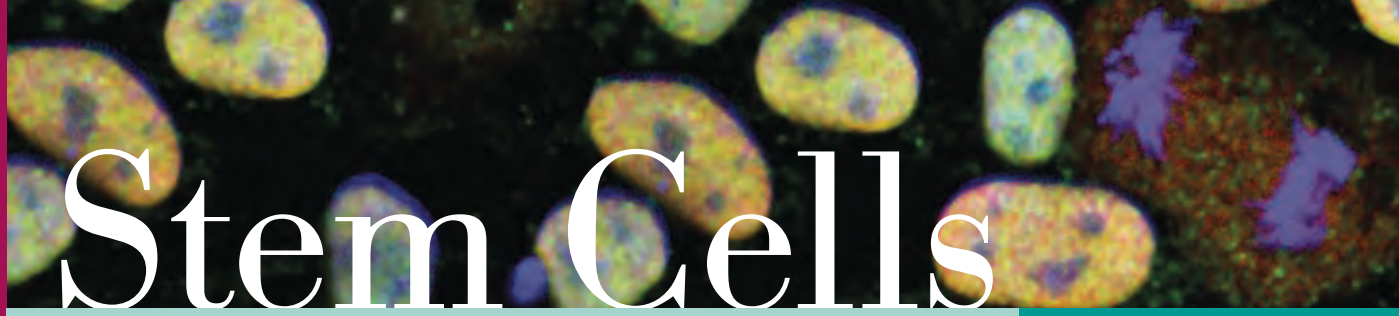
From the Bench



Stem Cells

CURRENT RESEARCH AT THE SALK INSTITUTE

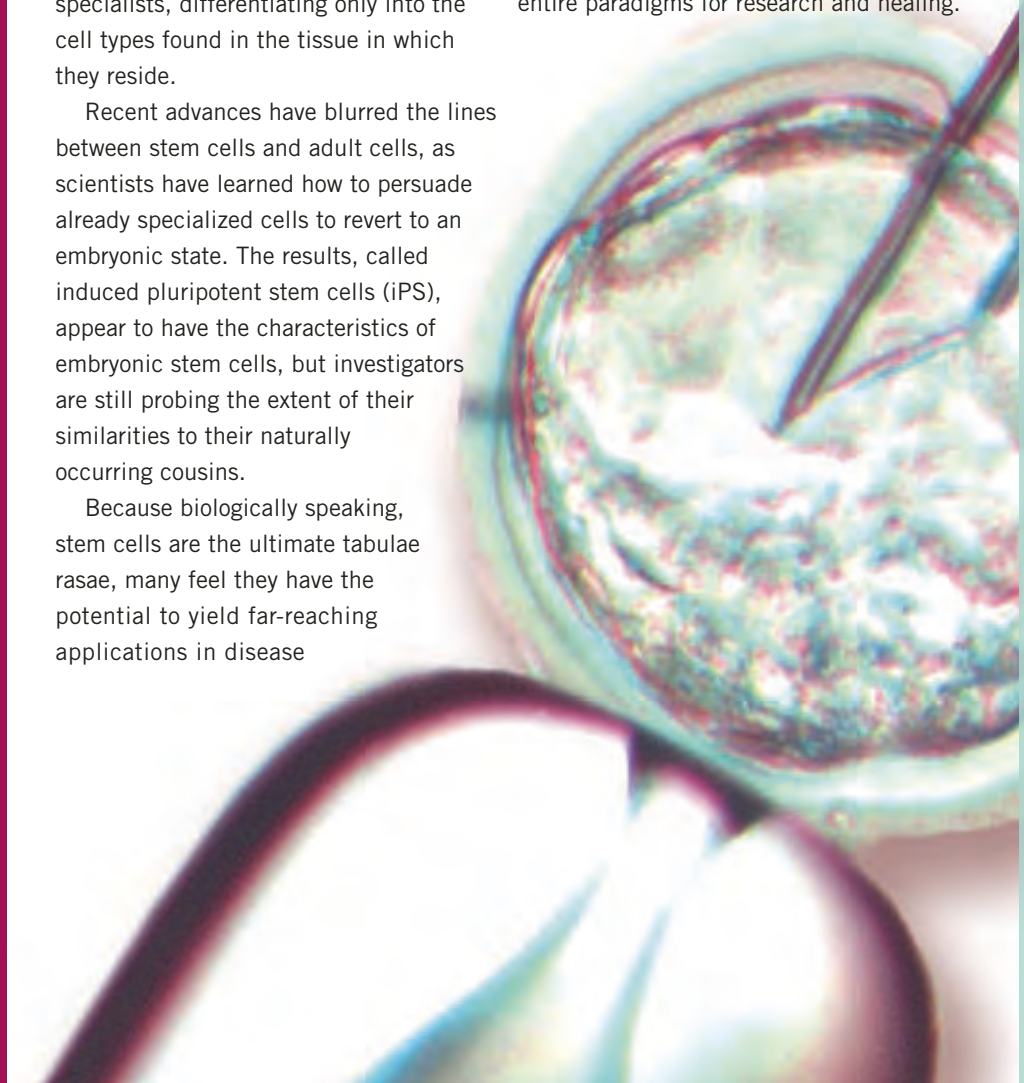
SUMMER 2009

THERE ARE, BY MOST ESTIMATES, AN AVERAGE OF 100 trillion cells in the adult human body. Yet all that we are, all that we may become, derives from only about 30—the embryonic stem cells that were formed roughly five days after conception.

Common to all multicelled organisms, embryonic, or pluripotent, stem cells enjoy almost limitless prospects, able to differentiate into the roughly 200 cell types that make up our blood, muscles, bones, fat, connective tissue, brain, heart, skin, and other organs. By contrast, adult somatic, or multipotent, stem cells, which are found in developed tissue, are specialists, differentiating only into the cell types found in the tissue in which they reside.

Recent advances have blurred the lines between stem cells and adult cells, as scientists have learned how to persuade already specialized cells to revert to an embryonic state. The results, called induced pluripotent stem cells (iPS), appear to have the characteristics of embryonic stem cells, but investigators are still probing the extent of their similarities to their naturally occurring cousins.

Because biologically speaking, stem cells are the ultimate tabulae rasae, many feel they have the potential to yield far-reaching applications in disease modeling, diagnostics, and therapeutics, if only scientists can discover how to harness them. Salk Institute faculty are leaders in that effort, exploring how stem cells function and developing novel ways to direct their behavior. Their research, recent examples of which appear in the following pages, promises an enormously powerful tool for discovery that may ultimately shift entire paradigms for research and healing.



Glossary

Stem cells—Cells with the ability to divide for indefinite periods and to give rise to specialized cells.

Differentiation—The process whereby a stem cell acquires the features of a specialized cell such as a heart, liver, or muscle cell.

Embryonic stem cells—Cells derived from early embryos that can divide indefinitely and develop into a wide variety of specialized cell types.

IPS cells (induced pluripotent stem cells)—Adult cells that have been reprogrammed into a stem cell-like, pluripotent state.

Neurons—The basic working units of the brain, neurons are designed to transmit information to other nerve cells, muscle cells or gland cells.

Pluripotency—Ability of a single stem cell to give rise to all of the various cell types that make up the body.

Regenerative medicine—A treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cell populations or tissues.

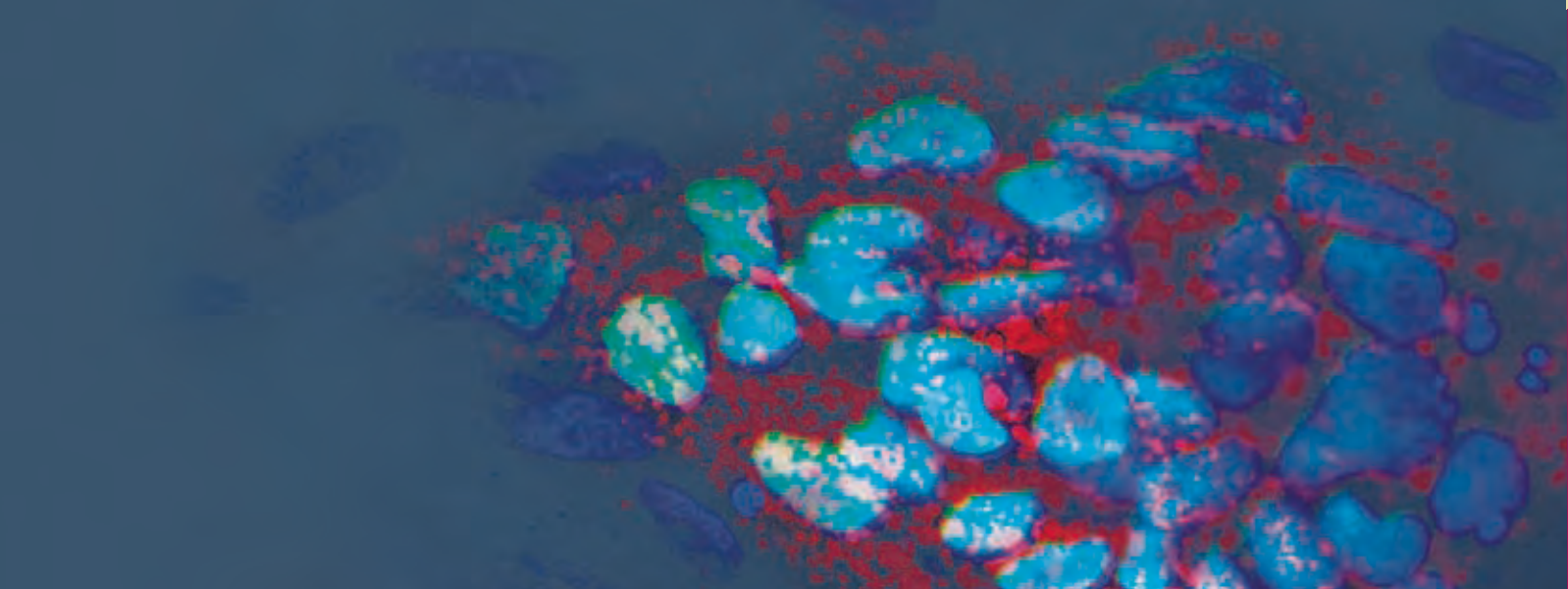
Self-renewal—The ability of stem cells to renew themselves by dividing into the same non-specialized cell type over long periods.

A different breed of cells

HUMAN EMBRYONIC STEM CELLS (HESCS)—FAMED FOR their capacity to transform themselves into any type of specialized cell—are a temperamental bunch. The slightest provocation causes them to disavow their almighty abilities and start differentiating into whatever cell type they feel like. **Senyon Choe** is developing a chemically defined nutrient solution, which not only prevents hESCs from determining their own destiny but allows scientists to steer the cells along a defined differentiation path.

Like ordinary cells, stem cells are grown in plastic laboratory culture dishes that contain a nutrient-rich broth known as culture medium—with one important difference: The surface of the dish is typically coated with so-called mouse feeder cells that provide an unspecified mix of growth factors. For therapeutic applications, however, stem cells need to be grown without the company of feeder cells, which can skew results or contaminate the stem cell sample. "And if you want to control differentiation, you need to know exactly which growth signals the cells are receiving," says Choe.

He and his team devised a novel and efficient method to produce TGF beta and FGF 2, two growth factors that are indispensable ingredients in any chemically defined medium that aims to keep stem cells chugging along happily without the support provided by non-stem cells. In another project, they are developing custom molecules based on a mix-and-match approach. These differentiation factors are designed to nudge stem cells down the endodermic pathway, which results in liver, pancreatic, and intestinal cells. A few molecules have already proved their mettle in tissue culture experiments, and the researchers are currently fine-tuning their effects.

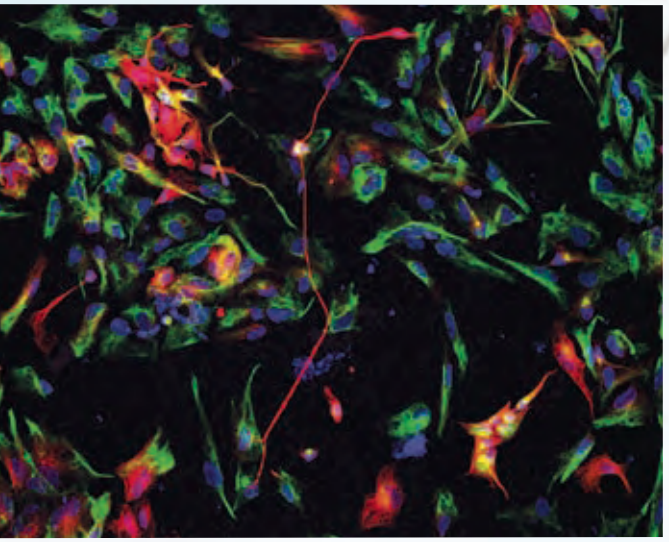


Too much of a good thing

DOWN'S SYNDROME, THE MOST FREQUENT CAUSE OF mental retardation, is caused by an extra copy of chromosome 21, but the exact genetic details largely remain a mystery. New research in the Cellular Neurobiology Laboratory, however, may offer important clues.

Among the anomalies found in patients' brains are a greater than normal number of glial cells, which function as personal assistants to neurons. Using a new mass spectrometry technique and stem cells that can be made to produce either neurons or glia, a team of Salk researchers in the lab of **Dave Schubert** identified a molecular signaling pathway that is required for the production of glial cells. Led by research associate Federico Herrera, the investigators then sought to determine whether the pathway was more active in Down's syndrome patients and in a mouse model of the condition. What they found was that the level of a protein called synaptojanin-1, which is encoded on chromosome 21, is much higher in both and is strongly correlated with more numerous glial cells.

"Given the required balance between the numbers of neurons and glia in a normal brain, an excess of glia may contribute to the cognitive deficits that characterize Down´s syndrome," says Herrera. "This is a critical first step to identifying drugs that specifically block the excess proliferation of glial cells associated with Down´s syndrome and perhaps promote the production of more neurons."



A niche of their own

IT'S ONE THING TO FIND ONE'S NICHE; IT'S ANOTHER TO actually create it. But like the most enterprising businesspeople, it turns out that some stem cells not only thrive in their own niche—a specialized environment that provides the factors necessary to keep the cells young and vital—but may also be responsible for maintaining it.

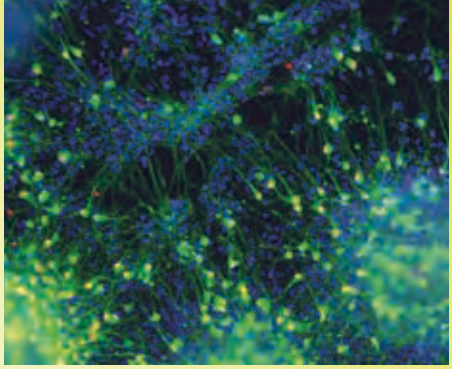
Investigators long thought that the cells making up a fruit fly's niche were allocated at birth and meant to last a lifetime. But **Leanne Jones** found that this may not always be the case. Imaging specific cell types over time, Jones' team caught a testis stem cell population in the act of turning into their own niche cells, known in the fly testis as the hub. The hub contains approximately ten cells that produce factors that promote self-renewal of two stem cell populations—germline stem cells, which become sperm, and somatic stem cells (SSCs), which develop into a structure that encapsulates maturing sperm. After tracking individual cells, the researchers saw that SSCs have the remarkable ability to generate other SSCs as well as their own niche support cells.

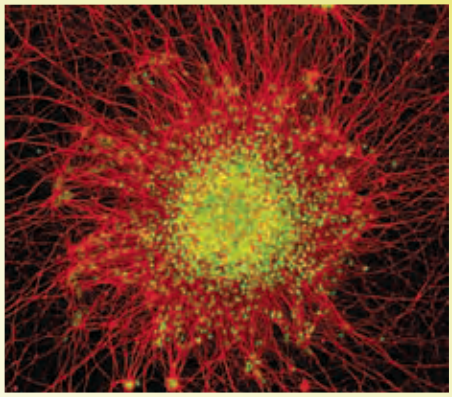
This finding has implications for regenerative medicine, aging research, and cancer therapeutics. If the same phenomenon exists in mammals, it may point to strategies for proposed regeneration therapies or provide new targets for cancer treatments.

Team players

AMYOTROPHIC LATERAL SCLEROSIS (ALS), also known as Lou Gehrig's disease, is best-known for bringing down the legendary New York Yankee slugger and for afflicting renowned physicist and author Stephen Hawking. Usually fatal, the neurodegenerative disease attacks motor neurons controlling voluntary movement, leading to progressive paralysis and muscle atrophy. Although ALS was first classified as a disease over 140 years ago, there are still few clues as to its cause. An important step toward understanding the condition came when scientists discovered that astrocytes—long thought of as mere bystanders in the nervous system—are crucial for the survival and well-being of motor neurons. In fact, defective astrocytes can lay waste to motor neurons and are now considered the main suspects in the development of ALS.

Sam Pfaff, a leading expert in motor neuron biology, has assembled a multidisciplinary team including ALS and human embryonic stem cell specialists, spinal surgeons, and biotech companies with expertise in ALS-specific clinical trials, to develop a stem cell-based therapy that halts the progression of ALS. In a novel approach, the team is planning to transplant healthy astrocytes rather than motor neurons themselves. Transplanted motor neurons not only exhibit a limited capacity for establishing functional connections in adults, but due to the toxic environment within ALS patients' spinal cords, are likely to degenerate just like the diseased motor neurons they are replacing.





"With their support crew intact, even challenged motor neurons may be able to keep doing their job," says Pfaff.

When good glia go bad…

THE STAR-SHAPED GLIAL CELLS CALLED ASTROCYTES ARE crucial for the survival and well-being of motor neurons. When they're defective, however, astrocytes can lay waste to their charges and are implicated in the muscle-wasting disease amytrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease.

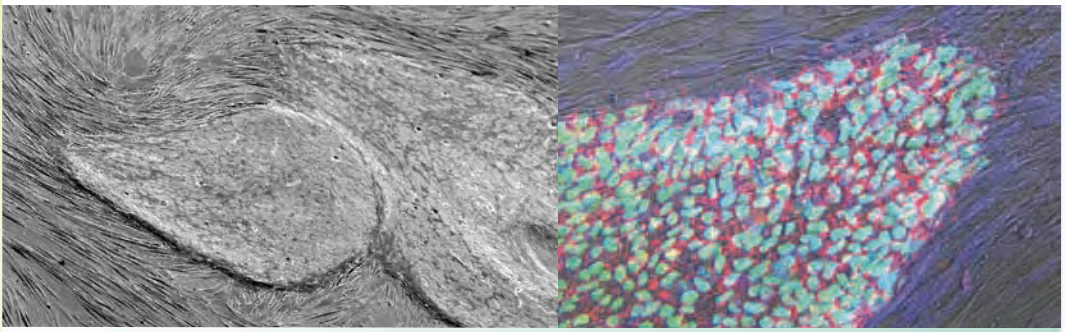
ALS can be induced by inherited mutations in the gene encoding the SOD1, or superoxide dismutase 1, enzyme, which protects the body from damage caused by free radicals—highly reactive molecules produced by cells during normal metabolism. To get to the root of the complicated relationship between neurons and astrocytes, M. Carol Marchetto, a postdoctoral researcher in the laboratory of **Fred H. Gage**, coaxed human embryonic stem cells (hESCs) to differentiate into motor neurons. She then co-cultured the neurons with human astrocytes expressing a mutated form of SOD1. The number of motor neurons alive in the Petri dish plummeted, and the astrocytes activated an inflammatory response and started producing the reactive ions or small molecules that are a hallmark of ALS.

When Marchetto treated the cells with known antioxidants, the percentage of astrocytes churning out harmful reactive molecules decreased significantly. What is more, the motor neurons treated with apocynin, which is found in many plants, were able to withstand their no-longer-supportive environment.

In addition to providing new insights into the toxic pathways contributing to ALS, the hESC-based system Gage's team devised opens up new possibilities for drug-screening experiments and clinical interventions using astrocyte-based therapies.

Cell lines to bloodlines

DESPITE THE RAPIDFIRE BREAKTHROUGHS IN THE CELL-REPROGRAMMING field, regenerative medicine is still several years away from curing a disease, at least in humans. But recent results from the laboratories of **Juan-Carlos Izpisúa Belmonte** and **Inder Verma** moved the field a giant step closer to the ultimate goal. Using skin cells taken from a human patient with Fanconi's anemia, a genetic disorder characterized by bone marrow failure, the investigators developed patient-specific stem cells or iPS cells, genetically engineered them to correct the genetic defect, and then successfully reprogrammed them into healthy blood cells. Now they are hard at work to find the right conditions under which genetically engineered stem cells will set up home in the bone marrow of mice suffering from Fanconi's anemia and start supplying the missing protein. While curing mice will be an important milestone, the scientists have set their sights much higher: "If we can demonstrate that a combined iPS-gene therapy approach works in humans, then there is no limit to what we can do," says Verma.



Starting over

HISTORICALLY, HUMAN EMBRYONIC stem cells (hESCs) have been derived from the inner cell mass of mammalian blastocysts—the balls of cells that develop after fertilization and go on to form a developing embryo. Not surprisingly, a media stir accompanied the first report that adult human cells (such as skin cells) had been reprogrammed back into so-called induced pluripotent stem (iPS) cells that appear to mimic hESCs in terms of appearance and behavior.

Despite the hope that reprogramming might fulfill the promise of patient-specific hESCs in research and medicine and bypass the ethical minefield of working with human eggs and early embryos, several challenges remain: For one, the reprogramming process is woefully inefficient.

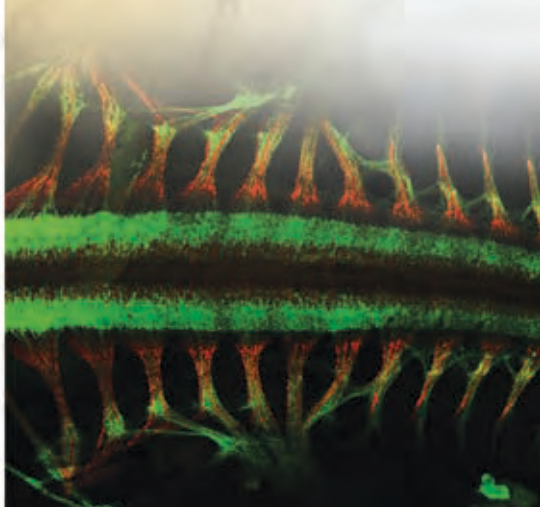
Researchers have to slip several genes inside the cells, and after three to four weeks, only a tiny fraction will transmogrify into cells that look and act like pluripotent human embryonic stem cells. Unfortunately, adding extra genes also carries the risk of inducing cancer, and most importantly, it is still not clear whether these cells really have the same properties and potential as embryonic stem cells.

Last year, **Juan Carlos Izpisúa Belmonte** and his team discovered that starting with keratinocytes attached to a single human hair instead of a skin biopsy increased about a hundredfold the reprogramming efficiency using the standard set of genes, sparing patients invasive procedures to collect suitable starting material. The researchers then successfully differentiated the resulting iPS cells into the many cell types that constitute our bodies, including cardiomyocytes (heart cells), showing that iPS cells could be used to generate mature cell types that would not be rejected by the patient's immune system after transplantation.

Fast-tracking drug development

SPINAL MUSCULAR ATROPHY (SMA), the leading genetic cause of death in infants and toddlers, is caused by an inherited defect in the SMN1 gene, short for Survival Motor Neuron Gene 1. Without a functional SMN1 gene, motor neurons, which instruct individual muscle fibers to contract, shrink and die. As the motor neuron network breaks down, muscles progressively weaken and waste away. There is no cure, and treatment is limited to managing the symptoms and preventing complications.

Instead of using stem cells to replace withering motor neurons in children suffering from SMA, **Sam Pfaff** and his team are banking on these versatile cells as a means to find new drug-based treatments. The researchers have isolated embryonic stem cells from mice carrying a mutation in the SMN1 gene and are currently coaxing them into developing into mature motor neurons. Once the stem cells reliably do as they are told, Pfaff plans to use the resulting SMA motor neurons to screen large compound libraries for drugs that keep them alive and healthy in a Petri dish. "We believe that we can use this system as a rapid drug screening test to identify the best candidates for further preclinical studies," he says.



Reverse engineering

DESPITE ITS CONCEPTUAL SIMPLICITY—CURING genetic diseases such as hemophilia by replacing or "repairing" defective genes—gene therapy never took off. Hampered by inefficient gene delivery, inadvertent activation of cancer-causing genes, deleterious immune reactions, and insufficient gene expression, human clinical trials could never replicate the promising results obtained in animals.

To circumvent these obstacles, **Inder Verma** and his team are combining gene therapy with the latest stem cell technology. They are planning to isolate skin cells from patients with genetic diseases and reprogram them into an embryonic-like state. After fixing the genetic defect with well-established methods in a Petri dish, they will coax the stem cells to develop into the desired cell type, then return them to the patient, where the now fully functional cells will supply the missing protein.

For now, the researchers are focusing on mice with hemophilia. The disease is an attractive model for gene therapy because just small amounts of the deficient protein can cure the condition. What's more, most of the clotting factors are manufactured in liver cells, called hepatocytes, so the technique Verma's lab develops may have broader applications. "Once we've learned how to reliably produce healthy hepatocytes and infuse them in recipients' livers, we can tackle a host of metabolic diseases that originate in defective hepatocytes," says Karl-Dimiter Bissig, who spearheads the project.

A time-stamp for memories

REMEMBER WHEN...?" IS HOW MANY A TRIP down memory lane begins. But how the brain keeps tabs on what happened and when is still a matter of speculation. A computational model developed in the laboratory of **Fred H. Gage** now suggests that the thousands of newborn brain cells generated by neural stem cells each day add a time-related code, which is unique to memories formed around the same time.

To ascertain the newcomers' job in adult brains, graduate student Brad Aimone took every piece of available biological information and fed it into a computer program designed to simulate the neuronal circuits in the dentate gyrus, the entryway to the hippocampus, which plays a key role in the formation of memory. It quickly became clear that young cells respond indiscriminately to incoming information, becoming the links between independent events that had nothing in common except the fact that they occurred around the same time—explaining why discussing the movie we saw a couple of months ago might bring back the name of the café we visited afterward, which had been eluding us. As these cells mature, they quiet down and take their place in the existing circuitry, leaving the next generation of newborn neurons to fire away at new events.

"Current thinking holds that when we bring up a certain memory, it passes back to the dentate gyrus, which pulls all related bits of information from their offsite storage," says Gage. "Our hypothesis suggests that cells that were easily excitable bystanders when the memory was formed are engaged as well, providing a hyperlink between all events that happened during their hyperactive youth."

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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