

Hepatitis in a test tube

Rockefeller researchers create the first strain of hepatitis C that can reproduce in captivity

BY JOSEPH BONNER

Charles M. Rice, head of Rockefeller's Laboratory of Virology and Infectious Disease, has spent decades studying the hepatitis C virus, the leading cause of chronic liver disease, including cirrhosis and liver cancer, in the United States.

But until this year, he and other researchers in the field have been hindered by the virus's stubborn
continued on page 4



IMAGE: RICE LAB

Viral explosion. Forty-eight hours after infection with the HCVcc strain of hepatitis C, a culture of human cells contains over 100,000 virus particles in a single milliliter.

A bacterium's sugar-free diet

Scientists find that fatty acids — not sugars — fuel infection

BY LYNN LOVE

As any parent can tell you, sugar can make kids do crazy things. For some, it leads to hyperactivity, moodiness and unpredictable behavior.

New research from **John McKinney's** Rockefeller laboratory shows it may have a similar effect on bacteria. In a study on *Mycobacterium tuberculosis* — one of the world's most pernicious pathogens — McKinney and his colleagues found that fatty acids, not sugars, are the key to understanding the organism's metabolism in mammalian lungs.

The finding is important because researchers tend to feed the bacteria, which cause tuberculosis and kill more than 5,000 people a day in the developing world, a fat-free sugar solution in order to study the organisms in test tubes.

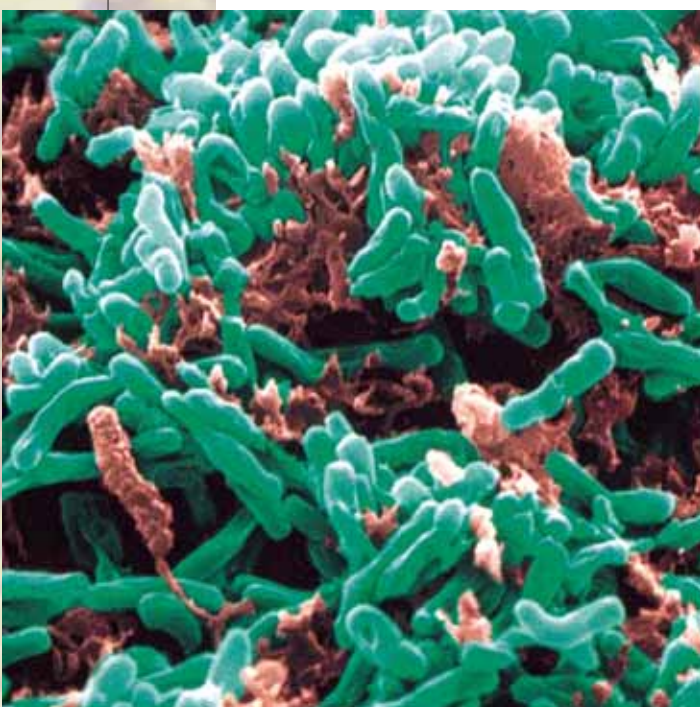
"Surprisingly little is known about the basic metabolism of pathogens living in their hosts," says McKinney. "Metabolic processes that we tend to focus on in lab studies are quite different from, and in some cases irrelevant to, the actual metabolism of microorganisms in their natural environments."

It was Rockefeller graduate student Ernesto Muñoz-Elías, in McKinney's lab, who made the surprising discovery that *M. tuberculosis* has to metabolize fatty acids in order to survive in a living host. He found that mutant bacteria that cannot metabolize fatty acids are rapidly eliminated, at least in mice. The results were reported in the June issue of *Nature Medicine*.

Muñoz-Elías's eye-opening research represents a promising new approach to developing treatments for tuberculosis that could work in weeks rather than the six to nine months that existing therapies require. Muñoz-Elías and McKinney's research provides a model for developing new defenses against microbes by understanding their metabolic Achilles heels. Their findings may also change textbook lore in microbiology.

Several years ago, McKinney, a devotee of the pre-Medline-era scientific literature, came across an interesting but overlooked finding. In 1956, William Segal and Hubert Bloch, at the Public Health Research Institute in New York, reported

continued on page 4



Just desserts. *M. tuberculosis* bacteria (green) thrives in a sugar-based culture, misleading scientists.

IMAGE: MCKINNEY LAB

INSIDE

- > Leukemia gene linked to deformed sperm
- > Making eyes (for flies)
- > A genetic link to appetite
- > Protecting a brain cell's undercoating



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A genetic link to appetite. Using a technique called laser-capture microdissection, in combination with microarray analysis, **Jeffrey Friedman's** laboratory has now identified a set of genes specific to the brain's ventromedial hypothalamic nucleus (VMH). Sometimes called the satiety center, the VMH is important for controlling appetite and keeping track of the blood's sugar level. How exactly it carries out these functions, however, has been hard to study because there were virtually no genes known to be specifically expressed by the VMH. The laser-capture technique involves placing a thin transfer film over a brain section and using a laser to bind specific cells to the film. When the film is lifted away, only the selected cells come with it. The study, led by first author Jeremy Segal, has identified genes that will aid researchers in analyzing the specific functions of the different types of nerve cells in the VMH. Their results also indicate that a similar approach could be used for other areas of the brain. Friedman is a Howard Hughes Medical Institute investigator, director of the Starr Center for Human Genetics, and the Marilyn M. Simpson Professor and the head of the Laboratory of Molecular Genetics.

The Journal of Neuroscience, April 20, 2005

How Alzheimer's destroys neurons. One small peptide, called amyloid- β , is believed to be responsible for the devastating effects of Alzheimer's disease. It is formed by a series of cuts to the amyloid precursor protein, the fragments of which then accumulate both within brain cells and along their exterior, diminishing the ability of neurons to send signals to each other. New research from **Paul Greengard's** laboratory shows that one way amyloid- β affects signaling between neurons is by actually eliminating the neurons' receptors. Cortical neurons, which play a large role in memory, use NMDA-type receptors to respond to the neurotransmitter glutamate. The new study finds that when amyloid- β protein is applied to cortical neurons in cell culture, it causes the NMDA receptors to disappear from the surface of the cells. With the receptors gone, the neuron's response to the glutamate signal is decreased. The effect of amyloid- β on the NMDA receptor may be related to the early cognitive impairment seen in Alzheimer's patients. The new finding may help scientists design treatments for Alzheimer's disease or help efforts to prevent its onset. Greengard is the University's Vincent Astor Professor, is the head of the Fisher Center for Alzheimer's Disease Research at Rockefeller University and is the head of the Laboratory for Molecular and Cellular Neuroscience.

Nature Neuroscience, July 17, 2005

Your brain, on stress. New research from the laboratory of **Bruce McEwen** shows that while chronic stress can change the brain, a single event, if powerful enough, can also influence the connections between neurons. When rats are subjected to the stress of being confined in a restrainer every day for 21 days, there is a growth of the neurons in the amygdala, the area of the brain devoted to fear and anxiety. McEwen and colleagues found that rats exposed to such chronic stress have a marked increase in dendrites (the tree-like receiving end of neurons), an increase in the density of synapses on spines (small protrusions on the dendrites) and an increase in fear, indicating strengthened connections between brain cells. But rats that suffered just one acute stressful event show a delayed increase in spines that takes 10 days, although their dendrites do not grow. They also show a delayed increase in fear. Besides cautioning investigators as to what is happening to their rats during ordinary laboratory procedures, McEwen hopes that studying the neurological changes that result from acute and chronic stress in the amygdala and other brain regions involved in memory will help scientists understand the neurological basis of depression and anxiety disorders, including post-traumatic stress disorder. He is the Alfred E. Mirsky Professor and head of the Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology at Rockefeller.

The Proceedings of the National Academy of Sciences, June 28, 2005

Protecting a brain cell's undercoating. In order for nerve cells to communicate properly with one another, they must be surrounded and insulated by a fatty substance called myelin. In the lab of **Sidney Strickland**, graduate fellow Wei-Ming Yu, research assistant professor Zu-Lin Chen and colleagues recently reported that Schwann cells, which are responsible for

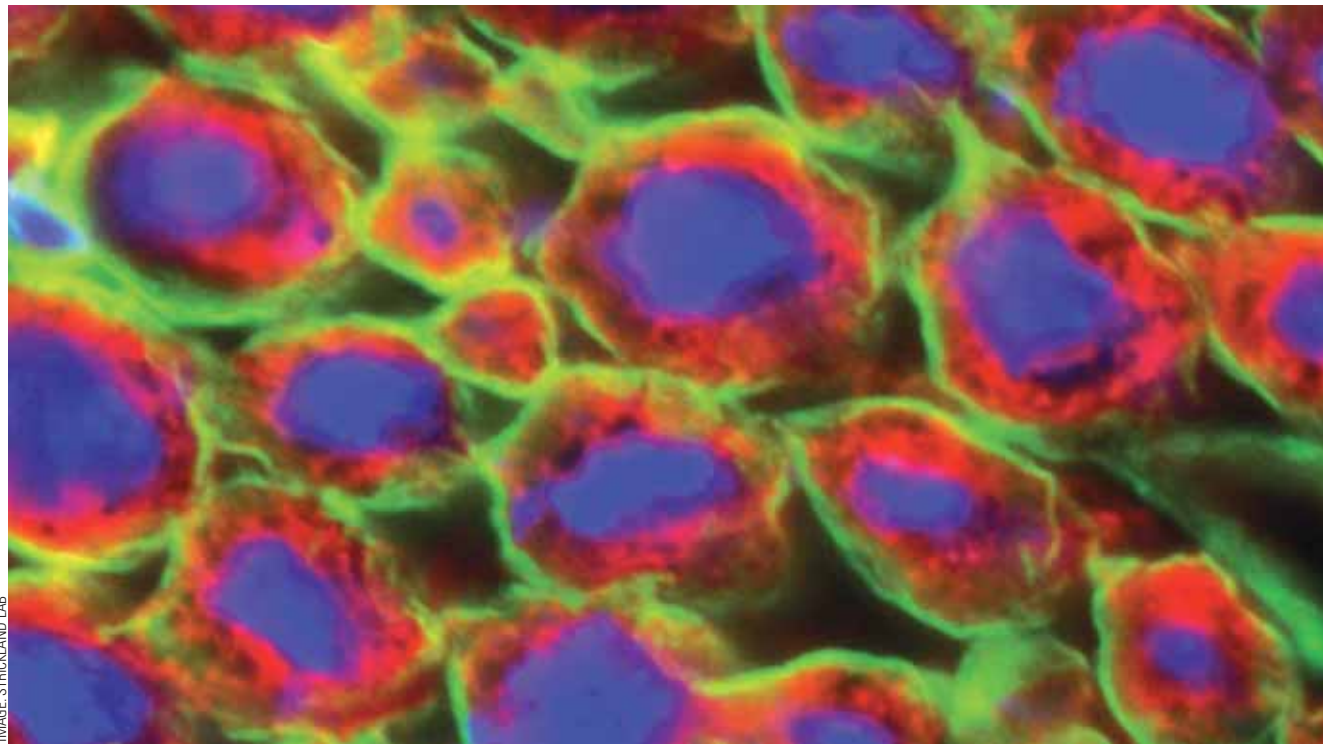


IMAGE: STRICKLAND LAB

Schwann's song. Schwann cells, which produce a fatty substance called myelin (stained red) that coats and insulates neurons (blue), require a protein called laminin in order to function. If laminin (green) is missing, myelination fails and the Schwann cells begin to die.

myelinating the nerve cells that run from the brain to different areas of the body, need the extracellular protein laminin in order to develop. When laminin is removed, mouse Schwann cells don't interact with nerve cells properly and myelination fails. Furthermore, the Schwann cells stop dividing and eventually begin to die. Strickland and colleagues hope that an understanding of the importance of laminin in myelination will contribute to new treatments for muscular dystrophy, which is caused by the demyelination of nerve cells throughout the body. A picture (above) of the myelinating Schwann cells was shown on the cover of *The Journal of Neuroscience*. Strickland is the head of the Laboratory of Neurobiology and Genetics.

The Journal of Neuroscience, May 4, 2005

Putting the brakes on cell division. Like a person getting ready to go on vacation, a cell goes over a checklist of must-have items before it begins to divide: is it big enough, does it have enough food, and is its DNA in good condition? New research from Janni Petersen, now a postdoc in the laboratory of **Paul Nurse**, shows how one protein, the Polo protein kinase, helps an *S. pombe* yeast cell run these checks. When all the checklist items are met, a stress response pathway interacts with Polo1 (causing phosphorylation of Polo1 at its serine 402) and triggers the yeast cell to begin division (see image, right). If the yeast cell senses damage, however, Polo1 remains untouched and reproductive processes remain stopped until the conditions improve. Once they do, Polo1 is phosphorylated and the cell returns to its normal cycle. Nurse is president of The Rockefeller University and head of the Laboratory for Yeast Genetics and Cell Biology.

Nature, May 26, 2005

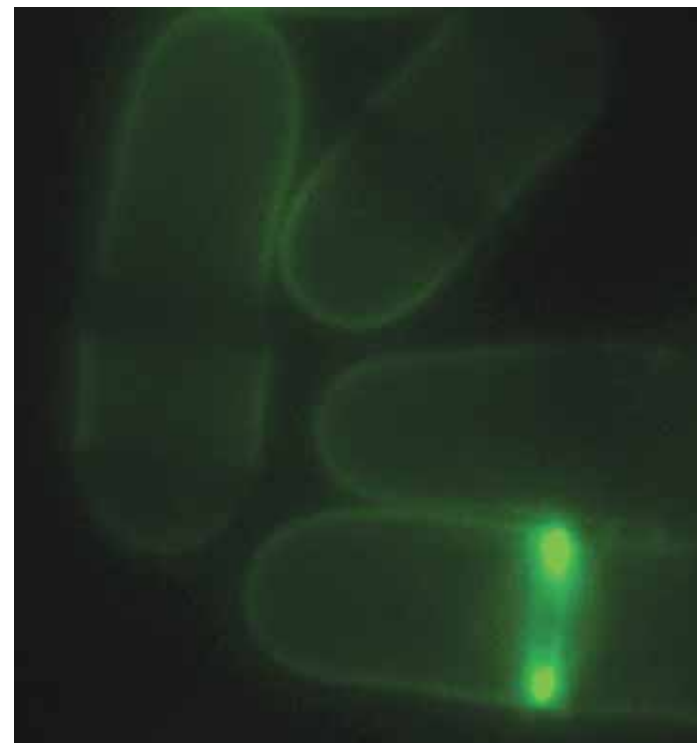
Keeping a stem cell a stem cell. One of the big mysteries of stem cells is how they can divide even as they remain immune to the molecular signals that induce their neighbors to become specialized. A new paper from the laboratory of **Elaine Fuchs** shows how a protein called beta-catenin — known to function in the differentiation and development of many different tissues — also regulates the activation step of stem cells. Beta-catenin proteins produced by stem cells are normally destroyed as they are made. But when a signaling protein called Wnt binds to the outside of the stem cell, a series of events helps to stabilize beta-catenin. Fuchs and colleagues have found that in hair follicle stem cells, beta-catenin activates a specific set of genes in the cell's nucleus that stimulate the stem cells to move from their dormant state to an activated state where they proliferate and subsequently differentiate to produce hair. Fuchs hopes that a better understanding of beta-catenin's role in stem cell maintenance and activation will provide new insight into how mutations lead to overactivation of stem cells may give rise to cancers. Fuchs is Rebecca C. Lancefield Professor, a Howard Hughes Medical investigator, and head of the Laboratory for Mammalian Cell and Developmental Biology.

Genes & Development, July 1, 2005

Trafficking plant growth. Change is scary, even for plants. The transition from one stage of development to the next, whether that is germination, growth or flowering, is influenced by the presence of a protein called ABI3. New research from **Nam-Hai Chua's** laboratory shows that ABI3, which stops a plant from changing stages when conditions are not ideal, is removed from the cells by a protein called AIP2. AIP2 tags ABI3 proteins for destruction, allowing the plant to advance in its development process. Controlling the levels of ABI3 would enable farmers to influence how and when their crops mature, helping them stall plant development in adverse conditions, such as drought and heat or excess rain. Chua hopes his research may lead to the development of plants that are better suited to respond to environmental stresses. He is the Andrew W. Mellon Professor and the head of the Laboratory of Plant Molecular Biology.

Genes & Development, July 1, 2005

Predicting proteins. Numerous neurodegenerative disorders, including Huntington's disease, are caused by a repetition of a



Splitsville. New cell walls (stained green) are built as fission yeast cells finish dividing they are big enough — and healthy enough — to complete the process.

specific amino acid — glutamine — within certain proteins. New research from the laboratory of **Robert Roeder**, aided by **Brain Chait** and researchers from two other universities, shows how this mutation, called a polyQ repeat, affects neurons. They studied a disorder called Spinocerebellar ataxia type 7, which is unique among polyQ-disorders in that it causes blindness. The scientists, led by Vikas Palhan, a former research associate at Rockefeller, found that the protein involved in this disorder, ataxin-7, plays a role in the activation of photoreceptor genes (as a component of a transcription coactivator complex called STAGA). Normally, ataxin-7 interacts with a specific histone acetylase, an enzyme that changes the proteins that organize DNA, to help the genes become active. The polyQ-expansion in ataxin-7 doesn't change its association with the histone acetylase, but acts as a dominant negative, preventing the histone acetylase from doing its job and the genes from being



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activated. The results show that the normal functions of proteins involved in polyQ disorders may provide clues to their actions after the polyQ-expansion, and ultimately help scientists design treatments for Spinocerebellar ataxia type 7, which currently has no cure, and other polyQ diseases. Roeder is Arnold and Mabel Beckman Professor, and head of the Laboratory of Biochemistry and Molecular Biology; Chait is Camille and Henry Dreyfus Professor and head of the Laboratory of Yeast Molecular Genetics.

Proceedings of the National Academy of Sciences, June 2, 2005

Molecular twister. STAT proteins, dormant cytoplasmic proteins that when activated serve to drive gene expression, often become persistently active and can contribute to a variety of human cancers. This March, **James Darnell, Jr.**, and colleagues published two papers, solving the crystal structure of the human unphosphorylated STAT1 transcription factor and exploring the role of that structure in the protein's function (while the phosphorylated state of both STAT1 and STAT3 had already been studied, the unphosphorylated state had not). Like the phosphorylated state, unphosphorylated STAT1 is found in a dimer, in which two STAT1 proteins interact together. However the conformation of the dimer was so different that Darnell calls it "antiparallel" to the "parallel" conformation found when the proteins are phosphorylated. The radical change in position within the dimer is made possible by a flexible chain connecting a domain in each STAT1 protein. Darnell's laboratory showed that when the antiparallel conformation is not allowed to form, through mutations that stop the domains from interacting, the STAT1 proteins are dephosphorylated very slowly, if at all. They hypothesize that the antiparallel conformation gives tyrosine phosphatases easy access to the STAT1 phosphorylated tyrosines, which inactivates the transcription factor. This is a particularly important step in STAT regulation, as perpetually active STAT3 protein is found in the majority of cancers. Darnell is the university's Vincent Astor Professor Emeritus and the Head of the Laboratory of Molecular Cell Biology.

Molecular Cell, March 18, 2005 and *Proceedings of the National Academy of Sciences*, March 15 2005

How motors move microtubules. As fission yeast divide, they grow longitudinally from their opposed tips, an action that partially relies on the organism's microtubules, a type of cellular scaffolding found along the long axis of the cells. New research from **Paul Nurse's** laboratory finds that during cell

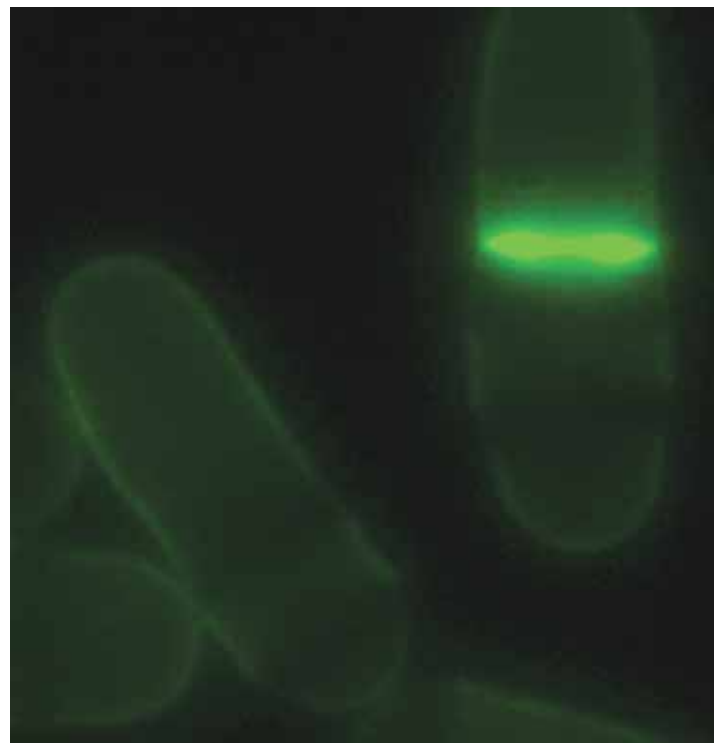


IMAGE: JANNI PETERSEN

ing, a process that occurs only after the cells have completed a checklist to ensure

division, these microtubules are distributed to their correct positions by a "motor protein" called Klp2. Like batteries, microtubules have a minus and a plus end, and they are normally aligned so that all the minus ends point toward the center of the cell and the plus ends face the periphery. Proteins are transported in the cell by motor proteins that move along microtubules in either a plus-to-minus or minus-to-plus direction. Nurse and colleagues found that during cell growth the Klp2 motor, which moves toward minus ends, allows new microtubules to slide along existing ones to create the microtubule scaffold necessary for the cell to grow in a polarized way. The finding makes sense in light of additional studies that suggest that in other cell types, including mammalian cells, minus-end motors are necessary to maintain correct microtubule organization during the process of division.

Science, July 8, 2005

Leukemia gene linked to deformed sperm

In the search for a killer's roots, scientists discover how good sperm go bad

BY KRISTINE KELLY

Making a knockout mouse is a lot like playing roulette. Instead of betting on numbers and colors, scientists gamble on specific genes, spending time and money to create a mouse that is missing that particular gene. Then they cross their fingers and wait six months to a year.

The prize, when they win, is a mouse with a specific defect. A bald mouse, for instance, or one that is prone to skin cancer. Then they know that the gene they "knocked out" is linked to that defect. At its best, one of these mice can tell scientists exactly what the gene is doing in the living mouse, and put them on the trail of which other genes it interacts with and when during the animal's life span it is important. Even cells from the mice can be useful: scientists can isolate them and test how they function and respond to different stimuli.

When the scientists lose, all they have is a regular mouse that couldn't care less that it's missing some of its DNA. Or, maybe, a mouse that has so many problems that there is no clear link between the gene and any particular function. Often, the developing embryo dies before it is even born, long before the researchers can glean any useful information from it.

When Holger Kissel and colleagues in **Hermann Steller's** laboratory went to create a knockout mouse, they placed all their chips on the *septin4* gene that they thought might be linked to leukemia. On one side, earlier experiments that made mice lacking the *septin5* gene had yielded a mouse with no detectable defects, when their *septin5* knockout mouse had been perfectly normal, Kissel and colleagues thought the *septin4* gene might be different.

"The *septin4* gene locus is unique from the other *septin* genes because it also encodes the protein ARTS, in addition to the Septin 4 protein," says Kissel, the lead author on the paper, published in the March issue of *Developmental Cell*. "ARTS has been suggested to play a role in children with acute lymphoblastic leukemia. This was our incentive to generate the *septin4* knockout mouse."

But, as is sometimes the case when you gamble, occasionally you win more than you expect. Although Kissel's *septin4* knockout mice did develop leukemia and other tumors, there was clearly something else wrong with them.

"One of the first things we realized right away was that male mice without the *septin4* gene were sterile," says Steller, head of the Strang Laboratory of Apoptosis and Cancer Biology. "When we looked closer, we saw that the sperm from these mice have problems similar to many defects seen in human sperm that contribute to infertility."

While the mutant mice produce the same volume and number of sperm as normal mice, the sperm from the mutant mice are unable to fertilize eggs. The sperm has several visible defects, including severely bent tails and large droplets of excess cellular material — cytoplasm — near the head. They actually look very similar to defective human sperm known as "droplet sperm."

In humans, as in mice, the majority of sperm that even fertile males produce appear to be abnormal in this way. The bulging droplet affects how the sperm swim, contributing to bent tails and making it unlikely that the sperm will ever be able to reach an egg. For 40 percent of the estimated six million American couples battling infertility, the problem lies with the man, and in many of those cases, too many droplet sperm are to blame.

"The sperm defects we are seeing in the mutant mice are probably an enhanced phenomenon that occurs in normal, healthy mice as well," Kissel says.

The sperm from mutant mice also reminded the scientists of another type of mutant sperm, not from

mice or humans, but from flies. In 2003 another postdoc in Steller's lab, Eli Arama, published a paper showing how caspases, enzymes that push a cell down the road to cell death, are essential for fly sperm to form properly. When the pathway was disrupted, the fly also

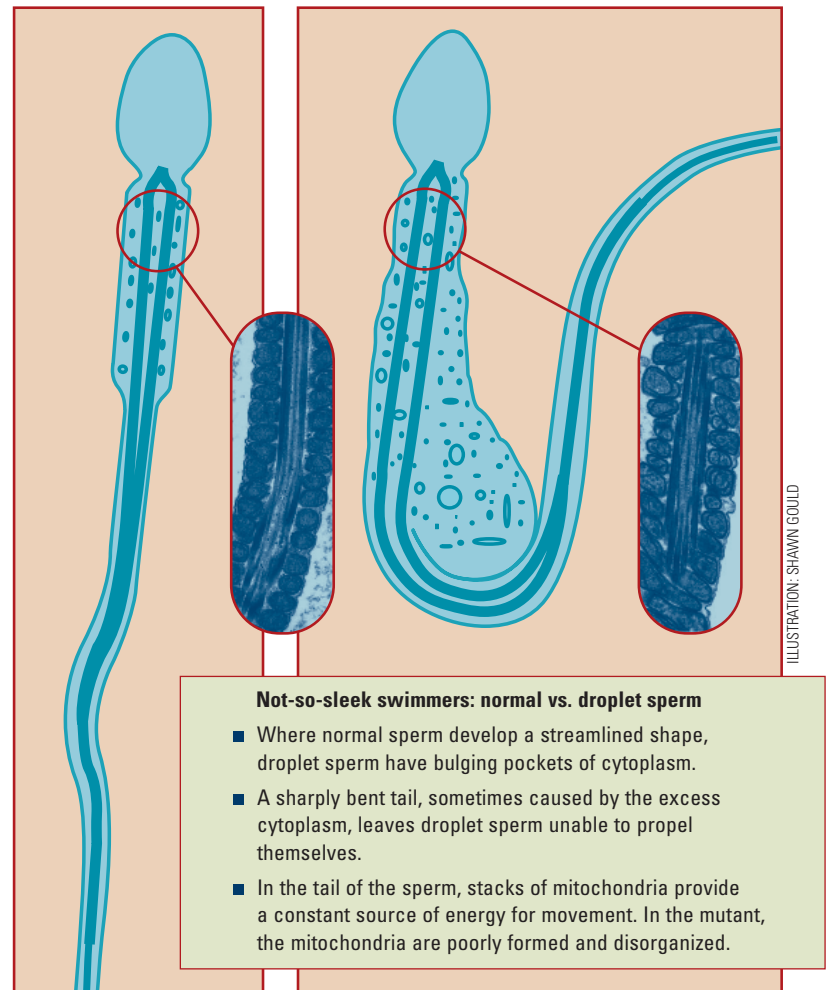


ILLUSTRATION: SHAWN GOULD

produced droplet sperm.

"Eli Arama had shown caspases were needed in flies to remove the majority of cytoplasm and organelles to make streamlined mature sperm," says Steller. "We also knew that in mice the ARTS protein helps regulate caspase activity. So the obvious question to ask was if the same death pathway was working in mice as it was in flies."

As it turns out, both the ARTS protein and the Septin 4 protein are needed for proper sperm development. While the ARTS protein controls the death pathway and removal of extra cytoplasm, the Septin 4 protein, part of a family of proteins first discovered in yeast, is important for making large cytoplasmic scaffolds, similar to construction scaffolding, in cells where proteins can assemble. The scaffolds provide a framework that helps to compartmentalize different proteins in the cell.

"The Septin 4 protein is normally found in a structure called the annulus in the sperm," says Steller. "The annulus was predicted to be a way to make different compartments in the sperm, and the mutant mice completely lack an annulus. If you normally have to keep all of the proteins in the right compartment, messing that up leads to a number of problems with the sperm, including bent tails and an inability to swim."

Research on the roles of both the ARTS and Septin 4 proteins in sperm maturation may eventually help to improve the fertility and quality of sperm in infertile men. Also, these proteins may prove useful in the development of male contraceptive drugs, because while the sperm are still produced in the mutant mice, they are unable to fertilize an egg.

"There are not many mutations that cause complete sterility without affecting the anatomy of the testis, as with our mouse," says Kissel. "The Septin 4 and ARTS proteins are great targets because interfering with them could accomplish inhibition of fertility without any negative side effects."

"This is a really great starting point," says Steller. "We were originally interested in the cell death aspects of these proteins, but the mouse has opened up many other nice opportunities—including insights into human sterility—that we are currently pursuing."

In roulette terms, that's what you call a big win.

Making eyes

For a fly eye to develop, a protein called spalt must turn on, then off, then on again

BY KRISTINE KELLY

Timing, as the saying goes, is everything.

By manipulating the timing of gene expression, Rockefeller researchers led by assistant professor Bertrand Mollereau, in the Strang Laboratory of Cancer Research, were able to change the development and function of cells in the fly eye — in one instance even causing the flies to become colorblind.

Like the common housefly, fruit flies have compound eyes, each of which contains about 800 light-sensitive clusters known as ommatidia. Each ommatidial cluster has eight photoreceptor cells, designated R1 through R8. The first six photoreceptors form an outer circle, and they detect motion and enable the flies to see in dim light. The inner two, R7 and R8, are required for color vision.

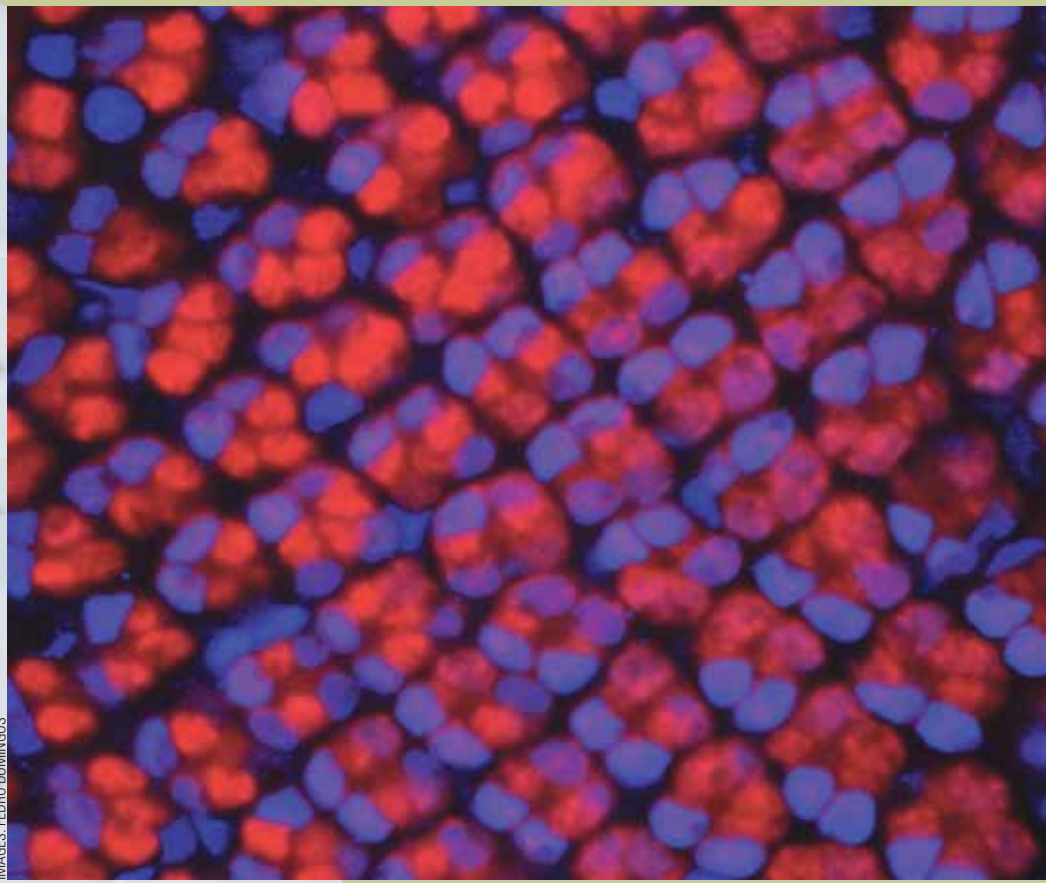
In previous research completed while he was a postdoctoral fellow at New York University, Mollereau discovered a protein that regulates gene expression — the transcription factor spalt — that controls the fly's ability to see color. By breeding flies that lacked spalt, the mutant flies developed eyes missing the R7 and R8 cells, rendering the flies colorblind. But two studies, done at Rockefeller, show that for the ommatidia to develop correctly, spalt must be present in several of the photoreceptor cells.

"Before spalt is used to establish the color vision cells R7 and R8, it is first needed for the proper development of two other photoreceptor cells — R3 and R4," says Pedro Domingos, a postdoc in Hermann Steller's lab who worked on the research. "However, it needs to be expressed at the right time in each of the cells; the spalt gene is actually turned on, and then turned off, several times during development.

"What this research shows is that depending on the timing, the same signal can be used over and over again to generate different cell specializations within the fly eye," says Domingos.

Beyond detecting motion, the R3 and R4 cells also help arrange the ommatidia into their final design: a spoked, circular pattern similar to a daisy flower. "The cells of the ommatidia need to rotate into position during development," Mollereau says. "The direction they rotate in is controlled by the R3 and R4 cells and is very specific." If the spalt protein isn't made, the ommatidia have no organization.

Normally, expression of spalt is fading in the R3 and R4 cells just as it is beginning to increase in the R7 color vision cells. Surprisingly, when the team artificially maintained spalt expression longer than normal during R3 and R4 development they were transformed into color vision cells. The scientists were able to change the fate and function of



IMAGES: PEDRO DOMINGOS

Compound interest. The developing eye of a fruit fly is composed of repeating units called ommatidia, each made of eight cells. The *spalt* gene (stained blue) in two of these cells helps direct the final organization of each ommatidium.

the cells just by controlling the timing of spalt gene expression. For R3 and R4 cells to form, the scientists realized that another protein must be capable of turning spalt off.

Mollereau and colleagues found that the protein responsible for shutting off spalt is called seven-up, a protein that has not been previously linked to spalt. "In the R3 cell, spalt actually induces seven-up expression after a while. Then seven-up represses spalt to keep the cells from turning into an R7," Mollereau says. "It's like a dog biting his own tail."

Mollereau's research, reported in the September 2004 issue of *Developmental Biology* and the November 2004 issue of *Development*, may eventually help scientists understand the mechanisms behind retinal degeneration and other sight disorders. More immediately, it may have implications for Townes-Brocks' syndrome, which has already been linked to spalt and results in hearing loss and limb deformities.

Hepatitis in a test tube *continued*

refusal to replicate outside of the human body. It's something that Rice's fellow virologists studying other viruses — those that work on HIV, for example — take for granted. To study the virus's entire life cycle, they can simply grow a sample in a culture of cells and watch through a microscope as the process unfolds. When you try to do that with hepatitis C, the virus withers and dies.

"The inability to reproduce aspects of the hepatitis C virus life cycle in cell culture has slowed research progress on this important human pathogen," says Rice, who is the Maurice R. and Corinne P. Greenberg Professor.

Earlier this summer, in a breakthrough that could hasten the development of badly needed drugs to fight hepatitis C around the world, Rice and colleagues from the Massachusetts Institute of Technology and Scripps Research Institute developed a method for producing an infectious form of the virus that can thrive in laboratory cultures.

Like all viruses, the hepatitis C virus cannot replicate by itself; instead it takes over the machinery of a host cell to make copies of itself. "The hallmark of viruses is their ability to exist in a form outside the host cell capable of infecting new cells," says Brett Lindenbach, a postdoctoral fellow in Rice's lab and first author of a *Science* paper in which the finding was reported. "Our method replicates and produces virus particles that can infect new cells, initiating replication in them and leading to the production of more virus particles."

Although little is known about the hepatitis C life cycle, researchers think that in humans the virus enters a liver cell and delivers its RNA and proteins into the cell cytoplasm. The hepatitis C virus carries its genetic information in its RNA, which is separated from the protein, copied, and then joined with new

protein components before being released from the liver cell to infect other cells.

Lindenbach, Rice and their colleagues named their infectious cell culture virus HCVcc. Already HCVcc is yielding new knowledge about the hepatitis C virus. In a separate set of experiments, the researchers used HCVcc to confirm that a molecule called CD81, which sits on the surface of the human cell membrane, plays a crucial role in the entry of the hepatitis C virus.

Scientists have known that a protein produced by the virus, called E2, binds to CD81, and they believed that this interaction is necessary for the virus to bind to target cells.

The Rockefeller researchers showed that CD81 molecules that are not attached to the surface of host cells compete with membrane-bound CD81 and inhibit entry of the virus into the cell. They also showed that HepG2 cells, which do not express CD81 but can support HCV RNA replication, could not be infected by HCVcc unless they were engineered to express CD81.

Liver failure due to hepatitis C is the leading cause of liver transplants in the United States, and about 25 percent of liver cancer cases in the country are associated with hepatitis C. Although about 85 percent of those who are infected develop chronic infection, the virus usually remains undetected for years, or even decades, until it causes advanced liver disease.

"This system lays the foundation for future test tube studies of the virus life cycle and may help in the development of new drugs for combating HCV," says Rice, who is the scientific director of the Center for the Study of Hepatitis C, a collaborative research and clinical effort of Rockefeller, Weill Medical College of Cornell University, and New York–Presbyterian Hospital.

A bacterium's sugar-free diet *continued*

that *M. tuberculosis* isolated from the lungs of chronically infected animals vigorously metabolized fatty acids, but ignored glucose — the opposite of the bug's behavior if it is cultivated on standard media in a Petri dish. Puzzled that no one had ever repeated the experiments, and intrigued by the possibility that *M. tuberculosis* defied conventional thinking about microbial metabolism during infection, McKinney decided to try a modern version of the experiment.

In 1999, Muñoz-Elías signed on as an ambitious new graduate student in McKinney's laboratory. "It was good timing," says Muñoz-Elías. "John had already identified a pathway involved in fatty acid metabolism in *M. tuberculosis*, called the glyoxylate cycle, and the complete genome sequence had just been published. This made it easier for us to identify additional genes that were likely to be involved in fatty acid metabolism."

In 2000, McKinney and Muñoz-Elías reported in *Nature* that the persistent form of *M. tuberculosis* infection required an enzyme of the glyoxylate cycle called isocitrate lyase 1 (ICL1), which is essential for the bacterium's ability to feed on fatty acids. A mutant strain of *M. tuberculosis* that lacked ICL1 grew normally in the lungs of mice during the early stages of infection, but failed to persist once the immune system rallied to fight the infection. These observations were intriguing because conventional tuberculosis drugs target processes involved in bacterial growth during acute infection, and are less effective against persistent bacteria. News of the bacterium's fatty acid requirements during persistence piqued the interest of the pharmaceutical company Glaxo-SmithKline. Using assays developed by McKinney, Muñoz-Elías, and colleagues, GlaxoSmith Kline scientists initiated high-throughput screens to identify ICL1 inhibitors as potential leads for drug development.

Now, pharmaceutical companies have even more reason to be interested. In their latest report, Muñoz-Elías and McKinney

take their earlier observations one step farther, by demonstrating that a second isocitrate lyase enzyme, ICL2, is jointly required, with ICL1, for *M. tuberculosis* growth and survival in vivo. Bacteria lacking both ICL1 and ICL2 were unable to establish infection in mice and were eliminated from the tissues almost immediately. Other metabolic pathways, including pathways for utilization of sugars such as glucose, were intact in the mutant strains, but failed to compensate for the loss of the ability to metabolize fatty acids. The new findings suggest that a dual-specific drug targeting ICL1 and ICL2 might kill the replicating bacteria responsible for acute disease as well as the non-dividing bacteria responsible for persistent infection. The new findings prompted GlaxoSmithKline to modify their screening strategy in order to identify compounds capable of inhibiting both ICL1 and ICL2.

"Making the ICL1/ICL2 double mutant gave us the answer as to what *M. tuberculosis* feeds on during active infection," says Muñoz-Elías. "Knowing this is important because people with TB don't get sick and die over a week or two; they get sick and die over months, years, or decades. As biomedical scientists, we will help doctors treat the disease more effectively if we understand how to attack TB when it is both active and latent," he says.

Meanwhile, other research groups have identified pathogens that also rely on fatty acid metabolism to survive in their hosts. "Following our 2000 report on the role of the glyoxylate cycle in TB pathogenesis, a remarkable number of bacterial and fungal pathogens, of both animals and plants, have been shown to depend on this pathway during infection — but the pathway is absent in humans and other higher eukaryotes, which is an attractive feature for drug development," says McKinney. "Studying alternate forms of microbial metabolism during infection as a strategy for developing new antimicrobials is turning out to be broadly important."