IN JUNE 1960, VOTERS IN THE CITY OF SAN DIEGO APPROVED A GIFT OF LAND TO HELP launch a promising new enterprise. By today's standards, the vote was little more than an act of faith. The venture, an institute for basic biological research, would not officially exist on paper for another six months, and it would be six years before the iconic twin buildings opened in the striking coastal location.

Nonetheless, the institute—the brainchild of polio vaccine pioneer Jonas Salk—was born of an audacious vision, one with the potential to reshape San Diego's physical and intellectual landscape and transform the way scientific research was carried out. It may have been a gamble, but its potential for payoff, both in advancing human health and in elevating the city's stature, was enormous, and that prospect quickly garnered the enthusiastic support of farsighted San Diego civic leaders and the March of Dimes.

Now, five decades later, we are celebrating the 50th anniversary of the fulfillment of that vision: the Salk Institute for Biological Studies. Widely recognized as one of the world's most influential centers for basic scientific research, the Institute has built its stellar reputation on the same cornerstones of imagination and daring upon which it was founded. In its latest international ranking, *Science Watch*, which measures the impact of research published worldwide, ranked the Institute number one in the category of Neuroscience and Behavior and number two in Molecular Biology and Genetics. When Joseph Ecker mapped the human epigenome, the achievement was named the Number 2 scientific discovery of the year by *Time* magazine.

Accompanying these achievements has been an exceptional level of philanthropy, which allowed us to broaden the Institute's research focus but also to deepen our commitment to existing areas. The Waitt Family Foundation contributed $20 million to fund the creation of an Advanced Biophotonics Center. Thanks to a $5 million gift from the Glenn Foundation for Medical Research, we launched a new center for studying the molecular basis of aging. A new Center for Nutritional Genomics was established with $5.5 million from the Helmsley Trust. A $3.8 million grant from the National Eye Institute (NEI) of the NIH established one of seven NEI-designated centers focused exclusively on basic research into vision—the first basic science facility created by the NEI in nearly a decade and the only one of its kind in the San Diego area. The Nomis Foundation, established by former trustee G.H. "Heini" Thyssen, made a gift of $11.5 million to launch the Immunobiology and Microbial Pathogenesis program.

Our scientists' extraordinary productivity has been recognized with some of the most prestigious awards in the world over this past year. Nonresident fellow Elizabeth Blackburn won the Nobel Prize in Medicine and Physiology, and Marc Montminy was elected to the National Academy of
Sciences. Reuben Shaw was named an Early Career Scientist by the Howard Hughes Medical Institute. Fred Gage was one of only three Americans elected an Associate Member of the European Molecular Biology Organization. Tatyana Sharpee was awarded the W. M. Keck Foundation Research Excellence Award and was one of only six scientists named 2009 McKnight Scholars, a program that supports young neuroscientists working on problems that, if solved at the basic level, would have immediate and significant impact on clinically relevant issues. Clodagh O’Shea received the Sontag Foundation’s 2009 Distinguished Scientist Award, which recognizes and supports the work of outstanding early career scientists whose research has the potential to generate new knowledge relating to brain tumors. Inder Verma received the 2009 Outstanding Achievement Award for the American Society of Gene Therapy and was also named the first holder of the Salk Institute’s Irwin Mark Jacobs Chair in Exemplary Life Sciences. Tony Hunter was appointed as the inaugural holder of the Frederick W. and Joanna J. Mitchell Chair, created in memory of their daughter Marian Mitchell. Joanne Chory was chosen as the inaugural holder of the Howard H. and Maryam R. Newman Chair and William R. Brody was appointed the inaugural holder of the Irwin M. Jacobs Presidential Chair, endowed by Qualcomm and its employees.

In recognition of their exceptional scientific achievements, three faculty members were promoted during the last year: Jeffrey Long and Martin Hetzer from assistant to associate professor and Richard Krauzlis from associate to full professor.

To ensure this level of accomplishment into the future, we welcomed three outstanding new assistant professors to our distinguished faculty: Sreekanth Chalasani, Björn Lillemoeier, and Ye Zheng.

While scientific breakthroughs are common around the Salk Institute, there is nothing commonplace about the people or the science conducted here. This year, as we contemplate the power of one person’s vision to inspire paradigm-changing scientific breakthroughs, we are also celebrating the determination of others to help bring that vision to fruition and to grow and sustain it for the betterment of humankind. Nowhere is that impetus more evident than in this Scientific Report, which documents key research findings from each faculty member’s lab.

We hope you will enjoy reading about some of the Salk Institute’s recent research highlights and new directions, and we look forward to celebrating our 50th anniversary with you throughout the year.

William R. Brody, M.D., Ph.D.
Irwin M. Jacobs Presidential Chair

William R. Brody, M.D., Ph.D.
Irwin M. Jacobs Presidential Chair

William R. Brody, M.D., Ph.D.
Irwin M. Jacobs Presidential Chair

William R. Brody, M.D., Ph.D.
Irwin M. Jacobs Presidential Chair

Marsha Chandler, Ph.D.
Executive Vice President

Marsha Chandler, Ph.D.
Executive Vice President

Marsha Chandler, Ph.D.
Executive Vice President
Hope lies in dreams, in imagination and in the courage of those who dare to make dreams into reality.

JONAS SALK
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An acclaimed physician-scientist, entrepreneur and university leader, William R. Brody joined the Salk Institute for Biological Studies on March 2, 2009 after 12 years as president of The Johns Hopkins University.

A native of Stockton, CA, Dr. Brody received his bachelor’s and master’s degrees in electrical engineering from the Massachusetts Institute of Technology. He received his doctorate (also in electrical engineering) and his medical degree (M.D.) from Stanford University. He continued his post-graduate training in cardiovascular surgery and radiology at Stanford, the National Institutes of Health, and the University of California, San Francisco.

Between 1977 and 1986, he held appointments at the Stanford University School of Medicine, including professor of radiology and electrical engineering; director, Advanced Imaging Techniques Laboratory; associate professor of radiology and electrical engineering; and director of research laboratories, division of diagnostic radiology. In 1987 he moved to The Johns Hopkins University School of Medicine where he held several appointments, including the Martin Donner Professor and director of the department of radiology; professor of electrical and computer engineering; professor of biomedical engineering; and radiologist-in-chief of The Johns Hopkins Hospital. After a two-year stint as provost of the Academic Health Center at the University of Minnesota, he was named president of The Johns Hopkins University in 1996.

Renowned for his achievements in biomedical engineering and the field of medical instrumentation, Brody is a member of the National Academy of Engineering and the Institute of Medicine. He has authored more than 100 articles in U.S. medical journals, holds two U.S. patents in medical imaging and is the co-founder of three medical device companies. Brody has made significant contributions to the fields of medical acoustics, computed tomography, digital radiography, and magnetic resonance imaging.

Brody is a member of the policy-making Scientific Management Review Board of the National Institutes of Health and has been a national figure in efforts to encourage innovation and strengthen the U.S. economy through investments in research and education. Most recently, he has written and spoken extensively around the country to promote a fuller discussion of health care reform.
The founding Fellows, resident and non-resident, in February 1966.

From left to right: Francis Crick, Edwin Lennox, Jacques Monod, Jonas Salk, Leslie Orgel, Melvin Cohn, Salvador Luria, Jacob Bronowski, Renato Dulbecco
One of the world’s pioneers in genetics and molecular biology, Sydney Brenner has devoted his career to conducting groundbreaking basic research and promoting science around the world.

Born in Germiston, South Africa, Brenner earned degrees in medicine and science in 1947 from Johannesburg’s University of Witwatersrand before moving to England, where he received a doctorate in chemistry from Oxford University in 1954 and began taking part in leading-edge research into DNA, molecular biology, and developmental genetics. By 1956, he was sharing an office in Cambridge with the late Francis Crick, an alliance that lasted 20 years. Along with Crick, Brenner proposed that a single amino acid was coded by three nucleotides, a triplet, of RNA. He further demonstrated that the triplet combination of uracil, adenine, and guanine—the “nonsense,” or “stop,” codon (a term he coined)—signifies the end of a translation process. In the early 1960s, Brenner co-discovered the existence of messenger RNA and demonstrated that the nucleotide sequence of mRNA determines the order of amino acids in proteins. This work led to his first Lasker Award in Basic Medical Research; he later received a second Lasker Award in honor of his outstanding lifetime achievements.

It was Brenner’s pioneering research with Caenorhabditis elegans, however, that led to his Nobel Prize. Beginning in 1965, he began to lay the groundwork to make C. elegans, a small, transparent nematode, a major model organism for genetics, neurobiology, and developmental biology research. As a direct result of his original vision, this tiny worm became the first animal for which the complete cell lineage and entire neuronal wiring were known. Today more than 1,000 investigators are working on C. elegans, and Brenner’s work was further honored when a closely related nematode was named Caenorhabditis brenneri.

Beyond his own research, Brenner has been a driving force in advancing scientific research worldwide. He was instrumental in guiding Singapore toward biomedical research and founded the Molecular Sciences Institute in Berkeley, CA, in 1996, serving as its president and director of science. He is also founding president of the Okinawa Institute of Science and Technology. Brenner, who previously had served as a scholar-in-residence at The Scripps Research Institute, has been a member of the Salk Institute faculty since 2000.
A Founding Fellow at the Salk Institute, Renato Dulbecco has made fundamental contributions to understanding the uncontrolled growth of cells that occurs in cancer. Dulbecco, a native of Catanzaro, Italy, received his M.D. from the University of Turin in 1936; in 1947, following World War II, he came to the United States, initially to Indiana University, where he worked on bacteriophages. Two years later, he moved to the California Institute of Technology; there he started work on animal oncoviruses. In 1963, he joined the nascent Salk Institute.

Dulbecco's studies of viruses that cause disease led him to develop a method, used universally since then, to assess their activity. While working on viruses that cause tumors in animals, he developed what is now a widely used technique to study their effects using cells grown in laboratory containers. Best-known of Dulbecco's discoveries was that tumor viruses cause cancer by inserting their own genes into the chromosomes of infected cells—a finding that was one of the first clues to the genetic nature of cancer and led to his Nobel Prize in 1975. Dulbecco's study provided a basis for precise understanding of the molecular mechanisms by which cancer cells propagate.

Dulbecco subsequently turned to the study of the origins and progression of tumors of the breast, using monoclonal antibodies, which can identify cells by their chemical signatures, to characterize the tumor cells.

In 1972, he moved on to the Imperial Cancer Research Fund in London (now the Cancer Research UK London Research Institute), where he served as deputy director of research until 1977, when he returned to the Salk Institute as a distinguished research professor. He served as president of the Institute from 1988–93.

In 1986, Dulbecco initiated the idea of studying all human genes, helping to launch the worldwide Human Genome Project. Currently, he studies the genes that are important in the normal development of the breast and in the tumors that arise in it.

The recipient of numerous honors in addition to the Nobel Prize, including the 1964 Lasker Award, Dulbecco is a member of the National Academy of Sciences, the Royal Society of London, and the Academia dei Lincei of Italy.
Considered the founder of the field of neuroendocrinology, Roger Guillemin, M.D., Ph.D., is a scientific pioneer whose research into brain hormones has led to treatments for disorders ranging from infertility to pituitary tumors.

A native of Dijon, France, Guillemin graduated from the University of Lyon medical school in 1949, then pursued an interest in endocrinology at the University of Montreal’s Institute of Experimental Medicine and Surgery, receiving his Ph.D. in 1953 and subsequently accepting an assistant professorship at Baylor College of Medicine in Houston, Texas.

In 1969, Guillemin made his first groundbreaking discovery. Although researchers had long suspected that the brain controls the function of endocrine glands, they did not know how these interactions occurred throughout the body. They believed the brain’s hypothalamus released a substance that activated these glands, but no one could find evidence for it. After manipulating 1.5 million sheep brains, Guillemin’s group eventually isolated a molecule called TRH (thyrotropin-releasing hormone), which ultimately controls all the functions of the thyroid gland. In the following years, he and his colleagues isolated other molecules from the hypothalamus that control all functions of the pituitary gland—for instance, GnRH (gonadotropin-releasing hormone), a hypothalamic hormone that causes the pituitary to release gonadotropins, which in turn trigger the release of hormones from the testicles or ovaries. This discovery led to advancements in the medical treatment of infertility and is also used to treat prostate cancer.

In 1970, Guillemin joined the Salk Institute to head the newly established Laboratories for Neuroendocrinology, where he and his group discovered somatostatin, which regulates the activities of the pituitary gland and the pancreas and is used clinically to treat pituitary tumors. He was among the first to isolate endorphins, brain molecules that act as natural opiates, and his work with cellular growth factors (FGFs) led to the recognition of multiple physiological functions and developmental mechanisms, including molecules such as inhibins and activins.

The recipient of numerous honors, Guillemin was awarded the 1977 Nobel Prize for Medicine or Physiology for his work with hypothalamic hormones. He is also a member of the National Academy of Sciences and the American Academy of Arts and Sciences, and has received the Lasker Award in Basic Sciences and the National Medal of Science, among many others. He was selected for the Hall of Honor at the National Institute of Child Health and Human Development (NICHD) for exceptional contributions to advancing knowledge and improving maternal and child health, and is listed as one of the most “highly cited” scientists from 1981–99 by the Institute for Scientific Information. As interim president of the Institute from 2007–09, he was instrumental in bringing art exhibits to the Salk Institute, fulfilling Jonas Salk’s vision of a facility that blends science and art.
We live in a dynamic environment. Optimal encoding of sensory information requires that sensory systems be continuously tuned to the prevailing environment, much like fine-tuning our cars for the current driving conditions. Yet while it is important that sensory information be represented with high fidelity, the brain has only limited resources available. Albright and his team are interested in how neural systems reconcile these conflicting demands in the primate visual system.

Using their working hypothesis that sensory systems resolve the dilemma by way of compromise, the scientists examined the effects of sensory resource reallocation—a phenomenon commonly known as sensory adaptation—on the perception of visual motion. The initial theoretical work led to predictions about the patterns of perceptual and neuronal change expected in a system that reallocates its resources to dynamically optimize perception in a changing environment. Behavioral studies put these predictions to the test by revealing the perceptual changes induced by adaptation. Physiological studies uncover the neuronal mechanisms of sensory reallocation.

The results reveal that sensory systems enhance the neuronal representation of those aspects of the environment that are frequently encountered and/or are significant for successful behavior. The enhancement comes at the cost of reduced sensitivity to those stimuli that are less commonly encountered. Specifically, the observed perceptual recalibration is mediated by changes in motion-selective neurons in the visual cortex. Some of the neurons change their sensitivity to particular stimuli, and others shift their sensitivity to other stimuli. These findings indicate that the neuronal mechanisms of perception can only be understood by studying how sensitivity is dynamically distributed across neurons tuned to the entire range of visible stimuli.

Taken together, the observations of Albright and his colleagues reveal that sensory processing is markedly adaptable. This adaptability enables us to optimize perception and behavior in a world that presents us with varying sensory demands, caused by changes in the environment, behavioral goals, and age-related decline in sensory function.
Ursula Bellugi
Professor and Director
Laboratory for Cognitive Neuroscience

“Williams syndrome is a perfect example of where a genetic predisposition interacts with the environment to sculpt the brain in unique ways. It provides a unique window for understanding how missing genes and the resulting changes in brain structure and function ultimately shape behavior.”

Autism spectrum disorders (ASD) and Williams syndrome (WS) are both neurodevelopmental disorders, but their manifestations couldn’t be more different. While autistic individuals live in a world where objects make much more sense than people do, people with WS are social butterflies who bask in other people’s attention. Despite myriad health problems, generally low IQs, and severe spatial problems, they are irresistibly drawn to strangers, look intently at people’s faces, remember names and faces with ease, and are colorful and skillful storytellers.

In recent studies, Ursula Bellugi and her team compared brain response patterns linked to face and language processing between individuals with ASD and WS to gain novel insights into their polar opposite social and communication profiles. To this end, they measured so-called event-related potentials, or ERPs for short, which are brainwaves directly reflecting electrical activity of the brain occurring in response to discrete events. They found that when viewing human faces, individuals with WS respond with a unique brain signature not found in ASD or any other group.

The observed differences may underlie gaze avoidance in ASD and reflect the increased interest in and attention to human faces in WS. Similarly, individuals with WS exhibited an abnormally large ERP response when a typical sentence finishes with an odd ending (“I take my coffee with sugar and shoes”), indicating that they are particularly sensitive and attuned to semantic aspects of language. In contrast, individuals with ASD did not show this negativity, suggesting that the inability to integrate lexical information into the ongoing context may underlie their communicative and language impairments.

To gain a better understanding of the neural and genetic correlates of social behavior in different social phenotypes, Bellugi’s team is now integrating these findings with the exquisitely mapped genetic profile of WS. They are hypothesizing that specific genes in the WS region may be involved in the dysregulation of specific neuropeptide and hormonal systems, which could explain the observed hypersocial behavior.

For more information, please visit salk.edu/faculty/bellugi.html
Ever since the first adult cells were converted into induced pluripotent stem cells (iPSCs), they have generated excitement as an alternative to embryonic stem cells and a potential source for patient-specific stem cells. Unfortunately, reprogramming adult cells into iPSCs is a slow, inefficient, and costly process and carries the risk of cancer, limiting the cells’ therapeutic value. Two recent studies in Izpisúa Belmonte’s lab, however, offer the prospect of safer, faster, and more efficient approaches to coaxing cells back in time.

The most widely used technology involves the forced expression of four transcription factors in fully committed adult cells: Oct4, Sox2, Klf4, and c-Myc. Because only a tiny fraction of cells transmogrify into iPSCs that look and act like embryonic stem cells, Izpisúa Belmonte wondered whether the process used to reprogram the cells was inducing a response that stopped them from growing. Izpisúa Belmonte and his team showed that adding Klf4 and c-Myc, which are oncogenes, activated the pathway for the tumor suppressor p53. In cells genetically engineered to lack p53, reprogramming efficiency increased at least tenfold, clearly demonstrating the important role that p53 played in reining in cells trying to revert back into a stem-like state. Because iPSCs generated with the full complement of reprogramming factors can turn malignant, Izpisúa Belmonte and his team also tried reprogramming mouse cells lacking p53 using only Oct4 and Sox2. The cells readily converted into iPSCs and gave rise to healthy, full-term mice that were able to reproduce, passing the ultimate test for pluripotent embryonic stem cells.

In related work, Izpisúa Belmonte’s group set out to transform immunologically immature hematopoietic stem cells isolated from umbilical cord blood into iPSCs. They not only successfully converted them using only Oct4 and Sox2, but did so in less time than any previously published methodology. The resulting iPSCs were indistinguishable from human embryonic stem cells and passed all standard tests for pluripotency, establishing the possibility of a comprehensive bank of tissue-matched, cord blood–derived stem cells.

For more information, please visit salk.edu/faculty/belmonte.html
Suzanne Bourgeois
Professor and Founding Director
Regulatory Biology Laboratory

“Today we take the Salk Institute and its success for granted. It is edifying—and makes a good story—to explore what it took to get us here and to be what we are: the idea, the people, the circumstances, the location, the building.”

After a scientific career researching bacterial cell regulation and gene expression in cancer cells, Bourgeois has recently turned her attention to the history of science—specifically, the early history of the Salk Institute. Because she was a witness to the Institute’s history since before its inception, Bourgeois is uniquely qualified to bring that story alive. She also had the privilege, while working in New York and Paris in the 1950s and 1960s, of knowing many of the pundits of molecular biology, some of whom became the founders of the Salk Institute. She therefore has begun work on a book-length history of the Salk Institute, which will be a well-documented personal chronicle that is based on extensive research encompassing archival material, interviews, and her own diaries.

Jonas Salk’s original concept for an institute evolved under many influences, including those of the physicists Robert Oppenheimer and Leo Szilard. In the late 1950s and early 1960s, he contacted the scientists who were to become the Institute’s founders: Melvin Cohn, Francis Crick, Renato Dulbecco, Edwin Lennox, and Jacques Monod. At the same time a mathematician and humanist, Jacob Bronowski, joined the group, and another mathematician and remarkable man became the first chairman of the Board of Trustees: Warren Weaver, who coined the term “molecular biology.” The success of the polio vaccine earned Jonas Salk the respect and friendship of Basil O’Connor, the founding president of the March of Dimes, whose support ultimately made the Salk Institute a reality.

The Institute’s founders belonged to the generation of World War II and the Manhattan Project and, afterward, the Cold War. The original faculty and several of the early presidents and trustees had actively participated in those events, which shaped what they wanted to do, how they operated, and how they saw the future. Most importantly, that background distinguished them as members of an impressive network of outstanding achievers. That legacy of accomplishment remains the foundation of the Salk Institute to this day.
Neuroscientists have identified dozens of different neuronal cell types in the brain that work together in distinct networks. But the circuits are intermingled, and even neighboring neurons of the same type differ in connectivity and function. Without access to a “wiring diagram”—a map of the neuronal connections—attempting to grasp how the brain lets us understand language, recognize faces, and schedule our day is akin to trying to discern how a computer chip works simply by looking at it.

Recently, Callaway and his team of researchers have jumped what many believe to be a major hurdle to preparing that diagram: figuring out single connections between neurons. They successfully modified the rabies virus, turning it into a tool that can cross the synaptic space of an infected nerve cell just once to identify all the neurons to which it reaches out. Viruses that naturally spread between neurons have previously been used to outline the flow of nerve cell communication, but without a way to stop them in their tracks, over time, they will light up the whole brain.

Callaway’s team deleted a gene required by the virus to spread across synapses, marooning the virus inside a cell. Supplying the missing gene in that same cell, however, allowed the virus to slip into all the cells that were directly connected to it. Since these neighboring cells lacked the gene supplied in the first cell, the virus was now permanently stuck. To restrict the viral infection to a certain cell type or even to single cells, they festooned these neurons with avian surface molecules and equipped the rabies virus with a homing device specifically for neurons “disguised” as bird cells.

While the first, published experiments were conducted using slices of brain, more recent studies are using transgenic mice to allow targeting of a specific class of neurons. The rabies virus then identifies the inputs to just that one neuron class. With these tools, the wiring map can then be constructed step by step as subsequent populations of cells are visualized. And once scientists can identify a neural circuit, they can then correlate it with such brain functions as perception and behavior.

For more information, please visit salk.edu/faculty/callaway.html
Our brain contains roughly 100 billion cells, each connected through thousands of contact points adding up to at least a quarter of a million miles of wiring—enough to reach from here to the moon. This marvel of evolutionary engineering allows us to navigate an ever-changing environment, to learn, and to remember, but its stunning complexity makes it difficult to trace how information travels from one neuron to another. Chalasani uses the nematode *Caenorhabditis elegans* as a model to understand how neural circuits transform sensory input into behaviors. *C. elegans* has exactly 959 adult somatic cells and 302 neurons connected by precisely 6,000 electrical and chemical connections. Despite its simplicity, this animal displays a number of sophisticated behaviors, making it the perfect model to explore how a simple, well-defined nervous system is able to integrate information from multiple sensory neurons and remember it for long periods of time.

Wild-type worms spend about 15 minutes searching for food when they are moved from a plate with food to a food-free plate. The duration of this search time is a function of the quality of the food and the amount of time they have spent feeding on it before being moved (more nutritious food or longer times elicit longer searches and vice versa). Chalasani found that the worms’ main olfactory neurons, known as *AWC* neurons, are primarily responsible for this food-seeking behavior. They are activated upon removal of a stimulus and respond with the release of chemical signals, which in turn activate one target neuron (*AIB*) and inhibit a second target neuron (*AIY*). These opposite connections resemble the first connections in the visual processing system in our own eyes—photoreceptors connecting to “ON” and “OFF” bipolar cells.

In the future, Chalasani plans to extend his studies to zebrafish larvae to test whether vertebrate and invertebrate circuits use similar mechanisms to process information, hoping to gain new insight into how the human brain functions.

For more information, please visit salk.edu/faculty/chalasani.html
About 1.25 million retinal ganglion cells—each of which views the world only through a small jagged window called a receptive field—collectively form the seamless picture we rely on to navigate our environment. There are 20 or so distinct ganglion cell types, and because the receptive fields of each type form a regular lattice covering visual space, each lattice is thought to transmit a complete visual image to the brain. Within any given regular lattice, however, the individual cells’ receptive fields have irregular and inconsistent shapes, which could potentially result in patchy coverage of the visual field and pose a problem for high-resolution vision.

To understand how the visual system overcomes this problem, Chichilnisky and his team used a microscopic electrode array to record the activity of ganglion cells in isolated patches of retina. After monitoring hundreds of ganglion cells over several hours, they were able to distinguish between different ganglion cell types based on their light response properties. With this information, they were able to deduce that receptive fields fit together like pieces of a puzzle, preventing “blind spots” and excessive overlap that could blur our perception of the world.

This finding brings scientists one step closer to the ultimate goal of vision research: the development of visual prosthetics that could one day restore vision to people whose retina has been damaged by disease (e.g., macular degeneration or retinitis pigmentosa). In principle, retinal implants could bypass the damaged retina with the help of tiny electrode arrays that mimic the electrical signals sent to the brain in response to light. In order to engineer these prosthetics, however, scientists need to understand how neurons in the retina function as a network to produce an image. Chichilnisky’s work is focused on building the foundation for that understanding, with an emphasis on distinguishing one cell type from another when studying sensory encoding by a population of neurons.

For more information, please visit
salk.edu/faculty/chichilnisky.html

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E. J. Chichilnisky
Associate Professor
Systems Neurobiology Laboratories

“Visual information is transmitted from the eye to the brain in just 1.25 million optic nerve fibers—about as many fibers as there are pixels in a cheap digital camera. Somehow we exploit this information to navigate busy highways, something even the most advanced robots cannot do. I want to decipher how the retina, the tissue lining the back of the eye, encodes visual information so the brain can use it to produce visual experience.”
Senyon Choe

Professor
Structural Biology Laboratory

“We are interested in understanding how biological messages are written and delivered between cells by messenger molecules in the body. The two messenger systems we are focusing on are called ion channels (for e-mails) and protein hormone receptors (for snail mail). By visualizing these messengers to better understand how such messages are coded for specific delivery, we can create brand-new messages on our own.”

The premise that “form follows function” became a mantra for numerous leading architects and industrial designers during a good part of the last century. In biology, evolution operates according to a similar premise because forms with better functionality are likelier to be selected. Trying to understand the relationship between a molecule’s fine structure and the functions it carries out, Choe and his colleagues use x-ray crystallography and NMR spectroscopy to zoom in on ion channels and receptors in the cell membrane to visualize how they interact with messenger proteins. Recent work focused on analyzing the three-dimensional structure of a whole protein complex to illustrate how TGF-beta, a messenger molecule that plays a role in cancer, the immune system, and heart disease, binds to its receptor molecules on specific target cells to instruct them to do its bidding. An extension of this work explores the possibility of designing new messages to instruct cells to carry out non-natural processes such as coaxing differentiated cells back into an immature, pluripotent state. These types of newly created messages will have tremendous clinical potential as guiding molecules.

Receptors and ion channels are both membrane-embedded proteins, which are hard to produce and hence notoriously difficult to study. Therefore, Choe and his team are keenly interested in developing new techniques that allow them to penetrate the elusive world of membrane proteins, which keep the lines of communication open between cells and thus are popular targets for the majority of blockbuster drugs. Lately, they discovered a “partner” molecule called Mistic that allows the widespread production of membrane proteins, enabling scientists to determine their atomic structure and design drugs that interfere with disease processes involving membrane proteins. Choe’s group has also done pioneering work on the molecular structure of an ion channel, which is important to many physiological functions ranging from heart rate to nerve cell communication. By understanding the atomic details of how channel proteins assemble into an ion-conducting pore and how such a pore is regulated by biological signals, scientists will be more likely to understand fundamental mechanisms of various neurological disorders and come up with a new strategy to treat them.

For more information, please visit salk.edu/faculty/choe.html
Stuck where the seed germinates, plants have to make the best of their real estate. They rely on an impressive arsenal of light-sensitive photoreceptors to decide when to germinate and flower to ensure the next generation of seeds. For more than 20 years, the Chory laboratory has studied the signaling pathways plants use to detect changes in the sunlight that hits their leaves, not only when seasons change but also when they grow in shady, crowded conditions. During the course of their studies, she and her group have assigned specific functions to a number of photoreceptors that regulate plant growth, identified components of the light signaling pathways, and shown that photoreceptors link the local light environment to hormone biosynthesis and signaling pathways within the plant.

Lately, Chory and her team have been expanding their studies to a collection of natural isolates of the thale cress Arabidopsis thaliana, which have been gathered all over the Northern Hemisphere, to investigate how genetic variation in light-sensitive pathways ensures that plants in light-starved northern latitudes are more sensitive to light than their counterparts growing in the sun-drenched Mediterranean. Using a reference strain of Arabidopsis, they have embarked on a systematic genome-wide reverse genetics screen to assess the contribution of almost every gene to light sensing and signaling in a variety of light environments.

Knowing the full spectrum of genes that can be altered in the laboratory to affect an adaptive trait—and how this compares with the genes that affect plants’ appearance in the wild—will advance understanding of how genes evolve together to make an efficient, coordinated network. This prospect is of direct and obvious importance not only to evolutionary biologists and plant breeders, but also to human biology, where similar experiments cannot be carried out. Moreover, Chory’s research may eventually enable researchers to develop plants that are particularly well-adapted to challenging environments, boosting the yields of agricultural crops.

For more information, please visit salk.edu/faculty/chory.html

Joanne Chory
Howard Hughes Medical Institute Investigator
Professor, Plant Molecular and Cellular Biology Laboratory
Howard H. and Maryam R. Newman Chair

“Our lab is interested in identifying the mechanisms that plants use to respond to changes in their environment, particularly light. Our hope is that by discovering the molecular triggers that determine whether a plant matures into a spindly or robust specimen, we can contribute to efforts to increase crop yield and alleviate hunger.”
Unable to predict which of the diverse array of pathogens it will encounter, the immune system must nevertheless respond promptly to defend the host organism from that invader. Complicating matters, pathogens evolve at a rate that is vastly more rapid than that of their invertebrate hosts. Cohn’s solution was to establish a set of basic immunologic rules based on the immune system’s evolutionary origins.

Invertebrates invented a number of biodestructive and ridding mechanisms to deal with pathogens, but their limited flexibility was not enough to keep up with the rapidly changing landscape of disease-causing agents. This created a selective pressure to invent a mechanism that generated a large and random repertoire of molecules able to recognize foreign invaders, which in turn required two new regulatory mechanisms: 1) a somatic decision mechanism to sort the repertoire into anti-self (the portion that needs to be inactivated to avoid autoimmune diseases) and anti-nonself (the activated portion that is now available to recognize invading pathogens and protect the host) and 2) a germline-selected decision mechanism to control the kind and magnitude of the immune response.

The rules Cohn developed cover most of immune behavior: the Combinatorial Theory of the nature of the repertoire, the Associative Recognition Theory of the Self-Nonself discrimination, Trauma Theory for the determination of the magnitude and effector class of the response, the B-Protecton Theory of humoral responsiveness, and the T-Protecton Theory of cell-mediated responsiveness. These theories are linked together by a computer program based on cellular automata principles (i.e., SIS, the Synthetic Immune System). Available online, the synthetic immune system allows Cohn and others to test their assumptions about how the real immune system works, facilitating understanding and predictability.

While understanding how the immune systems functions is Cohn’s primary goal, being able to predict the consequence of any given antigenic input would be an invaluable guide for the development of new vaccines, the treatment of autoimmune and allergic disorders, as well as the enhancement of the body’s response to infectious disease.

For more information, please visit salk.edu/faculty/cohn.html
Beyond age 65, the number of people with Alzheimer’s disease doubles every five years. Centenarians, however, seem to escape most common age-related diseases, including the ravages of Alzheimer’s. One of the telltale signs of Alzheimer’s disease is the buildup of toxic clumps of beta amyloid plaques in the brain. Beta amyloid production probably occurs in all brains, but healthy cells clear away excess amounts. Brains of people with Alzheimer’s disease, by contrast, are unable to control beta amyloid accumulation. The same is true for Alzheimer’s mouse models, which are genetically engineered to overproduce beta amyloid.

To determine whether modulating the aging process could influence the onset of Alzheimer’s, a team of investigators in Dillin’s lab slowed the aging process in an Alzheimer’s mouse model by lowering the activity of the IGF-1 signaling pathway—a highly conserved pathway that plays a crucial role in regulating lifespan and youthfulness across many species and is linked to extreme longevity in humans. Mice with reduced IGF-1 signaling live up to 35 percent longer than normal mice, and some very long-lived humans carry mutations in components of the IGF-1 pathway.

Dillin’s group then employed a battery of behavioral tests to find out whether it was simply the passage of time or aging per se that determined the onset of the disease. Chronologically old but biologically young animals appeared nearly normal in the tests long after age-matched, normal-aging Alzheimer’s mice exhibited severe impairments. When Dillin and his team looked at their brains, however, they found that those of the long-lived mice were riddled with highly compacted plaques.

These results clearly support the emerging theme that the plaques have a protective function and that as mice age, they become less efficient at stashing toxic beta amyloid fibrils in tightly packed aggregates. This work validates the hypothesis that genetic and pharmacologic changes to create a healthy lifespan can greatly reduce the onset of some of the most devastating diseases afflicting mankind.

For more information, please visit salk.edu/faculty/dillin.html
Sascha du Lac
Howard Hughes Medical Institute Investigator
Associate Professor, Systems Neurobiology Laboratory

“We all know that practice makes perfect, but surprisingly little is known about how new motor skills are etched into the brain. In my lab we try to understand how the brain learns from experience to improve performance.”

Motor learning is an intricate process whose outcome—a smoothly executed backhand, let’s say—is easy to spot. The underlying cell biological changes, however, are much harder to identify, not least because the neuronal circuitry for almost all behaviors is poorly understood. Trying to understand the molecular and cellular changes that underpin behavioral learning, Du Lac and her team focus on a simple type of learning: How does the brain learn to stabilize an image on the retina and use eye movement to compensate for a moving head? This so called vestibulo-ocular reflex, or VOR, needs to be fast. For clear vision, head movements must be compensated for almost immediately; otherwise, our vision would resemble an undecipherable blur. To achieve the necessary speed, the connection involves only three types of neurons: sensory neurons, which detect head movement; motor neurons directing eye muscles to relax or contract; and neurons in the brainstem that link the two.

To glean meaningful information from in vitro physiological measurements taken from individual neurons, scientists need to know where they fit into the in vivo circuitry. However, unlike the clearly defined layers of the cortex—the brain’s powerful central processing unit responsible for higher functions—the brainstem, which controls automatic functions such as breathing and swallowing, resembles a uniform jumble of neurons, making it difficult to even distinguish between different cell types. To overcome that limitation, Du Lac developed a battery of techniques and tools that allows her to manipulate molecular and genetic components of specific neurons within the VOR circuitry. Recently, she and her team identified two classes of neurons within the VOR circuitry: superfast neurons that rely on glutamate or glycine to transmit signals between cells and can sustain firing rates of up to 600 spikes/second, and GABAergic neurons, which are much slower but still faster than any neuron in the cortex. Their findings demonstrate that these microcircuits are tuned for speed while the whole system is tuned for resilience.

Gaining a better understanding of the neurobiological and molecular mechanisms underlying learning might lead to the development of preventive and therapeutic approaches for strokes, learning and movement disorders, as well as balance problems.

For more information, please visit salk.edu/faculty/dulac.html

Left to right:
Krista Perks, Shiloh Guerrero, Richard Jacobs, Kristine Kolkman, Lauren McElraine, Sascha du Lac, Michael Fuentez, Jeff Bush, Michael Faulstich, Minyoung Shin, Takashi Kodama

Not pictured: Seti Moghadam, Alexandra Sakatos
“Nature vs. nurture, genes vs. environment—what is more important? My group is interested in understanding the roles of genetic and ‘epigenetic’ processes in cell growth and development. By understanding how the genome and epigenome talk to one another, we hope to be able to untangle the complexity of gene regulatory processes that underlie development and disease in plants and humans.”

Although the human genome sequence lists almost every single DNA base of the roughly 3 billion bases that make up a human genome, it doesn’t tell biologists much about how its function is regulated. That job belongs to the epigenome, the layer of genetic control beyond the regulation inherent in the sequence of the genes themselves. Being able to study the epigenome in its entirety promises a better understanding of how genome function is regulated in health and disease, as well as how gene expression is influenced by diet and the environment.

One of the ways epigenetic signals can tinker with genetic information is through DNA methylation, a chemical modification of one letter, C (cytosine), of the four letters (A, G, C, and T) that comprise our DNA. In the last couple of years, Ecker’s laboratory started to zero in on genomic methylation patterns, which are essential for normal development and associated with a number of key cellular processes, including carcinogenesis.

To ascertain how the epigenome of a differentiated cell differs from the epigenome of a pluripotent stem cell, his team used an ultra high-throughput methodology to determine precisely whether or not each C in the genome is methylated and to layer the resulting epigenomic map upon the exact genome it regulates. The study revealed a highly dynamic, yet tightly controlled, landscape of chemical signposts known as methyl groups and resulted in the first detailed map of the human epigenome, comparing the epigenomes of human embryonic stem cells and differentiated connective cells from the lung called fibroblasts. The head-to-head comparison brought to light a novel DNA methylation pattern unique to stem cells, which may explain how stem cells establish and maintain their pluripotent state.

Now that they are able to create high-resolution maps of the human epigenome, Ecker’s group will begin to examine how it changes during normal development as well as in a variety of disease states.

For more information, please visit salk.edu/faculty/ecker.html
The growth of cells is tightly controlled, but cancer cells turn a deaf ear to signals that cause normal cells to stop dividing. Eckhart and his team identified and characterized genes in tumor viruses—so-called viral oncogenes—that override normal cell cycle controls. The viral genes he studied stimulate cellular growth signaling pathways, allowing the cells to divide continuously. Identification of growth signaling pathways has led to the development of drugs that inhibit the growth of cancer cells.

Cancer cells also lose the ability to communicate with each other through the exchange of materials through channels called gap junctions. This communication is important for coordinating the activities of cells in tissues, including normal regulation of cell growth. The proteins that form the channels are called connexins. Eckhart found that the addition of a phosphate molecule to a particular site in the connexin protein caused the channel to close. Some cancer-causing genes stimulate this addition, thereby shutting off communication between adjacent cells and disrupting normal growth. Restoration of communication allows the cells to grow normally again, suggesting that agents that regulate cellular communication might help in reversing cancer. Eckhart also studied the effects of a growth factor, insulin-like growth factor-1 (IGF-1), on human mammary epithelial cells growing in a three-dimensional culture system that mimics the environment of the body. These cells spontaneously develop into hollow structures resembling tiny milk ducts, the most common site where invasive breast cancer arises. Abnormal signaling by IGF-1 changed the clearly defined hollow tubes into a misshapen blob of cells, similar to what happens during early tumor development. Studies like these can help in understanding how changes in genes and growth signaling can lead to breast cancer.

Eckhart recently phased out his laboratory research program. He became professor emeritus in 2010.
Our genome is a patchwork of neighborhoods that couldn’t be more different: Some areas are hustling and bustling with gene activity, while others are sparsely populated and in perpetual lock-down. But once in while the wrong gene ends up in a quiet zone, often with disastrous results.

A cell’s DNA, if stretched out, would form a very thin thread about six feet in length. To fit it neatly inside a cell’s nucleus, the DNA molecules are threaded around histone proteins and coiled up in a highly condensed structure called heterochromatin. In areas of gene activity, the tightly packed heterochromatin is unfurled just enough to make the DNA accessible to regulatory proteins. In many different types of cancers, however, the gene for the tumor suppressor p16 gets buried deep inside heterochromatin. Why the particular stretch of DNA that houses p16 is flagged with chemical marks and wound up so tightly that it becomes inaccessible until recently had remained a mystery.

Most people had been looking for clues within the immediate vicinity of the gene but came up empty-handed. When Michael Witcher, a researcher in Emerson’s team, extended his search further upstream, however, he discovered a binding site for a protein known as CTCF. This protein forms the centerpiece of the molecular fence posts that separate heterochromatin from the rest of the genome. A closer look revealed that CTCF is lost from several binding sites in numerous types of cancer cells, leading to the collapse of the molecular boundary. Once the boundary was gone, the adjacent heterochromatin encroached and silenced the nearest gene.

For a long time scientists have been trying to understand how tumor suppressor genes get silenced in cancer. Understanding one of the key molecular events that leads to their inactivation might allow them to exploit this mechanism to develop novel therapies.

For more information, please visit salk.edu/faculty/emerson.html
Ronald M. Evans

Howard Hughes Medical Institute Investigator
Professor, Gene Expression Laboratory
March of Dimes Chair in Molecular and Developmental Biology

“Our lab studies how control of chronic inflammation can be used to devise new cures to battle obesity, diabetes, heart disease and cancer.”

Our body’s activity levels fall and rise to the beat of our internal drums—the 24-hour cycles that govern fundamental physiological functions, from sleeping and feeding patterns to the energy available to our cells. The clocks themselves keep time through the rhythmic waxing and waning of circadian gene expression on a roughly 24-hour schedule that anticipates environmental changes and adapts many of the body’s physiological functions to the appropriate time of day. Since the body’s circadian rhythm and its metabolism are closely intertwined, the risk for metabolic disease shoots up, when they are out of sync. Shift workers, for example, face a 100 percent increase in the risk for obesity and its consequences, such as high blood pressure, insulin resistance, and an increased risk of heart attacks.

Whereas the master clock in the brain is set by light, the pacemakers in peripheral organs are set by food availability, but the underlying molecular mechanism was still unclear. In probing the relationship between metabolism and circadian cycles, Evans’s team discovered that a metabolic master switch, when thrown, allows nutrients to directly control peripheral clocks. The switch, an enzyme known as AMPK, acts like a gas gauge by sensing how much energy a cell has. If a cell runs on empty, AMPK is turned on to activate the clock and reset rhythm. Evans has been able to show that the exercise drug AICAR (known as exercise in a pill) also works on the clock AMPK switch, providing a close link between circadian rhythm and exercise.

In addition to helping to reset the circadian clock, this work provides a better understanding of how nutrition acts on genes, helping to create new ways to treat obesity, diabetes, and cancer by controlling DNA.

For more information, please visit salk.edu/faculty/evans.html

Left to right:
First row: Liming Pei, Henry Juguilon, Henriette Uhlenhaut, Mingxiao He, Li-Jung Tai, Corinne Ocampo, Ester Banayo, Katja Lamia, Ronald Evans, Ruth Yu, Estelita Ong, Samantha Kaufman, Ann Atkins, Sally Ganley, Vihang Narkar, Erin Dunn
Second row: Jamie Whyte, Angkang Li, Xuan Zhao, Jaem Yung Suh, Michael Downes, Shigeki Sugii, Ning Ding, Han Cho, Sungsoon Fang, Tao Li, Elliot Williams, Malith Karunasiri, Yunqiang Yin
Third row: Grant Barish, Russell Nofsinger, Ling-Wa Chong, Johan Jonker, Suk-Hyun Hong, Yasuyuki Kida
Like a frenzied file clerk, the hippocampus, a small seahorse-shaped area located deep within the brain, processes and distributes memory to appropriate storage sections after readying the information for efficient recall. As it happens, the first relay station in the hippocampus, a part called the dentate gyrus, is also the brain’s hotbed of neurogenesis. Neurogenesis, the process by which new neurons are added to the brain, declines with aging and is thought to be associated with some of the cognitive changes that take place as we grow older. What precise purpose these newborn neurons serve has been the topic of much debate, but apart from studies showing that they somehow contribute to hippocampus-dependent learning and memory, their exact function has remained unclear. A study in Gage’s lab, however, has shed new light on their function.

While passing through the dentate gyrus, incoming signals are split up and distributed among ten times as many cells. This process, called pattern separation, is thought to help the brain separate individual events that are part of incoming memories. Since the dentate gyrus also is where neurogenesis principally occurs, Gage and his team hypothesized that adding new neurons could help with the pattern separation. In experiments that specifically challenged this function of the dentate gyrus, researchers used different behavioral tasks and two distinct strategies to selectively shut down neurogenesis in this structure in mice. Those without neurogenesis had no problem recalling spatial information in general but were unable to discriminate between locations that were close to each other. This observation led Gage and his group to theorize that new brain cells help us to distinguish between memories that are closely related in space.

Contributing to pattern separation might not be the only function of new neurons in the adult brain, however; a computer model simulating the neuronal circuits in the dentate gyrus suggests an additional function: “time-stamping” memories by forming a link between individual elements of episodes occurring closely in time.

For more information, please visit 
salk.edu/faculty/gage.html
Martyn D. Goulding
Professor
Molecular Neurobiology Laboratory

“We are focusing our efforts on how different types of spinal cord ‘interneurons’—neurons that bridge communications between sensory and motor neurons—control how we move and how we respond to touch and pain. Knowing more about how these cells develop and function is a critical step in devising new therapies to regenerate and activate circuits in the spinal cord following injury.”

Once a toddler has mastered the art of walking, it seems to come naturally for the rest of her life, but walking and running require the coordinated activity of at least 200 muscles. The choreographer is a specialized network of interneurons in the spinal cord—commonly referred to as CPG, short for central pattern generator—that functions as a local control and command center for the rhythmic movements that underlie locomotion.

Although scientists had known about the locomotor CPG for a long time, they were unable to identify the nerve cells that make up these circuits. When Goulding’s lab and others began to break the molecular code that makes these different interneuron cell types, they could start to unravel the wiring of the spinal cord to see how it works. Previously, Goulding and his team discovered that a subset of inhibitory interneurons, the V1 neurons, control the speed of motor rhythm and thus set the pace at which animals walk, while a second group of inhibitory neurons, called V0 neurons, govern the left-right alternating pattern of activity that is needed for stepping, as opposed to hopping, movements.

In their latest study, they set their eyes on a group of elusive neurons whose job it is to ensure that flexors and extensors don’t get in each other’s way: When you flex your arm, the biceps need to contract, while the triceps need to relax, and vice versa when you extend your arm. Their experiments revealed that the reciprocal pattern of flexor and extensor muscle activity is generated by the composite action of the V1 neurons and a second class of inhibitory neurons of a different embryonic origin. Strikingly, they found that the same neurons are present in the cords of swimming vertebrates, leading Goulding to propose that the “walking” CPG is an evolutionary adaption of the “swimming” CPG circuit.

The findings mark an important milestone in understanding the neural circuitry that coordinates walking movements, one of the main obstacles in developing new treatments for spinal cord injuries.

For more information, please visit salk.edu/faculty/goulding.html

Left to right:
Back row: Jingming Zhang, Floor Stam, Olivier Britz, Tim Hendricks
Front row: Aurore Giraudin, Chris Padilla, Lidia Garcia-Campmany, Martyn Goulding, Becky Hensley, Ying Zhang, Marta Garcia del Barrio, Tommie Velasquez
For close to a decade, pharmaceutical researchers have been pursuing compounds to activate a key nicotine receptor that plays a role in cognitive processes. Triggering it, they hope, might prevent or even reverse the devastation wrought by Alzheimer’s disease. Researchers in Heinemann’s lab, however, whose group first identified the brain receptors that respond to nicotine, have discovered that when the receptor, alpha-7, encounters beta amyloid, the toxic protein found in the disease’s hallmark plaques, the two may actually go rogue. In combination, alpha-7 and beta amyloid appear to exacerbate Alzheimer’s symptoms, while eliminating alpha-7 seems to nullify beta amyloid’s harmful effects.

Alpha-7 is expressed all over the brain, in all mammals, which means that it is probably essential, but investigators have not yet discovered for what. Intrigued by earlier studies showing that beta amyloid seemed particularly drawn to the alpha-7 nicotinic receptors, Heinemann and his team hypothesized that the receptors mediate beta amyloid effects in Alzheimer’s disease. To test their theory, they crossed mice engineered to lack the gene for alpha-7 with a mouse model for Alzheimer’s disease, which had been genetically engineered to overexpress amyloid precursor protein (APP), an antecedent to beta amyloid. They then put the offspring through a series of memory tests. Surprisingly, those with both mutations—too much APP and no gene for alpha-7—performed as well as normal mice. The Alzheimer’s mice, however, which had the alpha-7 gene and also overexpressed APP, did poorly on the tests. Pathology studies revealed the presence of comparable amounts of plaques in the brains of both types of mice, but in those lacking the alpha-7 gene, they appeared to have no effect. Similar disparities were evident in measurements of the synaptic function underlying learning and memory.

These findings, which suggest a completely different target for potential Alzheimer’s drugs than those that have been tried, could have important implications for researchers seeking to combat the disease.

For more information, please visit salk.edu/faculty/heinemann.html
Ordinarily, the proteins known as nucleoporins are among the 30 gene products serving as the bricks and mortar of nuclear pore complexes, the communication channels that regulate the passage of molecules to and from a cell’s nucleus. But recent research in Hetzer’s laboratory suggests that some also have a second job regulating specific genes during development, and that they sometimes stray from the straight and narrow to play a role in cancer as well.

For more than a decade, scientists have known that when the nucleoporin NUP98, which should be turned off during cell differentiation, abnormally fuses with certain proteins that regulate gene expression, the marriage causes leukemia. Moreover, in many cancers, the nucleoporins NUP214 and NUP88 are misregulated and in particular are associated with very aggressive forms of lung cancer.

Investigators have long questioned why these components of the cell’s transport channel are implicated in cancer and have theorized that the connection relates to a problem in the conveyance of molecules in and out of the nucleus. But Hetzer offers a different explanation. He and his team believe these proteins also function as a new class of gene transcription regulators, which turn specific genes on and off during cell differentiation or tissue development. They found that nuclear pore proteins are not only part of the transport channels but also play a role in the organization of the genome and a very direct role in gene expression.

This finding is especially significant because one of the hallmarks of cancer is abnormal organization of the cell nucleus. This nuclear pathology can be used diagnostically to determine what type of cancer someone has, but no one really knows if it is a cause or consequence of tumor formation. Hetzer and his group are now seeking a molecular understanding of aberrant nuclear organization that they hope will provide the answer.

For more information, please visit salk.edu/faculty/hetzer.html

Left to right:
Front row: Martin Hetzer, Maya Capelson, Jessica Talamas, and Filipe Jacinto
Middle row: Yun Liang, Christine Doucet, Brandon Toyama, Maxi D’Angelo
Back row: Jesse Vargas, Sebastian Gomez, Robbie Schulte, Tobias Franks
Cell cycle checkpoints act like molecular tripwires for damaged cells, forcing them to pause and take stock. The DNA damage checkpoint, for example, is triggered by DNA damage and blocked replication—the process that copies DNA—buying time to repair damage and recover from stalled or collapsed replication forks. If not repaired, these errors can either kill a cell when it attempts to divide or lead to genomic instability and eventually cancer. A key role in this process is played by the checkpoint protein Chk1, which responds to stressful conditions induced by hypoxia, DNA damage–inducing cancer drugs, and irradiation. These same conditions set the protein up for eventual degradation, which allows the cell to resume cell cycle progression after the damage has been repaired. But just how the cellular protein degradation machinery knows that it is time to dispose of activated Chk1 had been unclear.

In their experiments, Hunter and his team discovered that activation of Chk1 exposes a so-called degron, a specific string of amino acids that attracts the attention of a protein known as Fbx6, short for F box protein 6. Fbx6, in turn, brings in an enzyme complex that flags Chk1 proteins for degradation, allowing the cell to get rid of the activated checkpoint protein. Once Chk1 is eliminated, cells can exit the checkpoint or, in the prolonged presence of replication stress, undergo programmed cell death. Yet some cancer cells keep dividing even in the presence of irreparable damage. A closer look at some cancer cell lines resistant to camptothecin, an FDA-approved cancer drug that induces replication stress, pinpointed defects in the Chk1 destruction machinery as the underlying cause. As a result, the checkpoint tripwire stays in place longer, allowing cells to recover and press on regardless of the damage.

A better understanding of this crucial process may lead to the identification of biological markers that predict patients’ responsiveness to chemotherapy drugs such as irinotecan, platinum compounds, and gemcitabine, as well as the development of new cancer drugs with fewer side effects.

For more information, please visit salk.edu/faculty/hunter.html
“The search for unified theories is not limited to those who study physics or the cosmos. A ‘unified theory’ of gene expression is required to explain how transcription—the complex, multistep process that generates messenger RNA copies from protein-encoding genes—is coordinated within the cell.”

If stretched out, the DNA of a single human cell would form a thin thread about six feet in length. To fit inside the cell nucleus, the DNA is threaded around histone proteins and coiled up in a condensed structure called chromatin. When genes are actively transcribed, the tightly folded chromatin must open up just enough to give the transcriptional complex access to the DNA. Recently, Jones and her team discovered that Spt6, a histone “chaperone” protein that functions to move nucleosomal histones out of the path of the oncoming transcription complex, interacts directly with RNA polymerase II (RNAPII), the central catalytic enzyme responsible for copying genes to RNA. Surprisingly, the complex assembled by Spt6 on RNAPII does not include factors required for transcription, but instead includes enzymes that work with Spt6 to modify the histones during transcription, as well as other proteins that transfer onto the newly made RNA to negotiate its export from the nucleus. They are currently identifying new proteins in this complex that integrate transcription with chromatin organization and downstream steps in gene expression.

The lab has also discovered that transcription elongation and histone methylation at highly induced genes is controlled by RNA-binding proteins that mediate alternative splicing of RNAs. Interestingly, these positive elongation factors become inactivated in cells that are subjected to various kinds of stress, resulting in the rapid shut-off of many cellular genes. However, within the HIV-1 genome and at a handful of cellular genes, transcription is induced by environmental or genotoxic stress, suggesting that stress might also destroy negative regulators of transcription. The lab recently discovered that a central human RNA-binding protein plays a hitherto unsuspected role in the repression of stress-regulated genes. This transcriptional inhibitor protein is removed from the nucleus upon stress, releasing transcription at HIV-1 and other responsive genes. Transcription elongation requires either the inactivation of this negative elongation factor by stress, or, in normal cells, its release from the promoter via positive-acting elongation factors. Therefore under stress, transcription elongation may be uncoupled from the Spt6-RNAPII complex.

For more information, please visit salk.edu/faculty/kjones.html

Left to right:
Stem cells, with their defining characteristics—extensive proliferative potential and an ability to give rise to one or more specialized cell types—are common in early embryos. But by adulthood, only a few stem cells remain, tucked away in their own private niches. They have, nonetheless, retained a remarkable capability: They can operate at a “steady state” to maintain and repair tissues with no apparent limit throughout life.

In the *Drosophila* testis, the stem cell “ecosystem” Jones studies, the stem cells sit at the tip of the testis, cradled in their niche, which is also known as the apical hub. As a stem cell divides, one daughter cell moves out of the niche to generate mature sperm cells. The remaining daughter cell stays put and retains its stem cell identity. In an earlier study, Jones and her team had shown that the hub cells send out a local signal, which supports neighboring stem cells, making hub cells an essential component of the stem cell niche.

More recently, they explored how stem cells respond to body-wide circulating signals in addition to local signals emanating from the stem cell niche. The insulin/IGF pathway, which is best known for controlling blood glucose, serves as a “nutrient sensor” and plays an important role in aging in many organisms, including fruit flies. When the researchers fed their flies a “poor,” proteinless diet, the levels of circulating insulin-like peptides plummeted, and stem cell numbers started to decline. Upon re-feeding, insulin-like peptide expression and stem cell numbers recovered quickly. The study revealed that stem cells can sense changes in available nutrients and respond by maintaining only a small pool of active stem cells for tissue maintenance. When favorable conditions return, stem cell numbers multiply to accommodate increased demands on the tissue.

Elucidating the mechanisms by which the insulin/IGF pathway influences stem cell behavior under normal conditions and in response to stress has provided important insights into the use of stem cells in regenerative medicine, during wound repair, and in individuals experiencing metabolic stress.

For more information, please visit salk.edu/faculty/jones.html

Leanne Jones
Assistant Professor, Laboratory of Genetics
Emerald Foundation Developmental Chair

“The behavior of stem cells is regulated both by intrinsic factors within the stem cells and extrinsic factors from the surrounding environment, which is known as the stem cell niche. I am interested in how the relationship between stem cells and their environment changes during development, aging, and tumorigenesis.”

Left to right:
Seated: Anthony Essex, Sharsti Sandall, Anne Conway, Pedro Resende, Leanne Jones
Standing: Chris Koehler, Lei Wang, Justin Voog, Darrell Tran, Chihunt Wong, Thomas Fellner, Cecilia D’Alterio, Will Ansari, Severine Landais, Mariano Losa-Coll
"Safeguarding the ends of linear chromosomes, known as telomeres, is essential for any animal’s survival. We are trying to understand how cells keep tabs on their telomeres and prevent catastrophic meltdowns to gain a better understanding of the interrelationship of aging and cancer."

Like slow-burning fuses, telomeres—the protective ends of chromosomes—become shorter each time a cell divides. Eventually they are depleted, and the cell enters a permanently arrested state called senescence. This process has long been correlated to aging, but how cells recognize that their telomeres are getting shorter and how that affects the cell on a genome-wide scale has remained a mystery. Karlseder and his group have recently cracked the case, finding a direct connection between telomere shortening and histones, the protein “spools” that DNA winds around and that control access to DNA. Collectively known as heterochromatin, the histone packaging can be modified by enzymes that leave chemical signals and instructions behind.

In their study, they hypothesized that senescence is an epigenetic adaptation to chronic changes in the chromosomal architecture of telomeres as they shorten over successive cell divisions. To the team’s surprise, the data revealed that a decline in histone biosynthesis is a central feature of cellular aging.

When telomeres become shorter, they start to emit a chronic signal that alerts the DNA damage machinery to the presence of potential problems at the chromosome ends. This signal is not strong enough to induce cell cycle arrest, but it directly affects the synthesis of two core histones, leading to an imbalance in the composition of chromatin. In response, methyl and acetyl groups connected to individual amino acids in histones that monitor cell division and integrity are reshuffled. This redistribution amplifies the signal emitted locally by shortening telomeres and turns it into a nucleus-wide response. The signal amplification cycle continues until a threshold is exceeded, and the cells respond by entering senescence.

This study explains for the first time how a local event at the chromosome ends gets translated into a signal affecting the entire cell. By providing a link between telomere shortening, histone synthesis, and chromatin maintenance, Karlseder’s lab is helping to address a fundamental question: how telomeres determine the lifespan of human cells.

For more information, please visit salk.edu/faculty/karlseder.html

Jan Karlseder
Associate Professor
Molecular and Cell Biology Laboratory
Tiny hairlike structures called motile cilia sweep mucus and dirt out of our lungs, propel the egg from the ovary through the Fallopian tube into the uterus, and move fluid through the brain’s ventricles. A lot is known about the structural details of cilia: An array of microtubules arranged in nine doublets around a central pair is anchored through the so-called basal body inside the cell. But how cilia form in epithelial cells and how they coordinate the direction of their stroke along a common polar plane is far from clear.

The Kintner lab has identified the gene FoxJ1 as a key factor required for motile cilia, but on its own, FoxJ1 can only induce the formation of a single cilium. By searching for genes that are only expressed in cells that make hundreds of motile cilia, Kintner and his team identified a novel gene, called multicilin. When expressed in nonciliated cells, it both activates FoxJ1 and a program required to form hundreds of basal bodies, resulting in multiciliated cells that are indistinguishable from those found in the lung. Ongoing studies are elucidating the genetic mechanisms that allow multicilin to convert epithelial cells into multiciliated cells.

In a separate study, Kintner’s group tracked the orientation of hundreds of cilia in Xenopus frog larvae, whose skin is covered with multiciliated cells. They found that during early embryonic development, cilia point more or less in the general direction of the body’s back end and start creating a weak flow. During the following refinement phase, all cilia get in line and trim their sails to the prevailing winds. When they analyzed the planar cell polarity (PCP) pathway, which is widely used as a mechanism to orient structures within cells and tissues, they found that the PCP pathway has several functions in ciliated cells: It not only orients cilia in a specific planar direction through cell–cell interactions but also positions basal bodies within cells so that cilia can form.

Identifying the components involved in cilia-specific functions and in the molecular mechanisms underlying the various ciliopathies is likely to facilitate the development of novel therapeutic strategies.

For more information, please visit salk.edu/faculty/kintner.html

“We are studying the genetic and developmental mechanisms that guide the formation of ciliated cells, with the goal of using this information to better diagnose and treat ciliopathies, which can cause respiratory problems, middle ear infection, and infertility.”
Richard J. Krauzlis

Professor
Systems Neurobiology Laboratories

“My laboratory investigates the brain mechanisms that link perceptual and cognitive processing to behavioral responses. The long-term goal of our research is to understand how neural circuits distributed across multiple brain regions coordinate even simple motor outputs like eye movements to higher-order processes such as attention, perception, and executive control.”

Our eyes are in constant motion. Even when we attempt to stare straight at a stationary target, our eyes jump and jiggle imperceptibly. For several decades, scientists have debated the function, if any, of these unconscious flicks, also known as microsaccades. Wondering whether the command center responsible for generating these quick darts resides within the same brain structure that controls how our eyes scan the lines in a newspaper or follow a moving object, the Krauzlis laboratory decided to measure neural activity in the superior colliculus before and during microsaccades. The superior colliculus is a highly conserved brain region that helps orient the head and eyes either toward or away from the sights and sounds in our environment.

Krauzlis’s group not only discovered that the superior colliculus is an integral part of the neural mechanism that controls microsaccades, but also found that individual neurons in the superior colliculus are highly specific about which particular directions and amplitudes they command—even for these smallest of detectable eye movements, which redirect our line of sight by about the width of a sewing needle held at arm’s length. Because images on the retina fade if they are perfectly stabilized, discovery of this mechanism explains how the central nervous system generates these miniature movements to constantly shift the scene ever so slightly, thus refreshing the images on our retina and preventing us from going “blind.”

Microsaccades, however, do more than prevent the world around us from vanishing from view. When we avoid looking directly at an object of interest—for reasons of propriety, for example—our microsaccades betray our true attraction to the object because their direction is biased toward the object. On the other hand, during some tasks, such as threading a needle, microsaccades tend to be less frequent. By showing that the superior colliculus is involved in generating microsaccades, Krauzlis and his team now have an explanation: Microsaccades provide a snapshot of our priorities at any given moment, even when we try not to move our eyes.

For more information, please visit salk.edu/faculty/krauzlis.html

Left to right:
Lee Lovejoy, Natalie Dill Moursund, Zhongchau Liao, Eileen Boehle, Sam Nummela, Rich Krauzlis, Shaun Mahaffy, Karine von Bochman, Kristina Nielsen, Alex Zenon
Proteins, like people, are often judged by the company they keep. p75, for instance, belongs to the same family as tumor necrosis factor. In addition to regulating neuronal growth, survival, and degeneration and guiding nerve fibers in growing embryos to their final destinations, it was widely thought to mediate cell death in some context.

Various in vitro studies have examined p75 in combination with beta amyloid, seeking evidence that it helps induce nerve cell death in Alzheimer’s disease. A team of scientists in Lee’s laboratory, however, found that p75 instead has a neuroprotective effect on the sympathetic nervous system.

Scientific interest in the peripheral nervous system has been growing as investigators studying neurodegenerative diseases seek new insights into disease progression. To gather evidence about p75 and the sympathetic nervous system, Lee’s group crossed a mouse model for Alzheimer’s disease with a line of mice genetically modified to lack the gene for p75. Without p75, they theorized, the neurotoxic effects of beta amyloid would be reduced, and the mice would show fewer Alzheimer’s symptoms.

Along with profound motor problems, the p75-deficient mice exhibited severe defects in the wiring of nerves to multiple organs, and the majority died within just three weeks. (Mice normally live up to two years.) But when the researchers scaled down the production of toxic beta amyloid by deleting one copy of BACE1, which encodes the molecular shears that make the first cut in the production of beta amyloid fragments, the nerves in the sympathetic nervous system of p75-deficient mice were substantially restored.

This was the first time the interplay between p75 and beta amyloid in the peripheral sympathetic system has been demonstrated. Lee’s findings not only challenge the prevailing view of p75’s harmful role in Alzheimer’s but could lead to new insights and, ultimately, new protocols for managing the secondary deficits that accompany the condition’s hallmark dementia and memory loss.

For more information, please visit salk.edu/faculty/lee.html

“Communication happens at many different levels and in many different ways, but all instances have one thing in common: They need underlying hardware to convey the information. I am interested in how the nervous system wires up the body during embryonic development so the brain can send and receive signals.”
Greg E. Lemke
Professor
Molecular Neurobiology Laboratory

“In biology, the ability to turn something on is always coupled with a mechanism to turn it off. In the absence of this regulation, a biological system is akin to the ‘Sorcerer’s Apprentice,’ in that it sets in motion a chain of events over which it has no control. Our work on the TAM receptors has revealed that they play this role in the immune system: They regulate the innate immuneresponses to bacteria, viruses, and other pathogens, ensuring that it is strong enough to defeat these threats, but not so strong that endangers the host.”

Antigen-presenting cells or APCs, which provide the body’s first line of defense against disease-causing bacteria and viruses, are constantly on the prowl in search of pathogens. When they encounter foreign invaders, they unleash a “cytokine storm”—a wave of chemical messengers that jumpstart the T and B cell response. When the invaders have been successfully vanquished, the APCs need to shut down; otherwise, a chronic inflammation ensues, overwhelming the regulatory mechanisms that normally distinguish “self” from “non-self,” leading to autoimmune diseases such as lupus and rheumatoid arthritis.

In their latest study, Lemke and his team explored how the so-called TAM receptors (Tyro3, Axl, Mer) in mice stop the immune system from mounting an out-of-control, destructive inflammatory response against invading pathogens. When receptors studded on the surface of patrolling APCs encounter a pathogen, the cells release an initial burst of cytokines, which is then amplified in a second stage via a feed-forward loop working through cytokine receptors. But this same activation pathway trips the fuse that is designed to prevent the inflammatory response from spiraling out of control.

The researchers found that an essential stimulator of inflammation—the type 1 interferon receptor (IFNAR)—turns on the expression of Axl, a TAM receptor. Axl and IFNAR then physically bind together and activate SOCS genes, whose products are potent inhibitors of pro-inflammatory signaling pathways. Without TAM receptors, they discovered, the APCs never shut down after their initial activation, but remain in a state of red-alert.

Knowing how important TAM receptors are to the control of inflammation in mice will not only aid our understanding of human immune system disorders but might enable researchers to manipulate the switch in ways that could be clinically beneficial. For example, a drug that inhibited TAMs in the short term could be given along with a therapeutic vaccine, in order to help the body mount a better immune response. Conversely, it may be possible to engage the TAMs early in an immune reaction to treat chronic autoimmune diseases such as lupus.

For more information, please visit salk.edu/faculty/lemke.html

Left to right:
Back row: Carla Rothlin, Michael Reber, Amy Blount, Nick Bevins, Asa Gardner, Patrick Burrola, Joe Hash
Front row: Thomas Vacik, Lawrence Fourgeaud, Erin Lew, Greg Lemke, Becky Hensley
In eukaryotes, the plasma membrane—a double layer of lipid molecules that encloses all cells—not only segregates the cell from its environment but also serves as the principal interface for communication between cells. Not surprisingly, the plasma membrane’s structure and properties impact many biological processes. T cells, whose main job is to fight infection, for example, utilize and reorganize their plasma membrane constantly during activation and effector functions. This is most dramatically seen in the establishment of signaling microclusters and the formation of the immunological synapse between T cells and antigen-presenting cells upon activation of the former by the latter.

Despite a lot of interest in the precise architecture of the plasma membrane in the past, studies of plasma membrane–associated signaling had been hampered by technical barriers such as cell lysis and limited resolution in microscopy. Lillemieier overcame these limitations through the use of novel high-resolution imaging techniques such as photo-activated localization microscopy (PALM) and dual color fluorescence cross-correlation spectroscopy (dcFCCS), which allowed him to observe directly the spatial and temporal distribution of membrane-associated molecules on a nanometer scale.

He discovered that all membrane-associated proteins in the cells that he examined are clustered into what he refers to as “protein islands,” which led him to postulate a novel concept for the general architecture of plasma membranes. Lillemieier also found that the T cell receptor signaling cascade is spatially and temporally controlled through the segregation and association of distinct membrane microdomains (protein islands) that contain specific subsets of T cell signaling molecules. He believes that this type of signal control may be a general feature of membrane-associated signaling and is probably used in a variety of signaling processes.

In the future, Lillemieier will expand his research to understand how this higher order in the plasma membrane is achieved and what molecular mechanisms are in place to utilize it during signal transduction. His studies will help to expand knowledge of spatio-temporal signaling control, which will suggest new approaches in manipulating the response of the immune system to pathogens and diseases.

For more information, please visit salk.edu/faculty/lillemieier.html
“A plant’s shoot system is responsible for all of the above-ground portions of the plant, such as leaves, branches, and flowers, and is the site of photosynthesis. The root system lies below the ground and provides water and nutrients to the plant. My lab’s research is focused on how a plant embryo sets up this apical/basal polarity.”

Controlled by a tightly regulated choreography that determines what goes up and what goes down, plant embryogenesis establishes a very simple structure that contains two stem cell populations: the shoot meristem, which will give rise to all the “above-ground” organs such as the stem, the leaves, and the flowers, and the root meristem, which gives rise to roots. While investigating why a defective TOPLESS gene messes with a plant’s basic architecture—mutant embryos develop into a seedling topped with a second root instead of a stem with leaves—Long and his team discovered that functional TOPLESS codes for a repressor protein that inactivates the genes that otherwise would cause root development in the shoot area of the plant. Their latest study revealed that these fate-transforming genes are two familiar characters: PLETHORA 1 and 2, which had been known to act as master regulators that determine the identity of the root meristem. Without TOPLESS to keep them turned off, the two PLETHORAs are free to impose their will on the top half of the plant embryo, causing the development of a second root instead of a shoot.

With the “below-ground” hierarchy worked out, the question of how the identity of the shoot meristem is determined was still unanswered. Trying to unearth the missing master regulators of shoot development, the researchers searched through tens of thousands of mutant plants till they hit on a member of the CLASS III HD-ZIP transcription factors, known as PHABULOSA, that fit the bill. When forcefully expressed in the traditional territory of the PLETHORA duo, PHABULOSA transformed the root into a shoot, resulting in a seedling with leaves on both ends. Further studies revealed an antagonistic relationship between the PLETHORA and HD-ZIP III genes, ensuring that they stay where they belong and don’t get in each other’s way.

Understanding these mechanisms at a molecular level is one of the key areas of fundamental plant biology, which could be used for developing agricultural plants with more desirable traits.

For more information, please visit salk.edu/faculty/long.html
Whether cells have a finite or infinite ability to proliferate is determined by the ends of the chromosomes, called telomeres, which are tended to by a dedicated enzyme called telomerase. In cells where telomerase is inactive, telomeres shorten with each cell division until they become so whittled away that they signal the cell to stop dividing; cells that retain telomerase are able to continue dividing indefinitely.

Although our knowledge of how telomerase is regulated in human cells remains incomplete, researchers do know that there is a telomerase complex, which was first identified in simple single-celled organisms. Lundblad’s group discovered the key protein subunit of telomerase in budding yeast, providing the tools to identify its human counterpart. In budding yeast, telomerase consists of three proteins, called Est (for ever shorter telomeres). The Est2 protein, together with a telomerase-dedicated RNA, does the heavy lifting in terms of telomere reconstruction, while Est1 and Est3 help orchestrate the process.

Lundblad’s lab is now looking for the proteins that tell telomerase when and how to act, again using budding yeast as the starting point. Earlier, her group found a clue when they showed that a small area on the surface of Est1 acted like molecular Velcro, by attaching Est1 (and thus the rest of the telomerase complex) to a telomere-bound protein, thereby ensuring that yeast cells continuously divide. Simply changing a single amino acid on this site prevented telomerase from reaching the ends of chromosomes, and the telomeres shortened.

Lundblad’s group postulates that there must be multiple docking points on the surfaces of the three Est proteins, each performing a distinct regulatory activity. To test this, they are surveying the entire surface of the telomerase complex. So far, her group has identified two additional molecular tethering points, on Est1 and Est3, and they are hot on the trail of the proteins that interact with these two sites.

For more information, please visit
salk.edu/faculty/lundblad.html

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“I am fascinated by the complex DNA transactions that chromosomes undergo—particularly the types of events that occur at telomeres, the very tips of chromosomes. When these telomere-associated DNA processes start to run out of steam, or conversely, become overly active, this can lead to either premature aging or cancer predisposition.”

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Left to right:
John Lubin, Bari Ballew, Michael Killoran,
Christine Killoran, Tim Tucey, Vicki Lundblad,
Ed Mandell, Margherita Paschini
Obesity, which is probably the most important factor in the development of insulin resistance—promotes insulin resistance through the inappropriate inactivation of a process called gluconeogenesis, where the liver creates glucose for fuel and which ordinarily occurs only in times of fasting. Yet, not all obese people become insulin resistant, and insulin resistance occurs in non-obese individuals, leading Montminy and his team to suspect that fasting-induced glucose production was only half the story.

It had been known that a condition known as ER (endoplasmic reticulum) stress is abnormally active in livers of obese individuals, where it contributes to the development of hyperglycemia, or high blood glucose levels. Glucose production is turned on by a transcriptional switch called CRTC2, which normally sits outside the nucleus waiting for the signal that allows it to slip inside and do its work. Once in the nucleus, it teams up with a protein called CREB, and together they switch on the genes necessary to increase glucose output. In insulin-resistant mice, however, the CRTC2 switch seems to get stuck in the “on” position, and the cells start churning out glucose like sugar factories in overdrive.

Surprisingly, when the researchers mimicked the conditions of ER stress in lean mice, CRTC2 moved to the nucleus but failed to activate gluconeogenesis. Instead, it switched on genes important for combating stress and returning cells to health. On closer inspection, they found that in this scenario CRTC2 did not bind to CREB but instead joined forces with another factor, called ATF6a. What’s more, like jealous lovers CREB and ATF6a are competing for CRTC2’s affection—the more ATF6a is bound to CRTC2, the less there is for CREB to bind to. This clever mechanism ensures that a cell in survival mode automatically shuts down glucose production, thus saving energy. Under the kind of persistent stress presented by obesity, however, levels of ATF6a go down, triggering aberrant glucose production in the liver, and explaining how obesity sets the stage for diabetes and why thin people can become insulin-resistant.

For more information, please visit salk.edu/faculty/montminy.html

Left to right:
Standing: Sam Van de Velde, Jose Paz, Kim Ravnskaer, Biao Wang, Younsgup Song, Jeong Ho Kim, Marc Montminy, Bing Luan, Hongbo Wang, Pankaj Singh, Naomi Goebel, Nina Miller, Jason Goode, Noel Moya
Seated: Motoyuki Igata, Yi Liu, Yiguo Wang, Liliana Vera, Susie Hedrick, Kristin Viste, Meghan Hogan
Trying to make the best of their real estate, plants dispatch an impressive arsenal of small molecules to communicate and interact with the outside world. Among them, terpenes—the oldest and probably most widespread group of natural products synthesized by plants—play a particularly important role. Examples of common terpenes are pine resins and the essential oils of myrrh, rosemary, and thyme, but two of the best-known terpenes are probably cholesterol and taxol.

Despite their extraordinary diversity, all terpenes are assembled from the same five-carbon isoprene building blocks and then modified by an armada of terpene synthases in thousands of ways. Despite the great variety in the terpene synthases’ substrate and product specificity, each enzyme falls squarely into one of two camps: cisoid or transoid, depending on how they prefer their raw material spatially configured. As a general rule, terpene synthases only produce cisoid products from cisoid substrates and transoid products from transoid substrates. In a recent study, however, Noel and his team discovered a notable exception: the transoid tobacco sesquiterpene synthase, which is in charge of catalyzing the first step in the biosynthesis of capsidiol, the main component of tobacco’s natural antifungal chemical defense.

When fed a non-natural (cis, trans) version of the enzyme’s natural (trans, trans) substrate, the enzyme very efficiently converted the alternative substrate into a new compound with a complex chemical structure and a pleasant woody scent. This finding hinted that the so-called non-natural version of the enzyme’s substrate may not be so non-natural after all. In fact, there is now evidence that it is made in small amounts in all living systems. During its evolutionary history, this particular plant enzyme may have taken advantage of the presence of this alternative substrate to produce a new chemical, with a function selected for during the course of evolution. With the compound now in hand, the search for its role in the plant is under way. Nevertheless, this new compound not only might be of great interest to the fragrance industry, it could become an important starting point for the development of new pharmaceuticals to treat disease.

For more information, please visit salk.edu/faculty/noel.html

Joseph P. Noel
Howard Hughes Medical Institute Investigator
Professor and Director, Jack H. Skirball Center for Chemical Biology and Proteomics

“Most people are familiar with the word biodiversity, but ‘chemodiversity’—the extraordinary tapestry of natural chemicals found in all organisms—is just as important for life and the survival of many different ecosystems on the earth. I am particularly interested in the chemical systems or biosynthetic pathways that give rise to these vital molecules.”
“We believe that identifying the mechanisms regulating developmental events is requisite for understanding the basis of most biological disorders and is essential both for prevention and the development of strategies to repair damage to the nervous system due to genetic defects, tumors, or injuries to the brain or spinal cord.”

The cerebral cortex, the outermost layer of neurons commonly referred to as gray matter, is the largest and most complex component of the brain. Although initially all stem cells in charge of building it are created equal, they quickly commit irrevocably to forming specific cortical regions. How the stem cells’ destiny is determined, however, has remained an open question.

During embryonic brain development, the stem cells that will give rise to the cerebral cortex pass through a series of tightly regulated stages. Early during neurogenesis, stem cell-like progenitor cells known as neuroepithelial cells undergo cell division to expand their own pool. Later, they differentiate into more mature progenitor cells called radial glia, which produce a constant stream of both progenitors and neurons, the latter migrating outward to establish the gray matter of specialized cortical regions. The defining characteristic of the progenitor cells that will go on to form the cerebral cortex is their expression of a transcription factor called Emx1.

After discovering that a specific member of the fibroblast growth factor family of morphogens controls the timing of the critical transition period bridging the early expansion phase of neuroepithelial cells and the later neurogenic phase of radial glia, O’Leary hypothesized that the regional identity of progenitors in the Emx1 lineage may involve one or more transcription factors that define unique subpopulations of progenitors via differences in their expression levels. A promising candidate was the LIM transcription factor Lhx2, which is expressed in all progenitors of the Emx1 lineage but at different levels in a graded regional pattern. By creating a new genetically engineered mouse line, his team deleted Lhx2 from neuroepithelial cells at different times during embryonic development and demonstrated that Lhx2 regulated their destiny.

These findings may help expand understanding of the genetic underpinnings of many neurodegenerative disorders, as well as eventually provide the means to direct stem cells to repair specific parts of the brain ravaged by disease or injury.

For more information, please visit salk.edu/faculty/o’leary.html
If a cell suffers non-repairable injury to its genetic material or cell growth starts to go astray, the tumor suppressor protein 53 pulls the emergency brake, a built-in “auto-destruct” mechanism that eliminates abnormal cells from the body before they can cause disease, including cancer. To sidestep the cell’s suicide program—a process called apoptosis—tumor cells need to inactivate p53, which turns on genes that mediate cell cycle arrest and apoptosis.

Similarly, adenovirus, which cause upper-respiratory infections, needs to get p53 out of the way to multiply successfully; therefore it brings along a viral protein, E1B-55K, which binds and degrades p53 in infected cells. Without E1B-55K to inactivate p53, adenovirus should only be able to replicate in p53-deficient tumor cells, making it the perfect candidate for oncolytic cancer therapy. Oncolytic viruses offer a novel and potentially self-perpetuating cancer therapy. Each time a virus infects a cancer cell and successfully multiplies, the virus ultimately kills the cancer cell by bursting it open to release thousands of viral progeny. The next generation seeks out remaining tumor cells and distant micro-metastases but leaves normal cells unharmed.

Clinical trials found such viruses to be safe and promising. Contrary to all expectations, however, patient responses did not correlate with the p53 status of their tumors. When O’Shea followed up on this unexpected finding, she discovered that the inability of the E1B-55K–mutant virus to replicate in normal cells was not because the virus failed to degrade p53. Instead, adenovirus brings along another protein, E4-ORF3, which neutralizes the p53 checkpoint through a completely different mechanism. It prevents p53 from binding to its target genes in the genome by modifying histone proteins, the “spools” around which DNA winds. With its access denied, p53 is powerless to pull the trigger on apoptosis. O’Shea and her team are now exploiting this new viral protein as a powerful tool to both pinpoint and connect critical new targets in the cellular p53 tumor suppressor network and to develop the next generation of oncolytic viruses.

For more information, please visit salk.edu/faculty/o’shea.html
“Going to bed at night and waking up in the morning is a seemingly simple routine that we follow in every season. I want to understand how our brain clock keeps track of time in all seasons and time zones and tells our body when to sleep, when to wake up, and when to eat.”

In mammals, the circadian timing system is composed of a central circadian clock in the brain and subsidiary oscillators in most peripheral tissues. The master clock in the brain is set by light and determines the overall diurnal or nocturnal preference of an animal, including sleep-wake cycles and feeding behavior. The clocks in peripheral organs, however, are largely insensitive to changes in the light regime. Instead, their phase and amplitude are affected by many factors, including feeding time.

All clocks keep time through the fall and rise of gene activity on a roughly 24-hour schedule that anticipates environmental changes and adapts many of the body’s physiological functions to the appropriate time of day. In particular, the oscillator in the liver—the body’s metabolic clearinghouse—helps the organism adapt to a daily pattern of food availability by temporally tuning the activity of thousands of genes regulating metabolism and physiology.

To investigate whether the circadian rhythms in hepatic transcription were solely controlled by the liver’s clock in anticipation of food or responded to actual food intake, Panda and his team put normal mice and mice without functional clocks on strictly controlled feeding and fasting schedules. Their experiments revealed that the daily waxing and waning of thousands of genes in the liver is mostly controlled by food intake and not, as conventional wisdom had it, by the body’s circadian clock. For example, the activity of genes that encode enzymes needed to break down sugars rose immediately after a meal, while the activity of genes encoding enzymes needed to break down fat was highest after a prolonged fast. Consequently, a clearly defined daily feeding schedule puts the enzymes of metabolism in shift work and optimizes burning of sugar and fat.

Their findings could explain why shift workers are unusually prone to metabolic syndrome, diabetes, high cholesterol levels, and obesity. It is not the shift work per se that wreaks havoc on the body’s metabolism but changing shifts and weekends, when workers switch back to a regular day-night cycle.

For more information, please visit salk.edu/faculty/panda.html
The neuromuscular circuitry that controls bodily movements relies on constant sensory feedback to fine-tune its commands to hundreds of muscles. Muscle fibers are each controlled by one motor neuron in the spinal cord that relays signals from the brain; sensory receptors send information from the periphery back to the central nervous system. These nerves are not unlike a roadway, with orderly traffic moving in both directions.

The traditional view has been that during embryonic development, sensory and motor neurons are able to incorporate into tightly coordinated pathways without getting mixed up because growth cones—structures that guide growing axons to their destination—are studded with Eph proteins that constantly search their environments for molecules called ephrins, which nudge them in the right direction. (Axons are long projections that conduct electrical impulses away from the nerve cell body.) Researchers in Pfaff’s laboratory, however, found that neurons not only carry both types of proteins, but that the role of Ephs and ephrins can change, raising the question of what happens when adjacent neurons bump into one another.

To find the answer, Pfaff’s team studied sensory and motor neurons, which extend their axons along the same pathway to the periphery. They found that with ephrin/Eph signaling intact, the axons sorted into separate fascicles containing either sensory or motor axons, but never both. When they deleted EphA3 and EphA4 in motor neurons, however, sorting between the fascicles of the motor and sensory axons broke down; instead of reaching for muscles, some motor neurons made a U-turn, joined the sensory lane, and headed back toward the spinal cord.

Ephrin and Eph both appear to control where the axons grow and to maintain the normal arrangement between the motor and sensory pathway. They also play a major role in preventing spinal cord neurons from regenerating after injuries. As scientists in the spinal cord field work to overcome the block preventing axonal growth within the central nervous system, Pfaff’s findings demonstrate that this research needs to be approached cautiously, lest it promote indiscriminate motor axon growth and cause severe problems.

For more information, please visit salk.edu/faculty/pfaff.html
The long-range goal of our laboratory is twofold—to understand the fundamental nature of the computations that are carried out by the neocortex, including the computations that enable us to attend to sensory stimuli, and to understand how and why these computations fail in brain disease.

The brain never sits idle. Whether we are awake or asleep, watch TV or close our eyes, waves of spontaneous nerve signals wash through our brains. To be reliably processed, incoming sensory information has to stand out from this ongoing background activity. In his latest study, John Reynolds and his team wondered whether attention, which so efficiently tunes out external distractions, does the same for the internal racket.

Researchers had known for some time that paying attention to visual details increases the firing rate of neurons tuned for attended stimuli. The stronger the neural signal, the better we are able to perceive a stimulus. But neurons are very noisy computing devices, and even under the most controlled laboratory conditions, neuronal responses vary over repeated presentations of an identical sensory stimulus. If each neuron produced random noise that is independent from what its neighbor is doing, the brain cell on the receiving end could simply pool all incoming signals and average out the noise.

But most of the brain’s background noise originates in waves of spontaneous nerve signals and can’t be simply averaged out. However, an interesting thing happened when the researchers measured the activity of populations of brain cells in animals trained to play a video game that required rapt attention. When attention was directed to a visual stimulus on a computer monitor, the internal fluctuations or shared noise quieted down, increasing the salience of the incoming sensory information. This noise reduction substantially increased the fidelity of the neural signal, an improvement four times as large as the improvement caused by attention-dependent increases in firing rate alone.

A hallmark of brain disorders such as Alzheimer’s disease, autism, and schizophrenia is the loss of our capacity to attend to behaviorally relevant stimuli. This newly discovered neural mechanism has enormous implications for treating diseases in which attention fails, and the Reynolds laboratory is now embarking on a series of studies to understand how failures of this neural mechanism figure in brain disease.

For more information, please visit salk.edu/faculty/reynolds.html

Left to right: John Reynolds, John Curtis, Emily Anderson, Tamara Berdyyeva, Jaclyn Reyes, Catherine Williams, and Jude Mitchell
The health of mammals depends on their ability to maintain the internal environment of their bodies within narrow and clearly defined limits in the face of physiological or psychological threats. Challenges to the body's homeostasis—whether perceived or real—are handled by the hypothalamic-pituitary-adrenal (HPA) axis, which involves the interaction of the brain structure known as the hypothalamus, the pituitary gland (just below the hypothalamus), and the adrenal glands (at the top of the kidneys). Together these three glands control reactions to stress and regulate many body processes, including digestion, the immune system, mood and emotions, and sexuality, as well as energy storage and expenditure.

Alcohol is one of the stimuli that activate the HPA axis in rodents, but the mechanisms responsible for it are not yet fully understood. Rivier's laboratory had shown earlier that the peptide corticotropin-releasing factor (CRF), which is produced in the hypothalamus, was essential for an appropriate HPA axis response to acute alcohol. While the ultimate effect of alcohol is binding of CRF to specific receptors on pituitary cells, and the ensuing release of ACTH and adrenal steroids, recent experiments by Rivier and her team revealed a more complex picture of alcohol's action on the brain. They found that alcohol increases the activity of dopamine b-hydroxylase, the enzyme directly responsible for the synthesis of norepinephrine. The latter contributes to the HPA axis’s response to alcohol, and this knowledge may help the development of specific therapies that counteract some of the deleterious effects of this drug.

Stress is thought to play a role in the ability of addicted individuals to maintain abstinence. Thus, a better understanding of the function of the HPA axis during the development of alcohol dependence, and how the activity of this axis differs between dependent and non-dependent animals, will be helpful in pursuing new therapies for the treatment of alcohol addiction.

For more information, please visit salk.edu/faculty/crivier.html
Jean E. F. Rivier

Professor, Clayton Foundation Laboratories for Peptide Biology
Dr. Frederik Paulsen Chair in Neurosciences

“Remissions leading to a full recovery or relapses progressing to recurrences are characteristic of a significant number of pathological conditions. We are concentrating our efforts toward understanding how the temporal restoration of the body’s homeostasis may induce remission and ideally lead to recovery.”

Like a central command center, the brain area known as the hypothalamus sends out “master” brain hormones, which regulate basic bodily functions. Many of these hypothalamic hormones, including the “stress hormones” corticotropin releasing factor (CRF) and the urocortins (Ucn), as well as their two receptors (CRFR1 and CRFR2), were characterized at the Salk Institute. Because of their broad distribution, CRFs and CRFRs mediate numerous complementary stress-related endocrine, autonomic, metabolic, immune, cardiovascular, gastrointestinal, and cutaneous pathologies.

To test whether molecules that block CRF’s functions may induce remissions, Rivier, in collaboration with Salk colleagues Wylie Vale and Catherine Rivier, developed a series of very effective CRF antagonists. Working with Lixin Wang, Mulugeta Million, and Yvette Taché at UCLA, Rivier and his team then tested the effects of a potent and long-acting version in mice that overexpress CRF. Without treatment, these mice develop symptoms that are typical of Cushing’s syndrome, such as thinning of the skin, loss of fur, and fat accumulation at the midsection of the body as they get older. When treated with a CRF antagonist, however, their fur immediately starts to regrow.

Under stressful conditions, the body responds initially by mounting a multipronged counterattack that normally deals successfully with acute challenges but more likely than not will fail in the face of chronic challenges. When unsuccessful, the body’s defense mechanisms such as the immune system become compromised, triggering a recurrence of the disease. If the chronic stress response is mitigated either psychologically or pharmaceutically, Rivier believes that those systems recover their ability to deal with the insult, thus triggering a remission—the first step toward full recovery. He is now testing this hypothesis in animal models, including in prematurely weaned pigs, which suffer from stress-induced diarrhea. Initial promising results indicate that CRF antagonists could play an important role in treating irritable bowel syndrome and, hypothetically, most other conditions initiated by or relapsing as a result of stress.

For more information, please visit salk.edu/faculty/rivier.html
The typical signs of an infection—fever, listlessness, lack of appetite—are orchestrated by the brain in response to inflammatory cytokines, which are the immune system's signaling molecules. Cytokines are generated at the site of infection, then circulate in the blood and communicate with neurons in the brain to engage the hypothalamic-pituitary-adrenal (HPA) axis, an integral part of the brain's stress response machinery. But cytokines are big molecules, and how their reach extends beyond the almost impenetrable blood-brain barrier has been the topic of much dispute.

Earlier research by Sawchenko and others suggested a vascular route whereby cytokines interact with vessel walls to generate secondary messengers, which then engage the relevant circuitry in the brain. Tightly packed endothelial cells, which line narrow capillaries throughout the brain, are perfectly positioned to record circulating immune signals, but they require a very strong signal to become activated. Perivascular macrophages, on the other hand—specialized white blood cells that digest cellular debris and pathogens and are lined up along the blood-brain barrier—are more sensitive but lack direct access to the bloodstream.

To determine the role of these two cell types, Sawchenko's group recently injected liposomes containing clodronate, a drug that can cause cell death, into the lateral cerebral ventricle of rodents. The liposomes were taken up by the macrophages, which were killed off. Without perivascular macrophages, the animals were unable to respond to blood-borne interleukin-1, which is a cytokine, and initiate the brain's so-called acute phase responses, which help the body tackle the challenge at hand but also cause the feeling of "being sick." To their surprise, however, Sawchenko's team found that the same cells suppressed the pro-inflammatory activities of endothelial cells, which are only stirred to action when they encounter lipopolysaccharide, a key component of the cell walls of certain bacteria.

The identification of a potent anti-inflammatory mechanism in the brain may pave the way for new therapies for chronic neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's, Alzheimer's and prion diseases, in which central inflammatory mechanisms play an important role.

For more information, please visit
salk.edu/faculty/sawchenko.html
David R. Schubert
Professor and Laboratory Head
Cellular Neurobiology Laboratory

“Our research group is studying how cells live or die in the context of Alzheimer’s disease, Parkinson’s disease, stroke, ischemia, and other degenerative brain disorders, with the ultimate goal of identifying drugs that inhibit cell death and getting them through the initial stages of drug development and on to the clinic.”

There are currently no treatments for the nerve cell death associated with chronic neurological diseases, including Alzheimer’s, Parkinson’s, Huntington’s, and ALS, or the neurological complications of diabetes. There are many reasons for this deficiency, but the main hurdle is the innate complexity of the diseases themselves. In most cases, the nerve cells die from several types of insults, not one specific stress or toxin. Therefore, drugs that effectively treat these conditions will have to block the different pathways that together lead to the nerve cell’s demise.

Because plants lack an immune system, they instead synthesize drug-like molecules to defend themselves against infectious pathogens and insect predators. Most of these molecules have numerous biological activities, and they are the basis of much of the current pharmacopoeia. They have not, however, been exploited for the treatment of chronic neurological diseases.

Schubert’s lab and that of Pamela Maher in the Cellular Neurobiology group are using a series of innovative biological assays to identify plant products that are broadly neuroprotective. They then modify the structure of the compounds through medicinal chemistry to improve their properties as drugs. The goal is to make drugs that inhibit the pathways that cause nerve cell death and are safe because they are based upon edible plant material. To date, they have identified two compounds that meet the above criteria. The first, discovered by Maher, is fisetin, found in strawberries, which is orally active in animal models of memory enhancement, Huntington’s, and Parkinson’s. It is also very active in a rigorous rabbit stroke model. Schubert has made potent synthetic derivatives of curcumin, the major component of the Indian spice turmeric, that work well in rabbit stroke models and memory enhancement.

Currently, this work is being extended in three areas: refinement of the chemical structures, identification of the multiple molecular targets, and testing in additional animal models where they are likely to be effective, such as the complications of diabetes and Alzheimer’s. The ultimate aim is to get these compounds through the initial stages of drug development so they can be advanced to the clinic.

For more information, please visit salk.edu/faculty/schubert.html
Multiple sclerosis affects an estimated 400,000 Americans and more than 2.5 million people worldwide. A chronic, often disabling disease that attacks the central nervous system, it is characterized by a baffling range of neurological symptoms, including numbness, tingling, motor weakness, paralysis, and vision loss. It is thought to result when the immune system attacks the myelin sheath that insulates axons, the nerve fibers that conduct electrical impulses to and from the brain and between neurons within the brain. Ordinarily, the myelin speeds up the signals the axons transmit, but when axons lose their insulation, either signal conduction fails because the demyelinated axons are unable to generate an impulse, or the axons become hyperexcitable and overcompensate by firing even in the absence of an input.

The first computer model of axonal transmission, developed in the 1950s for the giant axon of the squid, which lacks myelin, tracked positively charged sodium and potassium ions, whose movements across the neuronal membrane generate the necessary electrical signals. Building on that model, Sejnowski and his team included myelin in their own model, then demyelinated one of the sections and incorporated all the changes known to take place as a result. Most prior studies had focused on the sodium channel because it is responsible for initiating the electrical signal, but to everyone’s surprise, Sejnowski’s group found that it was the ratio of densities between the sodium channel and a previously ignored but ubiquitous voltage-insensitive potassium current, called the leak current, that determines whether neurons can fire properly. If the sodium level drops, an accompanying drop in the leak current will maintain the signal, whereas if the sodium drops but the leak current doesn’t, signal transmission may fail. Conversely, if the sodium level is too high and the leak current doesn’t increase, a patient may experience twitching.

Sejnowski’s model not only offers an explanation for many of the bizarre symptoms that multiple sclerosis patients experience but could also provide a new target for drugs that increase or decrease the potassium leak current to maintain a constant ratio and offer relief.

For more information, please visit salk.edu/faculty/sejnowski.html
"Neurobiologists are on a perennial quest to understand how the brain codes and processes information. In the past, scientists had to rely on simplified objects on a computer screen. I try to take it a step further and analyze how brain cells respond to natural stimuli because some neurons only respond when a certain object comes into view. Scenes from our environment provide a rich ensemble of various object combinations sure to drive any sensory neuron at some point."

Circuits in the nervous system, built from cells and the connections between them, cannot be made as regular as circuits in engineered man-made systems. Yet animals can detect and act on signals in the environment with precision that not only rivals that of engineered systems, but consumes much less energy (the brain is estimated to “run” on 12 Watts of power). Neurons in the retina only respond when a stimulus appears within an approximately round window covering a small part of the visual field that the eye sees. Theoretically, one would expect to obtain the best resolution if these windows, known as receptive fields, were circular and arranged on a perfect triangular lattice. Indeed, receptive fields are roughly circular and are positioned on a roughly triangular lattice, but imprecisely so. In collaboration with Charles Stevens, Sharpee and her team were surprised to find that the combination of these two types of irregularities yielded a near perfect performance. By comparison, performance dropped by a third when receptive fields either were made perfectly circular or irregular receptive fields were adjusted to follow an ideal lattice.

These results suggest new strategies for improving the performance of retinal implants that could help restore vision in blind people. Retinal prosthetic devices rely on an array of electrodes implanted near the retina to send electrical signals to the brain through remaining neurons in the retina. Although the implants themselves are regular arrays, irregularities arise at the interface with the neural tissue, in part because cells can move from their original positions over time. Thus, visual performance of the implant can be reduced when signals derived from a given portion of visual space are sent to cells that normally respond to a somewhat different part of the visual field. The same algorithms that Sharpee and her colleagues used to predict receptive fields in a healthy retina can now be used to find the optimal outlines of the regions of visual space that should be associated with a particular electrode.

For more information, please visit salk.edu/faculty/sharpee.html

Left to right: Jeffrey Fitzgerald, Adam Calhoun, Sophie Liu, James Jeanne, Alfred Kaye, Ryan Rowekamp, Tatyana Sharpee, Saeed Saremi
People with Peutz-Jeghers syndrome, a rare inherited cancer syndrome caused by a mutation in the tumor suppressor LKB1, develop gastrointestinal polyps and are predisposed to colon cancer. Currently there is no treatment for Peutz-Jeghers; patients must undergo continual surgeries to remove the polyps and tumors as they arise. During earlier work, Shaw had discovered that LKB1 activates a metabolic master switch known as AMPK. If a cell runs on empty, LKB1 turns on AMPK, which puts a damper on cell growth and proliferation. When LKB1 is absent or disabled, cells facing starvation never get the message and continue to divide. AMPK operates via the mTOR pathway, short for “mammalian target of rapamycin.” Rapamycin is a powerful immunosuppressant that binds and inactivates mTOR.

Since a loss of LKB1 results in a hyperactive mTOR signal, Shaw and his team hypothesized that rapamycin could be used to treat the tumors that arise as a result of Peutz-Jeghers. When administered to mice that had intestinal polyps because of an LKB1 mutation, rapamycin shrunk their polyps and in most cases eliminated them altogether. The researchers then wondered whether they could visualize the drug’s effectiveness using a technique called FDG-PET, which reveals the uptake of radioactively labeled glucose into cells. Normally, heart cells are the most ravenous consumers of glucose, but in patients with cancer, tumors light up. Most people assumed that polyps weren’t far enough along on the road to malignancy to be visible on an FDG-PET scan, but Shaw’s experiments revealed that the LKB1 mutation resulted in altered glucose metabolism in cells and tumors, allowing even benign LKB1 polyps to be clearly visible.

Their findings suggest that FDG-PET could be used to detect when polyps arise in people with Peutz-Jeghers syndrome, but also to monitor the therapeutic response to treatment. These findings also suggest that the subset of human lung cancers harboring alterations in the LKB1 gene may show altered glucose uptake, perhaps allowing for their early diagnosis and helping to dictate their therapeutic treatment.

For more information, please visit salk.edu/faculty/shaw.html

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For more information, please visit salk.edu/faculty/shaw.html

Left to right:
Front row: David Shackelford, Dana Gwinn, Annabelle Mery, Debbie Vasquez, Laurie Gerken, Reuben Shaw
Back row: Pierre-Damien Denechaud, Dan Egan, Maria Mihaylova, Rebecca Kohnz, Jonathan Goodwin

Reuben J. Shaw
Howard Hughes Medical Institute Early Career Scientist
Hearst Endowment Assistant Professor, Molecular and Cell Biology Laboratory

“When a normal cell runs low on energy, it won’t divide, but in some cases, cancer cells can override the built-in shutoff. The same cellular brake helps cells and organisms adapt their glucose metabolism. I am particularly interested in understanding the molecular link between cancer and metabolism since it embodies a critical intervention point for future therapeutics.”
Drugs of abuse can produce long-term changes in the electrical activity of neurons in the brain. Recently, we have been researching a new role for GIRK potassium channels—proteins that control the movement of potassium ions in the brain—in drug addiction. Our studies may provide new insights into the cellular mechanisms of drug addiction as well as some mental disorders, such as schizophrenia and attention deficit hyperactivity disorder (ADHD).

Alcohol’s inebriating effects are familiar to everyone. But despite its long history and the widespread use of ethanol—the alcohol in intoxicating beverages—when it comes to alcohol’s impact on brain activity on a molecular level, it remains among the least understood of psychoactive drugs. Although alcohols had previously been shown to lead to the opening of GIRK (short for G-protein-activated inwardly rectifying potassium) channels, it was not known whether this was a direct effect or byproduct of other molecular changes in the cell.

When Slesinger and collaborators determined the three-dimensional structure of GIRK channels at high resolution, they discovered a molecular pocket that resembled confirmed alcohol-binding sites found in two other proteins (alcohol dehydrogenase, the enzyme that breaks down alcohol in the body, and LUSH, a fruit fly protein that senses alcohol in the environment). This finding allowed them to address the puzzle of how alcohol activates GIRK channels.

When they systematically introduced amino acid substitutions that denied alcohol molecules access to the potential interaction site, alcohol could no longer efficiently activate the channel, confirming that they had hit upon an important regulatory site for alcohol. The team further established that this pocket is a trigger point for channel activation since G protein activation was also altered. They believe that alcohol hijacks the intrinsic activation mechanism of GIRK channels and stabilizes the opening of the channel, perhaps by “lubricating” the channel’s activation “gears.”

A better understanding of how GIRK channels are activated could point to new strategies for treating human diseases. Using the protein structure as a starting point, for example, it may be possible to develop a drug that antagonizes the actions of alcohol to treat alcohol dependence. Alternatively, if a novel drug is identified that fits the alcohol-binding site and activates GIRK channels, this could dampen overall neuronal excitability in the brain and perhaps provide a novel pharmacological tool for treating epilepsy.

For more information, please visit salk.edu/faculty/slesinger.html
Virtually all of the neural circuits in the vertebrate brain possess what computer scientists call a scalable architecture, allowing them to increase their computational power by simply growing in size while conforming to a single basic design. The vast majority of computers currently in use have what is known as a von Neumann architecture, which is not scalable. For evolution to work, however, brains need to be able to adapt to increased demands without the need for significant reconfiguration. Research in Stevens’s laboratory is aimed at learning the design principles that endow vertebrate neural circuits with a scalable architecture.

How can the scalability of neural circuits be studied? Most vertebrate brains—for example, the human brain—are nearly the same size for a given species, so that circuits of very different sizes are not available for comparison. Brains across a series of evolutionarily related species, such as primates, have a range of sizes that can be studied, but these brains are not necessarily comparable because of adaptive specializations of specific species. Stevens is interested in discovering principles that govern all vertebrate brains, so he started by comparing the brains of a single species, goldfish, which come in a variety of sizes but are strictly comparable. Fish, unlike mammals, continue to grow throughout their lives and add new nerve cells to their neural circuits as they grow older and larger. Of course, any principles discovered in fish must be confirmed by studies in mammals.

Scalability implies that quantitative aspects of brain structure and function follow what are called scaling laws, orderly relationships that, for example, dictate for all brains how the number of neurons in one brain area is related to the number of neurons in a second area that receives information from the first one. These scaling relations embody general design principles, and so the job of a theorist is to figure out the computational significance of the scaling laws and learn how the brain grows in a way that conforms to the scaling law during the organism’s development.

For more information, please visit salk.edu/faculty/stevens.html
Growing nerve cells are great communicators, extending long projections called axons, which hook up with other specific nerve cells to form functional circuits within the developing nervous system. At the tip of each growing axon is the growth cone, which steers the axon to its target cells by responding to cues in the extracellular environment.

Capitalizing on the advanced genetics of the fruit fly *Drosophila*, John Thomas’s lab has identified key molecules in the axon’s navigation system that govern a basic event common to all nervous systems: making axons grow from one side of the brain to the other. Crosstalk between the two sides of the nervous system is essential for many behaviors, from simple coordinated locomotion to the integration of higher cognitive functions, and its importance is underscored by the large number of nerve cells that project their axons across the midline to the opposite side.

Using a set of axon-targeted reporter molecules, Thomas has identified a number of axon guidance molecules, including a receptor called Derailed and its ligand, Wnt5, which together control how axons cross the midline of the nervous system.

Axons, it turns out, do not cross the midline randomly. Instead, they choose specific routes, or tracts. In *Drosophila*, crossing axons choose one of two tracts. The Derailed receptor is expressed on the growth cones of all nerve cells that cross in one of the tracts. Wnt5 is secreted by nerve cells that cross in the other tract, and by binding to Derailed, it acts as a repellent to keep the Derailed-expressing axons from wandering off the beaten tract.

Both Derailed and Wnt5 belong to larger families of related molecules, and Thomas has found that their relatives control additional axon guidance events. Recent studies have revealed that in mammals, the molecule that corresponds to Derailed is also a receptor for Wnt proteins and that it too is involved in guiding axons. What that means is these guidance molecules are deeply rooted in who we are, whether we are a fly on the wall or a human being wielding a flyswatter.

For more information, please visit sailk.edu/faculty/thomas.html
Sex chromosomes and sexual selection strongly influence every aspect of biology, including development, behavior, and even ecological interactions between species. Umen’s research team has taken advantage of a unique group of green algae to begin answering a longstanding question in developmental and evolutionary biology: How do different sexes evolve?

While the single-celled alga *Chlamydomonas* has two sexes whose reproductive cells look identical, its close relative *Volvox* is multicellular and has evolved females and males that produce eggs and sperm, respectively. When Umen and his collaborators sequenced the sex-determining chromosomal region from both species, they found intriguing differences. Not only was the size of the sex-determining region from multicellular *Volvox* much larger than that of its unicellular relative *Chlamydomonas*, but the genes within this sex-determining region were found to evolve in a very different manner from those of the single-celled species. Unlike *Chlamydomonas*, the male and female versions of genes in the *Volvox* sex-determining region were so distinct that they looked as if they came from different species.

Using revolutionary “deep-sequencing” technology, which allows researchers to quickly identify genes and their expression patterns, Umen’s laboratory found a large number of newly evolved genes in the *Volvox* mating locus that do not have counterparts in *Chlamydomonas*. Moreover, they found sex-linked patterns of molecular evolution and expression for many of the genes in the locus, indicating that sex chromosomes are a rich source of new genetic material and can play a major role in driving changes in sexual development.

A recent study in humans has indicated that—similar to the *Volvox* sex-determining region—the human Y chromosome, long considered a place of slow evolutionary decay, instead is a hotspot of evolution that is changing more quickly than any other part of humanity’s genetic code.

These findings suggest that driven by common processes, sex chromosomes in species as diverse as algae and primates are evolving rapidly and dynamically.

For more information, please visit salk.edu/faculty/umen.html

**James Umen**
Assistant Professor
Plant Biology Laboratory

“My laboratory is interested in fundamental questions in cell biology—how do cells control growth and division, how do they determine their size, and how do the genetic pathways that control size and division evolve to generate life’s incredible diversity?”

Left to right:
Seated: Yubing Li, Jim Umen, Shigeki Stoner, Cristina Lopez
Standing: Sa Geng, Matt Zones, Peter De Hoff, Brad Olson
“Stress hormones give us added strength and endurance and make us more vigilant and ready to freeze, flee, or fight. But they can also contribute to mental and physical illness when stress is persistent. We study the roles that certain peptides discovered at Salk play in regulating the stress response and are particularly interested in conceiving therapeutic approaches to mitigate stress-linked diseases.”

The Vale lab studies peptide hormones and growth factors that provide communication between the brain and endocrine system. One of their programs involves the CRF network, comprising the peptide hormones CRF and three urocortins and two receptors that have broad and distinct tissue locations and actions. The first member of the family to be discovered, CRF, acts through CRF receptors within the brain to trigger stress-mediated behavioral, hormonal, and autonomic responses. Furthermore, CRF is suspected to play a role in various stress-related disorders, including anxiety, depression, irritable bowel syndrome, and the dysphoria associated with drug and alcohol withdrawal. Enabled by findings at the Salk Institute, drugs that modify CRF actions have been developed by pharmaceutical companies and are in clinical trials for these disorders.

The urocortins have complex effects on behavior and many important actions on the cardiovascular system, gastrointestinal tract, and metabolism. For example, urocortins acting through the urocortin receptor have beneficial effects on the injured heart and are currently undergoing proof of principle clinical trials in patients with acute congestive heart failure. Urocortin 3 is produced in the brain, where it regulates appetite, and in the beta cells of the pancreas, where it can increase production of insulin, particularly when blood sugar is too high.

Recently Vale’s group found CRF receptors in beta cells and has shown that CRF is also a powerful stimulator of insulin production. Mature, differentiated beta cells can divide, albeit slowly, but if they are exposed to CRF or urocortins, they will start to divide more rapidly. Type 1 diabetic patients are thought to have a few beta cells left in their pancreas, so those remaining beta cells, though not enough to control glucose levels, could seed a population of regenerating beta cells if the destructive autoimmune process behind Type 1 diabetes could be abated. These findings may provide new insights into the control of insulin production and suggest novel targets for disease intervention.

For more information, please visit salk.edu/faculty/vale.html
The mouse has long provided researchers with valuable insights about cancer. But the most commonly used technique for producing a cancer mouse model—transplanting human tumor tissue or cancer cell lines in immunocompromised mice—ignores the role of the immune system in cancer. Other animal models either express oncogenes in a tissue-specific manner or shut down the expression of tumor suppressor genes in the whole tissue. But tumors generally develop from a single cell or a small number of cells of a specific cell type, which is one of the major determinants of the characteristics of tumor cells.

To create a better mouse model, researchers in Verma’s laboratory turned to gene therapy techniques, using modified viruses to infect cells and ferry activated oncogenes into a small number of cells in adult, fully immunocompetent mice. After initial experiments confirmed that the approach was working, his team injected viruses carrying two well-known oncogenes encoding the tumor suppressor p53. They specifically targeted astrocytes, star-shaped support cells that are suspected of being the source of glioblastoma, the most common and deadly human brain cancer. Within a few months, massive tumors that displayed all the histological characteristics of glioblastoma developed in two of the regions.

To test whether the induced glioblastomas contained cancer stem cells, investigators isolated and cultured individual tumor cells in the lab, which looked and behaved just like neural stem cells. Less than 100 and as few as 10 cells were enough to initiate a tumor when injected into immunodeficient mice. These findings show that this cancer model will not only allow scientists to gain new insights into the biology of glioblastoma but will also help them answer many questions surrounding cancer stem cells. Verma and his team are currently using this methodology to investigate lung and prostate cancers.
Geoffrey M. Wahl
Professor
Gene Expression laboratory

“We are studying the genetic basis of the origin and progression of cancer and developing new strategies to tailor-make drugs based on the genetic signature of a patient’s tumor.”

Cancer is the second leading cause of death in the United States, but the revolution in our understanding of cancer etiology holds the promise for significant therapeutic advancements in the future. There are, however, two significant impediments to effectively treating cancer. First, cancer is often detected when it is very advanced. Second, cancer cells are often genetically unstable, enabling them to develop resistance to anti-cancer drugs at alarming rates. Wahl and his team showed that cancer cell genetic instability often involves disabling a stress response pathway controlled by the key tumor suppressor, p53. P53, a transcription factor that regulates the activity of genes, is activated by many of the stresses, such as DNA damage, that promote cancer cell development. Once activated, it stops cell division or, if the damage is too severe, induces a cell death program.

Recently, a collaborative study led by Geoff Wahl and Juan Carlos Ispizúa Belmonte uncovered that the tumor suppressor p53 not only stops cells that could become cancerous in their tracks but also controls somatic cell reprogramming. In cells genetically engineered to lack p53, reprogramming efficiency was increased at least tenfold compared to control cells, demonstrating that p53 clearly played an important role in repressing cells trying to revert back into a stem-like state. Their findings not only bring iPS cells technology a step closer to fulfilling its promise as a source of patient-specific stem cells but also are forcing scientists to rethink the development of cancer.

The idea that cancer arises through the de-differentiation of fully committed and specialized cells had been around for decades, but eventually it was discarded in favor of the currently fashionable cancer stem cell theory. Now that Wahl and his colleagues have shown that p53 prevents de-differentiation, they believe it is time to reconsider the possibility that reprogramming plays a role in the development of cancer since virtually all cancer cells lose p53 function in one way or another. They have begun to investigate whether mutation of p53 promotes acquisition of a stem-like state during tumorigenesis in vivo, with encouraging preliminary results.

For more information, please visit
salk.edu/faculty/wahl.html

Left to right:
Rose Rodewald, Jennifer Lin, Justin La, Samantha Cheung, Jennifer Green, Dannielle Engle, Geoffrey Wahl, Mark Wade, Vivian Wang, Daphne Chen, Benjamin Spike, Leo Li, Taneashia Morrell (at top)
Cells provide a dazzling variety of functions that cover all of our body’s needs, yet they make do with a very limited number of molecular building blocks. With few exceptions, all known forms of life use the same common 20 amino acids—and only those 20—to make all the proteins necessary to keep organisms as diverse as humans, earthworms, tiny daisies, and giant sequoias alive. During protein synthesis, amino acids are brought out one by one by molecules known as transfer RNAs (tRNA) and added to the growing protein chain according to the instructions spelled out in the body’s genes until a stop codon—for which no corresponding tRNA/ amino acid pair exists—lets everybody know that this particular job is done.

Each of the 20 amino acids is matched to specific transfer RNAs and by extension to nucleotide triplet codons in genes and messenger RNAs according to what has become known as the universal genetic code. Although different algorithms, or codes, were undoubtedly tested during a long period of chemical evolution, the modern code proved so robust that, once established, it gave birth to the entire tree of life. But how individual amino acids were assigned to specific three-letter codons during the evolution of the genetic code is still subject to speculation. Although different hypotheses abound, data were hard to come by.

When Wang and his team probed the genetic code in bacteria, they found evidence that direct interactions between amino acids and nucleotide triplet codons (or the matching anticodons carried by tRNAs, which “read” messenger RNAs) helped establish matching pairs. After only two waves of “matching,” all 20 amino acids were firmly assigned, setting the stage for the emergence of proteins with unique, defined sequences and unchanging properties. Once that critical step had been taken, the last common ancestor was ready to give rise, over billions of years, to the wonders of a planet teeming with life.

For more information, please visit salk.edu/faculty/wang.html

Lei Wang
Assistant Professor, Chemical Biology and Proteomics Laboratory
Frederick B. Rentschler Developmental Chair

“The genetic code, which is shared by plants, animals, and bacteria, includes 64 codons encoding 20 different amino acids and three stop signals. We are trying to expand the code and insert artificial amino acids into proteins in mammalian cells and multicellular organisms, which provides novel tools to address challenging questions that are insurmountable with conventional means. Currently, we are also studying the evolution of the genetic code.”
“Viruses are powerful model systems to study cell biology. Our lab has become particularly interested in how viruses are recognized by the cellular DNA damage pathway, and we have uncovered ways that viruses either exploit or manipulate the cellular machinery as they commandeer the cell for virus production. Our findings have important implications for understanding DNA repair and genomic instability.”

Like all viruses, herpes simplex virus (HSV)—the culprit behind cold sores—requires a living host in order to multiply. But before it can hijack the cellular machinery to produce scores of copies of itself, it needs to evade the cell’s security system. To the host cell, invading viral DNA looks just like the product of DNA damage, which must be repaired or removed in order for the cell to stay healthy. This led Weitzman to suspect that viral DNA would be recognized by the cell’s DNA repair machinery and that the virus must somehow override the cell’s response to this foreign DNA.

To test this hypothesis, Weitzman and his team looked at what happens in a virus-infected cell when its DNA is damaged. In a normal cell, DNA damage sensor proteins rushed to the site of damage. In cells infected with HSV, however, the cells’ emergency repair teams didn’t respond properly. They identified a single viral protein, known as ICP0, that takes out the DNA damage response in human cells. It attaches so-called ubiquitin marks to two important DNA “security guards,” the proteins called RNF8 and RNF168. Ubiquitin flags proteins for destruction, in this case instructing the cell to get rid of the very proteins that protect it. Others had recently reported that RNF8 and RNF168 play a central role in DNA repair, where they ubiquitinate a protein called histone H2A, which directs DNA damage response proteins to accumulate at the sites of damage. The Weitzman lab found that through the degradation of these security guards, the virus prevents ubiquitination at sites of cellular damage and therefore diminishes the cell’s ability to call in repair proteins.

Watching these battles unfold yields important insights into fundamental mechanisms that allow HSV to dismantle its hosts’ defenses and may suggest a common mechanism by which viruses can successfully infect host cells. It also points out the key steps in maintaining an undamaged genome, which is a continuous challenge and crucial to prevent a cell from turning cancerous.

For more information, please visit salk.edu/faculty/weitzman.html

Left to right:
Inigo Narvaiza, Brandon Lamarche, Nicole Orazio (seated), Liz Boice, Mira Chaurushiya, Matthew Weitzman (seated), Caroline Lilley, Marius Tham, Ayumi Kudo (seated), Sebastien Landry
HIV can remain “hidden” in a latent form for years, even after long-term suppression with highly active antiretroviral therapy. Moreover, viral drug resistance represents a formidable problem, creating an urgent need for new classes of antiretrovirals. HIV begins its assault by injecting its core, which contains single-stranded RNA, into a host cell. Once inside, the viral RNA is converted into double-stranded DNA—a process known as reverse transcription—and the original viral RNA is degraded. Another enzyme, integrase, mediates the final step of the genome conversion, where the viral double-stranded DNA slips into the host’s DNA, allowing it to take advantage of the host cell’s genetic machinery to replicate and propagate itself. During these early steps of infection, the virus relies heavily on its host cell to lend a helping hand, which makes it particularly vulnerable to antivirals and host defense mechanisms.

To identify cellular processes that participate during this critical time period, Young and his collaborators disrupted the function of individual genes in the genome of host cells. They then screened the mutagenized cells for their ability to resist infection with murine leukemia virus (MLV), a virus often used as a convenient, harmless stand-in for HIV.

Their experiments revealed a previously unknown regulatory step during MLV and HIV infection that uses sulfonation—a type of chemical modification—to boost the production of new viral particles. When sulfonation was impeded genetically or through chemical inhibitors during or shortly after viral DNA integration into a chromosome, the virus’s ability to reproduce was comprised. A closer look revealed that sulfonation was required to fully activate the viral long terminal repeat (LTR) promoters that flank the viral genome. LTRs function as regulatory regions that interact with cellular and viral factors to trigger gene expression as well as production of the viral genomes that are packaged into the next generation of virus particles.

This discovery might open up new avenues for the development of drugs that specifically interfere with this newly revealed aspect of retroviral biology and render host cells resistant to HIV infection.

For more information, please visit salk.edu/faculty/young.html

John A. T. Young
Professor
Nomis Foundation Laboratories for Immunobiology and Microbial Pathogenesis

“One of the major long-term goals of our work is to uncover the cellular protein machinery required for replication steps of viruses such as HIV, the cause of AIDS. Knowledge of this machinery provides brand-new insights into the cell biology of virus infections and could suggest new broad-spectrum antiviral approaches.”
Ye Zheng
Assistant Professor, Nomis Foundation Laboratories for Immunobiology and Microbial Pathogenesis
Emerald Foundation Developmental Chair

“The immune system must keep a delicate balance between effectively fighting foreign pathogens while minimizing collateral damage. Research in my lab is focused on generating and maintaining regulatory T cells, which prevent biological ‘friendly fire’ by ensuring that the immune system does not overly attack the body’s own tissues.”

The immune system is often described as a kind of military unit, a defense network that guards the body from intruders. Seen in this way, a group of white blood cells called T cells are the frontline soldiers of immune defense. The majority of T cells engage invading pathogens head on, while a smaller subset, called regulatory T cells, limit excessive immune reactions. Autoimmune diseases such as type 1 diabetes, Crohn’s disease, lupus, and rheumatoid arthritis occur when the balance of power between the two breaks down.

Regulatory T cells are controlled by a pivotal gene regulator called Foxp3. In fact, when Foxp3 stops functioning, the body can no longer produce working regulatory T cells. Until now, however, scientists had barely understood what signals lead to Foxp3 expression and how Foxp3 in turn controls regulatory T cells because they knew very little about the actual genes under Foxp3’s purview. Zheng identified several DNA elements in the Foxp3 locus that are directly involved in inducting and maintaining Foxp3 expression and that regulate the development and stability of regulatory T cell lineage. He then focused on the downstream targets of Foxp3. Using a genome-wide screen, Zheng mapped all genes directly regulated by Foxp3 and identified a small group of transcription factors—proteins that control the expression or “transcription” of genes—that drive the expression of genes involved in regulatory T cell function. One of them, IRF4, stood out as the key player in regulatory T cells’ ability to control type-2 T helper cells, which, if uncontrolled, can activate other immune cells and lead to allergy and asthma.

In the future, Zheng will expand his current studies to determine how regulatory T cells are generated and maintained. His experiments not only will provide a better understanding of regulatory T cells but will suggest new therapeutic approaches for treating a wide range of autoimmune diseases, improving organ transplant survival, and boosting the immune system’s response to tumors.

For more information, please visit salk.edu/faculty/zheng.html

Left to right:
Nina Miller, Ye Zheng, Oren Milstein
NCI Cancer Center

The Cancer Center, a National Cancer Institute-designated basic research center, was established in 1970 by Jonas Salk. Today, under the leadership of Tony Hunter, researchers in the Cancer Center probe the fundamental aspects of cancer biology, with the ultimate goal of reducing incidence, morbidity, and mortality.

Stemming from a philosophy that basic research has the power to illuminate underlying causes of cancer, often in unexpected ways, the center’s research is divided into three programs:

**METABOLISM AND CANCER**
- Andrew Dillin
- Ronald Evans
- Tony Hunter
- Marc Montminy
- Joseph Noel
- Satchidananda Panda
- David Schubert
- Reuben Shaw
- Wylie Vale

**MOUSE MODELS AND STEM CELLS**
- Senyon Choe
- Fred H. Gage
- Juan Carlos Izpisúa Belmonte
- Leanne Jones
- Christopher Kintner
- Kuo-Fen Lee
- Greg Lemke
- Samuel Pfaff
- John Thomas
- Inder Verma
- John Young
- Ye Zheng

**GROWTH CONTROL AND GENOMIC STABILITY**
- Beverly Emerson
- Martin Hetzer
- Katherine Jones
- Jan Karlseder
- Björn Lillemeier
- Vicki Lundblad
- Clodagh O’Shea
- James Umen

For more information, please visit salk.edu/faculty/cancer_center.html
The overall goal of the Crick-Jacobs Center, an interdisciplinary research unit named in honor of the late Nobel laureate Francis Crick and chairman of the Salk board of trustees, Irwin Jacobs, is to integrate experimental and theoretical approaches to understanding the organization of signaling systems and the functional neuroanatomy of the brain, from the molecular to the systems levels, and how behavior arises from the interactions between the brain’s many components.

Under the leadership of Terrence J. Sejnowski, the scientists who work at the Crick-Jacobs Center combine approaches from biology, physics, chemistry, mathematics, computer science, and engineering and exploit techniques that include computer simulations, imaging, viral vectors, and molecular genetics.

**MEMBERS**
Sreekanth Chalasani  
Salk
Tatyana Sharpee  
Salk

**SENIOR FELLOWS**
William Bialek  
Princeton
Sydney Brenner  
Salk
Edward M. Callaway  
Salk
Sean Eddy  
Janelia Farm
David Kleinfeld  
UCSD
Christof Koch  
CalTech

**SENIOR FELLOWS CONTINUED...**
Terrence J. Sejnowski  
Salk
Mel Simon  
CalTech
Larry Smarr  
UCSD
Charles F. Stevens  
Salk
Susumu Tonegawa  
MIT

**JUNIOR FELLOWS**
Tanya Baker  
Andrea Hasenstaub  
Mike McConnell

**VISITING FELLOW**
Rex Kerr

For more information, please visit  
salk.edu/faculty/crick-jacobs_center.html
Glenn Center for Aging Research

The Glenn Center for Aging Research was established in January 2009 with a $5 million gift from the Glenn Foundation for Medical Research. Led by Andrew Dillin, the center draws from ten of Salk’s leading laboratories specializing in genetic analysis, stem cell biology, and metabolism research to address the overarching goal of defining a healthy lifespan, or healthspan, and to answer one of the most elusive questions in biology: Is there a defined biological process of aging that is universal to all organisms?

GENETIC ANALYSIS GROUP
Andrew Dillin  
Martin W. Hetzer  
Jan Karlseder  
Vicki Lundblad

STEM CELL GROUP
Fred H. Gage  
Juan Carlos Izpisúa Belmonte  
Leanne Jones

METABOLISM GROUP
Ronald M. Evans  
Marc R. Montminy  
Reuben J. Shaw

For more information, please visit salk.edu/glenn/
The Center for the Neurobiology of Vision was designated as a basic research center by the National Eye Institute in 2009. Directed by Thomas Albright, the center comprises 15 independent investigators, whose research programs span nearly the full range of visual processing stages, from the retina through object recognition and visual-motor control. The development and plasticity of these processing stages is addressed at multiple levels of experimental analysis, ranging from theoretical and molecular genetic investigations through studies of individual cells and their interactions, small neuronal circuits, larger neuronal systems, and behavior. Emphasis is also placed on clinical disorders of visual perception and visually guided behavior, such as Williams syndrome and autism.

MEMBERS
Thomas D. Albright
Ursula Bellugi
Edward M. Callaway
E.J. Chichilnisky
Sascha du Lac
Sergei Gepshtein
Richard J. Krauzlis
Greg E. Lemke
Dennis D. M. O’Leary
Satchidananda Panda
John H. Reynolds
Terrence J. Sejnowski
Tatyana Sharpee
Charles F. Stevens
Gene Stoner

For more information, please visit salk.edu/cnv/
Nomis Center for Immunobiology and Microbial Pathogenesis

The Nomis Center for Immunobiology and Microbial Pathogenesis was launched in 2008 with a gift of $11.5 million received on behalf of the Nomis Foundation, a European foundation established by Salk trustee G. H. “Heini” Thyssen.

The center aims to shed light on the molecular mechanisms that cause infectious diseases, define key molecules involved in the body’s response to injury or infection, and understand why inflammatory processes spin out of control under some circumstances.

An increasing number of reports show that chronic inflammation is the culprit behind the most common illnesses of middle and old age. It’s capable of bursting plaques in coronary arteries, leading to heart attacks, and damages nerve cells in Alzheimer’s patients. It drives autoimmune disorders and is intricately linked with the early stage development of cancer and diabetes.

MEMBERS
Melvin Cohn
Ronald M. Evans
Tony Hunter
Katherin A. Jones
Greg E. Lemke
Björn F. Lillemeier
Clodagh O’Shea
Inder M. Verma
Matthew D. Weitzman
John A.T. Young
Ye Zheng

For more information, please visit salk.edu/faculty/nomis_center.html
Salk Center for Nutritional Genomics

Founded in 2009 with a $5.5 million grant from the Leona M. and Harry B. Helmsley Charitable Trust, the Salk Center for Nutritional Genomics employs a molecular approach to nutrition and its impact on the role of metabolism in diabetes, obesity, cancer, exercise physiology, and lifespan, thereby increasing the understanding of how nutrients affect health. It includes a metabolic core facility and an interdisciplinary fellows program.

The center approaches fundamental aspects of medical physiology and endocrinology from the perspective of the genome. Its members look at metabolic control as a product of the regulated activity of metabolic genes, which undergo dramatic shifts, not only in response to fasting or feeding, but also in aging and disease.

These metabolic shifts provide a critical underpinning in many disease processes, and loss of their control underlies the development of both type 2 diabetes and obesity, as well as other insulin-resistant conditions, such as polycystic ovary syndrome. In addition, deregulated glucose metabolism is a long-known hallmark of tumor cells, and recent links have connected the pathways controlling glucose and lipid metabolism in tissues such as liver and muscle to the processes deregulated in many human cancers.

MEMBERS
Ronald M. Evans
Marc R. Montminy
Reuben J. Shaw

HELMSLEY FELLOWS
Nathan Baird
Dillin Lab
Weiwei Fan
Evans Lab
Mark Huising
Vale Lab
Bing Luan
Montminy Lab
Yeddula Narayana
Verma Lab
Erin Quan Toyama
Shaw Lab
The Sloan-Swartz Center for Theoretical Neurobiology

The principal objective of the Sloan-Swartz Center for Theoretical Neurobiology, which was established in 1994 with major financial support from the Alfred P. Sloan Foundation and continuing funding from the Swartz Foundation, is to develop a firm theoretical infrastructure for modern experimental neurobiology. To accomplish this goal, the center’s members promote the application of theoretical concepts and techniques, drawn from the physical sciences, to a wide range of problems in neurobiology.

Their areas of study span a wide range of critical levels of analysis, including molecular characterization of ion channels, synaptic transmission and plasticity, developmental events in circuit formation, characterization of the properties of cortical neurons, and the relationship of the latter to sensory, perceptual, and cognitive experience.

The center also seeks to educate conventionally trained neurobiologists in modern theoretical tools.

MEMBERS
Thomas Albright
Ursula Bellugi
Edward Callaway
E.J. Chichilnisky
Sasha du Lac
Martyn Goulding
Stephen Heinemann
Christopher Kintner
Richard Krauzlis
Greg Lemke
Dennis O’Leary
John Reynolds
Terrence Sejnowski
Charles Stevens
John Thomas

SLOAN-SWARTZ FELLOWS
Sergei Gepshtein
Samat Moldakarimov
Ruadhan O’Flanagan

For more information, please visit sloan-swartz.salk.edu/
Waitt Advanced Biophotonics Center

Founded in 2008 with a $20 million challenge grant from the Waitt Family Foundation, whose president and founder, Ted Waitt, is also the vice-chair of Salk’s board of trustees, the Waitt Advanced Biophotonics Center aims to develop highly sophisticated imaging tools and methods that will allow scientists to “see” and “record” a living cell’s inner workings at the molecular level.

This transformative initiative will ultimately allow us to understand how single molecules function in real time, shed light onto molecular and cellular circuits, and investigate how neurons connect with one another in the brain in healthy and diseased states. The center will exist when completed in two symbiotic parts: the biophotonics core facility and the biophotonics faculty.

The core facility will be equipped with the latest commercial imaging and data analysis technologies and will provide logistical access and technical support to Salk faculty so they can integrate cutting-edge imaging tools into their individual and collaborative research programs. Examples of instrumentation that will be available to all Salk faculty research labs are confocal microscopy, TIRF microscopy, two-photon microscopy, electron microscopy, and super-resolution imaging. In addition, the core will operate an advanced data analysis suite (using both commercial and custom-designed software) that will allow sophisticated modeling to be undertaken using image data acquired on the facility’s imaging systems. The data core will also provide access for up to 500TB of processing and storage space.

The biophotonics faculty, when hired, will consist of three assistant professors. Each faculty lab will engage in both technological development research projects as well as focus on applying new technological advances to significant biological problems that are consistent with the Salk’s broader research areas.

Both the biophotonics faculty and the core will work together in a close collaborative relationship to further advances in imaging technology and apply and integrate those new methods into the biological research community at Salk.

MEMBERS

Core Facility:
James Fitzpatrick
Director

Faculty:
Active faculty search under way
Scientific Core Facilities
Razavi Newman Center for Bioinformatics

Gerard Manning, Director

“We provide a gateway for Salk scientists to use computational methods and databases to deepen and integrate their research. In an era of whole genome sequences and high-throughput experiments, there is almost no area of research that cannot benefit from bioinformatics analysis of internal data and mining through the growing public databases of genomic and experimental data.”

Services
The center’s mandate is to enable the use of bioinformatics approaches throughout the Salk Institute. To that end, Manning and his team collaborate, consult, and help with both choosing bioinformatics approaches and carrying them out. The center has particular expertise in analysis of gene sequences and evolution and in signaling and cancer, but works across the full range of bioinformatics techniques. The center also operates a number of bioinformatics servers and can help in design and creation of tools and databases.

Research
Manning and his team don’t have a “wet lab.” Instead, they try to understand the results of the greatest experiment of all time: the more than 2 billion years of evolution and selection that have played out across the entire planet, and whose results are written in the genomes that are now being read at such a remarkable and growing pace. Their specific research focus is on protein kinases, a set of genes that control most activities of the cell and are an important and successful cancer drug target. Other research areas are emerging from collaborations with other Salk and outside groups, including a large collaboration with the Dillin lab to address age-related diseases, and a project with the Craig Venter Research Institute to understand bacterial evolution and diversity.

The center is named in honor of Salk trustee Howard Newman and his wife Maryam Razavi, who provided generous funding to establish the center.

For more information, please visit cores.salk.edu/bioinformatics/

Left to right:
Eric Scheeff, Scott Becker, Ana Rodrigues, Gerard Manning, Aaron Legler, Brian Baker, Mike Dacre

Not shown: Yufeng Zhai, Mark Jinan Chen, Thi Pham, Anna Luan
Center for Cytometry and Molecular Imaging

David Chambers, Director

“Fluorescence is key tool in today’s research, and Salk’s Center for Cytometry and Molecular Imaging (CCMI) provides access to sophisticated fluorescence technology and expertise in related areas. We train and assist investigators in the use of our equipment, assist with data analysis and presentation, and are also available for advice concerning experimental design and optimization.”

Flow cytometry
Flow cytometry may be used to analyze large numbers of cells in a short time and optionally isolate cells of interest. The facility’s two analytical flow cytometers are capable of resolving up to six fluorescent colors simultaneously, while the high-speed digital cell sorter (FACSVantage DiVa) offers up to eight colors; cells separated by the latter may be used for subsequent analyses or may be further expanded in culture. The cell sorter allows investigators to isolate pure populations of rare (<0.1% abundance) cells with a throughput of up to 50 million cells per hour.

Confocal microscopy
Our digital confocal microscope is a high-end Leica TCS SP2 spectral unit with four lasers. This machine provides high-resolution images of cells at the sub-micron level, or overviews of large tissue sections, using up to four simultaneous fluorescence colors. One of this machine’s major advantages is that it features freely programmable fluorescence collection, rather than using traditional fixed color filters. This not only saves time because color overlaps can easily be eliminated, it also helps eliminate the unwanted autofluorescence that many samples exhibit.

Two-dimensional imaging
Extracts from cells tagged with fluorescent or radioactive compounds may be separated by one-or two-dimensional electrophoresis, and the resultant gels or blots analyzed using our 2-D imagers. In the case of radioactively labeled probes, a radiation-sensitive screen is exposed to the sample, and then imaged while fluorescent labels are detected immediately. The facility has two imagers—a Molecular Dynamics Typhoon, and a Fuji FLA 5100. The Fuji instrument is newer and can analyze more colors than the Typhoon.

Microplate reading
The CCMI also offers a multi-well plate reader, which is able to quantitate fluorescence or absorbance in standard plate formats ranging from six wells to 384 wells per plate.

For more information, please visit cores.salk.edu/ccmi/

Left to right: Jonna Barrie and David Chambers
Functional Genomics Core

Ling Ouyang, Director

“The Functional Genomics Core was established at the Salk Institute in 1999 to provide a shared resource for analysis of gene expression using Affymetrix Gene Chip technology and the real-time detection of PCR products for genes of interest. Our mission is to provide Salk investigators with high-quality, state-of-the-art gene expression services in a timely manner.”

Gene Expression Analysis
The Functional Genomics Core provides instrumentation and expertise for RNA transcript profiling. DNA microarrays provide a highly parallel means of measuring the abundance of RNA for targeted genes in a biological sample. The facility supports two microarray formats: oligonucleotide arrays synthesized by Affymetrix, Inc. and custom arrays of probes printed in house on glass slides.

SNP Genotyping
The core offers genotyping service using Affymetrix Genechip technology and Applied Biosystems real-time PCR instruments.

Qualitative and Quantitative analysis of DNA, protein, and RNA samples
The core recently purchased the Agilent 2100 Bioanalyzer, which is a microfluidics-based platform to support the use of the quantitative and qualitative analysis of DNA, RNA, and proteins. The results are shown in gel-like images, electropherograms, and tabular formats.

Quantitative PCR
The core relies on the ABI PRISM® 7900HT Sequence Detection System to offer reliable real-time detection of PCR. It uses fluorescent primers and probes (Taqman) or Sybr green to quantify the accumulation of nucleic acid sequences.

In addition, the Functional Genomics Core offers an automated miniprep service for rapid handling of DNA samples—a cost-effective and convenient way for laboratories to analyze large numbers of samples.

For more information, please visit cores.salk.edu/microarray

Left to right
James Nguyen and Ling Ouyang
Peptide Synthesis Facility

Jill Meisenhelder, Director

“We have been providing a reliable, cost-effective source of peptides for Salk investigators to use in their research since 1992. In addition to synthesizing custom peptides, we also provide advice and training for investigators in the design of appropriate peptides, use of protocols, and the interpretation of peptide characterization profiles.”

The current peptide synthesizer, a Model PS3 from Protein Technologies, was purchased in June 2006 and is operated by Jill Meisenhelder, whose role has expanded to include providing advice on the design of peptides and protocols in which they will be used. She arranges for the crude peptides to be analyzed by mass spectrometry in the proteomics facility and evaluates the results to gauge the fidelity of the synthesis.

Peptides synthesized by the facility are frequently utilized for developing antibodies that can be used for immunoprecipitation or immunoblotting. Synthetic peptides are also used to identify regions of protein–protein interactions by testing whether peptides representing defined regions of a protein can block interaction between two full-length proteins in competition assays. They are also employed to block functional interactions between proteins and for use in biological assays to stimulate cells. Hydrophobic peptides that readily form aggregates, such as those found in the brains of Alzheimer’s patients, have been used for structural studies.

During a typical year, more than 100 peptides are synthesized, including phosphopeptides, peptides with chemical modifications such as acetylation or biotinylation, as well as peptides incorporating specially derivatized amino acids to control peptide folding.

For more information, please visit cores.salk.edu/peptide/
The Vincent J. Coates Foundation Mass Spectrometry Center

Wolfgang Fischer, Director

“The Mass Spectrometry Center supports Salk researchers by using mass spectrometry to identify proteins and their post-translational modifications.”

The Mass Spectrometry Center routinely identifies small amounts of proteins that are separated by gel electrophoresis. In addition, the facility is equipped to analyze complex protein mixtures by two-dimensional chromatography followed by mass spectrometric identification. It is equipped with a hybrid Fourier-transform mass spectrometer with an electrospray source. In addition, a time-of-flight mass spectrometer that employs matrix-assisted laser desorption ionization is available.

**Protein Identification**

During the past year, a number of projects requiring mass spectrometric protein identification were completed. These include the characterization of factors involved in the regulation of follistatin expression. Proteins bound to the gene’s regulatory element were isolated and identified by mass spectrometry. The proteins identified included the known regulator Smad3 and the forkhead family transcription factor FoxL2. In another study, proteins interacting with the CREB co-activator CRCT2 during ER stress were identified. This led to the identification of activating transcription factor 6 alpha, which provides a link to glucose homeostasis. In an extensive investigation of phosphoproteins involved in gliogenesis, the inositol phosphatase synaptojanin was identified as playing an important role in the generation of astroglia under normal and pathological conditions. These findings are relevant in the pathology of Down’s syndrome.

**Identification of Post-Translational Modifications in Proteins**

The facility routinely determines the disulfide linkage in extracellular proteins. The disulfide bridge arrangement is an important feature in proteins and stabilizes the structural topology. Most recently, the disulfide pattern of several variants of the ligand-binding domain of a peptide hormone receptor was determined. A more thorough understanding of the interaction between neuropeptides and their receptors is essential for the development of therapeutic approaches in a variety of diseases.

Protein phosphorylation is an important modification that plays an essential role in the regulation of many biological processes. Recently, a phosphorylation site in a transcription factor was identified that was only present when cells were treated with the growth factor Activin-A.

For more information, please visit cores.salk.edu/massspec/

Left to right
Jessica Read, Ratindra Rastogi, Wolfgang Fischer, William Low
Stem Cell Core Facility (STEM)

Travis Berggren, Director

“The Stem Cell Core Facility is dedicated to promoting research involving human ES and iPS cells. We maintain existing human embryonic stem (hES) cell lines and newly derived human iPS cell lines. The STEM Core provides the physical space, dedicated equipment, and hands-on training for scientists to use these pluripotent cells for their research goals.”

The STEM Core Facility is ideally suited for Salk scientists to perform studies involving existing hES and iPS cell lines (provided by the core) and to support the derivation and propagation of reprogrammed induced pluripotent stem (iPS) cells.

Travis Berggren and lab manager Margaret Lutz provide individualized training in proven techniques for successful culturing of these cells. The core staff is available for consultation on experimental design and can assist in getting projects involving hES or iPS cells moving quickly.

All the requisite equipment and resources for successful stem cell culture are found in the Stem Cell Core, including: two cell culture rooms, benchtop research space, a microscope room, and cryo-storage equipment. One of the cell culture rooms is designated for existing, preapproved hES and iPS cell lines only. The other cell culture room is designated for work with viral vectors, for reprogramming adult cells to stem cell–like iPS lines, and has dedicated hoods and incubators for quarantined cell culture. The segregation of these spaces is part of a mechanism to provide flexibility for new research projects while protecting the existing cells from external contamination.

The facility is centrally located on the Salk campus, making it convenient for Salk researchers to drop in while staying within easy reach of their home laboratories. It is free of federal funding, making it a safe haven for research involving either the NIH registered or the newer non-NIH registered human ES cell lines. The Stem Cell Core operates on a recharge rate basis to cover supplies used by researchers.

Left to right: Krystal Sousley, Margaret Lutz, Travis Berggren
Transgenic Core

“Research at the Salk Institute depends to a high degree on the use of mouse models. The Transgenic Core is dedicated to providing access to cutting-edge technologies to create these models. We offer quality and efficiency in transgenic and knock-out model production, IVF, embryo cryopreservation, and rederivation of mouse strains by embryo transfer for Salk Institute investigators.”

Transgenic and knockout mice have become an integral part of the research programs at Salk. Established in 1994, the Transgenic Core provides a variety of services, including microinjection of embryonic stem (ES) cells into blastocysts, microinjection of DNA into one-cell embryos, lentiviral infection, in-vitro fertilization (IVF), cryopreservation, and rederivation of mouse lines, providing neo-resistant mice for generating feeder cells for ES cell culture, providing inbred strains of mice, and establishment of ES cell lines from wild-type and mutant mice. It also is able to develop new techniques and applications, provide immediate access to individuals with knowledge of dealing with transgenic mice, and offer the potential for collegial interactions.

Over the last year, the microinjection laboratory has generated more than 120 transgenic and knockout mouse lines and rederived and cryopreserved more than 100 lines for investigators at the Institute. These mice are not only important resources for Salk researchers but also for investigators at other institutions. More than 50 publications have been based on genetically altered mouse lines created by the Transgenic Core during the last year.

Operated by a core director and two research assistants who are skilled in the appropriate techniques, the Transgenic Core is located within a pathogen-free barrier facility in which only animals from standard sources are allowed. It maintains a mouse colony in four holding rooms adjacent to six procedure rooms.

The Institute ensures that all employees dealing with the animals understand their individual and collective duty and embrace their ethical obligation to provide the highest level of care, conforming to all relevant regulations and rules concerning laboratory animal husbandry. The activities of the transgenic core facility are overseen by the Institutional Animal Care and Use Committee (IACUC), which is chaired by Thomas Albright.
Viral Vector Core

Daniel Gibbs, Director

“The Viral Vector Core was established at the Salk Institute in 2008 as part of the NIH Blueprint for Neuroscience research program and the NEI Center for the Neurobiology of Vision. Our mission is to provide a resource for Salk investigators and researchers at external institutes, to promote and support their use of recombinant viral vectors as research tools.”

Recombinant viral vectors are rapidly becoming the method of choice for targeted, quick, and regulated gene delivery in many experimental systems. The Viral Vector Core provides access to multiple viral vector platforms and offers expert consultation on the safe and effective use of viral vectors. Its staff can advise on all areas of viral vector use, from the selection of a specific vector through plasmid construction, vector production, experimental design, biosafety, and vector delivery techniques. The core facility maintains stocks of reporter viral vectors that allow investigators to quickly and cheaply identify the vector system best suited to their research needs and currently offers in vivo grade, custom production of the following vector types:

Lentiviral vectors
Lentiviral vectors can deliver large transgenes to both non-dividing and dividing cells. The transgene stably integrates into the host cell genome, allowing long-term expression in vivo and in vitro. Available vectors are based on second- and third-generation HIV-1 packaging systems developed at the Salk Institute by Inder Verma and colleagues. Non-human lentiviral vectors are also available, based on the equine infectious anemia virus (EIAV) system developed by John Olsen and colleagues.

Retroviral vectors
Retroviral vectors are based on MLV and can deliver large transgenes to dividing cells, where they will stably integrate to facilitate long-term expression. Both lentiviral and retroviral vectors can be pseudotyped with a range of glycoproteins to alter viral tropism, including VSV, ASLV EnvA and EnvB, and rabies glycoproteins.

Adeno-associated viral vectors (AAV)
AAV vectors are non-pathogenic in humans and under normal circumstances will not integrate into the host cell’s genome. Available AAV vectors are based on AAV2 and can cross-package transgenes into a range of AAV capsids with differing patterns of tissue-specific infectivity.

Adenoviral vectors
Adenoviral vectors are non-integrating and can be generated in producer cells either by infection from viral stocks or from transfection with linearized Ad5 transfer plasmids. Available vectors are based on the Ad5 E1 deleted vector system.

The vector core, in conjunction with the Salk Stem Cell Core, also offers validated off-the-shelf retroviral iPSC reprogramming vectors on a recharge basis.

Left to right: Juan Velasquez, Helen Fang, Daniel Gibbs, Dominika Rainey
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SALK NON-RESIDENT FELLOWS

The Nonresident Fellows partner with the research faculty to lead the Institute. They play a major role in shaping Institute policy, although their primary responsibilities are at research institutions throughout the world.

David Baltimore, Ph.D.
Nobel Laureate, 1975
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