

Biophotonics

CURRENT RESEARCH AT THE SALK INSTITUTE

SPRING 2011

THE YEAR WAS 2006. THE SALK INSTITUTE faculty, a diverse assemblage of biologists studying everything from cancer to cognition, from stem cells to aging to the structure of molecules, had come together to discuss future endeavors. And in a rare consensus, nearly all agreed that one thing, more than any other, would revolutionize their research: the ability to peer into a cell and observe its functions in real time.

ADVANCES IN PHOTONICS—THE science and technology of the generation, manipulation and detection of light—had made the notion more than just a dream. Quantum-like particles of light called photons were already being used to transmit, process and store information. But achieving the kind of breakthrough that the faculty had in mind would take a special initiative, spearheaded by two visionary faculty members, **Inder Verma** and **Ron Evans**, and one visionary donor, Salk trustee **Ted Waitt**, who ultimately joined forces to bring advanced biophotonics to the Salk Institute.

Through his foundation, Waitt provided funding for the Salk faculty to meet with the world's leading experts in the fledgling field, who visited the Institute to help Salk investigators both understand the possibilities of the technology and home in on what they needed. From those meetings, a vision emerged, one that was grounded in instrumentation and

encompassed biology, physics and engineering, facilitated by staff capable of working across those disciplines to give researchers a front-row peer into the inner workings of cells. And once again Ted Waitt helped make it a reality by pledging \$20 million to establish the Waitt Advanced Biophotonics Center.

The biophotonics facility that Verma, Evans and Waitt had envisioned just a few years before opened quietly in early 2010, equipped with state-of-the-art microscopes and imaging instruments, and Salk faculty have wasted no time in exploiting its remarkable capabilities. The following pages showcase some of their discoveries and the images that helped make them—images that are, in a very real sense, windows into a scientific revolution.



Evolution of light microscopy

Light microscopy has come a long way since the first experiments in the seventeenth century by Robert Hooke, who became famous for his elegant drawings of microscopic objects, and by Antonie van Leeuwenhoek, who was a poor artist and actually had someone else draw as he described what he was seeing through the microscope.

Today, Salk scientists walk into the Waitt Advanced Biophotonics Center, flip a switch on one of the million-dollar microscopes and after a couple of mouse clicks to choose the settings, they are ready to place their samples on the stage and adjust the lens. One more click starts a fully automated imaging procedure that can last up to several days.

In the intervening centuries the steady march of technology, particularly the invention of stains and fluorescent dyes, new light sources such as lasers and LEDs, CCD cameras and last, but not least, computers, had a huge impact on microscopy. As a result, the practical use of optical imaging has exploded over the last 100 years, despite the fact that the conceptual basis and design of the microscope has remained almost constant.

The first simple microscopes revolutionized our understanding of how life operates on a minuscule scale. As new technologies continue to push the boundaries of the visible world, microscopes will continue to profoundly impact biological thinking.

Salk Trustee Ted Waitt (left) and James Fitzpatrick, Director Biophotonics Core

Modeling autism in a dish

IN THE PAST, SCIENTISTS HAD BEEN limited to studying the brains of people with autistic spectrum disorders via imaging technologies or postmortem brain tissues. Now the ability to obtain induced pluripotent stem (iPS) cells from patients' skin cells, which can be encouraged to develop into the cell type damaged by the disease,

has given **Fred Gage** and his collaborators an unprecedented view of autism.

Carol Marchetto, a member of Gage's team, started with skin biopsies taken from patients with Rett Syndrome, the most physically disabling of the autism spectrum disorders. After she had patiently coaxed the iPS cells into developing into fully

functioning neurons—a process that can take up to several months—she was able to discern disease-specific cellular defects, such as fewer functional connections between Rett neurons. The symptoms were reversible, raising the hope that one day, autism may turn into a treatable condition.

“It is quite amazing that we can recapitulate key elements of a psychiatric disease in a Petri dish.”

FRED GAGE

Rett patient-derived neurons (in green) grow on top of feeder cells (shown in red).

Image: Carol Marchetto

Looking on the bright side

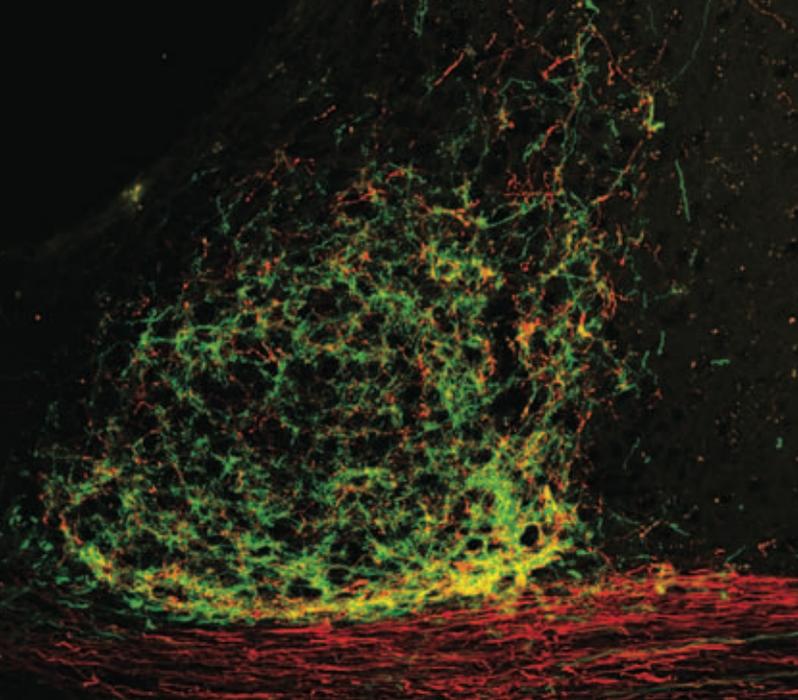
FOR THE GREATER PART OF 100 YEARS, IT WAS THOUGHT that the ability to convert light into electrical signals in the retina was restricted to only two types of photoreceptors: rods and cones. This view changed dramatically when **Satchindananda Panda** discovered the existence of a third type of photoreceptor, melanopsin, which is only present in a few thousand cells embedded in the deeper layers of the retina. These cells send their signals directly to the human circadian pacemaker, where they set the body's biological clock.

When Panda and his collaborators traced individual nerve fibers extending from the retina, they discovered that melanopsin-expressing retinal ganglion cells reach out to visual processing centers

in the brain, where they relay information about the brightness of incoming light. The findings reveal a new role for melanopsin during image-forming vision and suggest that it could make a significant contribution to assessing the intensity of light and supporting vision even in people with advanced retinal degeneration.

Axons projecting from melanopsin-expressing cells in the left (red) and right (green) eye intermingle freely in the brain's circadian pacemaker before they split up again and continue largely on the opposite site.

Image: James Fitzpatrick



Increasing insulin production

THE NEUROPEPTIDE CORTICOTROPIN-RELEASING FACTOR (CRF), in concert with its receptor, CRFR1, has long been known as key to the body's response to various forms of stress, but the pair is also involved in many more processes, including a number with direct ties to metabolism. As early as the 1980s, studies had suggested that pancreas cells can respond to CRF, but the few limited observations did not demonstrate the nature of the response or which cells or receptors were involved.

Recently, **Mark O. Huising**, a senior research associate in **Wylie Vale's** lab, showed that beta cells in the pancreas actually carry CRF receptors on the cells' surface. When activated by CRF, these receptors increase insulin secretion and promote the division of insulin-producing beta cells. These findings provide new insights into diabetes, particularly type 1, as well as suggest novel targets for drug intervention.

The cell membranes of beta cells, whose nuclei are shown in blue, are studded with CRF receptors, shown in red.

Image: Mark O. Huising

Staying alive

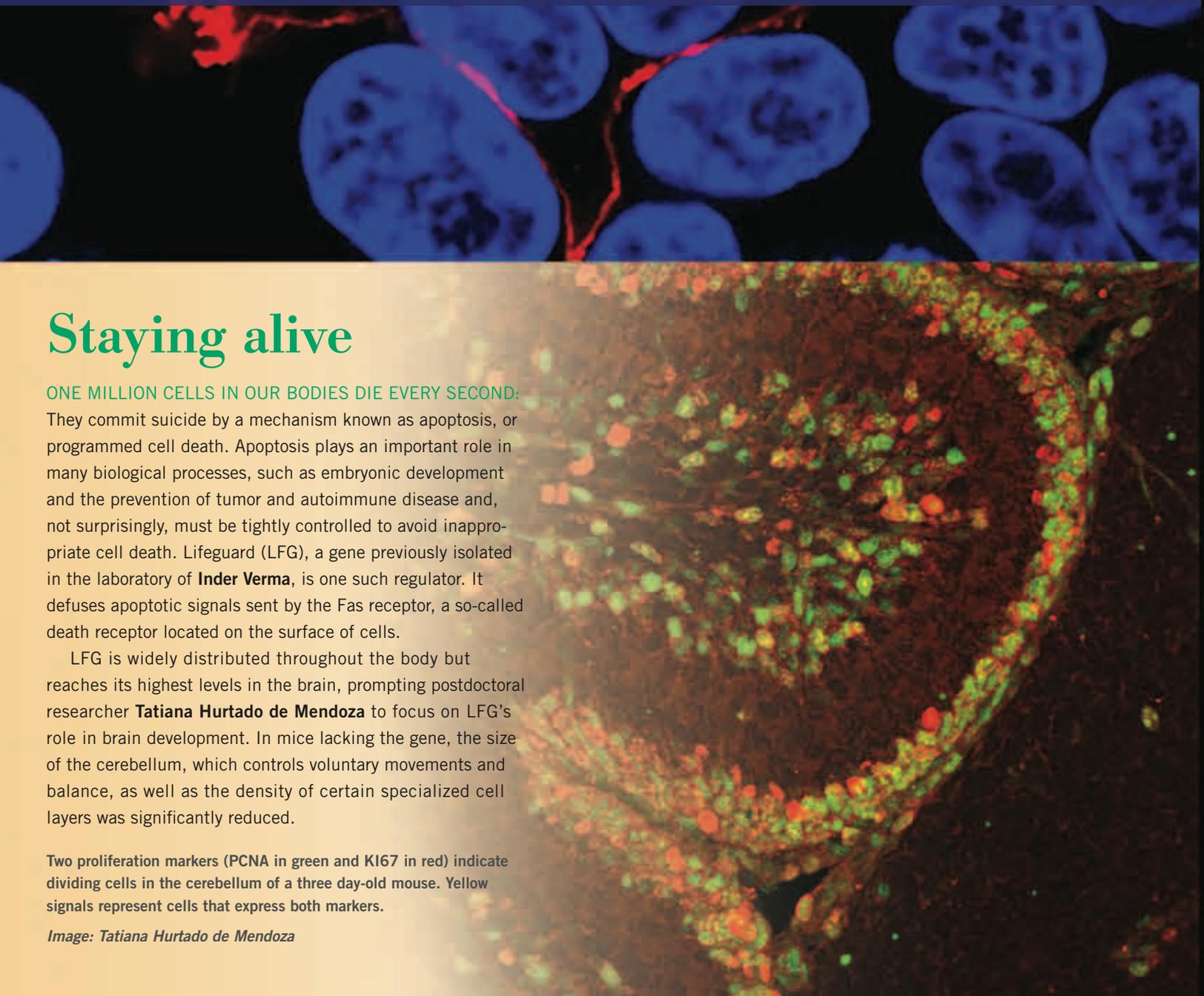
ONE MILLION CELLS IN OUR BODIES DIE EVERY SECOND:

They commit suicide by a mechanism known as apoptosis, or programmed cell death. Apoptosis plays an important role in many biological processes, such as embryonic development and the prevention of tumor and autoimmune disease and, not surprisingly, must be tightly controlled to avoid inappropriate cell death. Lifeguard (LFG), a gene previously isolated in the laboratory of **Inder Verma**, is one such regulator. It defuses apoptotic signals sent by the Fas receptor, a so-called death receptor located on the surface of cells.

LFG is widely distributed throughout the body but reaches its highest levels in the brain, prompting postdoctoral researcher **Tatiana Hurtado de Mendoza** to focus on LFG's role in brain development. In mice lacking the gene, the size of the cerebellum, which controls voluntary movements and balance, as well as the density of certain specialized cell layers was significantly reduced.

Two proliferation markers (PCNA in green and KI67 in red) indicate dividing cells in the cerebellum of a three day-old mouse. Yellow signals represent cells that express both markers.

Image: Tatiana Hurtado de Mendoza



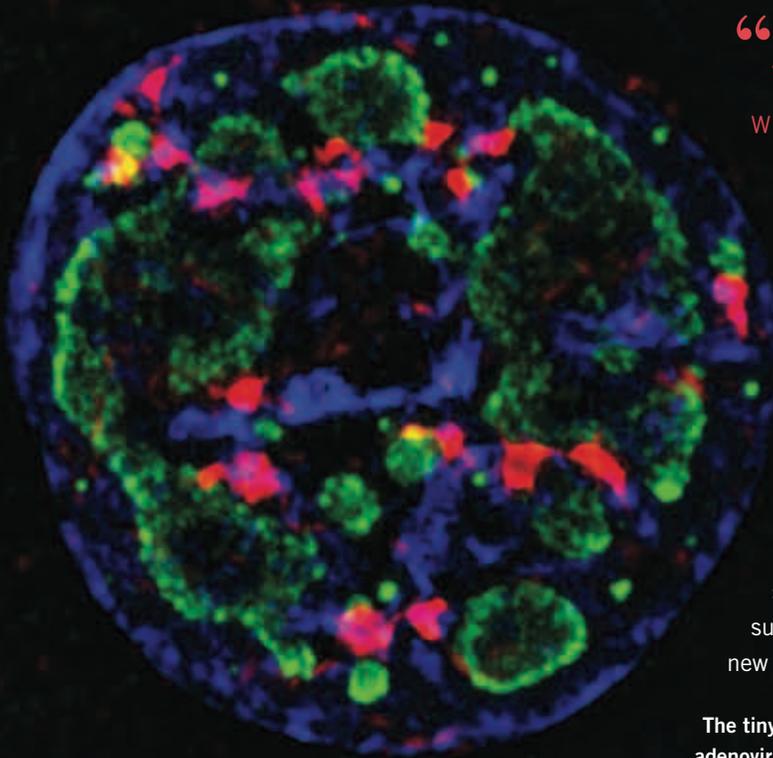
“Zipping” the genome

WHEN A CELL IS UNDER STRESS, THE TUMOR

suppressor p53 springs into action, activating an army of foot soldiers that initiate a built-in “auto-destruct” mechanism that eliminates virus-infected or otherwise abnormal cells from the body. Just like tumor cells, adenoviruses, which cause upper-respiratory infections, need to get p53 out of the way to multiply successfully.

Clodagh O’Shea and her team found that instead of inactivating p53 directly, adenovirus renders the “guardian of the genome” powerless by targeting the genome itself. “It literally creates “zip files” of p53 target genes by compressing them till they can no longer be read,” she says. The novel mechanism used by adenovirus to sidestep the cell’s suicide program could go a long way toward explaining how tumor suppressor genes are silenced in tumor cells and pave the way for a new type of targeted cancer therapy.

The tiny adenovirus protein known as ORF3 (shown in red) clears the way for adenovirus replication (replications centers shown in green) by creating “zip files” of genes (blue) that help the cell defend itself against the virus. *Image: Horng Ou*



Stem cells on a diet

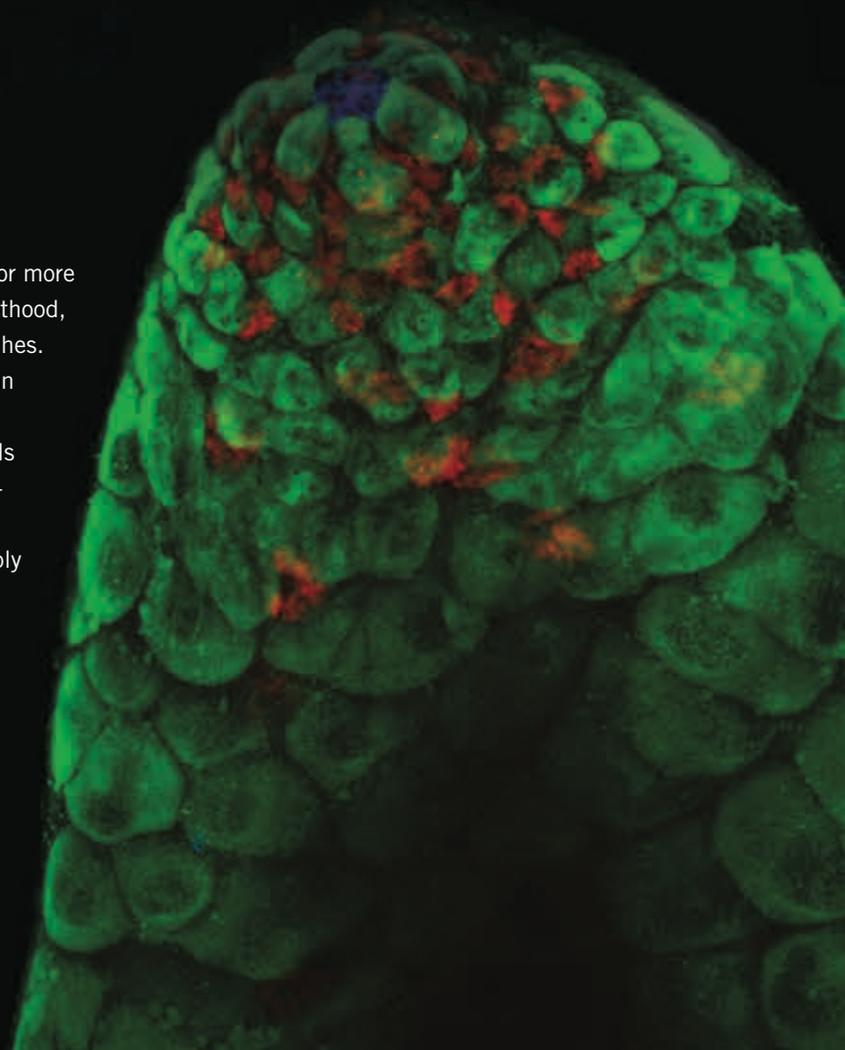
STEM CELLS, WITH THEIR DEFINING CHARACTERISTICS—

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.

Recently, **Leanne Jones** and her team discovered that stem cells can sense a decrease in available nutrients and respond by retaining only a small pool of active stem cells for tissue maintenance. When, or if, favorable conditions return, stem cell numbers multiply to accommodate increased demands on the tissue. Elucidating the mechanisms by which hormonal signaling influences stem cell behavior under normal conditions and in response to stress provides important insights into the activities of stem cells in regenerative medicine, during wound repair and in individuals experiencing metabolic stress.

Male germ line cells (green) at the tip of *Drosophila* testes and somatic stem cells (red) are in contact with hub cells (blue), the stem cells’ own private niche.

Image: Pedro Resende



Running on empty

THE METABOLIC MASTER SWITCH KNOWN AS AMPK HAS been extensively studied for years since it is both activated in response to exercise and is triggered by drugs used for treating type 2 diabetes. It also puts a damper on the cell proliferation that underlies the growth of tumors in the body by depriving cancer cells of the extra fuel they need to survive. When **Daniel Egan**, a graduate student in the lab of **Reuben Shaw**, searched for novel routes through which AMPK exerts control over a cell's household utilities, he discovered a direct molecular link to autophagy.

The process of autophagy, or self-cannibalization, allows cells to stay alive under nutrient-poor conditions by degrading their own organelles to generate desperately needed metabolites. Shaw believes that these findings will have implications for a variety of human diseases characterized by defects in the autophagy process, including a number of neurodegenerative conditions and cancer.

When the process of autophagy is defective, cells are unable to recycle cellular organelles such as mitochondria (shown in red) to generate molecular building blocks under starvation or stress conditions. Cell nucleus shown in blue.

Image: Daniel Egan

Ticking of the clock

LIKE CATS, HUMAN CELLS HAVE A FINITE NUMBER OF LIVES. Once they have divided a certain number of times, they slow their pace, change their shape and eventually stop dividing altogether, a phenomenon called "cellular senescence." Biologists knew that a cellular clock composed of structures at the ends of chromosomes, better known as telomeres, records how many "lives" a cell has expended, yet they had been unable to define how the clock's ticking signals the approach of cellular oblivion.

When **Roddy O'Sullivan**, a member of **Jan Karlseder's** team, undertook exhaustive "time-lapse" comparisons of DNA packaging proteins, or histones, in young versus aging cells, he found that as cells count down to senescence and telomeres wear down, their DNA undergoes massive changes in the way it is wrapped up. These changes likely trigger what we call "aging."

Each time a cell divides, the protective "caps" (red and green dots) at the tip of chromosomes (blue) erode a little bit further.

Image: Roddy O'Sullivan

Degenerating motor neurons

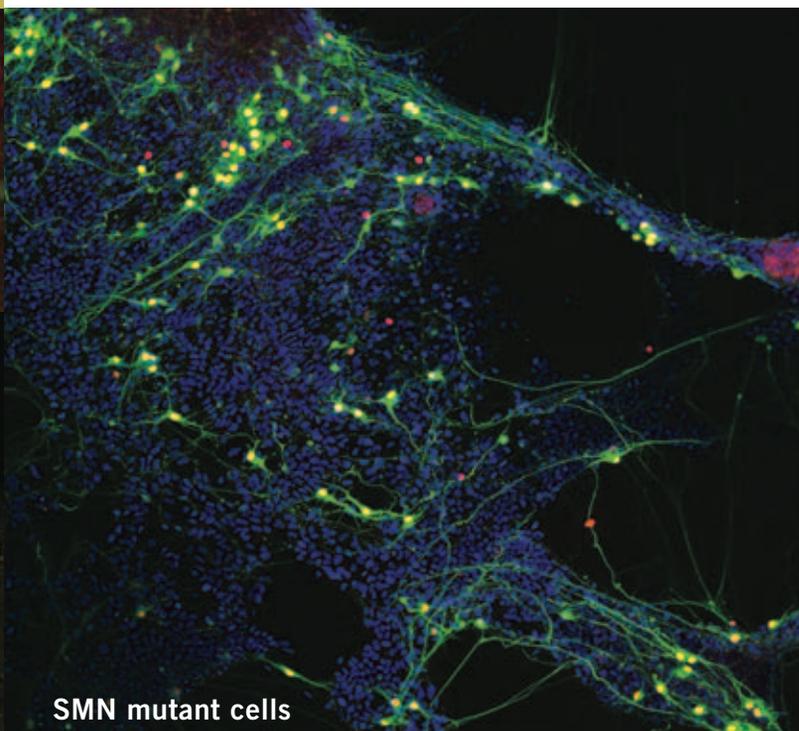
SPINAL MUSCULAR ATROPHY (SMA), THE LEADING GENETIC cause of death in infants and toddlers, affects about one in 6,000 newborns. The disease is caused by an inherited defect in the SMN1 gene. Without a functional SMN1 gene, motor neurons, which instruct individual muscle fibers to contract, shrink and die. **Samuel Pfaff's** goal is to define what makes spinal motor neurons particularly vulnerable to a lack of functional SMN protein and to develop an effective drug-based therapy to treat this debilitating and often deadly disease.

In a first step, his research team recapitulated the human disease in a mouse model by replacing the mouse SMN gene with a partially functional human SMN gene. Because Pfaff's group has specialized

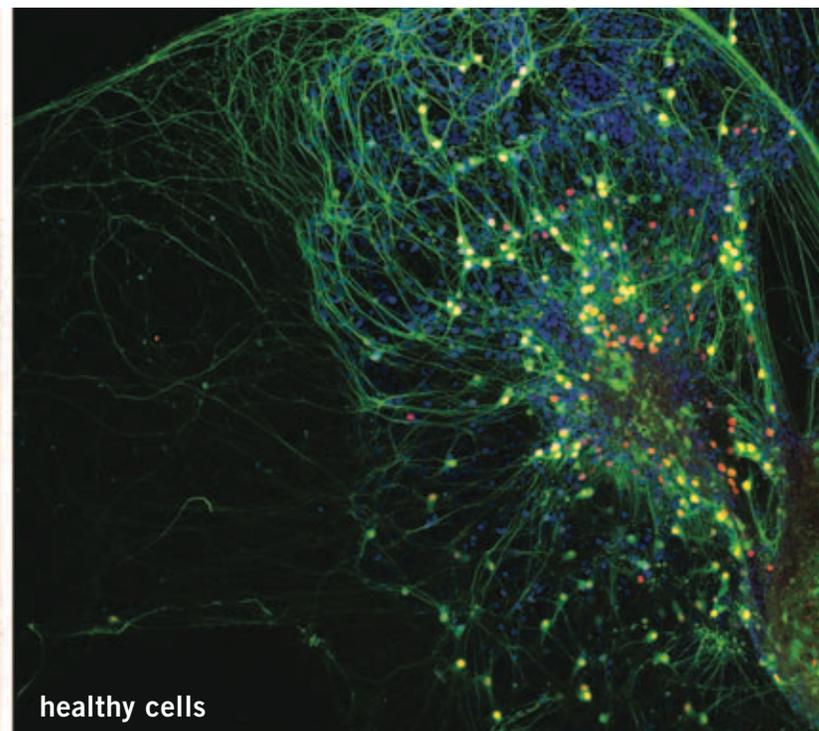
in defining the genetic pathways controlling the embryonic formation of motor neurons, they were able to isolate embryonic stem cells from these mice and drive them toward motor neuron differentiation. Embryonic stem cells from non-mutant mice readily differentiated into motor neurons, but mature motor neurons carrying mutant SMN soon started to degenerate and die.

“From a research standpoint, spinal muscular atrophy is a genetic disorders that offers great hope for a treatment in the near future.” SAM PFAFF

A green fluorescent protein reporter gene becomes activated when mouse embryonic stem cells differentiate into motor neurons. Cell nuclei are shown in blue. *Image: Matthew Pankratz*



SMN mutant cells



healthy 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. All images in this issue were taken in the Waitt Advanced Biophotonics Center.

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.

Rebecca Newman
Vice President, Development & Communications

James Fitzpatrick
Director, Biophotonics Core

Gina Kirchweger
Interim Sr. Director, Communications Editor

Sarah Lifton
Science Writer

Joe Belcovson
Photographer

Sarah Loffler
Print Production Coordinator

Studio L
Graphic Design



SALK INSTITUTE
FOR BIOLOGICAL STUDIES

10010 North Torrey Pines Road
La Jolla, California 92037-1099

Post Office Box 85800
San Diego, California 92186-5800

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