Major Discoveries and Technological Advances in Biochemistry at the Beckman Center

By Krista Conger

It was a seismic shift in the geographic center of gravity for a relatively new scientific field. In June of 1959, six young researchers uprooted their families and moved from Washington University in St. Louis to create a new department of biochemistry at the Stanford University School of Medicine. Another joined them from the University of Wisconsin in Madison.

"At the time, DNA was a buzzword, microbiology was blossoming," recalled Paul Berg, PhD, one of the department's founding members. "There was a feeling of hyper-excitement about science and medicine among students and faculty members who understood the field."

The researchers, led by the department's new chair, Arthur Kornberg, PhD, upended traditional ideas about how science should be practiced and encouraged unprecedented degrees of collaboration and resource sharing. They also immediately transformed how biochemistry was taught at Stanford.

"I vividly recall our first class," said Berg, who would go on to share the 1980 Nobel Prize in Chemistry for his work on the biochemistry of nucleic acids and recombinant DNA. "Sixty students had enrolled, but the room, which seated 120, was jam-packed."

That sense of excitement and comradery has persisted for more than 50 years, from the discovery of the molecular underpinnings of biology's "central dogma," which touted a linear path from DNA to RNA to protein, to more recent discoveries and technological advances that have begun to turn these long-held biological principles inside out and upside down.

"We now have access to incredible technology that allows us to study living cells, and to do biochemistry almost in vivo," said Beckman Center director and developmental biologist Lucy Shapiro, PhD. "Researchers in the department explore the biology of living organisms and tissues with absolutely exquisite biochemistry to answer critical biological questions."

At the time of the department's founding, all of the researchers were wholly focused on enzymes, studying pure protein molecules to determine how exactly they interacted with one another to carry out complex biological processes. Now, decades later, younger faculty members are true to that legacy while also extending the ideals and principles of the early department to address a nearly unimaginable variety of biological questions.

Regions of exploration and discovery include whole genome sequencing; protein folding, structure and targeting; chromosomes and telomeres; and the molecular processes that govern how cells cycle, divide, move and die. Researchers are plumbing the depths of RNA, learning that these molecules are far more than just couriers of information, but can instead act as unexpectedly powerful enzymes and regulatory molecules that control many of a cell's functions. They've learned that proteins can interact with ribosomes to create new protein molecules in the absence of RNA, and they are beginning to identify the functions of the vast portions of "dark" matter in the human genome.

They are also studying the developmental pathways necessary to create the branching tubules of the mammalian lung, and they've come up with ways to simulate billions of years of evolution in a test tube. Others are using statistical and computational methods to learn how proteins fold in three-dimensional space and how genes are regulated.

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Department researchers are also increasingly turning their attention to how these processes contribute to diseases, including HIV, malaria, celiac sprue, and food-borne bacterial illnesses.

"Many of us want to understand the implications of biochemical processes on human health and disease," said Peter Kim, PhD, the Virginia and D. K. Ludwig Professor of Biochemistry and a former graduate student in the department. "We have the opportunity to connect our basic science research with ideas and knowledge applicable to human health. That's really quite exciting."

It is a panoply of research interests as impressive for its breadth as for its depth, with its roots in the painstaking analysis of molecular interactions throughout the cell.

"We've always been interested in understanding how molecules make life happen," said department chair Suzanne Pfeffer, PhD. "That's been the key "glue" that holds this department together. But one of the most unique aspects about our department is the philosophy of collaboration and sharing established by our founding fathers. Since the late 1950s, we've kept up the tradition of talking to each other and sharing ideas about science."

DEPARTMENT OF BIOCHEMISTRY 1959-60

First Row: S. Kornberg, M. Morris, M. Dieckmann, A. Kornberg, D. Kaiser, D. Smith, P. Berg, J. Josse, K. Aveleno, J. Fleischman

Second Row: D. Hogness, E. Sherberg, E. Jaunzemis, M. Stetson, F. Noel, A. Pratt, C. Radding, R. Inman, G. Bugg, G. Wake, R. Baldwin, V. Aposhian, H. Fancher, J. Johnson, R. Lehman

Third Row: M. Chamberlin, W. Wood, J. Butzow, F. LaBar, J. Yeates, H. Morales, J. Simmons, J. Preiss, R. Barrand, M. Cohn, J. Adler, E. Elson, G. Roussos, S. Zimmerman

The tradition has clearly been effective: Members of the biochemistry department have been recognized with two Nobel Prizes, Albert Lasker Basic Medical Research Awards, , two National Medals of Science, a National Medal of Science, MacArthur Fellowships, an NIH Pioneer Award and multiple National Academy of Sciences memberships, among many other honors.

The biochemistry department is often described as a uniquely close-knit family unusual in a world where researchers often compete for funding and race one another to publication. Faculty members share reagents, ideas and even laboratory space. “I call it the ‘sociology’ or ‘style’ of the Stanford biochemistry department,” said Berg, “and it’s something that’s never been successfully replicated elsewhere.”

Berg and others credit Kornberg with the idea that science in the department should be a collaborative effort. At the time of the move from St. Louis to Stanford, Kornberg had recently elucidated the workings of DNA polymerase I, an essential enzyme for faithfully replicating the DNA within living cells. The discovery was remarkable in part due to the fact that the structure of DNA itself had been determined by James Watson and Francis Crick only six years earlier.

Kornberg had been the chair of Washington University’s department of microbiology, and he insisted as part of his agreement to come to Stanford that he be allowed to bring any members of his department who wished to populate the new biochemistry department.

After deliberation, Berg, David Hogness, PhD, Dale Kaiser, PhD, Melvin Cohn, PhD, and I. Robert Lehman, PhD, accepted the offer. In addition, Robert (Buzz) Baldwin, PhD, agreed to to join the group from his position as an assistant professor of biochemistry at the University of Wisconsin in Madison. Once the decision was made, the planning began.

“We came here because we were going be an honest-to-goodness biochemistry department,” said Berg, “We were all roughly the same age, and we were excited to be in close contact with our sister science departments, such as chemistry and physics. In St. Louis, the microbiology department was across town from the main university.”

Together the researchers created a biochemistry course and prepared to launch a graduate program in the field.

The excitement of the young department manifested itself as a ripple effect throughout the school of medicine. Joshua Lederberg, PhD, from the University of Wisconsin in Madison promptly accepted an offer to head the university’s first department of genetics. Lederberg had received the Nobel Prize in Physiology or Medicine in 1958 for his discovery that bacteria could mate and exchange genes. And noted researcher Charles Yanofsky, PhD, who would become known for showing that the nucleotide sequence of DNA is reflected by a change in the amino acid sequence of the protein that it encodes (a concept called co-linearity), arrived from Western Reserve University Medical School in Cleveland to join Stanford’s department of biological sciences.

In addition to transforming the way biochemistry was taught at Stanford, the fledgling department was also unique in its approach to lab work.

"The department's trademark collaborative spirit is one reason why many current faculty members are themselves former biochemistry graduate students or post-docs."

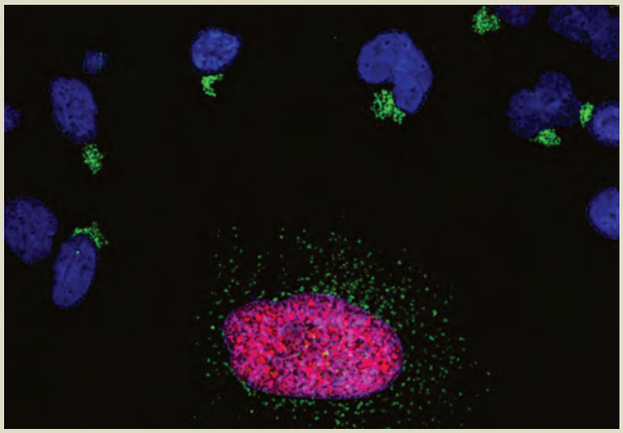
"We shared all the laboratory space," said Berg. "Any one lab might have people from four different research groups. This created a system where information could be distributed very quickly because our students acted as a kind of antennae. We also shared all the research money. This meant that a young, starting assistant professor could function at a level far beyond the financial capacity of his own laboratory. We didn't want to hamper anyone's creativity or ideas just because he didn't have the money he needed. Finally, we agreed to keep our own labs to about 10 to 12 people to be sure to always have room for students and postdocs. This is the way we grew up together."

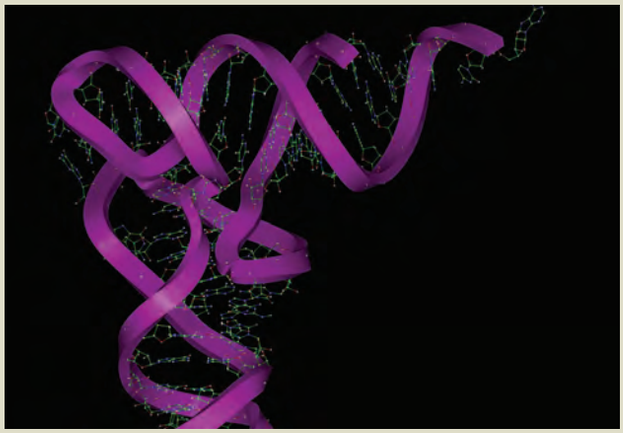
The department's trademark collaborative spirit is one reason why many current faculty members are themselves former biochemistry graduate students or post-docs.

"I was delighted to have an opportunity to return to Stanford," said Kim, who completed a PhD in the department in 1985 in the laboratory of Buzz Baldwin. After spending time at MIT's Whitehead Institute, Kim served from 2001 to 2013 as the executive vice president and then president of Merck Research Laboratories before returning to academia. "In particular, I was really attracted by the very close proximity of world class scientists, engineers and physicians, who are all within a five minute walk."

Other alumni-turned-faculty members include Philip Beachy, PhD, a professor of biochemistry and of developmental biology and the Ernest and Amelia Gallo Professor in the School of Medicine; Mark Krasnow, MD, PhD, professor of biochemistry and former chair of the department; and James Spudich, PhD, the Douglass M. and Nola Leishman Professor of Cardiovascular Disease and former chair of the biochemistry department. Beachy and Krasnow are both also investigators for the Howard Hughes Medical Institute.

The 1950s were a time of explosive learning in biology. Francis Crick and James Watson had published the





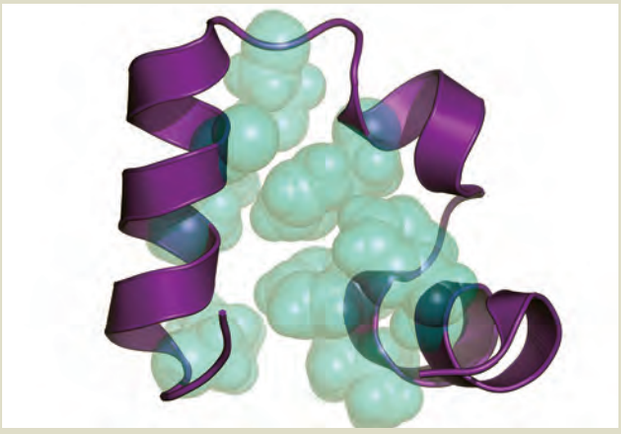
structure of DNA in 1953, and Crick coined the term "central dogma" in 1956 to describe the flow of genetic information in a living cell from DNA to RNA to protein. At the time, Kornberg was deeply immersed in dissecting how DNA was copied prior to cell division to ensure no loss of genetic information from generation to generation. His discovery of DNA polymerase I would win him the Nobel Prize in 1959 shortly after his arrival at Stanford.

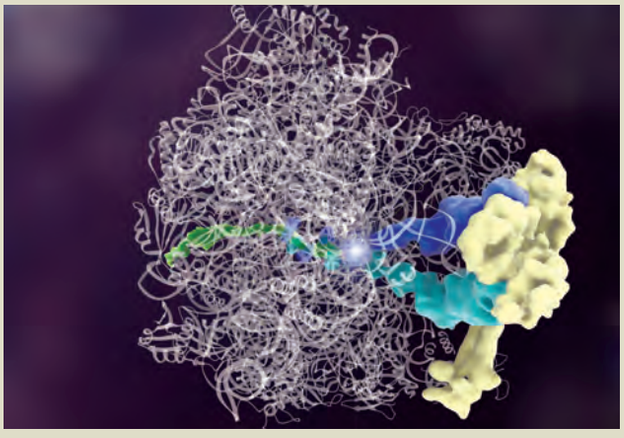
Many of the researchers in today's biochemistry department study components of that central dogma — DNA, RNA and protein — but in ways and combinations that Crick and his compatriots would have been hard pressed to understand at the time. Entire genomes have been sequenced, not just those of humans, but also those of many other species, shedding light on the functions of individual genes and also on what's been known as the 'dark matter' of the genome.

These non-coding areas were designated as 'junk' in the framework of the central dogma, but are now proving to be rich in regulatory regions that control gene expression across great distances in diverse tissues and disease states.

Ronald Davis, PhD, a professor of biochemistry and of genetics and director of the Stanford Genome Technology Center, has contributed much to this understanding. His group devised new technologies to drastically lower the cost of generating the oligonucleotides necessary for whole genome sequencing projects. In the 1990s the center began a collaboration with Affymetrix to generate some of the first DNA microarrays to aid in the generation of clinically relevant data during development and disease.

In 2013, Davis was named one of today's greatest inventors by *The Atlantic* magazine, which noted that





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"A substantial number of the major genetic advances of the past 20 years can be traced back to Davis in some way." Davis' ongoing studies of the genome of the yeast *Saccharomyces cerevisiae* have led to the generation of over 21,000 haploid and diploid strains with a complete deletion of each of the one-celled organism's 6,000 genes. He and his colleagues grew the strains under a variety of stressful conditions to show that nearly all the genes contribute in some way to the fitness of the organism. The study may help identify the function of each gene in yeast and shed light on the corresponding genes in humans.

Researchers in the department are interested not just in the nucleotide sequence of genes and genomes, however. They've also turned their attention to understanding the processes of cell division and damage repair. Aaron Straight, PhD, an associate professor of biochemistry, studies how chromosomes are segregated during cell division to ensure genome stability, and Gil Chu, MD, PhD, professor of biochemistry and of medicine focuses his research on how DNA damaged by ionizing radiation and ultraviolet light is repaired by specialized enzymes.

Steve Artandi, MD, PhD, professor of biochemistry and of medicine, meanwhile, focuses his research on the DNA caps called telomeres on the ends of chromosomes. These caps shorten a bit with each cell division, providing a kind of internal molecular clock that signals an aged cell to stop dividing or die. In stem cells and in some types of cancers, however, a protein called telomerase stops the countdown clock by adding nucleotides to the telomeres and effectively making the cells immortal. Researchers believe that understanding telomerase activity could help clinicians battle disease, or even unlock the mysteries of human aging.

RNA was once believed to be little more than a handy molecular telegram to bring the instructions encoded in the nucleotide sequence of genes in the nucleus to the protein-making machinery in the cell's cytoplasm. Read from one end to the other by ribosomes, the RNA carefully dictates the sequence of amino acids in a growing protein. New research has shown that's far from true. RNA has been shown to be an enzyme in its own right and a powerful regulator of gene expression. Assistant professor of biochemistry Julia Salzman, PhD, for example, recently discovered the fact that many RNA molecules in humans actually exist as circular, rather than linear, molecules.

"My research takes place at the boundary of physics and biochemistry," said Salzman, whose PhD is in statistics and who completed a postdoctoral position in the laboratory of Patrick Brown, PhD, an HHMI investigator and an emeritus professor in the biochemistry department. "As part of my work, my lab and I generate and mine massive biological data sets to better understand the regulation and function of alternative RNA splicing. Pat taught me that it's possible to study gene regulation and function in high dimension."

Similarly, assistant professor of biochemistry Rhiju Das, PhD, uses a degree in physics to investigate exactly how individual RNA molecules fold in three-dimensional space to form molecular structures able to carry out diverse tasks far beyond coding for proteins. He and his lab crowd-source some of the data crunching with computer software they've devised to let interested members of the public practice their RNA folding skills. They then synthesize the most promising candidates in the lab and watch to see if they fold as predicted. The "winners" of the game are used to inform future folding efforts.

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Daniel Herschlag, PhD, a professor of biochemistry, of chemistry and of chemical engineering, is also working to better understand how RNA and protein molecules fold and catalyze reactions within the cell.

Proteins have always been considered to be the workhorses, responsible for gene expression, metabolism, signaling within and among cells and even disposing of the cellular trash.

"We're interested in learning what happens to cells when they are under stress," said assistant professor Onn Brandman, PhD, who also has a background in computer science. "What happens when translation from RNA to protein fails, when something goes wrong?" A recent discovery by Brandman and colleagues at the University of California, San Francisco reveals a protein synthesis pathway outside the central dogma. The researchers showed that when a ribosome stalls, a protein directs a sequence of amino acids to be added to the growing chain — perhaps to designate a stalled protein for destruction or to check whether the ribosome is capable of functioning properly.

"Onn is changing the way we think about how proteins are created inside a cell," said Pfeffer, "and he's transforming our understanding of how ribosomes work."

Once made, proteins can fold into a dizzying combination of three-dimensional structures that allow them to carry out complex tasks within the cell. In 2005, associate professor of biochemistry, Pehr Harbury, PhD, was recognized with both a MacArthur Fellowship and a NIH Director's Pioneer Award for his studies of protein structure and iterative small molecule design using unique DNA tags, encapsulating billions of years of evolution in a single test tube. Harbury uses high-throughput mass spectrometry to analyze shifting, dynamic protein structures inside living cells as they catalyze reactions, and he's designed a computer program capable of accurately predicting the structure of proteins that have never existed in nature.

"We have so many young stars in the department now," said Berg. "Any biochemistry department in the world would be happy to have these researchers in their ranks."

Researchers in the laboratory of James Ferrell, PhD, professor of biochemistry and of chemical and systems biology, study how regulatory proteins guide the cell cycle as a way to learn more about biological oscillations, while Philip Beachy, PhD, professor of biochemistry and of developmental biology, focus on how protein members of the Hedgehog family — which govern critical developmental pathways — affect cellular differentiation and contribute to the development of cancers. He and his lab members recently showed that a single cancer stem cell and its progeny can quickly colonize the bladder lining, crowding out normal cells with others primed to invade surrounding tissue and become invasive bladder cancers.

Assistant professor of biochemistry and of medicine Rajat Rohatgi, MD, PhD, also studies the intersection between development and cancer, using novel optical probes to visualize the dynamics of signaling within cells. His group aims to find ways to manipulate these pathways to create new cancer therapies. And other researchers, like those in Suzanne Pfeffer's laboratory, study proteins called Rab GTPases that are the master

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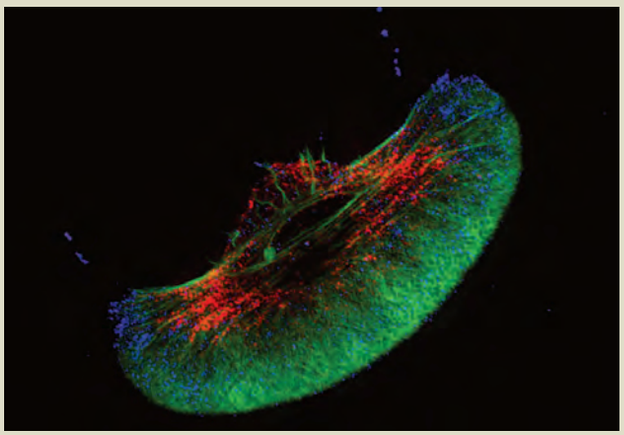
traffic controllers that govern how receptor proteins are transported and localize to specific intracellular membranes. These receptors govern countless biological processes.

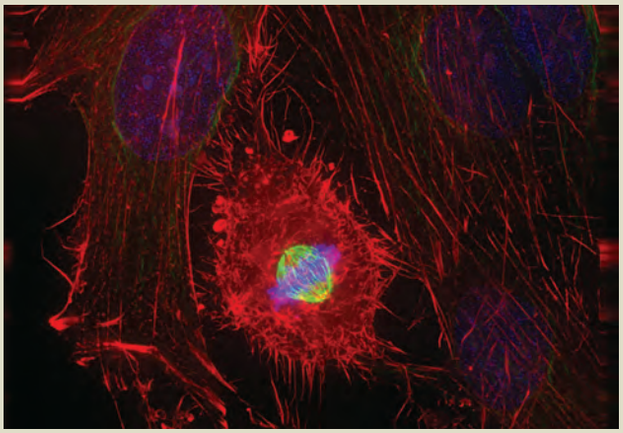
James Spudich, PhD, the Douglass M. and Nola Leishman Professor of Cardiovascular Disease was awarded the Albert Lasker Basic Medical Research Award in 2012 for his work in understanding the precise molecular interactions between actin and myosin, coupled with the expenditure of the energy molecule ATP, enables muscles to contract and cells to transport molecular payloads and other molecules via an actin cytoskeleton.

Food-borne bacteria like *Listeria monocytogenes*, which can cause serious illness in pregnant women, young children and others with weakened immune systems, and *Shigella flexneri*, one of the leading bacterial causes of diarrhea in the world, also capitalize on the actin cytoskeleton to move within an infected cell and even to spread to neighboring cells. Julie Theriot, PhD, a professor of biochemistry and of microbiology and immunology, received a MacArthur Fellowship in 2004 for her studies of the biochemistry and biomechanics of bacterial spread among cells and the evolutionary origin of pathogenesis. Theriot is also a Howard Hughes Medical Institute investigator.

The evolution of research in the department also includes investigations into how organisms including humans develop from a fertilized egg into a smoothly functioning unit that can walk, eat, think and breathe.

"The original focus of our department was the study of DNA replication and how chromosomes divide," said A. Dale Kaiser, PhD, emeritus professor of biochemistry and one of the department's founding members. "Now we're focused more on understanding developmental pathways. This is a very interesting problem. Whereas a lot of the ideas about DNA replication could be predicted once the chemistry and structure of the molecule was understood, that's not true in development. It's in no way obvious how cells





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of the developing trachea slither around one another to form the neuroepithelium capable of sensing a lack of oxygen."

Former chair of the department, Mark Krasnow, MD, PhD, professor of biochemistry and a Howard Hughes Medical Institute investigator, studies not just how lungs develop, but also what goes awry in lung disease and cancer in mice and humans. He's also spearheaded investigations into the genetics and genomics of one of the world's smallest primates, Madagascar's grey mouse lemur. He and colleagues in Madagascar are working together to identify key genes in the animal's development, physiology and behavior to better understand the diseases, ecology and conservation biology efforts that affect the country's lemurs. Much of the work is being done in Madagascar, including a citizen science project in which the researchers are helping to revise the curriculum of the local high school to include active exploration of mouse lemurs and other wonders of the local environment right outside the schools.

"Looking at the list of research interests of our faculty members, it would be hard to find anyone doing what would have been considered traditional biochemistry when we founded the department," said Berg.

Department of Biochemistry

Row 1: (L to R): R. Das, R. Rohatgi, D. Kaiser, P. Berg, O. Brandman, R. Lehman, R. Baldwin, P. Kim, S. Pfeffer **Row 2:** D. Nunez, R. Sircar, J. Li, G. Pusapati, C. Petersen, W. Kong, S. Cao, R. Gomez, T. Habebo, J. Ferrell, J. Salzman, V. Lamba **Row 3:** M. Pinney, P. Cheung, J. Bell, M. Williams, S. Barrett, B. French, L. Harris, M. Gebala, B. Alford **Row 4:** J. Aniversario, K. Amberg-Johnson, J. Gisselberg, M. Boucher, J. Jenkersen, E. Moscatelli, I. Jarmoskaite, S. Yang, B. Stoner, R. Norris, R. Taylor, E. Yeh, M. Kinnebrew, T. Johnson **Row 5:** L. Szabo, J. Metzger, G. Liew, C. Brown, J. Park, S. Nag, F. Ortega, P. Vaidyanathan, J. Caldwell, P. Lee, P. Saha, J. Lee, R. Dubey **Row 6:** J. Mesa, M. Morck, Q. MacPherson, G. Luchetti, A. Nabhan, D. Jukam, A. Natarajan, C. Chan, C. Cheng, L. Kozar, E. Koslover, C. Khosla, J. Mathews **Row 7:** D. Riordan, P. Cordero, J. Ouadah, B. Allred, F. Yabukarski, T. Yewdell, F. Westhorpe, A. Straight, M. Footer **Row 8:** M. Miell, C. Geniesse, B. Schmidt, P. Harbury, C. Sitron, D. Weigt, C. Galvin, G. Camus, L. Milla

In 1959 the Stanford University School of Medicine was undergoing its own transition as it moved from downtown San Francisco to join the main Stanford campus on "The Farm," a much more rural location in Palo Alto. The move was instigated by a belief on the part of Henry Kaplan, MD, then a leading radiologist, and Frederick Terman, Sc.D., an electrical engineer often credited with being the 'father of Silicon Valley' that the coming decades would focus heavily on advances in biology and medicine and that Stanford University would benefit by more closely integrating its medical school with the rest of the faculty and departments.

In addition to attracting the founding members of the biochemistry department, the concept led to a dynamic, active relationship between the medical school and fields as diverse as chemistry, physics, electrical engineering and computer science. Researchers are encouraged to actively pursue collaborations with other disciplines.

"All our faculty members are collaborating with others outside the department," said Pfeffer. "I'm working with Hideo Mabuchi, PhD, in applied physics, for example. Additionally, many of our newest members have been trained in other disciplines, and several of our faculty have joint MD and PhD degrees."

In fact, the Beckman building itself, which houses the departments of biochemistry, developmental biology, molecular and cellular physiology, as well as investigators for the Howard Hughes Medical Institute, was designed in the late 1980s to encourage interaction within and among departments. And in the late 1990s James Spudich and Stanford physicist Steven Chu, PhD, launched Stanford's Bio-X program, which brings together researchers of different skills and disciplines to devise creative ways to approach biological questions.

"The department is very interdisciplinary," said Shapiro. "They are applying biochemistry to critical questions in cell biology in much broader ways than ever before."

For example, Ellen Yeh, MD, PhD, is an assistant professor in biochemistry, in microbiology and immunology and in pathology. Her work focuses on the apicoplast, which is an organelle unique to plasmodium parasites such as those that cause malaria and a key target for anti-malarial drugs.

With the exception of Melvin Cohn, who left Stanford to join the fledgling Salk Institute for Biological Studies in La Jolla, California in 1963, and Kornberg, who died in 2007, all of the original founding members are still actively involved in the department. Baldwin, Berg, Lehman, Kaiser and Hogness have since retired, but frequently attend the department's Wednesday lunches, where researchers gather to discuss their latest findings.

"We continue to tell each other what we are doing, and what we are thinking about," said Pfeffer, "just like we've done every Wednesday for the last 50 years. It's a wonderful opportunity for suggestions and feedback."

Clearly the legacy of sharing and collaboration lives on, even as the department continues to evolve and change with the addition of younger members. Pfeffer credits the original group of seven for not just launching the department, but also for actively moving it forward through the decades.

"There was always the possibility that having such a famous group of founding fathers could crimp our style and stifle our growth, but that's absolutely not been the case," said Pfeffer. "Instead they've been tremendously supportive of our newest members and of the ways in which the field has evolved during the past decades. The future is very exciting."

Photo credits*:*

Page 9 (left): Albert Lu and Suzanne Pfeffer showed that the Golgi-localized protein, RhoBTB3 (green), targets Cyclin E for ubiquitylation and regulates Golgi morphology. A RhoBTB3-depleted cell at the bottom of the image shows a larger nucleus (blue), fragmented Golgi (green), and higher Cyclin E levels (red) compared to control cells above. *Image courtesy of* Albert Lu.

Page 10 (left): Villin headpiece. Villin is an F-actin bundling protein involved in the maintenance of microvilli of the absorptive epithelia. The 76 amino acid residue headpiece is the smallest known example of a cooperatively folded domain of a naturally occurring protein. *Image courtesy of* Robert (Buzz) Baldwin.

Page 10 (right): CryoEM structure of the 60S ribosome bound to Rqc2p (yellow). The nascent chain is in green, A-site tRNA cyan, P-site tRNA in blue. Rqc2p recruits tRNAs charged with alanine and threonine to the ribosome to facilitate elongation of the nascent chain. *Image courtesy of* Onn Brandman.

Page 13 (left): Zebrafish epidermal keratocyte. Green is filamentous actin, redi is myosin II, blue is phosphotyrosine (adhesion complexes). *Image courtesy of* Sunny Lou.

Page 13 (right): HeLa (human cervical adenocarcinoma) cell in mitosis. Acquired by first year students as part of the Biochemistry Department Bootcamp to train incoming students in optical microscopy - a training program run in conjunction with the Cell Sciences Imaging Facility (CSIF). *Image courtesy of* Aaron Straight