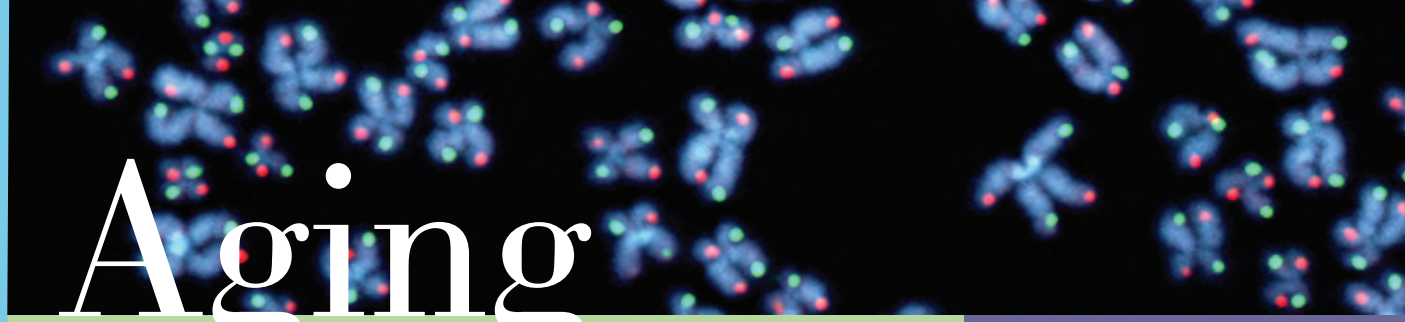
From the Bench



Aging

CURRENT RESEARCH AT THE SALK INSTITUTE

WINTER 2009

IN 1513, THE SPANISH EXPLORER PONCE DE LEON explored Florida in search of a mythic spring reputed to turn back the clock for anyone who drank its waters. He never found the Fountain of Youth he sought, but the legend, which dates back to antiquity, has retained its grip on the popular imagination.

Today the explorers are scientists, and the uncharted territory they're searching is confined to the laboratory, but their goal is similar in spirit to that of their predecessors: to uncover the mechanisms that cause us to age, with an eye toward counteracting a host of debilitating conditions that rob humans of their health, productivity, and quality of life.

While the outward manifestations of growing older—gray hair, wrinkles, stiffening joints, slower response times, "senior moments"—are as well-known as the many products advertised to address them, scientists actually know little about how we age. Yet the incentive to uncover the secrets of aging is powerful: Advancing age is the single greatest risk factor for most human diseases, from arthritis and cancer to diabetes and neurodegenerative disorders.

At the Salk Institute, faculty are particularly focused on the causes and possible treatments for age-related neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases, as well as on genetic and metabolic changes that take place as organisms grow old. Working individually and collaboratively across labs and disciplines, they are continuing to uncover novel insights that expand knowledge and promise to enhance quality of life for an aging population. This issue of *From the Bench* offers a sampling of their recent findings.

The Glenn Center for Aging Research

BY ITS VERY NATURE, AGING research is an interdisciplinary pursuit. In January 2009, catalyzed by a $5 million gift from the Glenn Foundation for Medical Research, the Salk Institute took a major step forward when it organized its diverse aging research programs within a new entity, the Glenn Center for Aging Research. Under the leadership of **Andrew Dillin**, the center facilitates aging research from nine of the Institute's leading laboratories to address fundamental questions in the field, including what constitutes a healthy life span and whether there is a defined biological aging process that is universal to all organisms.

Glenn Center members specialize in three main areas of investigation. The Genetic Analysis Group capitalizes on faculty expertise in a variety of cell types to explore new questions about key genetic pathways involved in cell maintenance and aging and to investigate how newly defined genes alter the aging process. The Stem Cell Group explores the specific molecular components associated with aging in stem cells, elucidating how stem cells stay healthy, which could explain why and how humans age. The Metabolism Group seeks to understand the molecular underpinnings of decreased metabolism and the aging process—specifically, how aging affects metabolism across key organ systems and how restrictive diets can alter genetic expression.

In coming years, their combined capabilities promise to add significant momentum to aging research at the Institute and beyond, expanding scientific understanding of a universal, and inevitable, part of life.

A nurturing environment

A STEM CELL'S IMMEDIATE NEIGHBORHOOD, A SPECIALIZED environment known as the stem cell niche, provides crucial support for stem cell maintenance. But just as an older house grows less efficient as its windows and doors weather and its appliances wear out, over time, the level of support in the stem cell niche drops off, diminishing stem cells' ability to renew themselves.

In a study of the testis in the fruit fly *Drosophila*, a team led by **Leanne Jones** focused on the influence of aging on the behavior of stem cells in the male germ line. They compared the number of stem cells in young, middle-aged, and old flies, and found that over time, stem cell numbers fell from an average of 8.3 in young flies to 5.1 in old flies. (The average fruit fly lives around 40 days.) When the researchers scrutinized the molecular signals that govern the behavior of stem cells in fruit flies, they found a sharp decline in a growth factor necessary to maintain stem cells. Conversely, forced expression of the growth factor within niche cells delayed the loss of germline stem cells in older males.

"The notion that the stem cell microenvironment is aging will certainly influence how we think about using stem cells in regenerative medicine," says Jones. "You can manipulate stem cells and propagate them in a dish, but many recipients of stem cell replacement therapies will be older individuals. If the stem cell niche has aged, it might not be capable of supporting the transplanted stem cells."

Identifying the reasons for reduced expression of the growth factor could reveal how aging leads to changes in stem cell behavior. Counteracting these changes may slow the loss of adult stem cells during aging and help ensure successful stem cell therapies in the future.

Growing, growing, gone

AS EMBRYOS, EACH OF US CAN REGENERATE our limbs and organs, but after birth, we lose that ability. Other species, however, are more fortunate; if part of an organ is removed, they have the uncanny capacity to grow it back. In fact, nearly every animal phylum has examples of species with the power to regenerate different body parts.

Juan Carlos Izpisúa Belmonte is studying one of those animals, the zebrafish, to learn more about the mechanisms behind its regenerative abilities. "When the fish is young, you can cut out part of its liver, fins, or even up to 20 to 30 percent of the ventricular mass in its heart, and the fish will regenerate it," he explains. "It is currently unknown whether it does this by activating a reservoir of stem cells or by reprogramming adult, committed cells." He and his team have found, however, that its remarkable powers are fleeting; the fish loses the ability to regrow its organs over time. They are currently trying to determine what happens in the interim—to uncover the genes involved and understand why the regenerative properties become impaired as the fish ages. If they can figure it out, they may have achieved a milestone toward understanding the aging process.

Red light, green light

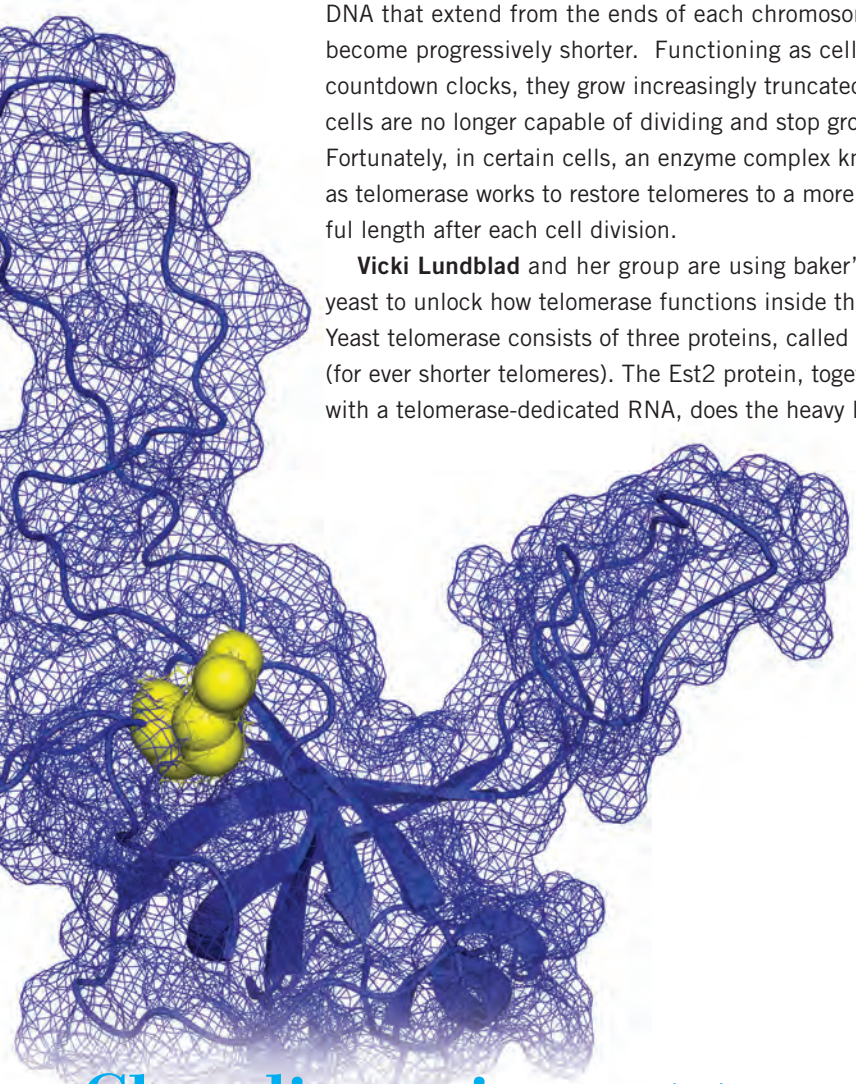
THINK OF THEM AS THE ULTIMATE SECURITY GATES, responsible for traffic control on the world's busiest thoroughfare. Nuclear pore complexes, which penetrate the membrane separating the cell's nucleus from its cytoplasm, each mediate approximately 1,000 transport events a second. Since they are as essential to nondividing cells as they are to dividing ones, a team led by **Martin Hetzer** wanted to determine what happens to them over time. Because most of the cells in our bodies are not actively dividing, the answer would have implications for aging and age-related diseases.

Approximately half the proteins in the nuclear pore complex make up a central scaffold, or core, while the other, peripheral proteins attach to the scaffold. Using *C. elegans*, a tiny roundworm that as an adult consists entirely of nondividing cells, Hetzer and his group found that while the peripheral proteins are continually exchanged, the proteins comprising the scaffold remain in place for the life of the cell—in some cases, for the entire lifespan of an organism.

When they set out to ascertain how these proteins hold up over time, the researchers found that in aging cells, one of the proteins composing the scaffold becomes damaged, and molecules that should be restricted to the cytoplasm invade the nucleus. In particular, a protein called tubulin, which belongs in the cytoplasm, shows up as long filaments that co-opt a large part of the nucleus.

Associated with several neurodegenerative diseases, the filaments have been found particularly in the substantia nigra of many Parkinson's patients, the part of the brain that is affected by the condition. Hetzer hypothesizes that it is the age-dependent defects in the scaffold proteins that allow the leakage. By finding ways to prevent or reverse it, he and his team may be on course to identify novel approaches to treating these perplexing, devastating, and costly age-related conditions.

A cellular fountain of youth



WHEN CELLS DIVIDE, TELOMERES—THE TAILS OF DNA that extend from the ends of each chromosome—become progressively shorter. Functioning as cellular countdown clocks, they grow increasingly truncated until cells are no longer capable of dividing and stop growing. Fortunately, in certain cells, an enzyme complex known as telomerase works to restore telomeres to a more youthful length after each cell division.

Vicki Lundblad and her group are using baker's yeast to unlock how telomerase functions inside the cell. 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. Telomerase is exquisitely regulated—not every telomere enjoys its attention each time a cell divides—but how its action is coordinated has been unclear. Lundblad's group has now developed the key to unravelling this mystery.

An earlier study in Lundblad's group provided a clue: It showed that a small area on the surface of Est1 was used to drag telomerase to telomeres. This spot 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. "Amazingly, just changing a single amino acid on this particular site on Est1 means that telomerase cannot get to the ends of chromosomes, and thus telomeres shorten," she explains.

But this only explains one aspect of the complexities of telomerase regulation. 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, using a mixture of computer predictions, yeast genetics, and old-fashioned brute force. 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.

Cleanliness is next to…youthfulness

IT'S A DIRTY JOB, BUT CELLS HAVE GOT to do it. The job in question is autophagy, a cleanup mechanism for removing the damaged molecules that accumulate during cellular aging. Scientists have known for some time that suppressing autophagy can accelerate the buildup of protein aggregates that leads to neural degeneration. But a team of researchers in the lab of **David Schubert** has demonstrated that the opposite is true as well: Boosting autophagy in the nervous system of fruit flies prevents the age-dependent accumulation of cellular damage in neurons and promotes longevity.

All cells undergo autophagy—literally, "self-eating." Specialized vesicles surround damaged cellular proteins or structures and then shuttle the "bagged garbage" to a second group of vesicles called autophagosomes, which dispose of the trash with the help of digestive enzymes. This process can be enhanced when animals are placed on a calorie-restricted diet, a regime known to extend lifespan.

When initial experiments indicated that the expression of several autophagy genes decreased over the normal lifespan of fruit flies, **Kim Finley**, a staff scientist in Schubert's group, focused on one particular protein, Atg8a, which is essential to the formation of new autophagosomes. She found that levels of Atg8a were significantly reduced by four weeks, when the flies are considered middle aged, and protein aggregates were not efficiently cleared and started to accumulate.

In flies genetically engineered to lack Atg8a, damaged proteins started to pile up early, and life expectancy plummeted. Moreover, the abnormal accumulation of protein aggregates had striking similarities to those seen in the most common human neurodegenerative diseases. When the researchers kept the neuronal levels of Atg8a high, however, the flies were spared the accumulation of protein aggregates, and their average lifespan was significantly extended. All of this suggests that regulating autophagy may be a key factor in controlling the aging process.

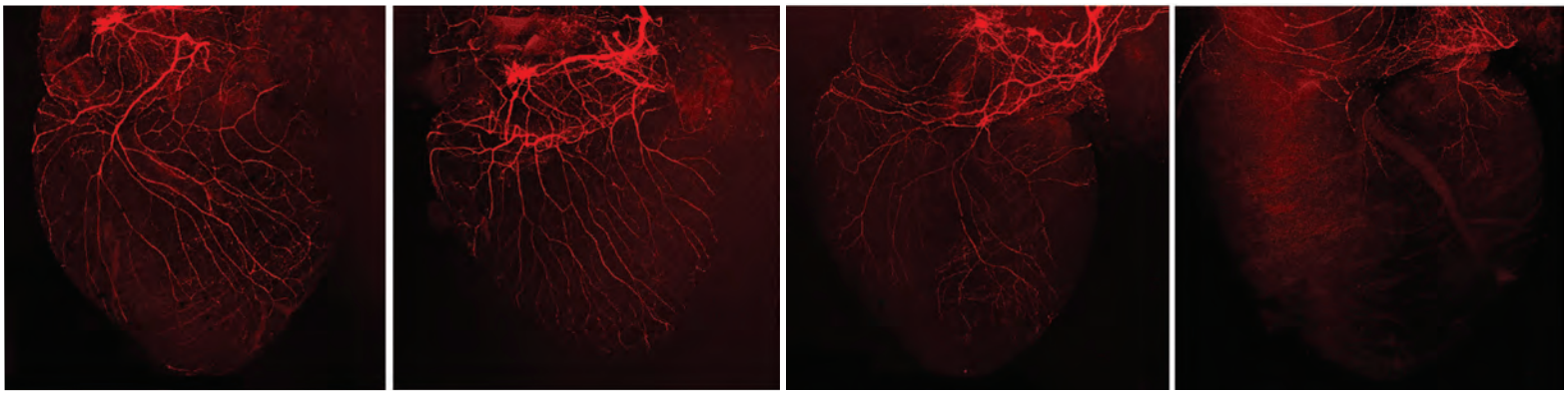
In related work, Schubert's lab has recently made a novel family of drugs that stimulate the removal of damaged proteins from aged mouse brains and enhance memory in the process.

Repairing a bad reputation

PROTEINS, LIKE PEOPLE, ARE OFTEN judged by the company they keep. The protein known as p75, for example, which regulates neuronal growth, survival, and degeneration and guides nerve fibers in growing embryos to their final destinations, belongs to the same family as tumor necrosis factor. As such, it has widely been thought to mediate cell death in some context. Some studies have suggested that it also exacerbates the neurotoxicity associated with beta amyloid deposits, which litter the brains of Alzheimer's disease patients. According to a study in the laboratory of **Kuo-Fen Lee**, however, p75's bad reputation may be unwarranted.

Scientific interest in the peripheral nervous system has been growing as investigators studying neurodegenerative diseases seek new insights into disease progression. "How a disease damages the peripheral nervous system could add a great deal to our understanding of its process, possibly leading to applications down the line that impact patient management and quality-of-life issues," says Lee. To gather evidence about p75 and the sympathetic nervous system, his team 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. Instead, the opposite was true. 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.) When the researchers scaled down the production of toxic beta amyloid, the nerves in the sympathetic nervous system of p75-deficient mice were substantially restored.

This is the first time the interplay between p75 and beta amyloid in the peripheral sympathetic system has been demonstrated, and the findings may help lead to new treatment strategies for Alzheimer's disease.



An Alzheimer's trigger?

FOR CLOSE TO A DECADE, PHARMACEUTICAL RESEARCHERS have been in hot pursuit of compounds to activate a key nicotine receptor in the brain 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 the lab of **Stephen F. Heinemann,** however, have discovered that when the nicotinic 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.

Intrigued by earlier studies showing that beta amyloid seemed particularly drawn to the alpha-7 nicotinic receptors, Heinemann and his team sought to determine whether the alpha-7 receptors modulate the effects of beta amyloid in Alzheimer's disease. 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. Both types of mice had comparable amounts of plaques in their brains, but in those lacking the alpha-7 gene, the plaques appeared to have no effect.

These findings could have important implications for researchers seeking to combat the Alzheimer's disease. "An Alzheimer's epidemic is threatening to swamp the medical system within 20 years, but all clinical trials for targets that the field has identified have failed because of side effects or because they don't work," says Heinemann. "This is a completely different target."

When less is more

FORGET WHAT YOUR MOTHER TOLD YOU. Cleaning your plate may not make you grow up strong and healthy. In studies going back to the 1930s, mice and many other species subsisting on a severely calorie-restricted diet have consistently outlived their well-fed peers by as much as 40 percent. Just how near-starvation extends lifespan has remained elusive, but recent studies by **Andrew Dillin** offer key clues, identifying a critical gene and enzymes that specifically link calorie restriction to longevity.

Initially, researchers thought that the effect of calorie restriction on aging was mediated through insulin-like signaling pathways. But experiments in Dillin's lab using the roundworm *C. elegans*, suggested otherwise, so his team set out to test a specific class of proteins to see if any of them delayed aging in the calorie restriction response. After knocking out the gene for each protein separately and observing the genetically altered worms, they found that loss of the gene encoding the protein PHA-4 nullified the lifespan-enhancing effect of calorie restriction in worms. The opposite experiment—overexpressing PHA-4 in worms—enhanced the longevity effect.

In collaboration with **Andrea C. Carrano,** a postdoctoral researcher in **Tony Hunter**'s laboratory, Dillin next went on to identify additional players in the same genetic pathway, including an enzyme linking aging and stress.

"After 72 years of not knowing how calorie restriction works, we finally have genetic evidence to unravel the underlying molecular program required for increased longevity in response to calorie restriction," says Dillin. Identifying key links between calorie restriction and aging also opens the door to development of drugs that mimic the effects of calorie restriction and might allow people to reap its health benefits without enduring near starvation.

Too close for comfort

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.

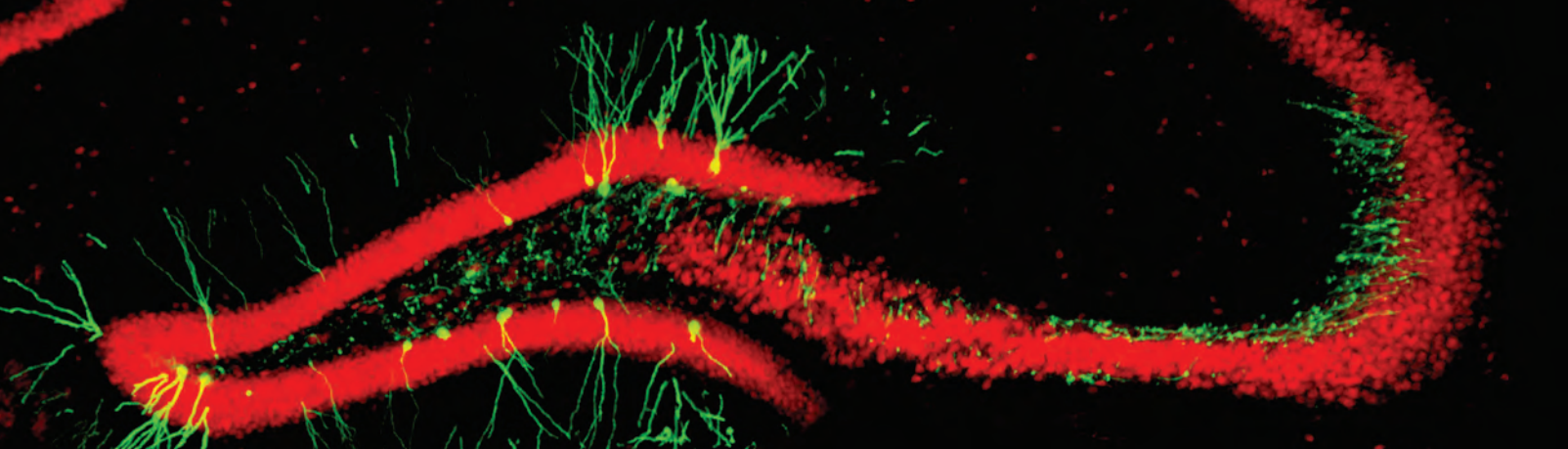
As it happens, the hippocampus—or more specifically, its first relay station, a part called the dentate gyrus—is also the brain's hotbed of neurogenesis. Neurogenesis is a process by which new neurons are added to the brain, and this process, which declines with aging, is thought to be associated with 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 **Fred H. Gage**'s lab, however, has shed new light on their purpose.

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 happens to be the place where neurogenesis is occurring, we originally thought that adding new neurons could help with the pattern separation," says Gage.

Graduate student **Claire Clelland** designed experiments that would specifically challenge this function of the dentate gyrus, using different behavioral tasks and strategies to selectively shut down neurogenesis in the structure. In two sets of experiments, mice without neurogenesis had no problem recalling spatial information in general but were unable to discriminate between locations that were close to each other.

"We believe that new brain cells help us to distinguish between memories that are closely related in space," says Gage.

In a separate study, Gage and his team have shown that exercise can increase neurogenesis in aged mice and reverse some of the learning and memory deficits.



A recipe for treatment?

AS IF THEIR RICH COLOR AND JUICY FLAVOR weren't enough, now there's another reason to enjoy strawberries. **Pamela Maher**, a senior scientist in the laboratory of **David Schubert**, has identified a natural substance in the fruit that has neuroprotective and cognition-enhancing properties. In her studies, fisetin, which is also found in lesser amounts in tomatoes, onions, oranges, apples, peaches, grapes, kiwifruit, and persimmons, promoted the survival of cultured nerve cells that had been subjected to a variety of toxic insults and boosted memory in healthy mice.

Most recently, she has studied its effects on Huntington's disease, an inherited, progressive, and ultimately fatal neurodegenerative disorder that is characterized by psychiatric, cognitive, and motor symptoms. Huntington's, which typically shows up in midlife, is caused by a mutation in the huntington (Htt) protein, and identification of the mutation has led to the development of a number of cellular and animal models of the disease. These have revealed the complexity of mechanisms underlying disease development and progression, which may be why no effective treatment is yet available.

Because of fisetin's multiple beneficial effects on nerve cells, Maher decided to test fisetin in three progressively complex models of Huntington's disease. In cultured nerve cells, she was able to show that fisetin prevents changes in specific signaling pathways associated with Htt-mediated cell death. In flies expressing the disease-causing form of Htt, she found that fisetin could prolong lifespan. In a Huntington's mouse model, she determined that fisetin both helped to maintain normal motor function and prolonged life span: The fisetin-fed mice lived more than 30 percent longer than the mice fed a control diet. These promising results just might lead to a fresh approach to slowing the disabling—and deadly—progression of Huntington's disease.

Cellular countdown

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. **Jan 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 chromatin, the histone packaging can be modified by enzymes that leave chemical signals and instructions behind.

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 redistributed. This redistribution amplifies the signal that the shortening telomeres emit 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.

This issue of *From the Bench* is part of a series of updates on key areas of scientific research conducted at the Salk Institute for Biological Studies. Our goal is to keep you informed of Salk researchers' most recent findings in areas such as stem cells and regeneration, vision, plant biology, neuroscience, behavior, and more. We are very interested in your feedback regarding this update.

For more information, or to share your comments, please contact Judy Hodges at the Institute's Office of Development at 858.453.4100 x1882 or email hodges@salk.edu.

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