Beckman Women Scientists

Women Scientists of the Beckman Center

BY ANNA AZVOLINSKY

"Science was an evolution of curiosity for me. My father was a chemist and in grade school, I spent time on the weekends in his lab, watching dry ice skate on water. In high school, I got into writing code with my friends. My high school was in Bethesda, Maryland, and a lab investigator came to our high school and asked if anyone likes to write code because he wanted students to come to his biophysics lab and write code for his research. My friend and I put our hands up to volunteer and after school, and in the summer, we worked in his lab. The lab needed a computer program to automatically analyze their data from a spectroscopy instrument. Today, the instrument would come with its own software, but that was not the case back then. The scientist gave us a scientific paper on how to implement a Fourier transformation and we had no idea what that meant, but we said, 'Ok, let's figure this out.' I loved solving puzzles and through that experience became fascinated by biology. The friend, Felasfa Wodajo, as it turns out, also came to Stanford. He worked as a computer programmer in Bill Newsome's laboratory before going to medical school and is now a musculosketal tumor surgeon."
–Miriam Goodman, Professor of Molecular and Cellular Physiology

"When I was seven, my dad took me out in the backyard with a yellow kitchen stepstool, a few jars and tubes: we put a jar full of water on each step and he taught me how to make a siphon. That was my formal introduction to physics. Later, in high school I loved how my physics course in Newtonian mechanics explained the things we see in our everyday world and decided to major in physics in college. After a year, I realized I wanted to do experimental rather than theoretical work, but right or wrong, I felt like there was always a big machine required between you and the questions. My sophomore year, I took an introductory biology course and asked one of the professors if I could work in his lab over the summer. He said yes and then went away on sabbatical. When left basically to my own devices in the lab, I discovered something, I realized that in biology, you could ask and answer questions with just your mind, your eyes, some test tubes and a water bath. I was hooked."
–Margaret "Minx" Fuller, Professor of Developmental Biology and Genetics

"I had lots of unscheduled afternoons growing up and would get lost in the natural world for hours and hours, roaming and exploring. It's the same free-roaming mental space as becoming lost, now, at the microscope for hours and hours. I would find dead animal carcasses, bring them home and preserve them in mason jars in the basement. In hindsight, my parents were so tolerant of it all. Not all kids would have been allowed to bring rotting carcasses into the basement and keep them there."
–Lucy O'Brien, Assistant Professor of Molecular and Cellular Physiology

"The single person that altered my life trajectory was my high school chemistry teacher who was an extremely inspirational woman and incredibly dedicated. She led a chemistry club where we spent afternoons, evenings, and weekends sitting in the back of our chemistry classroom, doing experiments and exercises. She prepared us for the chemistry Olympiad which I made twice to the international competition, representing Poland. This is how everything started for me. Those international competitions opened up my worldview. I saw that there was a bigger community of scientists around the world. Then I got to participate in a countrywide summer science camp in Poland that provided this amazing geek community for me where all of us, I think, saw that there were other strange kids like ourselves who liked science and that we were not alone. After that, I had little doubt that I would end up in science in some capacity although I couldn't even begin to imagine at the time that I would be a professor at Stanford University."
–Joanna Wysocka, Professor of Developmental Biology and of Chemical and Systems Biology

"I was always a good student, but it wasn't until college that I decided to focus on science. I majored in chemistry and in my senior year decided to take microbiology, where we learned about recombinant DNA and how to cut and paste genetic information. There was also a genetics course that I almost didn't take because I went to the University of Notre Dame where it was really cold, and I would have to walk across campus to the lab late at night to collect virgin fruit flies. But fortunately, I took the course and there, I learned that genetics was my natural language. There was also a sense I got that there was much in biology remaining to be discovered. Those genetics and microbiology classes made me realize that there were new tools available to help answer important questions in biology and that I wanted to do that."
–Anne Villeneuve, Professor of Developmental Biology and Genetics

All these different women had unique paths that led them into becoming experimental biologists and shaping them into being an integral part of a talented group of scientists that has been the backbone of the Beckman Center for Molecular and Genetic Medicine. One commonality that these women of science did share; however, was the desire to be part of what each one describes as the collegiate, collaborative, and inquisitive culture at the Beckman Center and Stanford University.

A Matter of Balance

As a young professor in the 1980s, Margaret Fuller's first job was at the University of Colorado at Boulder. There, she was assigned the task of teaching the section of an undergraduate developmental biology course on how blood stem cells differentiate to form every blood cell in the body. "The idea at the time was that there was a local stem cell niche, a microenvironment where the stem cells self-renew, but no one had proven that it existed," says Fuller. It was hard to study the niche when the first step commonly used in the field was to take the cells out of the body and sort them in preparation for transplantation. Thinking back to her days as a postdoc studying the genetics of cytoskeletal proteins during fruit fly spermatogenesis, Fuller realized that in the fruit fly, the adult stem cells that differentiate into sperm are known and that they could be studied within the body, in the context of their normal anatomy. The fruit fly could also be genetically manipulated, which means that this humble insect could be used to identify the factors necessary for both stem cell maintenance and the switch to differentiation.

And she did just that. Fuller began to use the fruit fly male germline to understand how adult stem cells are maintained and what governs their ability to differentiate, after she came to Stanford's Beckman Center in 1990. Then graduate student in the Fuller lab, Amy Kiger, now an associate professor at the University of California, San Diego, performed genetic screens to find mutants that affected the ability of the male germline stem cells to either maintain their 'stemness' or to initiate differentiation. Based on the structural arrangement of cells in the region of the fruit fly testis where the germline stem cells reside, Kiger surmised that communication between the stem cells and surrounding somatic cells might be crucial for the male germline to retain its ability to self-renew. Kiger, postdoctoral fellow Helen White-Cooper, and Fuller found that a key to the balance of maintaining the fruit fly's male germline stem cells is the activator

Margaret "Minx" Fuller, PhD
Professor of Developmental Biology and Genetics

of transcription (JAK-STAT) pathway in neighboring germline and somatic stem cells that is needed to preserve the two stem cell populations.

A few years later in 2003, Fuller and then postdoctoral fellow, Yukiko M. Yamashita, now professor at the University of Michigan, were looking for additional ways to ensure that balance is maintained between stem cell self-renewal and onset of differentiation in the cell lineage that leads to production of sperm. Yamashita had discovered that the germline stem cells go through an asymmetric cell division, in part, by relying on two intracellular factors, the centrosome and the Adenomatous Polyposis Coli (APC) tumor suppressor protein. The stem cell uses these two factors to align its mitotic spindle perpendicular to the niche, ensuring that one daughter cell maintains its attachment to and localization within the protective environment of the niche and so retains stem cell identity. The other daughter cell, meanwhile, is displaced away from the niche and begins its transition to differentiation. Then, Yamashito did what Fuller calls, "an amazing study." Yamashito labeled the mother and the daughter centrosomes—components of the cell that organize the spindle fibers that are necessary for cell division— that duplicate before a cell divides. Yamashito found that the older centrosome stays anchored near the niche while the younger centrosome migrates to the opposite side and goes off to the cell that will begin to differentiate. Thus, the stem cell retains the mother centrosome, a so-called, "centrosomal Eve," possibly through the life of the fly. The anchoring of the mother centrosome, likely to the protein complex that anchors the germline stem cell in the niche, may provide the mechanism that orients the spindle to make sure that each germline stem cell division has an asymmetric outcome, according to Fuller. This allows the germline stem cell lineage to persist throughout the fly's reproductive lifespan.

Anne Villeneuve, PhD
Professor of Developmental Biology and Genetics

"The best part about being a scientist is the hunt—the bringing together bits of disparate data together into a plausible model," says Fuller. For her, that hunt wouldn't be possible without the, "incredible students and postdocs at Stanford that have been part of her laboratory and that have been true partners in generating questions and ideas."

A Window into Meiosis

Anne Villeneuve came to Stanford in 1989 as an independent fellow after having just completed her graduate training at the Massachusetts Institute of Technology. "Instead of having a single mentor, I had multiple mentors including Lucy [Shapiro] and Minx [Fuller] who were both invested in my success," she says. Villeneuve's lab now studies the process of proper homologous chromosome segregation during meiosis in the nematode, *Caenorhabditis elegans*, but she came to the topic by serendipity.

During her second year at Stanford, Villeneuve was helping a graduate student friend apply for fellowships to study chromosome pairing during meiosis—the specialized cell division program that allows organisms with two sets of chromosomes to generate the sperm and ova reproductive cells that contain only one set of chromosomes. "As a graduate student studying *C. elegans*, I had learned about the interesting properties of chromosomal rearrangements and genetic recombination in nematode meiosis. As I talked to my friend, suddenly a light bulb went on in my head and I realized that I was getting more excited about these questions than what I was actually studying in the lab," she recalls. Villeneuve gave herself a deadline for progress on her original project after which she took a few days to formulate her plan for studying meiosis in the worm.

As a geneticist, she naturally started with a genetic screen—looking for mutants that had an increased frequency of embryos with aberrant chromosomal numbers due to errors in separation of chromosome pairs during meiosis. That initial screen and other screens resulted in mutants that led her lab to identify both chromosomal regions that facilitate homologous chromosome pairing and the protein machinery that is responsible for establishing and maintaining the pairings. Part of this machinery is the synaptonemal complex, a scaffold-like structure that assembles between homologous chromosome pairs during meiosis. Since that screen, Villeneuve's lab has investigated every aspect of meiosis, from chromosomal pairing, to assembly and function of the synaptonemal complex, to the DNA recombination process that creates crossovers between the chromosome pairs, and to the cell division processes that separate them.

More recent experiments in the lab has led them to a detailed and systems-level understanding of how the synaptonemal complex and recombination function together. Postdoctoral fellow Alex Woglar, developed methods to visualize parts of the process that had not been observed previously, revealing that the synaptonemal complex plays multiple roles in promoting and regulating recombination. In a related study, the lab also discovered that the synaptonemal complex morphs from a more dynamic and fluid state to a more stable one as the germ cells progress through meiosis and showed that this state transition is dependent on the DNA events of recombination. "What we are seeing is that there are multiple engineering principles at play in this system, including negative feedback, quality control and fail-safe mechanisms, that work in concert to yield a robust outcome," says Villeneuve.

Looking back, Villeneuve cannot forget that Lucy Shapiro took a chance on her. "She believed in me and that was really important," Villeneuve recalls. Another facet of her success comes from the Beckman Center's resources. Because her research relies heavily on visualizing the steps of meiosis in the optically transparent *C. elegans*, the Cell Sciences Imaging Facility has been integral to her lab's work. The microscopy tools have allowed Villeneuve's lab to continue to push the technology to see meiosis in ever greater detail, both using fixed preparations and also imaging of live worms to see

Villeneuve ascribes another facet of her success to Beckman Center resources. Because her research relies on visualizing the steps of meiosis, the Cell Sciences Imaging Facility has been integral to her lab's work.

the dynamic process of meiosis unfold over time. "The director of the facility, Jon [Mulholland] is not only terrific in the technical aspects of the microscopes, but takes a keen interest in the scientific questions and is constantly on the lookout for new technologies that can enable us to drive our science forward, which is special," she says. "The microscopy tools, over the years, have improved and they have allowed us to identify additional components of meiotic structures. It's a positive feedback loop where we are seeing a level of detail that we could never have seen before." And the aesthetic beauty of the images and movies that the lab generates, enabled by the Beckman Center, is not lost on Villeneuve. "The ability to look at the chromosomes and see what is happening through meiosis progression is one of the most amazing features of combining *C. elegans* with the latest microscope technology."

Gut Behavior

As a budding scientist, Lucy O'Brien had the untethered creativity and ability to endlessly explore. In graduate school, however, O'Brien was grounded with the realization that besides creativity and curiosity for the unknown that had driven her to do her own experiments, to be a successful biologist, she needed to incorporate strategy, planning and being able to fit in as part of a scientific team.

These characteristics of a scientist—taking cues from your environment, being able to adapt, and working as a team—it turns out, parallel those of cells within an organ. Organs are not locked into a single state, but rather can grow or shrink to meet changing physiological demands. This adaptation of adult organs to their environment is distinct, from the growth and maturation of organs during development and, O'Brien realized as a postdoctoral fellow, that the flexibility and adaptation of adult tissues is mostly unknown.

Lucy O'Brien, PhD
Assistant Professor of Molecular and Cellular Physiology

At the Beckman Center, O'Brien now uses the fruit fly midgut as a model system to uncover the rules that govern how adult tissues sense and respond to change. Why the midgut? "It is one of the simplest somatic tissues that undergoes continuous stem cell-based renewal," says O'Brien. The midgut is comprised of about 10,000 cells and about 2,000 stem cells and the numbers are even smaller for the midgut compartment that O'Brien studies—just about 2,000 cells and about 400 stem cells. In other words, it's knowable.

Recently, O'Brien's lab tackled the link between a cell dying within an organ and a new cell being made. In 2017, graduate student Jackson Liang, O'Brien and their colleagues found that it's the differentiated cells, rather than the stem cells that are, "leading the dance," as O'Brien puts it, of when a new differentiated cell is needed in the organ. The healthy somatic cells in the midgut inhibit the resident stem cells from dividing, but if a somatic cell dies, it releases a short-lived smoke signal of

sorts—a "puff" of EGF. This signal triggers the EGFR on the surface of the stem cells nearest the dying cell, prompting them to divide. "This mechanism, if we extrapolate it across an entire tissue or organ, could explain how tissues get to be the precise size that they are," says O'Brien. The team is now testing their model, to see if they can expand or shrink the reaches of the EGF signal and in turn, either grow or shrink the fly midgut.

O'Brien's lab also continues to push already leading-edge technology. "Many labs have tried and failed to live image the fruit fly midgut and I was not planning on tackling this right away in my lab." But, Judy Martin, a research technician in the lab, had seen a talk on how to cut a window in the fly brain and live image neuron recording. She told O'Brien that she wanted to see if the technique could be applied to the midgut. To both of their initial surprise, the test runs worked! Martin managed to keep the tiny fly alive, to make an incision in its back and image the still digesting creature. The live movie reel that emerged showed O'Brien and Martin that the midgut's stem cells move around much more so than the researchers had expected. "We knew that stem cells can respond and move to the site of an injury, but we found that the stem cells are actually moving around all the time. Martin, along with postdoctoral fellow XinXin Du, found that the stem cells are not randomly distributed but rather, space themselves to face away from each other, akin to the way people will space themselves out away from others in an elevator. "We think that the movement is helping to place the stem cells throughout the tissue so that if a somatic cell dies, there will be a stem cell nearby that will get that EGF signal and respond by making a new cell," says O'Brien.

O'Brien arrived on the Stanford campus six years ago. A big draw to the Beckman Center for O'Brien was the strong female leadership, including Lucy Shapiro. Since arriving on campus, "I've sensed the collective presence of strong, well-respected female faculty," she says, "which creates a positive environment at the Beckman Center and that has been wonderful for me as a young female faculty member."

Long-Distance Reach

Ending up at Stanford was a pleasant surprise for Wysocka who had never spent a significant amount of time on the west coast. "My interview at Stanford was one of the most intellectually stimulating ones that I went through," says Wysocka. "I got the impression that the people here consistently have the most out of the box thinking. Talking to the faculty, I found that they had creative ideas that made me think about biological problems in completely new ways." Wysocka, herself a creative thinker, fit right in. "I have not been disappointed with my decision."

By the end of her time as a graduate student, Joanna Wysocka became interested in chromatin and how it controls gene expression. As a postdoc, she discovered that the plant homeodomain (PHD) finger domain on chromatin remodeling proteins mediates an interaction with specific histone modifications. When she started her lab at Stanford, Wysocka used the opportunity to take a risk and do something new—to

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take her expertise in chromatin to study gene regulation in developmental biology. Wysocka's lab studies gene regulation in human development and how it evolved and is distinct from that in primates. One big question is: how does the vast non-coding DNA—which makes up over 90% of the human genome—help orchestrate the turning on and off of combinations of genes needed at certain times and by certain cell types? In particular, Wysocka is studying DNA elements called enhancers that exert their effect on gene expression over long distances, and which are essential for genetic control during development in both time and space. The lab is using genomics and genetics, as well as live cell imaging to track the effects of enhancer DNA on various genes, most of which are nowhere near the genes they regulate. And her gamble to set up a system entirely new to her has paid off.

Wysocka and her colleagues recently developed a technique, using the CRISPR Cas9 enzyme to label enhancers and other gene regulatory elements in the genome and watch their movement within the genome during stem cell differentiation. Their initial observations are that an uptick in the mobility of these DNA elements coincides with increased transcription of the genes under the control of these loci. To study human-specific gene regulation, Wysocka's lab mainly works with human cranial neural crest cells, what Wysocka calls, "the most fascinating cell type there is." In the embryo, these cells originate in the neural tube—the group of stem cells in the embryo that will become the brain and spinal cord—dissociates, and travels a long way to then become parts of the skull and face, forming an unusual number of cell types including neurons, bone, cartilage and connective tissues. She recognized the unique plasticity of these cells and wanted to understand how this relates to developmental plasticity. Tying in the DNA regulatory elements, Wysocka's lab is asking how different gene expression can lead to different cell types that originate from the cranial neural crest cells. The importance of this stem cell type is underscored by the numerous human birth defects—almost one-third of these are linked to malfunctions in the neural crest. Because the neural crest is not accessible in the forming embryo, Wysocka's lab painstakingly developed an *in vitro* model that mimics embryonic neural crest development. In 2015, then graduate student, Sara Prescott, and colleagues established that they could recapitulate parts of higher primate embryo development *in vitro* using induced pluripotent stem cells. Searching for enhancer DNA elements in the chimpanzee and human genomes, they found many differences, suggesting the evolution of these regulatory DNA loci from higher apes to humans.

Joanna Wysocka, PhD
Professor of Developmental Biology, of Chemical and Systems Biology, and Howard Hughes Medical Institute Investigator

Following on the human genome evolution thread, the lab is also exploring how transposable elements—which make up almost half of the human genome—have facilitated human evolution. "What

is not well appreciated is that the majority of these transposable elements invaded our ancestral genome after the primate lineage separated from other mammals. This means that we can think of them as a source of uniquely primate DNA sequences. We thought that this is fertile ground for exploring how new primate-specific and human specific regulatory mechanisms evolved," says Wysocka. Using their CRISPR DNA-labeling technique, the lab found that specific transposable elements found only in primates could act as enhancers that can control human embryonic genes over long DNA distances.

Immunology Tools

Leonore (Lee) Herzenberg came to Stanford University's Department of Genetics in 1959, as a research assistant to her husband, Leonard (Len) Herzenberg, who was starting his laboratory there. Lee worked alongside her husband in his lab, which grew into their joint laboratory that they ran together for more than 50 years, until Len passed away in 2013.

"I don't remember when I first felt that I was a scientist in my own right. Basically, I just grew into a scientist. I didn't know how to be anyone else," says Herzenberg. In 1981, she received an equivalent to a Doctor of Philosophy degree in immunology from the University Paris V Sorbonne, where Len was doing a sabbatical, and was promoted to professor of genetics at Stanford in 1989.

"At Stanford, I was accepted as a scientist," says Herzenberg. "I was able to essentially do almost all of the things that a faculty member could do. There were projects that Len led and I helped manage and projects that were mine, but that Len helped manage. We basically passed the baton back and forth, depending on whose ideas and whose thoughts made up more of the project at the time," she says. "To us, our science was simply what we lived and did." As a cell geneticist, Len recognized the need to automate the counting, characterization and sorting of rare cells within a population of cells, for example, from blood. He and Lee assembled a team of biologists and engineers to create the first fluorescence-based cell sorter, the Fluorescence Activated Cell Sorter (FACS). Now a broadly used tool in biology and medicine, modern flow cytometry instruments can identify and sort living cells by size and molecular content. In essence, flow cytometry instruments identify cells as they stream first past lasers that "light up" fluorescently labeled cells, then move past detectors that sense the amounts of fluorescent labels bound by each cell, and finally pass by stream-steering electrodes that sort cells expressing pre-selected combinations of fluorescent labels.

Leonore "Lee" A. Herzenberg, PhD
Professor of Genetics

While Len worked more with the engineers tasked with developing the FACS hardware technology for identification and sorting of desired cells, Lee focused on functional studies with FACS-sorted cells and on the development of the software tools used to analyze FACS data.

"Very early on, I learned to program computers. Leonard never did take to computers, but I found that programming was fun," says Herzenberg. "When the Beckman Center was built," she recalls, "there were arguments about whether to increase costs by building computer wires into the walls. It was a new idea, but ultimately, we won. In fact, as far as I know, Beckman Center was the first building on campus to have in-wall data transfer wires to connect the computers in the basement to data users on the upper floors. The building's designers thought we were crazy, but the labs thanked us for years thereafter."

Len and Lee Herzenberg were also involved early on in the development and distribution of fluorescent-labeled monoclonal antibodies for biomedical purposes. César Milstein in Cambridge, England, succeeded in fusing an antibody-producing tumor cell with a normal antibody-producing cell, creating an indefinitely surviving, antibody-producing cell line. This technology enabled long-term production and broad distribution fluorescent-labeled monoclonal antibodies, such as those now commercially available for FACS clinical and research studies. Len had foreseen this use of monoclonal antibodies and had arranged for a year-long sabbatical with Milstein. Len and Lee worked as a team in Cambridge, and then brought the technology back with them to Stanford. There was no simple name for these "hybridized" antibody-producing cell lines until, at a party celebrating New Year's Eve with Cesar Milstein in Cambridge, Lee suggested the name 'hybridoma' and the name stuck.

Herzenberg now still works on the basic questions that she and her husband asked when they set up their laboratory: what are the ways immune cells develop and how do they regulate gene expression? And she still loves working with her computer colleagues to develop and distribute software, including "CytoGenie" which provides new and easier ways to find subsets of cells within FACS data.

Unpacking Parkinson's

Suzanne Pfeffer joined Stanford University's faculty in 1986 and remembers well the planning and deliberations that went into building the Beckman Center. "There was a lot of discussion of how to design the building to maximize interaction between all researchers," Pfeffer recalls.

Part of the Beckman Center ever since, her lab studies how human cells transport receptors to the correct cellular compartments. "Very few labs study receptor trafficking and there are still many fundamental mysteries about this process," says Pfeffer. She takes full advantage of the Beckman Center, collaborating with Rajat Rohatgi's neighboring lab on signal transduction pathways and with Pehr Harbury's protein structure laboratory. Four years ago, she began an international collaboration with labs in the United Kingdom and Germany. Biochemists

Suzanne Pfeffer joined Stanford University's faculty in 1986. "There was a lot of discussion of how to design the Beckman Center, so that the faculty and students would interact well and collaborate," Pfeffer recalls.

Suzanne Pfeffer, PhD
Professor of Biochemistry

Dario Alessi of the University of Dundee and Matthias Mann of the Max Planck Institute told her that a protein they were working on, leucine rich repeat kinase 2 (LRRK2), which is mutated in some forms of inherited Parkinson's disease, chemically modifies several Rab GTPases, small proteins that Pfeffer's lab studies. Now, Pfeffer, Alessi and Mann are working together to figure out how LRRK2 affects these GTPases, which Pfeffer previously found, direct the shuttling of membrane proteins to their appropriate spot in the cell. In the patients who have LRRK2-mutated, inherited Parkinson's disease, the protein, a kinase, is constantly active and inappropriately modifies a subset of GTPases. "Studying how LRRK2 changes Rab protein function is giving us important clues into what may cause Parkinson's disease in patients with these mutations," says Pfeffer. Recently, the laboratories have made inroads into just how the action of pathogenic LRRK2 may trigger Parkinson's disease.

Parkinson's disease is associated with loss of dopamine-producing neurons in the brain. In a healthy brain, when this type of neuron is stressed, it sends a signal to another region of the brain that in turn, sends back protective factors so that dopamine neurons can continue to thrive. "We think that in LRRK2 mutant brains, a specific subset of cholinergic neurons loses their ability to receive these important stress signals and therefore fail to trigger neural protection for the dopamine neurons," Pfeffer explains. The neuroprotection might be necessary in the aging brain as part of the pathology of Parkinson's disease. Now, the teams are continuing their collaboration to provide additional evidence of how the disruption of cell signaling in the brain may lead to Parkinson's disease.

Says Pfeffer, "We started studying the basic roles of how these GTPases work in normal cells and now we are able to apply everything that we have learned to understand the molecular basis of a human disease that afflicts more than 1 million people in the U.S. alone. This is exactly what Paul Berg had in mind when he established the Beckman Center. Our success demonstrates the importance of studying fundamental cell biology and biochemistry because such knowledge has important consequences for our understanding the underlying cause of all diseases."

Ribosomes, Front and Center

Once Maria Barna discovered laboratory research as an undergraduate, she fell hard. "I became bitten by the science questions of the virology laboratory I worked in as an undergraduate and was very productive. I didn't want to leave the

Maria Barna, PhD
Assistant Professor of Developmental Biology and Genetics

lab. It became a lot of effort for me to even be able to walk home at night. I was so excited by what was happening, I couldn't wait to get back to it the next day," she says.

As a graduate student at the Sloan Kettering Institute in New York City, Barna was interested in tissue patterning and how cells in an embryo acquire complex forms and structures, such as an exquisite array of skeletal element of a precise shape and size. It was also a moment in which her former mentor, Lee Niswander, had started some of the first large scale mouse mutagenesis screens.

Foregoing a traditional postdoctoral fellowship, Barna became an independent researcher at the University of California, San Francisco through the Sandler Fellow program—intended for young scientists to pursue big and bold scientific questions. Inspired by her interest in tissue patterning and using mouse forward genetics to map unsuspecting gene products, she characterized a spontaneous mutant mouse that was first identified in the 1940s that had profound changes to its body plan so that the skeletal structures were jumbled up in the wrong spots—an extra set of ribs in the neck, for example, and nobody knew why. In 2011, she and her colleagues mapped the gene mutation, and discovered that it was a rather mundane one—in a so-called housekeeping gene encoding a core ribosome protein. A single cell can contain as many as 10 million ribosomes—the protein plus RNA machines in the cell responsible for decoding messenger RNAs to synthesizing proteins.

"I was obsessed with trying to understand how this seemingly boring ribosomal protein could be functioning to control the formation of the mammalian body plan," says Barna. "For decades, the thought was that although these are among life's most important machines, ribosomes are just backstage participants in gene regulation."

The prevalent view of ribosomes is that they are all the same and that although every crevice of a cell's cytoplasm is filled with them, that they are passive machines, picking up and reading any messenger RNA to translate them into proteins. Barna's research is turning that view on its head.

In 2017, Barna's lab analyzed 15 ribosomal proteins in mouse embryonic stem cells, and showed that ribosomes are heterogeneous, with distinct subpopulations of ribosomes in a single cell. To do the analysis, the team had to employ a new tool sensitive enough to pick up slight differences in ribosomal makeup. The technique, called selected reaction monitoring, uses mass spectrometry to sort molecules by their weight and allows the lab to identify different flavors of ribosomes.

The work so far suggests that distinct populations of ribosomes are dedicated to transcribing metabolic messenger RNAs, RNAs that function in cell signaling, and other categories. The results are adding an additional layer of the genetic code that has previously been hidden from view. "Our

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results are hinting that there is regulation that is encoded in our genetic template that endows transcripts the ability to be translated by specific types of customized ribosome," says Barna.

The unexpected finding has spawned many new questions for Barna. Are there specific subcellular spaces dedicated to certain species of ribosomes? How is this ribosomal heterogeneity established? How did the distinct differences ribosomes evolve and what does that mean for species-specific differences in ribosome function? Does the presence of one ribosome species influence the characteristic of the proteome of a species?

Barna's latest data suggests that the way messenger RNAs get sorted to find their way to their appropriate ribosomal population is the way the RNA is folded. RNAs can form elaborate structures and the lab is now developing learning algorithms to try to detangle the RNA structure code. Like comparing a DNA or RNA sequence to a library of sequences to find a match, Barna's lab is trying to decode RNA secondary structure to find groups of RNAs with the same structure that might be translated by the same ribosomal population. "I think this is the missing link in that the instructions for these RNA structures are embedded in the transcripts and we just haven't seen them before," she says.

Barna's lab brings together many biology disciplines from biochemistry to developmental biology to informatics and uses a diverse set of model organisms to study the heterogeneity of ribosomes. "Stanford and the Beckman Center are probably the only places in the world where we can do this research. It's very easy to pick a direction by thinking creatively of what the problem is and finding expertise somewhere on campus. We can move our research quickly into directions whereas in other places this would be daunting. Everyone is extremely interactive. The students and postdocs drive the projects forward and start new ones which has been revolutionary for my science."

Old Disease, New Insights

One of the main research questions in Yueh-hsiu Chien's laboratory is the role that gamma delta (γδ) T cells play in host immune defense. These cells make up a relatively small proportion of T cells in the peripheral blood and express T cell receptors made up of gamma and delta chains rather than the more common alpha and beta chains of the T cell receptors found on $CD4^+$ helper and $CD8^+$ cytotoxic T cells. Research in Chien's lab has revealed several groundbreaking discoveries about these cells including that gamma delta T cells and alpha beta T cells are distinct in their antigen recognition and antigen-specific repertoire and also in activation requirements and effector-function development. These features allow gamma delta T cells to occupy unique niches both in space and time and initiate and regulate inflammatory responses.

Recently, the lab has begun to study the many components of the human immune system as a whole using a systems biology approach. Specifically, the lab is studying the immune system's response to a latent *Mycobacterium tuberculosis* (Mtb) infection. Tuberculosis is among the oldest and

most studied human diseases, affecting about one-fifth of the global population. Most infections with Mtb are clinically asymptomatic known as a latent infection. Fewer than 10% of the infected individuals eventually progress to an active tuberculosis disease state, which is the leading cause of death from infectious disease worldwide. Yet it's not known how the immune system of the vast majority of those infected people keeps the Mtb in check. And despite intense research efforts, how the immune system responds to a latent tuberculosis infection is not understood. Without this knowledge, the tried efforts to prevent a latent infection from advancing to active disease have so far not been successful.

"We are trying to understand how the immune system controls a latent tuberculosis infection," says Chien. Her lab has recently revealed some of the immune system changes that occur in those with dormant Mtb by analyzing blood samples from a cohort of South African adolescents, some of who had a latent Mtb infection and some that were not infected. Rather than focusing on just certain aspect of the immune system, the team used high-dimensional mass cytometry to cast a wide net over the many types of immune cells and identified distinct host immune states that distinguish individuals with a latent Mtb infection from those control individuals without an infection. The work was a collaboration with Dr. Thomas Scriba of the South African Tuberculosis Vaccine Initiative and his colleagues. Their analysis revealed that a latent infection results in systemic inflammation along with significant reduction of the levels of naive B cells and impaired ability for T cells to respond to stimulation. This state of immune deviation was coupled with an enhanced antibody mediated cytotoxic response that is mediated mostly by immune cells known as natural killer cells, suggesting that a latent Mtb infection is associated with a major mechanism of protective immunity.

Yueh-hsiu Chien, PhD
Professor of Microbiology and Immunology

Collaborating with Stanford's Purvesh Khatri, an associate professor of biomedical informatics and immunology to analyze publicly available gene expression data sets, the researchers found increased levels of circulating natural killer cells as a common factor, irrespective of the age, genetic background and geographic location of the individuals. The natural killer cell levels in those with an active tuberculosis infection fell below the levels of those in uninfected individuals and returned to baseline levels of uninfected controls upon successful treatment. And, the peripheral natural killer cell levels at the time of a tuberculosis diagnosis was inversely associated with clinical parameters indicative of disease severity the team found.

"These analyses offer critical insights into the underlying pathophysiology in tuberculosis progression and the factors

that control and influence disease outcomes," says Chien. "The results will be useful for generating hypotheses that could lead to new intervention strategies to prevent the switch," she adds.

Science as Form of Rebellion

Lingyin Li, an assistant professor in the biochemistry department, joined the faculty at Stanford in 2015. Li grew up in Xi'an, China, an ancient capital city of China where many of the Chinese dynasties lived. Li was fascinated by her surrounding history and Chinese literature. But her attention quickly pivoted to chemistry and math when her sixth-grade math teacher said that literature and history are subjects for girls and math is for boys; she was set on proving him wrong. Following a PhD in chemical biology from the University of Wisconsin-Madison, Li focused her chemistry training on cancer immunology, including showing that certain metabolites of plants and fungi known as flavonoids could act as stimulators of an important immune pathway known as STING (stimulator of interferon genes). At the Beckman Center, Li is homing in on the chemical biology of innate immunity to understand how to use the naturally made defense molecules in the body to develop potential cancer, autoimmune disease and neurodegenerative disorder therapies.

Lingyin Li, PhD
Assistant Professor of Biochemistry

Full Circle

In a given living cell, there are magnitudes more types of RNA species than there are genes. Julia Salzman, assistant professor in the biochemistry and biomedical data science department is exploring why that is. Her lab uses both computational and molecular biological tools that allow the team to study RNA—and DNA —at the single nucleotide level in species that range from single-cell bacteria to humans. Her lab has developed statistical algorithms to precisely detect circular RNA. More recently, the lab has developed newer, more precise methods for detecting RNAs specifically expressed in cancer and other diseases, producing both new insights for gene function in cancer and new diagnostic tools for cancer genomics, including those that could be used in liquid biopsies to detect minute amounts of cancer cell-specific DNA in the blood.

Previously thought to be rare, Salzman's lab has found that circular RNA species are surprisingly abundant in human cells. Salzman and her colleagues sequenced the breadth of RNA species in both wild type and malignant human cells and found that these RNA circles are as abundant as their linear counterparts, suggesting that the circles are the rule rather than the exception in mammalian cells. The team scanned RNA molecules for those where the nucleic acid sequences were scrambled, that is, were in a different order compared to the corresponding DNA sequence of the gene. The lab has since been studying how these circular RNAs

Julia Salzman, PhD
Assistant Professor of Biochemistry and of Biomedical Data Science

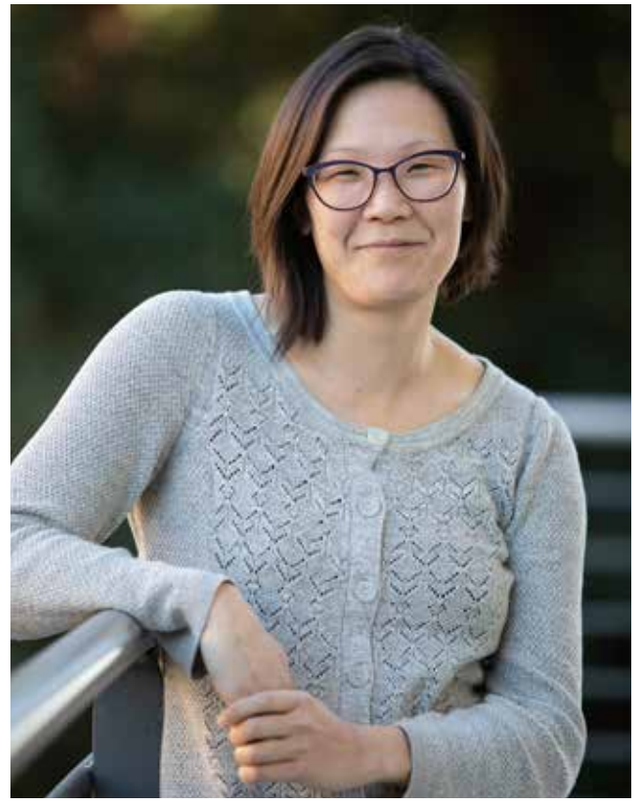
form *in vivo* and their biological functions. More recently, Salzman's lab found that circular DNA is not just a feature of mammalian cells, but are expressed across all eukaryotes tested, suggesting that the molecules are an evolutionary conserved part of gene expression.

Malaria's Achilles' Heel

Ellen Yeh, assistant professor in the Department of Biochemistry, first started working with *Plasmodium falciparum*, the parasite that causes malaria, as a postdoctoral fellow at the University of California, San Francisco (UCSF). Malaria is a major burden of infectious disease around the world, with more than 300 million cases per year and among the top three potentially lethal infectious diseases globally.

Yeh focused on the parasite's apicoplast, a unique and essential four-membrane organelle that was discovered in the 1990s. The organelle was acquired by parasites ancestral to *P. falciparum*: the ancient parasite got a two-for-one deal when it 'ate' an eukaryotic alga. That alga, it turned out, already possessed a plastid through the endosymbiosis of a free-living prokaryote, a cyanobacterium. Unlike plant plastids, the apicoplast had lost its ability to do photosynthesis and instead performed other essential functions in the parasite including protein synthesis. For Yeh and other researchers, the apicoplast was a good target for anti-malarial drugs. Because of its plant origins, some of the apicoplast processes and molecules might be unique to plants and targeting these would not harm human host cells. Yet, the function of the apicoplast during human infection had eluded researchers.

In 2011, at Joseph DeRisi's UCSF lab, Yeh showed that the essential role of the apicoplast during the human infection stage is to produce one metabolite, isopentenyl pyrophosphate (IPP), a precursor molecule to several molecules



Ellen Yeh, MD, PhD
Assistant Professor of Biochemistry, of Pathology, and of Microbiology and Immunology

essential for the parasite's ability to replicate in human red blood cells. At Yeh's own lab at Stanford, she used this finding as a starting point to identify multiple steps in this process that can be blocked by drugs and is working as part of the Stanford AIM with Takeda Pharmaceuticals to develop these into anti-malarial compounds. Yeh's lab is also not stopping at IPP; they are finding ways to disrupt the entire apicoplast irreversibly. The lab executed a mutagenesis screen to uncover essential genes required for making new apicoplasts and found several novel enzymes as promising drug targets.

Exciting Membranes

"I have always loved chemistry because it is tractable and because you can solve chemistry problems in a clear and quantitative way," says Merritt Maduke.

Yet, it was the biology questions that drew her into research. She has been using chemistry to pursue biological questions ever since. In graduate school, Maduke studied the physical and chemical basis of how proteins are imported from the nucleus into the mitochondrion, a membrane-enveloped organelle. She constructed artificial membranes without any protein receptors found intertwined within them and showed that the unique negative potential of the mitochondrial membrane can drive positively-charged helices-containing proteins right through the membrane to the inside of the mitochondria. The work demonstrated that a driving force, rather than energy-expending cargo-based import can be sufficient for charged peptides to traverse an organelle membrane.

Continuing the theme of applying biophysical and chemical properties to a biological system, Maduke focused on ion channels as a postdoctoral fellow in Christopher Miller's laboratory at Brandeis University in Boston. "I discovered that ion channels were, in 1995, a way to study a macromolecule at a single molecule level. We could look at an ion channel with ions opening and closing, and ions moving in and out very quickly. I found this to be completely fascinating," says Maduke. Potassium channels had recently been found in bacterial cells and Maduke wondered whether the one-celled organisms also contained chloride channels. She cloned what looked to be a chloride channel in *Escherichia coli*, purified the ion channel and added them to an artificial membrane. "It was amazing to see the first signal that told me this protein could shuttle chloride ions across this membrane. It seems so mundane now, but it was so exciting then," she says.

For Maduke, unlike the difficulty of choosing a graduate school and postdoctoral laboratory, choosing to come to Stanford University, as a faculty member

Merritt Maduke, PhD
Associate Professor of Molecular and Cellular Physiology

was an easy decision. "The students here are so fantastic. I love the naive curiosity they bring to our research questions and their innovative ideas."

She also loves the collaborative spirit of Stanford and the Beckman Center. "I was walking to get coffee one day and Stanford's William Newsome, a neurobiologist, approached with a question." Newsome had read recently published papers that showed ultrasound could be used to activate neurons in the brain and wanted to understand the mechanism of how this happened. "'I have no idea how this could be working but I know how I would approach the problem', I told him." Maduke told him that she would first want to pare down the question and ask what ultrasound does to an ion channel—the fundamental unit of excitability in the brain. The question turned into a collaboration among Maduke, Newsome, and other Stanford professors including Stephen Baccus, who studies the circuitry of the retina, and radiology researcher Kim Butts Pauly. Each lab tackles a different angle of the question. "It has been incredible to work with this team for the last 8 years within this still young field. We find results on the molecular and cellular level and then the other groups apply that to a whole organ and whole animal level," says Maduke.

Their initial result was surprising. Using the synthetic lipid bilayer system that Maduke had developed, the lab showed that when ultrasound radiation is applied to membranes themselves, without any ion channels, the membrane responds with an electrical current, likely as a result of the ultrasound radiation pressure pushing on the membrane. The lab also demonstrated that a mechanically-activated, but not a sodium channel was activated with ultrasound stimulation. The lab is now testing these effects in native tissue—brain slices with neurons that have multiple types of ion and mechanically sensitive channels. "What we're working towards is a unified theory to explain all of the effects of ultrasound at these different biological levels. Then, we can develop ultrasound better as an experiment and therapeutic tool," she says.

The Hazards of Motion

The long, spindly sensory neurons in the skin have the endless task of helping us sense the world through touch. Yet, how petting a dog or sensing running water from the faucet on our hands is registered by these neurons mostly unknown. Added to this complexity is the need for these neurons to move and stretch along with the skin and remain intact. Miriam Goodman is striving to understand just how these neurons can take on these never-stopping stresses—using the nematode *Caenorhabditis elegans*, a 1-millimeter worm. Recently, a postdoctoral fellow in the lab, Michael Krieg, who is now a professor at the ICFO Institute of Photonic Sciences in Barcelona, Spain, uncovered mutants in cytoskeleton proteins that results in neurons that crinkle,

Miriam Goodman, PhD
Professor of Molecular and Cellular Physiology

Besides her scientific accomplishments, Goodman is shining a spotlight on the underrepresentation of women and minorities in science and on gender research.

fracture, and stretch, resulting in neurons that over time, are beyond repair. One of the mutated proteins was spectrin, which forms cylinder-shaped scaffolds on the inside of a cell's plasma membrane. The protein, according to Goodman, has been implicated in brain and heart function and is important for helping red blood cells keep their shape. When the spectrin mutation is combined with a mutation in tau—a protein found in the brain and that has been linked to Alzheimer's disease—the neurons, instead of crinkling, form tiny coils as a result of mechanical tension, twisting, and bending. To explain how these proteins are affecting the physical properties of the long sensory neurons in *C. elegans*, Goodman collaborated with a computer science lab in Munich, Germany to develop a computational model that could predict the shape defects based on modifications in physical properties. "In the mutants, instead of the neurons lying flat, you get these strange shapes because there is no longer a balance when the neurons are forced to move along with the tiny worm," she explains. Now, Goodman's lab is identifying additional proteins in the cytoskeleton that help the neurons keep their shape in the face of mechanical movement and stress.

To better study the forces that cells and cellular components experience as the worm moves, Goodman's lab is also collaborating with Jennifer Dionne's material sciences laboratory which is also at Stanford University, developing nanoparticles that can go inside the animal and measure these microscopic-scale forces, by emitting different forms of light that correspond to different mechanical forces.

One of Goodman's favorite features of the Beckman Center? The ability to try out what she calls her "crazy ideas." I've had lots of ideas where I want to try something new and need access to a piece of equipment I don't have in the lab. Every time I reach out to someone to use their equipment for a pilot study, no one has ever not responded, and no one has ever said no. Almost none of these crazy ideas have turned into anything, but the freedom to try without these other social barriers is really invaluable."

Besides her scientific accomplishments, Goodman is also shining a spotlight on the underrepresentation of women and minorities in science and on gender research. She spent a year as a faculty fellow at Stanford's Clayman Institute for Gender Research studying gender inequality in science. Building on that experience, Goodman, along with neurobiology professor Jennifer Raymond, started a social sciences journal for students and postdocs to discuss studies on the underrepresentation of women in different fields. "What surprised me most about the discussions, which I thought might be depressing, is how energized the students were by having these conversations! We had fantastic discussions on what the research says, what does that mean about how our brains work and what does that mean about how science works?" says Goodman.

Goodman's energy to create open dialog on gender inequality issues in science appears to have no bounds. Following on from the journal club, for the last three years, Goodman has been running a mini-course called 'Diversity and Inclusion in Science' for graduate students and

postdocs. Goodman says that students are now much more aware of gender inequality issues and that the course is enabling students to, "gather evidence so that they can make arguments based on evidence that some things need to change and for them to take action to improve conditions, diversity and inclusion."

A team of graduate students who took the course came up with a new mentorship program called Solidarity, Leadership, Inclusion, Diversity (SoLID), that provides an additional faculty mentor for graduate students on issues besides their research such as advocacy, diversity and inclusion, academic activism, mental health and wellness, balancing social justice work and lab productivity, stereotypes and imposter syndrome. "The students built this program entirely themselves, recruited faculty across our graduate programs who were open to holding office hours once a month. The germ of the idea was in my classroom and I am extremely proud of that and the students that built it," says Goodman.

Thinking in 3D

In 1967, Lucy Shapiro was a newly minted assistant professor in the department of molecular biology at the Albert Einstein College of Medicine in New York. The chair of the department, Bernard Horecker, told her that she could work on whatever she liked. "This was amazing," says Shapiro. "I realized that I was clearly going to spend the rest of my life doing whatever I chose and that it had to be something that I deemed important." Shapiro took three months to read, think and formulate a plan. "I sat down and wrote out what I wanted to do in some detail based on my view of the biochemistry of the cell," she says. At the time, researchers were either bursting open cells to get to their enzymes which they then characterized biochemically in the lab, separate from the intact cell. Or, researchers were using genetics and observing mutants to infer what happens inside of a cell. "I saw both approaches as beautiful, but limited. Everyone seemed to be studying cell regulation outside of the spatial parameters of the cell but how does the cell inherit the information that tells it where to place everything in the cell? To me, the cell had to be considered as a three-dimensional entity. I also wanted to understand how all of these events in the cell were coordinated. In essence, how is the cell an effective machine? I visualized the cell as an integrated system and my goal was to go all the way from the chromosomes to the proteins that are made in three-dimensional space, to do essentially what I called *in vivo* biochemistry," says Shapiro.

For such a complicated task, Shapiro needed a relatively simple model organism, and one that had polarity and an asymmetrical cell division in which the

Lucy Shapiro, PhD
Professor of Developmental Biology and Beckman Center Director

mother and daughter cells were distinct from one another. This type of asymmetric division, as Shapiro saw it, is an essential event that generates diversity in cells, organs and organisms. "My plan was to study this model organism by including the three-dimensional organization of the cell as a function of regulatory mechanisms that result in the integrated genetic circuitry that makes a living cell run," Shapiro says.

Although she had no microbiology experience, after a lot of reading, Shapiro settled on the *Caulobacter crescentus*, a stalked bacterium that had all of the characteristics she had been looking for. Although researchers had figured out how to synchronize *Caulobacter* populations, there was no genetics or biochemistry worked out for her seemingly simple one-celled organism. Her colleagues and former advisors attempted to dissuade her, but Shapiro stayed her course, and has been using *Caulobacter* ever since, to learn how the cell distributes organelles and molecules in a polar way and how the various processes of the cell, such as DNA replication function as part of the larger integrated and three-dimensional network of the cell.

In 1996, while looking for mutant *Caulobacter* cells that prevented the formation of flagellum, an appendage that allows the bacterium to swim, Shapiro's graduate student, Kim Quon, made a discovery that, 30 years prior, Shapiro had envisioned when she set her lab's researchers goals. Quon found CtrA, a master transcription factor that controls many of the genes necessary to coordinate *Caulobacter*'s cell cycle. "We had this big 'ah-ha' moment in the lab when we found the promoter sequence recognized by this transcription factor and that the chemoreceptor genes, flagellar genes, DNA replication genes and many others all had promoters that harbored this sequence for CtrA to bind to. We realized that this single transcription factor was controlling a whole array of genes that were dynamically controlled throughout the cell cycle," Shapiro explains.

Soon after moving to Stanford, graduate student Mike Laub (now a professor at the Massachusetts Institute of Technology) and Shapiro showed that the simple bacterial cell has a hard-wired genetic circuitry by which hundreds of genes are turned on and off as a function of cell cycle progression. The study was among the first evidence showing that bacterial cells have regulatory circuitry that functions in time and space to coordinate the multiple, complicated events that drive the cell cycle.

Still paving the way and identifying novelties about the living cell, Shapiro's lab, more recently, showed a new way that prokaryotic cells organize their functions using membranelles organelles made up of bimolecular condensates that concentrate signaling proteins at the cell poles providing directionality to their signaling output. Her lab has also developed tools to study the three-dimensional structures inside the cell, which is enabling the study of protein interactions inside living cells.

Shapiro moved her laboratory to Stanford University in 1989, becoming the founding chair of the Department of Developmental Biology. In 2001, she became the director of the Beckman Center for Molecular and Genetic Medicine. Shapiro has had a steady confidence that has served her well in her career. Shapiro, for whom mentoring is an extremely important part of her job, believes that it is her duty to instill confidence and self-assurance into her well-deserved science trainees and to help ensure their future success. ■