Scientists at the Salk Institute have identified a novel pathway that regulates the body’s ability to store or burn fat, a discovery that suggests new ways to reduce obesity, diabetes and other fat-related human diseases.

Genetically engineered mice, in which the pathway was constantly revved up, were protected from the ravages of a high-fat diet, according to a study published in *Science* and led by Marc Montminy, professor in the Clayton Foundation Laboratories for Peptide Biology.

“These mice were able to deal with a high-fat diet much better than their normal counterparts,” Montminy says. “They stayed lean and were much more sensitive to insulin, even though they ate more.”

In humans, a high-fat diet — one heavy on red and processed meats like hot dogs and sausages, refined grains, fried foods and sweets — increases the risk of fatty liver disease, which may eventually lead to type 2 diabetes, especially in people who are overweight and out of shape.

*continued on page 2*
The engineered mice consumed an equally high-fat diet but did not gain weight, indicating that fat storage pathways can be tweaked.

“Maybe the most amazing finding is that these mice are protected from fatty liver disease, a serious problem in obese individuals with insulin resistance,” Montminy says.

Our body’s ability to store fat requires the activity of the enzyme acetetyl-coenzyme A carboxylase, or ACC. When we fast, our body starts to burn fat while simultaneously shutting down ACC through a chemical modification called phosphorylation.

The Salk researchers found that a critical protein called TRB3 orchestrates a second chemical modification of ACC, known as ubiquitination, which gets rid of the enzyme altogether.

“In this parallel pathway, TRB3 serves as a go-between for an enzyme that marks ACC for degradation,” says Jose Heredia, a graduate student in Montminy’s lab.

TRB3 levels in adipose tissue usually rise only during fasting, when ACC should be turned off. Heredia and co-first author Ling Qi reasoned that keeping the TRB3 pathway artificially “on” during both fasting and feeding might melt away fat depots.

And that’s exactly what happened. Mice genetically engineered to express permanently elevated levels of TRB3 protein in fat tissue were 10-20 percent skinnier than normal mice.

“Even when we put them on a high-fat diet, these mice just didn’t gain any weight,” Qi says. “Their physical activity was the same, but they were constantly burning fat.”

All this is good news for the fight against obesity and the disorders characterized by insulin-resistance known as “metabolic syndrome.” Most people with insulin resistance will develop type 2 diabetes within 10 years unless they lose 5-to-7 percent of their body weight — about 10-to-15 pounds for someone weighing 200 pounds. Defining the interaction of molecules that regulate fat storage could lead to novel measures to curb obesity.

“What really made this an interesting story is that the molecular mechanism and the biology intersected since we were able to explain how TRB3 made the mice lean,” he says. “That doesn’t happen very often.”

CIRM funds embryonic Stem Cell Training Program at Salk

The California Institute for Regenerative Medicine (CIRM) has provided funding for six postdoctoral fellows to conduct studies in various areas of human stem cell biology research at the Salk Institute over the next three years.

Each scholar will receive an annual stipend of $50,000 in addition to support for travel and lab equipment expenses. The program, which began in July, is designed to create a curriculum of study and experiences that provide high-quality training and ensures a continuing supply of scientists to conduct cutting-edge, health-related research in stem cell biology.

This year, CIRM-sponsored fellows are working in the laboratories of Drs. Juan Carlos Izpisúa Belmonte, Fred H. Gage, Inder Verma and Sam Pfaff pursuing studies on:

- Genomic characterization of cardiac progenitors.
- Human embryonic stem cells as tools for investigating the mechanisms of germ cell differentiation.
- Neuron differentiation and motor neuron specification from human embryonic stem cells.
- Gene silencing following viral transduction: Differences in mouse and human embryonic stem cells.
- The role of miRNA regulation during differentiation of human embryonic stem cells.
- Fate, function and dysfunction of human dopamine neurons in vitro and in vivo.

“Human embryonic stem cells represent an invaluable model for investigating the mechanisms that underlie cell and tissue differentiation,” says Belmonte, professor in the Gene Expression Laboratory and director of the Stem Cell Training Program.

“Therefore, they hold great promise for regenerative medicine. However, before human embryonic stem cells can be successfully utilized to develop new therapies for human diseases, a deep understanding of their biology will be essential,” he says.
The capacity of embryonic stem (ES) cells for endless division is limited. After a childhood spent dividing in a culture dish, even stem cells grow up and assume adult roles as nerve, muscle, or blood cells, never to return to their youthful state.

Salk Institute investigators now know how these differentiated cells can recapture their cellular innocence as explained in a recent study published in Proceedings of the National Academy of Sciences.

Those investigators, headed by professor Juan Carlos Izpisúa Belmonte of the Gene Expression Laboratory and including professor Fred H. Gage of the Laboratory of Genetics, show that a protein called Nanog keeps ES cells immature. Nanog is required for so-called “stemness,” which is defined by two qualities: the ability of ES cells to divide; and their plasticity in assuming the identity of almost any cell type.

Previously, the team showed that occasionally ES cells lost stemness and evolved into muscle cells. However, when those cells were forced to produce Nanog, they reverted to their naïve state.

In the latest study, they found that Nanog actually binds to proteins required for maturation and inactivates them. With those factors out of the game, cells revert to a forever-young state.

Atsushi Suzuki, a former post-doctoral fellow in the Belmonte lab and lead author of both studies, said that before this work, “nobody knew that ‘reverse differentiation’ occurred in ES cell cultures.”

This work has implications for regenerative medicine. While adult salamanders, for example, can regrow severed limbs, mammals can regenerate very few tissues. Replacement therapies for conditions like neurodegeneration may require the transformation of adult cells to a stem cell state to generate new cell types.

A collaboration between scientists at the Salk Institute and Stanford University suggests how nerve function could be preserved after injury.

The study, published in Neuron, combines data collected by Dennis D.M. O’Leary, professor in the Salk Molecular Neurobiology Laboratory, and Liqun Luo, professor at Stanford University and Howard Hughes Medical Investigator. Todd McLaughlin, a post-doctoral researcher in O’Leary’s lab, and Eric Hoopfer, a Stanford graduate student, were co-lead authors.

Normally when nerve cell branches, or axons, are cut in adult animals, the severed branches degenerate and die. Interestingly, a naturally occurring mouse mutant makes a protein known as Wlds that greatly slows degeneration following injury. The O’Leary team found that in the brains of newborn animals, Wlds protein doesn’t slow a desirable form of degeneration known as pruning, which is required to eliminate incorrect connections made by nerve cells in the developing brain.

However, when they severed the same axons in the developing brain in mutant Wlds mice, their degeneration was slowed, meaning that healthy developmental degeneration requires different activities than does neurodegeneration following injury or disease in both the developing and adult brain.

The Stanford team made similar observations in fruit flies. Adult flies carrying the Wlds gene showed slower degeneration in cut axons, but the gene had no effect on axon pruning during development, supporting the idea that axon degeneration following injury differs from the “good” form of degeneration required for neurons to wire up correctly.

“Wlds protein has been shown to delay degeneration not just after injury, but in diseases similar to Parkinson’s disease or motor neuron injury,” Luo says.
Bone marrow cells hand natural killer cells their license to kill dangerous invaders

A collaboration between scientists at the Salk Institute and the Pasteur Institute in Paris has identified molecular signals that trigger maturation of natural killer cells of the immune system into fully armed killing machines.

Prior to the study, published in Nature Immunology, scientists were familiar with the repertoire of surface molecules enabling natural killer cells to distinguish friend from foe, but how natural killers acquired their reconnaissance tool kit remained unclear.

Claude Roth, an immunologist at the Institut Pasteur, called on Greg Lemke, a professor in the Salk’s Molecular Neurobiology Laboratory, after discovering that low levels of a protein called Axl, which belongs to a class of molecules collectively known as receptor tyrosine kinases, were correlated with diminished killer activity in natural killer cells.

Qingxian Lu, a staff scientist in Lemke’s lab and co-lead author of the study, had previously deleted the Axl gene and its two cousins Mer and Tyro3 in mice. They discovered that mice lacking all three of these Tyro3 family genes, so-called “triple knock-outs,” developed autoimmune disease.

The Salk and Pasteur teams found that, in triple knock-out mice, natural killer cells are still armed with an arsenal of deadly enzymes used to eradicate invading cells, but they couldn’t access their weapons cache because they lacked the full spectrum of surface proteins that allow them to recognize virus-infected cells and tumor cells, and gives them a “license to kill.”

Signals released by bone marrow stromal cells, and received by the Tyro 3, Axl, and Mer receptors, give natural killer cell precursors the go-ahead to acquire and then activate the cell surface recognition proteins that allow them to attack with discrimination.

New roles for growth factors: Enticing nerve cells to muscles

During embryonic development, nerve cells extend tentacle-like protrusions called axons which sift their way through a labyrinth of chemical cues guiding them to their target.

Previous studies identified molecules that repel motor neuron axons. Now in a study published in Neuron, Salk Institute scientists pinpoint a molecule, the growth factor FGF, that actively lures growing axons to the right destination.

FGF family members regulate blood vessel formation, wound repair, lung maturation, and development of skeletal muscle, blood and bone marrow cells. The Salk study adds another task to that long list.

“Piece by piece, we are uncovering general principles that ensure that the developing nervous system establishes proper neuronal connections,” says senior author Samuel Pfaff, professor in the Gene Expression Laboratory.

Skeletal muscle consists of thousands of muscle fibers, each controlled by one motor neuron that pledges its allegiance to that specific fiber. Until now, scientists could only speculate how the invisible bond between nerve and muscle is formed.

Earlier studies suggested that muscles lining the spine sent chemical cues to a specific group of motor neurons known as MMCm neurons. Initially, the Pfaff team observed that FGF is expressed in target muscle and that FGF “sensors,” known as FGF receptors, are expressed on those MMCm neurons.

Then, using mice engineered to express a fluorescent protein in MMCm neurons, they showed that those glowing neurons extended axons toward cells expressing FGF, meaning that FGF was guiding MMCm axons to the correct target.

Scientists can already coax embryonic stem cells into developing into motor neurons in a culture dish. However, understanding how axons connect to the right muscle targets in an animal is required if movement is to be restored following spinal cord injury or motor neuron disease.

Spinal cord motor neurons (green) extend axons (yellow) through a labyrinth of chemical cues. Cells that will develop into spine and neck muscles (red) dispatch a growth factor to lure a specific subset of axons closer to their target. Other developing neurons are shown in blue.
Untangling steroid hormone signaling in plants

When given shots of the steroid hormone brassinolide, plants pump up like major leaguers on steroids. Led by Joanne Chory, professor in the Plant Molecular and Cellular Biology Laboratory and a Howard Hughes Medical Institute investigator, Salk Institute researchers recently mapped how these steroid hormones send signals from the cell surface to the nucleus, resulting in bigger plants.

Manipulating the levels or signaling pathways for plant steroid hormones, known collectively as brassinosteroids, could increase crop yields or make plants more resistant to drought, pathogens, and cold weather. Likewise, since low brassinosteroid levels are associated with dwarfism, changing hormone levels during dormant seasons may allow growers to control the height of grasses, trees or other plants — eliminating the need to constantly manicure gardens.

In a recent paper published in Science, postdoctoral researcher Xuelu Wang describes how brassinosteroid binding to a receptor on the cell surface initiates a chain reaction relaying the signal to a cell’s nucleus. Wang found that the signaling cascade revs up to full speed only after the receptor modifies an inhibitory protein that associates with its tail at the cell membrane. Once the receptor binds brassinosteroid, the inhibitory protein dissociates from the receptor — allowing the receptor to send a message to the nucleus. This message leads to the altered expression of hundreds of genes that regulate plant growth.

In a different study published in Nature, postdoctoral researcher Grégory Vert showed how the nucleus receives that message. He found that a critical factor activating gene expression required for growth was not standing in a cellular corridor outside the nucleus, as had been previously thought, but was poised for action inside the nucleus, just waiting for the signal to arrive. Modification of this factor allows it to bind DNA of growth promoting genes.

“Greg’s and Xuelu’s new studies, with those of former lab members, have better defined the mechanisms of the two ends of the brassinosteroid signaling pathway,” Chory says. “This turns our attention to the last mystery: the gap in our understanding of the events between steroid binding at the cell surface and these nuclear mechanisms.”

Scientists get to the root of plant cell fate

A plant that grows topless?

Researchers at the Salk Institute studying the wild mustard Arabidopsis thaliana recently determined why plants with a defective TOPLESS gene form an extra root in place of a shoot. Their findings, published in Science, suggest that it is possible to engineer a plant cell to develop in ways that better suit agricultural needs.

The study, which included lead author Jeff A. Long, assistant professor in the Plant Molecular and Cellular Biology Laboratory, UCSD graduate student Zachery R. Smith, and researchers from the laboratory of Elliot M. Meyerowitz at the California Institute of Technology, specifically focused on understanding mutations in the TOPLESS gene, which Long had previously identified in Arabidopsis thaliana.

Like animals, plants develop along a polar axis, with a root on one end and a shoot on the other. A defective TOPLESS gene, however, causes plant embryos to develop into a seedling with two oppositely oriented root poles — hence the gene’s name. Prior to the study, investigators did not understand why that happened.

Long found that the TOPLESS protein is a transcriptional co-repressor. In plants and animals, co-repressors regulate gene expression by inhibiting the activity of transcription factors — proteins that control gene activity by binding to DNA. Transcription factors can no longer activate their target genes when bound to co-repressors.

The Long study showed that the normal function of TOPLESS is to silence genes required for root development in the top half of a plant embryo, enabling that pole to develop shoots instead. This finding identifies a basic mechanism of gene regulation, conserved in plants and animals, that can be used to change a plant’s form in the future.
Neurons come in two flavors: excitatory neurons that transmit and amplify signals, and inhibitory neurons that inhibit those signals. Until recently, little was known about how developing cells choose inhibitory or excitatory fates. Researchers at the Salk Institute have now identified a critical pathway that regulates this decision.

Research efforts by a group of scientists led by Martyn Goulding, an associate professor in the Molecular Neurobiology Laboratory, have determined the origin of a group of “interneurons” in the spinal cord that help transmit sensory information from the surface of our bodies to the brain. These neurons can be either excitatory or inhibitory.

In a recent study published in *Nature Neuroscience*, Goulding and co-lead authors Rumiko Mizuguchi, a postdoctoral fellow, and graduate student Sonja Kriks showed that interneurons arise from a common precursor cell. The team then defined the transcriptional pathway that controls their development and, in so doing, found that a protein called Notch, which is known to regulate the orderly generation of neurons from neural stem cells, played a key role in determining whether cells differentiate as inhibitory or excitatory neurons.

Cells with high levels of activated Notch became excitatory, while cells with low Notch activity became inhibitory. “Notch acts as decision-maker,” Goulding says. “If Notch is up-regulated in one daughter cell, it will be down-regulated in its sibling.”

The neurons analyzed by the Goulding lab are known to transmit pain signals, and it is thought that chronic forms of pain often arise from an imbalance in the excitatory and inhibitory signals carried by these cells. As such, the findings by the Goulding group have important implications for the study of pain and for the development of new animal models to study pain pathways.

Connections between neurons act as information filters in the brain

Neurons are often considered the primary computational units of the brain. Until recently, it was unclear whether connections between neurons actively participate in computational processes or merely convey information.

For the first time, researchers at the Salk Institute have demonstrated that those cell-to-cell contacts in the brain, known as synapses, play an active role in processing information. A study published in *PLoS Biology* and led by Howard Hughes Medical Investigator Charles Stevens, professor in the Molecular Neurobiology Laboratory, shows that synaptic interfaces act as filters that sense whether information is important.

Brain cells signal by sending electrical impulses along axons, long, hair-like extensions that reach out and contact neighboring nerve cells via synapses. When an electrical signal reaches the end of an axon, the voltage change triggers release of a chemical messenger, known as a neurotransmitter, at the synapse.

Neurotransmitter molecules then travel across the synapse and either excite or inhibit the neighboring nerve cell. Relying on naturally occurring activity patterns recorded in living animals, Stevens, together with lead author and Salk post-doctoral researcher Vitaly Klyachko, stimulated isolated groups of neurons and measured which signals synapses transmitted to neighboring cells and which signals they ignored.

They found that both excitatory and inhibitory synapses, which were previously thought to always antagonize each other, are capable of acting in concert in response to specific patterns of stimulation. In response to high-frequency stimulation, for example, excitatory synapses get stronger, while the inhibitory ones get weaker to maximally amplify the signal. These findings show that synapses are more than conduits for information but play a filtering role, amplifying meaningful information and dampening irrelevant noise.

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Soon after President Bush vetoed a stem cell bill to allow NIH funded scientists to use human eggs destined to be discarded from fertility clinics, I was asked by a member of an audience, “What would you say to President Bush about stem cells if you had the opportunity to meet him?” The question got me thinking...

First, I would say, “With all due respect, Mr. President, your argument that ‘We shouldn’t take the life of an embryo to save the life of a person’ is based on outdated science.” I would explain that somatic cell nuclear transfer (SCNT), the technology of the future, doesn’t involve killing an embryo created by a sperm and egg. Rather, SCNT uses unfertilized human eggs, each with its DNA-containing nucleus removed. A patient’s DNA is then inserted into the “enucleated” egg, which generates stem cells that carry the patient’s DNA. Five days later, these stem cells are removed and the hope is probed for the genetics and/or drug sensitivity of the disease; some day they may even be used in transplantation.

I’d tell him, “Mr. President, scientists are now working to create alternative sources of stem cells that don’t involve human eggs at all, for instance, by de-programming cells that have begun developing into tissues. But ironically, these investigations are being thwarted by the very restrictions you’ve put on our research.”

I’d say, “President Bush, I respect your religious and moral beliefs, but you need to respect the needs of suffering people to get well.” I’d stress that the thrust of stem cell technology is to save and improve existing lives, and I’d illustrate this by describing a young woman I met whose chances of surviving beyond the age of 35 are remote, for she has cystic fibrosis. She endures constant medical crises and her life is on hold until an unknown day in the future when the next breath won’t come. Stem cell technology may be her only hope, allowing genetically engineered cells that produce mucous-clearing enzymes to be introduced into her lungs. I’d say, “Mr. President, I truly believe meeting this woman would expand your perspectives on life and death.”

I would tell Mr. Bush about the patient advocates I’ve met who devote their lives to supporting loved ones afflicted with injuries and diseases that may one day be solved with stem cell therapy; parents of children with type-one diabetes who yearn to replace non-functioning pancreatic beta cells with bioengineered stem cells that can sense circulating levels of glucose and pump out insulin in response. I would speak of the caregivers of thousands of young people paralyzed by spinal cord damage from accidents and sports injuries and say, “You’ve got children, Mr. President, and you seem like a devoted father. I’m sure you empathize.”

I’d point out, “Mr. President, no scientist wants to do morally unacceptable research. The research will be government regulated, with ethicists advising us on pitfalls and best practices. And the great majority of Americans support our work.”

And I’d be frank: “Mr. President, science will progress with or without your permission. Scientists are programmed to explore the unknown, especially when the end point is to create new knowledge that will improve the lives of fellow humans. Without your support, the road to progress will be longer and more difficult, but we will get there eventually. With your support, progress will come far more quickly, and the full potential of stem cell science for saving and improving human lives will be realized.”

And I’d end by saying, “Your veto of the stem cell bill was not a vote for ‘life,’ as you have framed it, but a serious setback for potentially life-enhancing, life-saving therapies. So please reconsider. And thanks for listening.”

Richard Murphy
President and CEO

Mr. President, please reconsider...
In 1960, just five years after developing the first safe, effective vaccine against polio, Jonas Salk, M.D., founded the institute that today bears his name. Home to 11 Nobel Laureates since its founding, the Salk Institute for Biological Studies is a world leader in basic research on the biological principles governing life at all levels, from the individual cell to an entire population. For more information: www.salk.edu.

Differentiation of dopaminergic neurons (red and yellow) from human embryonic stem (ES) cells is visible through staining techniques. The death of these neurons, which produce dopamine, causes Parkinson’s disease. Salk scientists are creating these neurons to study the mechanisms that lead to the development of Parkinson’s disease. Photo courtesy of Salk postdoctoral researcher Christian Carson in the Laboratory of Genetics.

Calendar

**JANUARY 11–14, 2007**

**Symposium on Biological Complexity: Diseases of Transcription**

The Salk Institute

**FEBRUARY 6–7, 2007**

**The Adler Symposium**

The Salk Institute

For additional information about these and other Salk events, please contact Institute Relations at 858.453.4100 x1200.

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