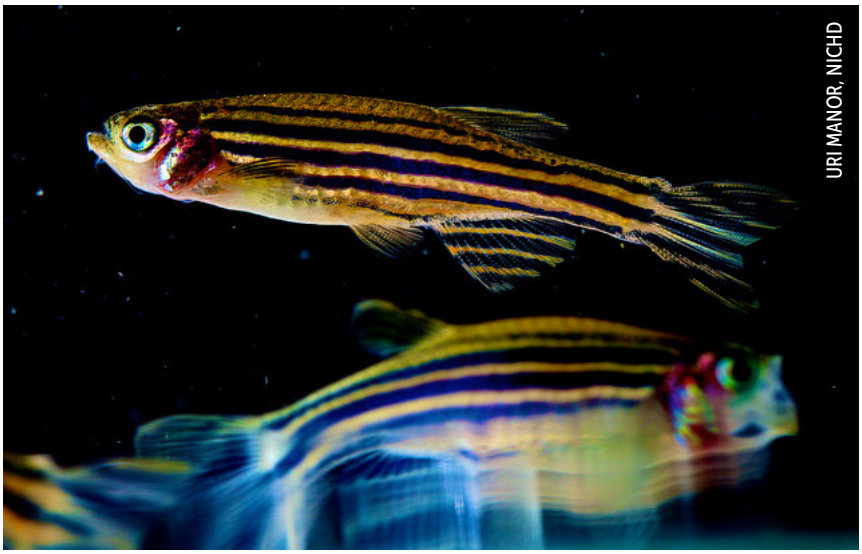
"After I got my Ph.D., I decided that I wanted to go after a big problem that has proved to be relatively intractable to solving, and sleep stood out as a mysterious and important problem. We still don't understand very well how sleep is regulated."

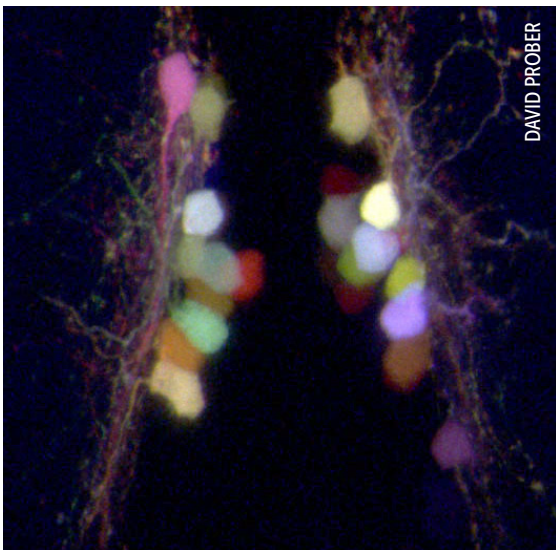
While most sleep research is conducted using laboratory mice, David Prober studies the molecular basis of sleep in the zebrafish, a small freshwater tropical fish that populates many pet store aquariums. Zebrafish have several surprising advantages for understanding how sleep works. Unlike mice, which are nocturnal and sleep in spurts throughout the day, zebrafish, like humans, are diurnal—active during the day—and spend most of the night asleep.

We spend a third of our lives sleeping, yet very little is known about why we need sleep or how sleep is regulated. According to Prober, one reason could be that until relatively recently—about 15 years ago—sleep was predominantly studied in rodents. Zebrafish, meanwhile, have anatomically similar brains to other vertebrates, including humans, but their brains are smaller—with tractable, easier-to-dissect neural circuits that can be readily imaged, thanks to their transparent bodies. "There are about 100-fold fewer neurons in the fish compared to the mouse. The zebrafish provides a much simpler system to figure out how neural circuits regulate behavior," Prober says.

Prober first got a taste of research as an undergraduate at the University of Manitoba in Canada, where he worked in three different laboratories. The experience made him want to pursue research as a career. As a graduate student, Prober joined the laboratory of Bruce Edgar (a 1995 Rita Allen Foundation Scholar) at the University of Washington in Seattle, where he examined the role of oncogenes such as *ras* and *myc* during development of the fruit fly. Prior tissue culture studies had suggested that mutated Ras and Myc cause cancer by promoting cell cycle progression and cell proliferation. Prober found that in the context of an intact tissue, Ras and Myc don't regulate the cell cycle directly, but rather promote cell growth, consistent with a role for these genes in driving the increase in tissue mass that underlies cancer.[1] This was not previously appreciated, according to Prober, because cell mass is not typically measured using cell cultures.

The research appealed to Prober in part, he says, because it addressed relatively old scientific questions using new methods that allowed for novel discoveries. "That really impressed upon me the power of looking at old problems using new





(Left) Prober uses zebrafish as a model for sleep, and, using a larval zebrafish-based screen, recently identified a novel neuropeptide regulator of the sleep/wake state. (Right) Individually labeled neurons in a 5-day-old zebrafish larva expressing the "Brainbow" transgene, which allows each hypothalamic wake-promoting neuron to be labeled with a different color. This technique allows Prober and his research group to trace each neuronal projection throughout the whole animal.

approaches to drive new discoveries." Prober has been applying that lesson throughout his career.

Prober then joined Alexander Schier's laboratory at the Skirball Institute of Biomolecular Medicine at New York University. There, Prober and his colleagues established zebrafish as a model for sleep and performed a drug screen on zebrafish, identifying molecules that perturb the normal sleep/wake cycle in zebrafish larvae.[2] Some of the drugs tested were already known to affect mammalian sleep, and most of these drugs altered fish sleep behavior in a similar manner, validating zebrafish as a model for the neurochemistry and molecular biology of sleep. This was the first drug screen in a vertebrate to examine effects on behavior.

In 2009 Prober established his own laboratory at the California Institute of Technology, and became a Rita Allen Foundation Scholar the following year. His group recently published results from a genetic screen that Prober began as a postdoc more than six years ago. This overexpression screen used a clever twist that overcomes some challenges of classic genetic screens, identified genes that regulate sleep and wake states, and revealed a novel neuropeptide that inhibits sleep in zebrafish larvae.[3] Prober's laboratory is now following up on other genes identified in this screen.

Here, Prober discusses how he came to study the molecular basis of sleep, the controversy over some of his findings, and why pursuing the question of why we sleep is not interesting to him.

Why did you decide to switch from studying oncogenes in fruit flies to the science of sleep in zebrafish?

After I got my Ph.D., I decided that I wanted to go after a big problem that has proved to be relatively intractable to solving, and sleep stood out as a mysterious and important problem. We still don't understand very well how sleep is regulated. At that time, the zebrafish was just starting to be seen as a useful model system for behavior. Because you can do genetic screens [which are difficult to perform in rodents], and because zebrafish have relatively simple and transparent brains, it seemed like a system that might help to break the logjam in the sleep field.

What aspect of your research is most exciting to you now?

We know that a circadian process that oscillates with the Earth's 24-hour cycle tells animals when they should be awake and when they should be asleep, and we know a lot about how the circadian clock itself works. However, we didn't know how

the circadian clock tells an animal when to sleep. My lab recently discovered that melatonin is the link between the circadian clock and sleep, at least in diurnal animals.[4]

We asked whether melatonin is important for the circadian control of sleep by knocking out the gene required to synthesize melatonin in zebrafish. We found that the circadian regulation of sleep is abolished in these mutant animals, demonstrating that melatonin is the key factor through which the circadian clock tells the animal to sleep at night.

This discovery gets a funny reaction from circadian biologists who work on rodents, who tell me that melatonin is not important because most lab rodent strains don't make melatonin and melatonin does not put these animals to sleep. However, there are good reasons to think that melatonin should not induce sleep in nocturnal animals. Similar to zebrafish, melatonin induces sleep in humans, but it is not as powerful as commonly used sedatives like Ambien. This actually makes sense, because you don't want the natural mechanism that regulates your sleep to be as powerful as Ambien, which puts you to sleep for eight hours no matter what, which would be very maladaptive. Having shown that melatonin is required for the circadian control of sleep, we now need to figure out how it does this.

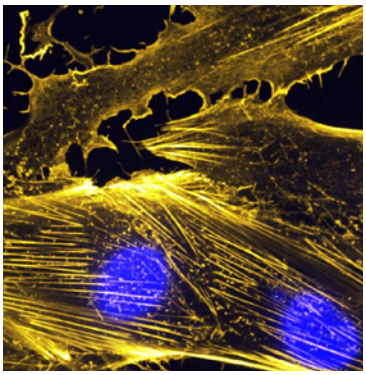
What is our understanding of why animals need sleep?

Researchers have come up with evidence for at least a half dozen possibilities. One suggestion is that sleeping cleans out waste that accumulates in our brains while we're awake. While this may be the case in advanced animals such as mammals, it seems unlikely to me that that's why simpler animals like roundworms or fruit flies sleep. I think the most compelling theory right now, proposed by Giulio Tononi and Chiara Cirelli at the University of Wisconsin, is that when we are awake, we take in information and learn, which builds new synapses. If this were to go on indefinitely, we would eventually run out of room for new synapses and be unable to learn. According to this theory, synapses that are generated during wakefulness, particularly those that are weak and not very important, are removed during sleep. This allows one to wake up the next day refreshed and able to learn again.

This theory would explain why any animal with even a simple nervous system would need to sleep on a daily basis. But it could be that many of the proposed theories are true, and it would be hard to figure out which is the most important. To me, the question of why animals sleep isn't a very satisfying research pursuit, because I don't know how you know when the question has been answered. In contrast, figuring out how sleep is regulated is concrete, with testable hypotheses and experiments that can provide clear answers.

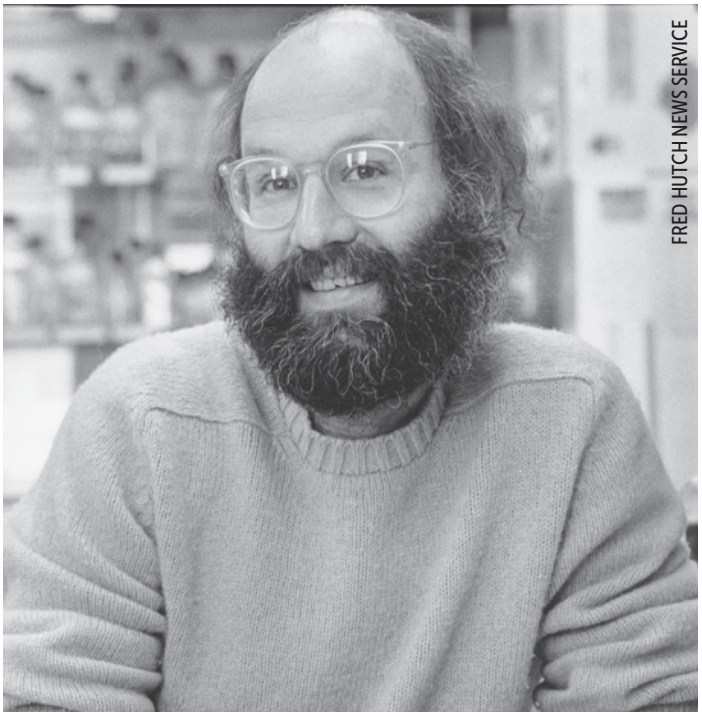
References

1. Prober, DA, and Edgar, BA. Ras1 promotes cellular growth in the *Drosophila* wing. *Cell* 100 (2000), 435-446.
2. Rihel, J, Prober, DA, Arvanites, A, et al. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science* 327 (2010), 348-351.
3. Chiu, CN, Rihel, J, Lee, DA, et al. A zebrafish genetic screen identifies neuromedin U as a regulator of sleep/wake states. *Neuron* 89 (2016), 842-856.
4. Gandhi, AV, Mosser, EA, Oikonomou, G, and Prober, DA. Melatonin is required for the circadian regulation of sleep. *Neuron* 85 (2015), 1193-1199.



IN MEMORIAM

Remembrances of Harold Weintraub, Paul Patterson and Stephen Udem


FRED HUTCH NEWS SERVICE

HAROLD (HAL) WEINTRAUB (1945–1995)

Professor of Genetics, University of Washington
Founding Member, Basic Sciences Division, Fred Hutchinson Cancer Research Center
1976 Rita Allen Foundation Scholar

A native of Newark, New Jersey, Harold Weintraub earned a bachelor's degree from Harvard College and an M.D.-Ph.D. from the University of Pennsylvania. He conducted postdoctoral research with Sydney Brenner and Francis Crick at the Medical Research Council in Cambridge, England, before joining the faculty of Princeton University. Weintraub moved to the Fred Hutchinson Cancer Research Center in Seattle in 1978. He is best known for the discovery of the *myoD* gene, which encodes a master regulator of muscle cell differentiation. He was a member of the National Academy of Sciences and was a Howard Hughes Medical Institute Investigator from 1990 to 1995.

Harold was my colleague at Princeton University. Probably the best way I can characterize him on a personal level, besides his acumen in basketball, is to say that when we had seminars I would sit near Harold. Why? Because Harold would ask a question in the seminar, and I would then spend the rest of the seminar trying to figure out why he asked that question, struggling away at a concept that he had picked up immediately. I would usually take a day or two to think about it before I was embarrassed enough to go to Harold and ask him why he had asked that question. When I got the answer, it was always insightful.

When he moved to the Hutchinson Cancer Center, he de-emphasized his studies on chromatin structure and began a study of tissue-specific transcription factors. This led to the discovery of MyoD, a transcription factor that is central to the synthesis of all muscle tissues. The idea that each tissue has a central transcription factor critical to its synthesis then flourished in the field. It led to the concept that a single protein could lead to the development of specific tissue types, and that every tissue type had such a protein, and this protein was fundamental for the direction of new tissue types. This is a great concept, and that's just one of the things Harold did. He kept doing insightful things like that, which changed the field all the time. He was a remarkable figure in 20th-century developmental biology and cancer biology.

–Arnold Levine, Honorary Chair of the Rita Allen Foundation Scholars 40th Anniversary Meeting and former Chair of the Scientific Advisory Committee

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[Weintraub's] efforts were driven by a creative intuition coupled with a courage to explore his ideas experimentally. One of his greatest strengths was his ability to conceive of simple experimental approaches that led to major advances in our understanding of complex biological phenomenon. His scientific persona was characterized by an odd mix of naiveté and confidence that led him in directions where others feared to venture.

The breadth and intensity of Hal's interest in science was strongly felt, not only by his students and fellows, but by an entire community of biologists. He listened to the efforts of others with excitement and contributed his thoughts and ideas generously.

Despite Hal's gentle and soft spoken manner and his aversion to the politics of administration, he brought together an exciting group of young scientists to create an exceptional research organization at the Fred Hutchinson Cancer Center.

–Richard Axel, University Professor and Professor of Biochemistry and Molecular Biophysics, of Neuroscience, and of Pathology at Columbia University College of Physicians and Surgeons; and Tom Maniatis, Vanderbilt University Chairman, Department of Biochemistry and Molecular Biophysics, Columbia University Medical Center (and 1978 Rita Allen Foundation Scholar) in an obituary published in the journal Cell*, May 5, 1995*

WIKIMEDIA/CPATTERSON1010

PAUL PATTERSON (1943–2014)

Anne P. and Benjamin F. Biaggini Professor of Biological Sciences Emeritus, California Institute of Technology 1979 Rita Allen Foundation Scholar

Paul Patterson was born in Chicago and completed his undergraduate studies at Grinnell College. He earned a Ph.D. from Johns Hopkins University, where he worked with William Lennarz. As a postdoc at Harvard Medical School, Patterson conducted pioneering research on neural plasticity with Edwin Furshpan and David Potter. He then became a faculty member at Harvard, before moving to Caltech in 1983. Patterson explored links between the immune system and the nervous system—he became known as a "neuroimmunologist"—and developed mouse models of schizophrenia and autism. He was a fellow of the American Association for the Advancement of Science.

Paul was a great friend. When I first got a lab at Harvard Medical School as an assistant professor, Paul was in the office next door. We had a communicating hatch between the offices. We would pass each other gin and tonics, and he would throw his discarded NIH grants into my office. He was just a dynamically tenacious, unorthodox individual. I often think of his ponytail, in wild forms...he was just an original, and an iconoclast in many ways.

He is best known for doing immaculate biochemistry to test the idea that neurons weren't preordained to use one neurotransmitter—they had the capacity to be plastic and switch transmitter. He designed a very incisive set of experiments in the 1970s, working with Ed Furshpan and David Potter and Story Landis, to demonstrate biochemically the transition of transmitter choice. There are people today who are basing their careers on these observations of neural plasticity.

–Thomas Jessell, Claire Tow Professor of Motor Neuron Disorders in the Departments of Neuroscience and of Biochemistry and Molecular Biophysics, Columbia University; 1984 Rita Allen Foundation Scholar

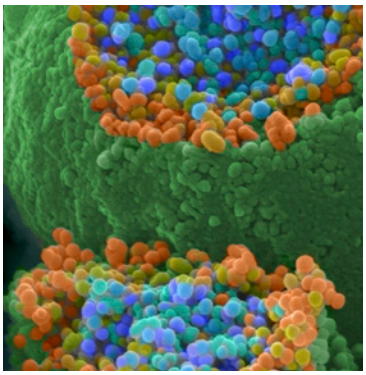
STEPHEN UDEM (1944–2014)

Consultant/Advisor for Vaccines, Biopharmaceuticals and Biotech R&D, UCT Advisors
Clinical Professor of Medicine, Division of Infectious Diseases, and Associate Professor of Microbiology and Immunology (Visiting), Albert Einstein College of Medicine
1979 Rita Allen Foundation Scholar

A lifelong New Yorker, Stephen Udem studied chemistry at City College, and earned M.D. and Ph.D. degrees in the Medical Scientist Training Program at Albert Einstein College of Medicine. He worked as an infectious disease clinician at several New York and New Jersey hospitals over the course of his career. Udem also made critical contributions to the fields of virology and vaccinology, first through academic research and later through his work in vaccine development in both industry and nonprofit settings.

Much of his research focused on mechanisms of viral persistence in the central nervous system. From 1994 to 2006, he held various leadership positions at Wyeth, where he managed the expansion of the company's vaccine programs. Udem then directed vaccine development efforts at the International AIDS Vaccine Initiative before establishing his own consultancy practice for vaccine R&D. He was a member of the American Medical Association and the American Association for the Advancement of Science.

Udem's obituary, published in *The New York Times* on March 9, 2014, includes this remembrance: "As a person he was warm, witty, incisive, empathic, care-taking, generous, brave and fiercely determined, but what was most characteristic was that he was always fully present with everyone he came into contact with."



PEOPLE

"The Scholars program... continues today to inspire scientific pursuit by supporting the aspirations of those who have a vision of what might be."
–Robert Campbell, Rita Allen Foundation Director Emeritus

Rita Allen Foundation Scholars 1976–2016

Award Years	Scholar	Institution
1976–80	James B. Lewis	Foresight Institute (Cold Spring Harbor Laboratory)
1976–79	Robert A. Weinberg	Massachusetts Institute of Technology
1976–78	Harold M. Weintraub †	Fred Hutchinson Cancer Research Center (Princeton University)
1978–82	Kathleen M. Foley	Memorial Sloan Kettering Cancer Center (Cornell University Medical College)
1978–81, 86	William W. Hall	University College Dublin (The Rockefeller University)
1978–79	Thomas P. Maniatis	Columbia University Medical Center (California Institute of Technology)
1978–82	Graham C. Walker	Massachusetts Institute of Technology
1979–83	John Condeelis	Albert Einstein College of Medicine
1979–83	Paul H. Patterson †	California Institute of Technology (Harvard Medical School)
1979–83	Stephen A. Udem †	Albert Einstein College of Medicine
1983–87	Bruce W. Stillman	Cold Spring Harbor Laboratory
1983–84	Luis P. Villarreal	University of California, Irvine (University of Colorado School of Medicine)
1983–86	Barbara J. Wold	California Institute of Technology
1984–85	Thomas M. Jessell	Columbia University Medical Center (Harvard Medical School)
1984–85	Carl F. Nathan	Weill Cornell Medical College (The Rockefeller University)
1984–88	H. Earl Ruley	Vanderbilt University School of Medicine (Massachusetts Institute of Technology)
1985–89	Bruce P. Bean	Harvard Medical School
1985–89	Brent H. Cochran	Tufts University School of Medicine (Massachusetts Institute of Technology)
1985–88	Stanley M. Goldin	Harvard Medical School
1985–89	Winship Herr	University of Lausanne (Cold Spring Harbor Laboratory)
1985–87	Carl S. Parker	California Institute of Technology
1986–90	Adrienne A. Brian	University of California, San Diego
1986–90	Charles D. Gilbert	The Rockefeller University
1986–90	Ronald D.C. McKay	Johns Hopkins University School of Medicine (Massachusetts Institute of Technology)
1988–92	Gilbert Chu	Stanford University School of Medicine
1988–92	Stephen L. Hauser	University of California, San Francisco
1988–92	Jon D. Levine	University of California, San Francisco
1989–93	Andrew Z. Fire	Stanford University School of Medicine (Carnegie Institution of Washington)
1989–93	Nouria Hernandez	University of Lausanne (Cold Spring Harbor Laboratory)

1989–92	Ronald D. Vale	University of California, San Francisco
1990–91	Peter S. Kim	Stanford University School of Medicine (Massachusetts Institute of Technology)
1990–94	Greg Lemke	Salk Institute for Biological Studies
1990–94	Marilyn D. Resh	Memorial Sloan Kettering Cancer Center (Princeton University)
1991–95	Elizabeth A. Komives	University of California, San Diego
1991–95	Jeffrey D. Macklis	Harvard Medical School
1991–95	David O. Morgan	University of California, San Francisco
1993–97	Jun Liu	Johns Hopkins University School of Medicine (Massachusetts Institute of Technology)
1993–97	Stephen L. Mayo	California Institute of Technology
1993–97	Christopher A. Walsh	Harvard Medical School
1994–98	Michael O. Hengartner	University of Zurich (Cold Spring Harbor Laboratory)
1994–98	Joachim J. Li	University of California, San Francisco
1994–97	James R. Williamson	The Scripps Research Institute (Massachusetts Institute of Technology)
1995–99	Stephen P. Bell	Massachusetts Institute of Technology
1995–98	Titia de Lange	The Rockefeller University
1995–99	Bruce A. Edgar	German Cancer Research Center (Fred Hutchinson Cancer Research Center)
1996–99	Andrew Chess	Icahn School of Medicine at Mount Sinai (Massachusetts Institute of Technology)
1996–99	Robert K. Ho	University of Chicago (Princeton University)
1996–99	Li-Huei Tsai	Massachusetts Institute of Technology (Harvard Medical School)
1998–00	Frank J. Hsu	Immune Design (Yale University School of Medicine)
1998–00	Peter Mombaerts	Max Planck Research Unit for Neurogenetics (The Rockefeller University)
1998–00	Ilaria Rebay	University of Chicago (Massachusetts Institute of Technology)
1998–00	Jon S. Thorson	University of Kentucky (Memorial Sloan Kettering Cancer Center)
1999–01	Susan M. Dymecki	Harvard Medical School
1999–01	K. Christopher Garcia	Stanford University School of Medicine
1999–01	Scott W. Lowe	Memorial Sloan Kettering Cancer Center (Cold Spring Harbor Laboratory)
1999–01	Yigong Shi	Tsinghua University (Princeton University)
2000–02	Yukiko Goda	RIKEN Brain Science Institute (University of California, San Diego; University College London)
2000–02	Gregory J. Hannon	Cancer Research UK Cambridge Institute (Cold Spring Harbor Laboratory)
2000–02	Michael P. Rout	The Rockefeller University
2000–02	Samuel S.-H. Wang	Princeton University
2001–03	Steven Artandi	Stanford University School of Medicine

2001–03	David C. Chan	California Institute of Technology
2001–03	Adrian R. Ferre-D'Amare	National Heart, Lung, and Blood Institute (Fred Hutchinson Cancer Research Center)
2001–03	Oliver Hobert	Columbia University
2001–03	Daniel L. Minor, Jr.	University of California, San Francisco
2002–03	Mark Henkemeyer	The University of Texas Southwestern Medical Center
2002–04	Xianxin Hua	Perelman School of Medicine at the University of Pennsylvania
2002–03	William Talbot	Stanford University School of Medicine
2002–03	Hao Wu	Harvard Medical School (Weill Cornell Medical College)
2003–05	Ajay Chawla	University of California, San Francisco (Stanford University School of Medicine)
2003–05	Leslyn A. Hanakahi	University of Illinois at Chicago (Johns Hopkins University)
2003–05	Christopher Lima	Memorial Sloan Kettering Cancer Center (Weill Cornell Medical College)
2003–05	Shai Shaham	The Rockefeller University
2004–07	Laura A. Johnston	Columbia University Medical Center
2004–07	Senthil K. Muthuswamy	Harvard Medical School (Cold Spring Harbor Laboratory)
2004–07	David M. Sabatini	Massachusetts Institute of Technology
2004–05	David A. Tuveson	Cold Spring Harbor Laboratory (University of Pennsylvania)
2004–07	Zheng Zhou	Baylor College of Medicine
2005–09	Hilary A. Coller *	University of California, Los Angeles (Princeton University)
2005–09	Elsa R. Flores	The University of Texas MD Anderson Cancer Center
2005–08	Johanna Joyce	University of Lausanne (Memorial Sloan Kettering Cancer Center)
2005–09	Joel L. Pomerantz	Johns Hopkins University School of Medicine
2006–10	Joshua T. Mendell	The University of Texas Southwestern Medical Center (Johns Hopkins University School of Medicine)
2006–10	Peter W. Reddien *	Massachusetts Institute of Technology
2006–10	Adrian Salic	Harvard Medical School
2007–10	Michael T. Hemann	Massachusetts Institute of Technology
2007–10	Tae Hoon Kim	The University of Texas at Dallas (Yale University School of Medicine)
2007–10	Lloyd C. Trotman	Cold Spring Harbor Laboratory
2007–10	Mark J. Zylka *	The University of North Carolina at Chapel Hill
2008–13	Steven J. Altschuler *	University of California, San Francisco (The University of Texas Southwestern Medical Center)
2008–11	Paul Chang	Ribon Therapeutics (Massachusetts Institute of Technology)
2008–11	Ian J. Davis	The University of North Carolina at Chapel Hill
2008–11	Ming Li	Memorial Sloan Kettering Cancer Center

2008–11	Emmanuelle A. Passegue	University of California, San Francisco
2008–11	E. Alejandro Sweet-Cordero	Stanford University School of Medicine
2009–14	Ben E. Black	Perelman School of Medicine at the University of Pennsylvania
2009–15	Jeremy S. Dittman	Weill Cornell Medical College
2009–12	Aaron D. Gitler	Stanford University School of Medicine (University of Pennsylvania)
2009–12	Steven Prescott #	University of Toronto (University of Pittsburgh)
2009–12	Theodore Price #	The University of Texas at Dallas (University of Arizona)
2009–14	Samara Reck-Peterson *	University of California, San Diego (Harvard Medical School)
2009–14	Daniel Stetson	University of Washington School of Medicine
2009–14	Sohail Tavazoie	The Rockefeller University
2010–13	Seena Ajit #	Drexel University
2010–13	Diana Bautista #	University of California, Berkeley
2010–15	Randy Bruno	Columbia University
2010–15	Maitreya Dunham	University of Washington
2010–15	David Prober *	California Institute of Technology
2010–15	Agata Smogorzewska	The Rockefeller University
2010–15	Ye Zheng	Salk Institute for Biological Studies
2011–	Briana Burton *	University of Wisconsin-Madison (Harvard University)
2011–	Elissa Hallem	University of California, Los Angeles
2011–	Rahul Kohli	Perelman School of Medicine at the University of Pennsylvania
2011–	Michael Lin	Stanford University School of Medicine
2011–	Axel Nimmerjahn	Salk Institute for Biological Studies
2011–14	E. Alfonso Romero-Sandoval #	Presbyterian College School of Pharmacy (Dartmouth Medical School)
2011–14	Yuanxiang Tao #	Rutgers New Jersey Medical School (Johns Hopkins University School of Medicine)
2012–	Sreekanth Chalasani	Salk Institute for Biological Studies
2012–	Christopher Hammell *	Cold Spring Harbor Laboratory
2012–15	Michael Jankowski #	Cincinnati Children's Hospital Medical Center
2012–	Xin Liu	The University of Texas Southwestern Medical Center
2012–	Michael Long	New York University School of Medicine
2012–	Luciano Marraffini	The Rockefeller University
2012–15	Sarah Ross #	University of Pittsburgh
2013–	Michael Boyce	Duke University School of Medicine
2013–	Sophie Dumont *	University of California, San Francisco

2013–	Dorothea Fiedler	Princeton University, Leibniz-Institut für Molekulare Pharmakologie
2013–	Elena Gracheva	Yale University School of Medicine
2013–	William James Greenleaf	Stanford University School of Medicine
2013–	Rebecca Seal #	University of Pittsburgh
2013–	Reza Sharif-Naeini #	McGill University
2014–	Lei Ding	Columbia University
2014–	Molly Hammell *	Cold Spring Harbor Laboratory
2014–	Sebastian Klinge	The Rockefeller University
2014–	Zachary Knight	University of California, San Francisco
2014–	Gregory Scherrer #	Stanford University School of Medicine
2014–	Lin Tian	University of California, Davis, School of Medicine
2014–	Tuan Trang #	University of Calgary
2015–	Minoree Kohwi	Columbia University
2015–	Yevgenia Kozorovitskiy	Northwestern University
2015–	Julie Law	Salk Institute for Biological Studies
2015–	John Schoggins *	The University of Texas Southwestern Medical Center
2015–	Robert Sorge #	The University of Alabama at Birmingham
2015–	Jeremy Wilusz	Perelman School of Medicine at the University of Pennsylvania
2015–	Yi Ye #	New York University
2016–	Steve Davidson #	University of Cincinnati College of Medicine
2016–	Camila dos Santos	Cold Spring Harbor Laboratory
2016–	Monica Dus *	University of Michigan
2016–	Katherine Hanlon #	University of New England
2016–	Alex Kentsis	Memorial Sloan Kettering Cancer Center
2016–	Bo Li	The University of North Carolina at Chapel Hill
2016–	Katharina Schlacher	The University of Texas MD Anderson Cancer Center

† Deceased
* Milton E. Cassel Scholar
# Rita Allen Foundation Award in Pain Recipient

Each Scholar's current research institution is shown first, with the original awarding institution(s) in parentheses.

A BIT OF HISTORY

Foundation Leaders Reflect on the Scholars Program

The Rita Allen Foundation Board of Directors in 2009, from left, William Gadsden, Elizabeth Good Christopherson, Moore Gates, Jr., Anne O'Neill Gates, Aristides Georgantas and Henry Hitch.

The idea of a Scientific Advisory Committee started back in the 1970s. The three of us on the Board of Directors obviously had no background in medical research. So we talked to Margaret Mahoney [Vice President of the Robert Wood Johnson Foundation]. She told us we should assemble a group of people who could make judgments about the science, and referred us to Howard Hiatt [Dean of the Harvard School of Public Health]. He was the key. He agreed to be on the committee and picked the other people, and it all fell into place.
–Moore Gates, Jr., Director, 1968–2010

The first Scholars selection meeting I attended was an awakening. Here were these incredibly—not just brilliant, but dedicated—men and women early in their careers. While they needed money for their labs, that was not their primary motivation. What was really important was to solve the big problem they were after. To listen to these young scientists was astonishing, as I realized that many of them were looking for breakthroughs in the biomedical field.
–Henry Hitch, Director, 1991–2014

I have been fascinated by the work of the Rita Allen Foundation's outstanding biomedical Scholars and proud to be part of the effort to support it. The Foundation's approach is unique: awarding funds without required results and placing a high value on curiosity-driven science. This perspective is grounded firmly on the premise that basic research advances the understanding of life for the protection of humanity. Because science depends on trial and error, even through "failure" our admirable Scholars find illumination.
–Aristides Georgantas, Director, 2003–2012

I joined the Rita Allen Foundation Board of Directors in early 2003. Shortly thereafter I attended my first Scholars program finalist presentations at the University Club in New York. I sat back and listened to the spirited interplay between the obviously gifted young researchers and our distinguished Scientific Advisors. There was one hitch: not being a scientist myself, I couldn't understand most of the discussions. By the end of the morning, I noticed that several of the finalists used the term "*C. elegans*," and I figured out that *C. elegans* was a worm. I knew this was the start of something wonderful.
–William Gadsden, Director, 2003—Present

One of the most appealing aspects of joining the Rita Allen Foundation Board was knowing that there was a cornerstone already in place on which to build a more expansive future. That cornerstone was the Scholars program, and it continues today to inspire scientific pursuit by supporting the aspirations of those who have a vision of what might be. The program also established a culture of risk-taking, which has been important in defining other pathways the Rita Allen Foundation has chosen to define and support.
–Robert Campbell, Director, 2009–2013

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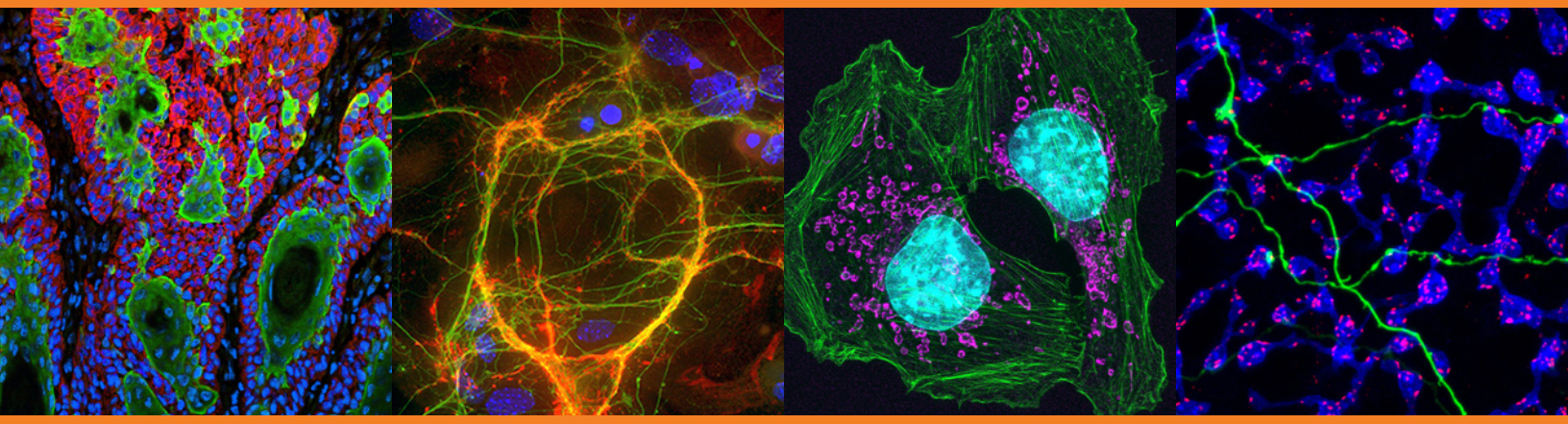

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