

## Defying death

A dying cell's legacy may be to stimulate new growth

BY RENEE TWOMBLY

Hermann Steller is fascinated by death. Death of cells, that is. He has dedicated his research to understanding cell suicide, has dubbed proteins that he has discovered Grim and Reaper, and speaks passionately about “undead” cells that should die, but fail to follow through with their own demise.

Now the Rockefeller cancer biologist has found that from the dying can come new life — not a transmigration of cellular souls, but a mechanism of cell generation that is completely novel and has important implications for cancer research and treatment.

In the October issue of *Developmental Cell*, Steller, Hyung Don Ryoo, a postdoctoral fellow in his lab, and senior technician Travis Gorenc report that as cells commit suicide in response to stress or injury, such as DNA damage, they emit signals that result in the growth of new cells.

In other words, cell death may not be as straightforward a matter — of weeding out the weak and ill — as scientists thought. Rather, Steller and his lab colleagues demonstrated that in their fruit fly model of cell death, a damaged cell's reaction to stress or injury is to trigger new growth nearby, ostensibly — and sensibly — to replace the dead cell.

Human cells seem to undergo the same process. “What we show is that the dying cells themselves make a signal, a mitogen that can induce division and proliferation in other cells,” says Steller, who is head of Rockefeller's Strang Laboratory for Apoptosis and Cancer Biology and a Howard Hughes Medical Institute investigator. “Anthropomorphically speaking, the cell is saying to its neighbors that ‘I am injured, I am going to die soon, and you have to do something to replace me.’”

While it has been known that in injured tissues cells can fill the gap created by those that have died, a process known as compensatory proliferation, “it has always been thought to be passive, that what was detected is the absence of cells which needed to be filled in,” he says. “What we have found is very different. We showed that it is the dying cells themselves that make a signal that pushes cells nearby to grow.”

Steller and Ryoo built upon their previous research showing how a fruit fly protein called Reaper triggers programmed cell death, or apoptosis. Reaper instructs the principal protein guarding against cell suicide to self-destruct. Once this protein, known as *Drosophila* IAP1, or DIAP1, is gone, proteins known as caspases are freed up; they are the key executioners of a cell.

Steller and his team knew that Reaper could remove DIAP1 in more than one way. So they next investigated whether it was important to apoptosis that DIAP1 destroyed itself. Ryoo looked at the biological role of this protein's degradation by using a mutant DIAP1 in *Drosophila* larvae that could no longer self-destruct as it should have. The researchers found that

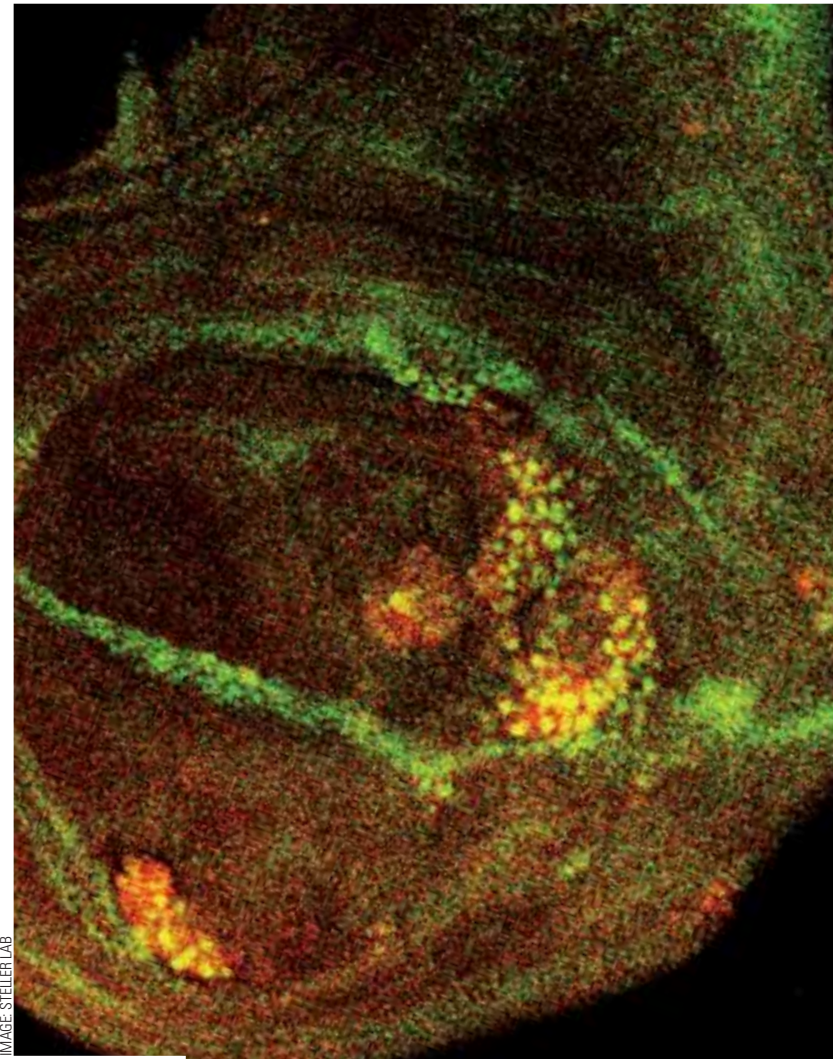


IMAGE: STELLER LAB

**Dying wishes.** Yellow fluorescence in the nucleus of this “undead” fruit fly cell indicates activity of the “wg” protein, part of the signaling pathway that triggers new cell growth.

cells with the mutant protein die. So Ryoo kept the cells alive by using proteins that inhibit caspases from destroying the cell.

“The idea was to prevent the death of cells that do not have DIAP1 and keep them alive — keep them undead — and see what happens,” says Steller. “But

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## Neurons on the move

How a structure called the centrosome leads a nerve cell's two-step migration in the brain

BY BETSY HANSON

“One of the most wonderful moments as a scientist comes when you feel your mindset shift,” says Rockefeller's Mary Beth Hatten. “In our case, we realized that the paradigm we used to design experiments was not going to yield the answer about what controls how brain cells migrate during development.”

Hatten studies how early nerve cells in the brains of developing mammalian embryos move outward from the central area of the brain where they are generated. “Scientists have spent the last 15 years focusing on specific adhesion systems as the most important aspect of cell migration,” she says, referring to the way nerve cells stick to, and later detach from cells called glia that guide them to their destinations in the developing brain.

As one aspect of the studies on surface receptors, Hatten and colleagues examined the cytoskeleton — the scaffold of elements called microtubules that support a cell's three-dimensional shape — of migrating neurons. That work showed a unique bingo-cage like structure around the nucleus that appeared to hold the nucle-

us as the cell moved. The importance of the cytoskeleton to brain development gained even more prominence as several genes that cause severe developmental defects in humans turned out to code for proteins that attach to the cytoskeleton.

Now Hatten, who is the university's Frederick P. Rose Professor and head of the Laboratory of Developmental Neurobiology, shows that it is not the relationship of the cytoskeleton to adhesion molecules, but a novel signaling protein within the cytoskeletal system that is critical to the pace of the cell's migration.

The new study, published in the November issue of *Nature Neuroscience*, shows that a surprising mechanism underlies the migration of nerve cells in the developing embryo. The Rockefeller researchers report that the cells move in a two-part “step” led by a structure within the cell called the centrosome. Once the centrosome, the key organizing point for the cell's internal skeleton, moves forward, the nucleus follows. The

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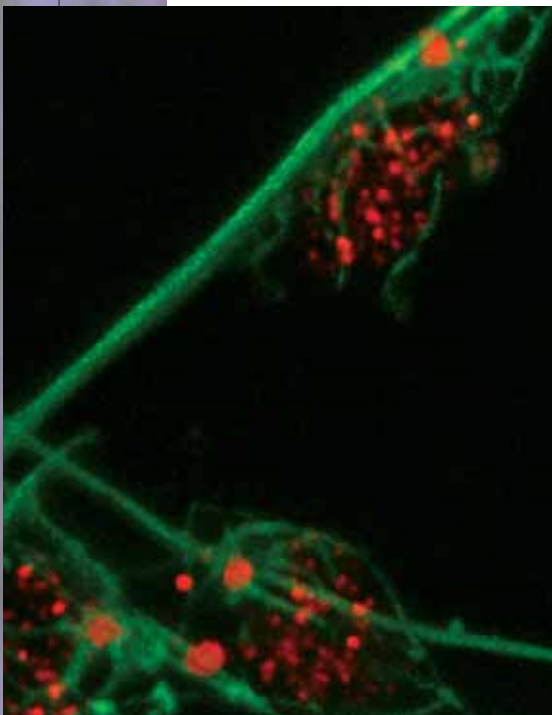


IMAGE: HATTEN LAB

**Line dance.** Developing mouse neurons migrate along glial nerve fibers (green) in a two-step pattern. For a movie of this process, go to [www.rockefeller.edu/scientist](http://www.rockefeller.edu/scientist).

### INSIDE

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**Balancing brain chemistry.** Scientists in **Paul Greengard's** laboratory have discovered a protein that plays a central role in integrating several major molecular signaling pathways in the brain's striatum, a region that controls movement and balance. Disruption of these pathways is associated with several diseases of the nervous system, including Huntington's and Parkinson's. The new protein, called regulator of calcium signaling (RCS), binds to another protein known as calmodulin, thereby inhibiting its activity. Calmodulin mediates the actions of calcium within cells, which are essential for cell signaling. The researchers found that, through its interaction with calmodulin, RCS mediates the balance between the neurotransmitters acetylcholine and dopamine. The finding opens the door to new therapies. Greengard is the university's Vincent Astor Professor and head of the Laboratory of Molecular and Cellular Neuroscience.

*Science*, October 23, 2004

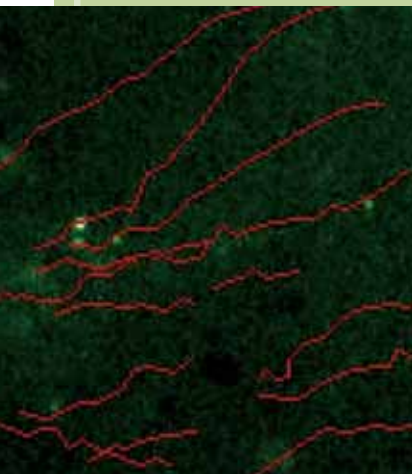


IMAGE: HEINTZ LAB / RU BIO-IMAGING RC

**Creating current.** Red paths trace the movements of particles as they flow through a mouse brain ventricle.

North, and German colleagues, explains the connection. In experiments with mice, they showed for the first time that, as in the respiratory system, there are cilia-studded cells that line the walls of fluid-filled cavities in the brain called ventricles and push fluid along in the proper direction. A mutation in the mouse gene *Mdnah5*, analogous to the human counterpart, cripples cilia in these brain cells as well as in respiratory cells. As a result, these mice develop hydrocephalus. Heintz is head of the Laboratory of Molecular Biology.

*Human Molecular Genetics*, September 15, 2004

**Chromosomal end game.**

Mammalian chromosomes end in long stretches of repeated DNA sequences known as telomeres. These telomeres, in turn, terminate in loops that protect the DNA from damage. Now scientists in **Titia de Lange's** laboratory have found that telomeres can be shortened dramatically and quickly by losing loop-sized stretches of DNA in a process called homologous recombination. The culprit is a mutant form of the TRF2 protein. In its normal form, TRF2 protects telomere loops, but a mutant called TRF2<sup>ΔB</sup>, in combination with other proteins, induces the large DNA deletions. The researchers propose that losing loops may be a normal part of the way cells regulate telomere length, one that could be harnessed in the treatment of certain cancers. de Lange is Leon Hess Professor and head of the Laboratory of Cell Biology and Genetics.

*Cell*, October 29, 2004

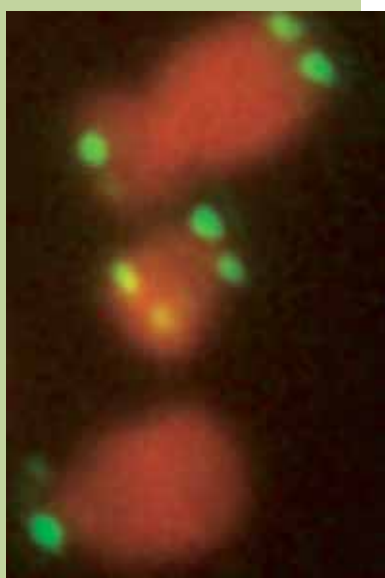


IMAGE: DE LANGE LAB

**Lost loops.** In cells with mutant TRF2 protein, DNA is deleted from telomeres (green) in random and unequal amounts. Some telomeres appear unaffected while others are missing completely.

**Hatten** *continued*

Rockefeller scientists produced time-lapse movies that show nerve cell migration in unprecedented clarity and detail.

"It's almost like a little inchworm in the way it moves," says Hatten, whose time-lapse movies first revealed this motion in 1987. With each step the cells travel a little more than a micron, or about half the width of a hair over the course of an hour. Translated to a human scale, they would travel as far as the distance between New York and Chicago in order to find their place in the right layer of

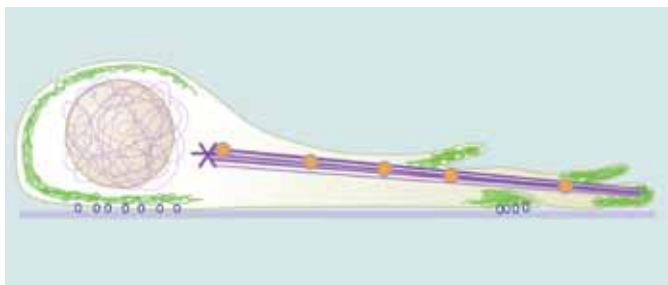


ILLUSTRATION: HATTEN LAB

**Stepping out.** As a young neuron moves along the glial fiber, it forms attachments (blue circles). But the key elements for migration are the cage of tubulin that surrounds the nucleus and Par6 protein which is in the centrosome (purple star). The nucleus stays in the cell body, which steps along the glial fiber every three minutes.

the developing network of the brain.

New microscopic techniques allowed the researchers to probe the internal dynamics of migrating nerve cells in real time in living cells. The scientists knew that disrupting the cytoskeleton with chemicals prevents the cell from moving and that problems with the cytoskeleton are implicated in human disorders in which nerve cells fail to migrate normally. But no one had previously watched the cytoskeleton in living neurons in real time. Also they knew that the nucleus is surrounded by a special cytoskeletal "cage," but they did not know the function of these elements.

The researchers took a new type of fluorescent dye called Venus, which glows 20 times brighter than other dyes, and tagged the microtubules of a type of mouse neuron called a granule cell. Rockefeller's Tarun Kapoor, an expert on the centrosome and on techniques for visualizing microtubules, collaborated with Hatten and her lab colleagues to create images showing these structures in extraordinary detail. Kapoor is head of Rockefeller's Laboratory of Chemistry and Cell Biology.

When the scientists looked at the cells with a spinning disk confocal microscope in the university's Bio-Imaging Resource Center, the dye revealed a cage-like structure around the cell's nucleus, proving the existence of this structure, whose role in cell migration has been debated by many scientists. The Rockefeller researchers settled the debate by showing that while the cage around the nucleus is essential for the cell to migrate because it holds the nucleus in place, it does not actually control cell movement.

Next the researchers looked at the protein Par6-alpha, referred to as mPar6-alpha in mouse cells. Par6 is one a group of proteins that signal cell polarity in the worm *C. elegans*. Recent studies in other laboratories located a mammalian counterpart of the worm gene, and led the scientists to theorize that mPar6-alpha might orient the young neuron on the glial wire during migration. Also, screens for genes that were enriched in migrating cells by a method called gene chips had indicated a high level of Par6 in migrating granule neurons. As a result, Hatten and her coworkers suspected that mPar6-alpha was active during migration.

Again, the researchers used Venus, this time to label mPar6-alpha protein. Its bright green glow concentrated in the centrosome, the organelle located just in front of the nucleus in migrating cells. "The centrosome has a large number of proteins that make up its structure. Most other known proteins in the centrosome are structural," says Hatten. "mPar6 is the one of the first signaling proteins found there."

But the brightly labeled centrosome led the scientists to an even more important discovery. Watching the cells with labeled mPar6-alpha as they moved along the glial fibers, the researchers could see a two-step process to every advance the cells made. First the centrosome slid forward, and then three minutes later the nucleus followed.

"The timing of the advance of the centrosome and then of the nucleus was exactly the same as the timing we measured 15 years ago that it takes for the nerve cell to adhere, let go and take a new step along the glial monorail," says Hatten. Additional experiments that tagged a different component of the centrosome confirmed these results.

To further investigate the function of mPar6-alpha, the researchers created cells with either too much or too little of the protein. In both cases the cells sat motionless on their glial fibers.

"In response to mPar6-alpha, the centrosome is playing a signaling role in setting the cadence for migration of the cell," says Hatten.

**Addiction** —

**Rockefeller's Mary Jeanne Kreek research into how illicit o**

BY CATHY YARBROUGH

It was a daring new research study that brought Mary Jeanne Kreek to Rockefeller University 40 years ago. During her medical training at New York Hospital-Cornell Medical Center, Kreek joined a project that challenged the then common notion that people addicted to heroin were immoral or behavioral deviants.

Along with Vincent P. Dole, who directed the landmark study, and Marie Nyswander, a psychiatrist who joined Dole at the same time, Kreek suspected that addicts may be victims of their biology, not their personalities. Their bold research pioneered the use of methadone in the chronic, or long-term, treatment of heroin addiction and established its medical safety and effectiveness for treatment to relieve a heroin addict's hunger for the drug — a major reason for relapse. They proved that methadone could prevent heroin from exerting its powerful effects on the brain, and years later at Rockefeller, Kreek led the research team that identified multiple genetic variants for the site of action on brain cells where heroin exerts its influence. Among the variants that she identified was a functional one with distinctly different activity from the normal gene.

When she joined the university in 1964, Kreek was participating in the first research elective that Rockefeller ever offered to Cornell medical residents. Today, Kreek, the university's Patrick E. and Beatrice M. Haggerty Professor, heads the Laboratory of the Biology of Addictive Diseases. To mark the 40th anniversary of the inception of the university's landmark addiction research, *Rockefeller University Scientist* spoke with Kreek.

**Is research on addiction taken more seriously today than it has been in the past?**

Yes, most scientists, particularly neuroscientists, do not doubt that addiction is a disease, and it's a disease of the brain. The research evidence comes from molecular biology, neurochemistry and genetics, as well as basic clinical and treatment research. This research has shown that alcohol, cocaine, heroin and other drugs of abuse can change the human brain in predictable ways, and that these changes contribute to the behaviors that we identify as addictions.

What's enormously exciting to scientists such as myself is how much we are learning today about the neurobiologic basis of how each of the specific addictions develops. This research will suggest effective approaches to early intervention and prevention as well as treatment.

**Treating alcoholics, one at a time**

The latest study from the Kreek lab suggests that new addiction treatments can be customized to individuals

BY JOSEPH BONNER



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# - 40 years later

## Mary Jeanne Kreek looks back on four decades of groundbreaking research on drug use impacts — and alters — the human body

### **You speak of addictions, plural. So there's not just one addiction?**

Correct. Research has revealed many similarities — but also profound differences — in the ways that the various drugs of abuse act in the brain. For instance, heroin acts only at one site in the brain, the mu opioid receptor of brain cells. In sharp contrast, cocaine acts at three similar but different sites.

### **Does that mean that cocaine addiction is more complex than heroin addiction?**

That's actually a provocative way of putting it. It's not really a more complex addiction, but the multiple sites of cocaine's action in the brain complicate the search for a single pharmacological treatment that will be as effective against cocaine as methadone has been for heroin addiction. In fact, the lack of a single treatment that is effective against each form of addiction is really profound, direct circumstantial evidence that these drugs of abuse cause different forms of addiction, at the level of brain cells.

### **When you worked on the methadone studies in the 1960s, no one could know where methadone was acting in the brain?**

No, we could not be at all sure of where it was acting. However, our laboratory hypothesized the existence on cells of specific binding sites, or "receptors," for heroin and its major metabolite, morphine. Further, we hypothesized that methadone would act at those same sites, and that its presumed and later proven long-acting qualities in humans would allow it to normalize the functions disrupted by the short-acting heroin. However, it was not until 1973 that three different research groups identified the specific cell receptors for opiates, which include heroin. We later found that there are three different kinds of opiate receptors and in the early 1990s the genes for these receptors were cloned. In 1994, our collaborator, Lei Yu, first cloned the mu opioid receptor gene, the site of binding of heroin and also methadone. We then identified five genetic variants, each containing the DNA code for the mu opioid receptors, the major molecular site for the action of heroin, and also methadone, in the brain. Two of these mu opioid receptors occur frequently — 10.5 percent and 6.5 percent — in the human population. Two years ago, my lab published a paper showing that some people who inherit a "high output" version of another opioid gene that makes the brain opioid peptide dynorphin, a neurotransmitter that is part of the brain's pleasure circuit, may be less likely to fall prey to the addictive powers of cocaine. So that's good news that we're pursuing in ongoing research.

### **Are people genetically prone to addictions?**

There's a genetic component to each of these addictions. The genetic factors contribute 30 percent to over 50 percent of the relative risk to develop addictions. A colleague at another institution found that heroin has the largest relative risk on a genetic basis — 54 percent — and probably has more unique genes contributing to this risk.

### **Do your genes excuse you from having an addiction?**

No. But they contribute to it. That's why it's inaccurate for anyone to argue that people with addictions to alcohol,

cocaine, heroin or other drugs of abuse have "brought it on themselves." Hypertension, diabetes, obesity and addiction are among the many diseases that are due to combinations of internal and external environmental factors as well as genes.

### **How powerful are genetics?**

Epidemiological studies of drug use and addiction over the last 50 years in the U.S. and Europe have found that about one in three to one in five people who ever self-expose — willingly inject or ingest heroin — will develop an addiction to it. One in 10 to one in 20 people who self-expose to alcohol become alcoholics. With cocaine, about one in 10 who self-expose to this drug will become addicted to it. These numbers are true in the United States, China and Europe. No country is immune to drug addictions.

### **Your studies revealed for the first time that people who were once addicted to drugs of abuse do not respond well to "stressors" — and those could be, I suppose anything from a traffic jam to being fired?**

At the level of their brains' neuroendocrine systems, both active drug-using addicts and former addicts who are no longer using drugs of abuse were found by my lab to respond atypically to stress. Their abnormal response, likely a product of the interplay of their genes and their environment experiences, may help explain why people become addicted in the first place, as well as why they are prone to relapse. The mu opioid receptor, which is the target of heroin, plays a major role in our body's stress response system, especially in the hypothalamic-pituitary-adrenal axis.

### **And, your studies related to HIV and AIDS?**

In 1984, my lab contributed to a crucial paper related to the then new disease that we now know as AIDS. In studies of blood samples from anonymous volunteer research subjects with addiction coming in for neurobiological or treatment research studies in my addiction research service at the Rockefeller University Hospital, we found evidence of the HIV antibody in 60 percent of untreated street heroin addicts. But importantly, only nine percent of former addicts who had entered an effective methadone program prior to 1978, when the AIDS epidemic hit New York City, and who had remained in treatment through the time of our study in 1985, had the antibody.

### **Why do you use "bidirectional" to describe your addiction research?**

We perform research both at the laboratory bench and at the Rockefeller University Hospital with volunteer subjects, including both active addicts and former addicts in effective treatment, as well as healthy control subjects. Our work at each influences the other. The addiction research in the 1960s began with studies of patients in the hospital, but that work inspired many different basic research studies at the bench, in the lab. What we've learned in the lab has led to basic neurobiological studies in humans. In parallel, observations made in humans with addictive diseases have led to our development of novel animal models, which allow us to conduct more meaningful studies of the neurobiology, neurochemistry and behavior related to addictions in rats and mice.

For instance, the "binge" animal model that we developed to study cocaine addiction in rodents in the lab was based on

in Kreek's lab, obtained DNA samples from 539 people living in central Sweden: 389 were dependent on alcohol, the remainder were healthy volunteers. The researchers chose central Sweden because this population has had a limited influx of non-Swedish immigrants and therefore has remained genetically stable for the last several hundred years.

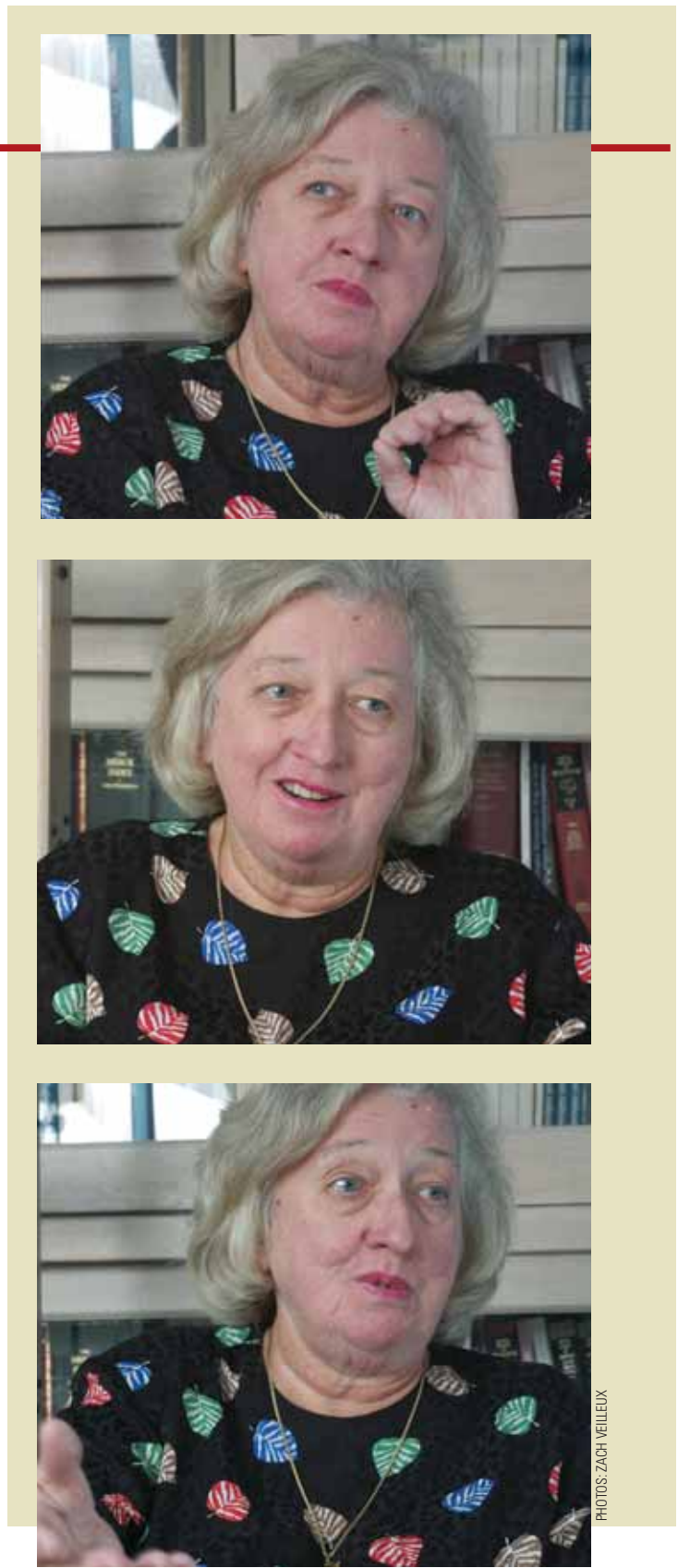
In collaboration with Jürg Ott, head of the Laboratory of Statistical Genetics, the scientists embarked on a molecular and statistical analysis of the DNA samples from the alcohol-dependent and control groups to calculate the attributable risk of A118G in developing alcoholism. Attributable risk estimates the extent to which a disease can be explained by having a specific SNP, in this case A118G.

What Ott found was surprising: the attributable risk for A118G in this population was 11 percent. A similar study by the Kreek and Ott labs of heroin addicts in central Sweden published last summer in *Molecular Psychiatry* showed that the attributable risk with the A118G variant for developing heroin addiction was 21 percent for people with two Swedish parents and 18 percent overall.

Mary Jeanne Kreek has a hypothesis about alcohol addiction: alcohol abusers are forever chasing the "good feeling" that non-alcoholics get from drinking a single drink. Studies have shown that the alcohol-dependence treatment naltrexone may mimic this good feeling by activating part of the stress responsive system, and thus reduce the craving for that second and third drink that alcoholics seek. Now, new research may explain why some alcohol abusers do better with naltrexone than others.

The key lies in a gene variant, or single nucleotide polymorphism (SNP), of the much studied mu opioid receptor gene, called A118G. In 1998, Kreek's lab identified A118G, as well as four other SNPs of the coding region of the mu opioid receptor gene. Each SNP derives from a single difference in how the building blocks of DNA pair with one another. Some of these differences may change the gene's protein products in ways that may confer susceptibility — or protection — against diseases.

In the new study, published in *Neuropsychopharmacology*, Kreek and first author Gavin Bart, director of clinical research



PHOTOS: ZACH VELLEUX

our observations of people's patterns of use of this drug. People also "binge" on alcohol, so our lab uses the "binge" model in rodent studies of alcoholism. People do not "binge" on heroin — it's intermittently used, so our animal model studies mimic that pattern. For cocaine and alcohol, the "binge" model has been a more reliable way of learning how drugs can act as DNA switches turning genes on or off. In lab animals, for instance, "binging" on cocaine reduces the binding at the cell receptors for dopamine, one of the neurotransmitters that cocaine increases in the brain and that is part of the brain's pleasure circuit. "Binging" on cocaine also increases gene expression and the numbers of mu opioid receptors, but with no increase in the neurotransmitter, beta-endorphin, which is commonly associated with the "runners' high" as well as pain relief. Other opioid gene products, the dynorphin peptides, reduce the levels of dopamine. If those changes persist for a long time, which we have shown does happen, it could lay the basis for relapse as an addict tries to compensate for a crippled pleasure circuit.

"Together, these two findings may indicate that the A118G variant contributes to a common susceptibility for developing two different addictive diseases," says Kreek. "This makes sense because all addictions probably share some genetic load, but also have unique variants."

This human genetic research has implications for developing new, customized treatments that target these unique variants. "We know that if a person drinks a shot, the levels of certain stress hormones in the HPA axis — the body's fight or flight center — increase," says Kreek. "In chronic alcoholics, this increase is lost. We hypothesize that alcoholics are chasing that increase in stress hormones, so they keep drinking in an attempt to get it."

Kreek cites a study she performed in collaboration with Stephanie O'Malley at Yale University that shows naltrexone, which can reduce the urge to drink alcohol in some patients, mimics alcohol's activation of the HPA axis. Kreek and Bart believe this effect may explain why patients with the A118G SNP may respond better to naltrexone treatments than people without this SNP.

# Biology, the aerial view

To understand the processes of life we must get past the school buses, says Sandy Simon

BY JOSEPH BONNER

Proteins in our bodies' cells have always been believed to zip around the cytoplasm, wriggling in and out of the nucleus and escaping through the cell membrane as they perform the array of tasks that keep us alive and well. Recently scientists have visualized these dances in the cell by fusing a cellular protein to a fluorescent tag from jellyfish. This has led to many insights into the cell, but comes with many cautionary tales.

"Our eyes catch the brightest star in the heavens — the signals from a cell that shine strongest," says Sandy Simon, professor and head of the Laboratory of Cellular Biophysics at Rockefeller. It is a classic example of what microscopists call the "yellow school bus" problem.

"Let's say you're a spy and you come to America to figure out what is the function of the yellow school bus," says Simon. "I give you a spy plane, and you fly over the country taking pictures of all the school buses. What you mainly get are pictures of

large parking lots filled with school buses. Every so often you'll see a single school bus on the street, but obviously it's a rare, stray event. You conclude it's not significant."

Scientists studying protein transport in living cells too often have tended to see only the school bus depots — the locations where proteins are synthesized. When they see something that associates with the protein they assume that it is an important co-factor or chaperone when in reality it may be a nearby but unrelated structure.

"In biochemistry and genetics, when you look at this macroscopic picture — focusing on 10,000 buses and ignoring two — you're averaging," explains Jyoti Kumar Jaiswal, a postdoctoral fellow in the Simon lab. By averaging, Jaiswal says, scientists may be missing important events that are happening inside the cell.

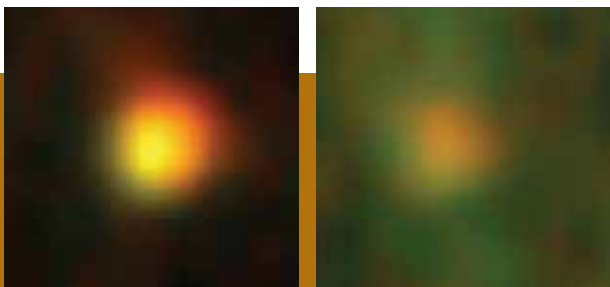
"The way around the conundrum is to observe the single school buses," says Jaiswal. "It's the one

that is actually performing the function it was built for: picking up and dropping off children. With patience, eventually you'll see that every bus leaves the depot."

(Of course, single events can also be misleading. "Each single event you observe is an anecdote," Simon says. "Let's say you were an alien spying on Earth, and you happened to pick Adolph Hitler as your subject to follow. The impression you get of the human race might not be the same as if you chose Winston Churchill.")

Simon's lab tackles the yellow school bus problem with the biophysicist's version of the spy plane: a special fluorescence imaging system called TIRFM, or total internal reflection fluorescence microscopy. Using TIRFM, scientists can track a protein of interest with a fluorescent tag and follow it as it travels near the cell membrane. Three important discoveries have recently emerged from the Simon lab's efforts to focus on a single bus.

## How vesicles unload their cargo



**Unloading dock.** In mouse cells lacking SytVII, vesicles release their cargo faster and more robustly (right) than in healthy cells.

A family of proteins called synaptotagmins was widely believed to trigger the release of a cell's cargo. But after using TIRFM to study the function of a specific synaptotagmin protein, SytVII, Simon, Jaiswal and their colleagues say it actually restricts release.

SytVII is one of 15 members of the synaptotagmin family of fusion proteins, which are associated with vesicles that contain hormones and nerve signaling proteins. In response to a rise in the level of calcium in the cell, synaptotagmins are believed to help trigger fusion of the vesicles with the cell membrane allowing them to release their contents, a process called exocytosis.

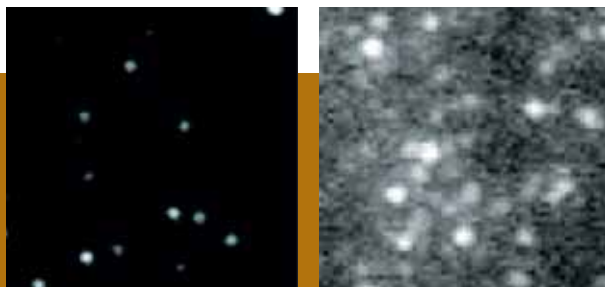
Jaiswal expected vesicles in SytVII-deficient cells to fail to release their cargo, or at least to delay it. But the exact opposite occurred: vesicle release was faster and more robust. The absence of SytVII caused the vesicles to release even cargo that would otherwise have been too big to pass through the fusion pore.

"The faster response to calcium argued against the assumption that SytVII was doing its job as a calcium-dependent trigger for fusion," says Jaiswal.

The findings, published in the August issue of *PLoS Biology*, help to explain how cells heal potentially lethal tears in their membranes by using cellular structures called lysosomes — regulated in part by SytVII.

"This finding may explain Jyoti's previous discovery in which he found that when a cell has a rupture in its surface the membrane will try to patch the surface by fusing lysosomes there, but preventing the intermixing of the lysosomal and plasma membrane proteins," says Simon. "SytVII may be a scaffold that holds all those membrane proteins together, so they can't diffuse out, and the cell has a way of putting a Band-Aid on to heal over."

## How SNAREs help membranes fuse



**Got calcium?** Vesicles containing v-SNARE proteins do not fuse to a t-SNARE enriched lipid bilayer (left) until calcium is added (right).

The secretion of insulin by a pancreatic cell, the transmission of a signal by a brain cell, and the growth of all types of cells depend on the proper fusion of cell membranes. In a May issue of *Proceedings of the National Academy of Sciences*, Simon, Jaiswal and postdoc Marina Fix set out to answer once and for all the question: what role do a class of membrane proteins called SNAREs play in membrane fusion?

According to Simon, cell biologists are divided into two camps: one that has argued that SNAREs are the key proteins necessary for membranes to fuse, and the other countering that SNAREs need to dock with vesicles before fusion can occur.

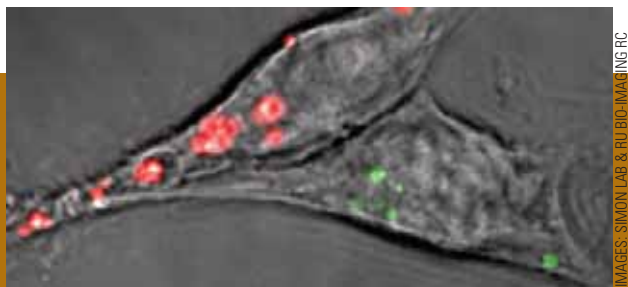
"To answer this question, we decided to start from scratch, build up a system and ask 'What does the cell need for fusion to occur,'" says Simon.

First, Fix recreated the plasma membrane by forming a "lipid bilayer" on the coverslip of a microscope slide. She then added two types of SNAREs, vesicle-based (v-SNAREs) and target-membrane (t-SNAREs), and found that they did not fuse to the bilayer. But when she added calcium to the mix, in a concentration designed to mimic what occurs naturally at the synaptic terminal of nerve cells in the body, she watched as individual vesicles released their contents as they fused to the lipid bilayer.

"Marina's findings are the first solid evidence that SNAREs are the minimal sufficient machinery to get physiologically relevant fusion," says Simon.

Jaiswal says that the *PNAS* paper validates the Simon lab's approach. "Our experiments used reagents that have been used by other researchers in many other experiments; it's not that we made these things up ourselves. The difference is that other studies were done as an average, in populations. No one looked at single vesicles."

## Watching as tumors glow



**Tracing cancer.** Cultured melanoma cells are tagged with two different quantum dots (red and green).

Proteins aren't the only biological structures that can be tracked by using fluorescence. Sandy Simon's lab was one of the first to use quantum dots, nano-sized fluorescent crystals, to simultaneously track multiple proteins in living cells for days at a time. Now, in research reported in the September issue of *Nature Medicine*, Simon's lab extended this technique to track cancer tumor cells in living mice.

Because the glow they emit is bright, long lasting and highly precise, quantum dots overcome some of the obstacles that limit the usefulness of other organic fluorescent molecules. Simply by altering their size, scientists can manufacture them to produce light in any color of the rainbow, and, additionally, only one wavelength of light is required to illuminate all of the different-colored dots. Thus, spectral overlap doesn't limit the number of colors that can be used at once in an experiment. In addition, quantum dots shine for an average of 1,000 times longer than most known fluorescent dyes.

In the *Nature Medicine* paper, Simon, Jaiswal and postdoc Evelyn Voura injected melanoma tumor cells tagged with quantum dots into the tail veins of mice. Using a two-photon microscope at Rockefeller's Bio-Imaging Resource Center, they tracked the tumor cells as they metastasized and traveled to the lungs. The tumor cells labeled with quantum dots were equally proficient as the unlabeled cells in metastasizing and forming tumors, and the cells did not have any detectable effect on the health of the mice.

The Rockefeller researchers say that quantum dot labeling of tumor cells could have an enormous impact on how scientists everywhere investigate the mechanisms of metastasis. "The ability to specifically tag cells with quantum dots can be a crucial tool that could enable oncologists to monitor quantitatively how well cancer cells have been eradicated after experimental treatments," says Jaiswal.

## Defying death *continued*

when we did this, tissue became several times its original size. This was a big surprise, because if you are saving cells that should normally die, the tissue should not be smaller, but it should certainly not be bigger." Repeated experiments found the same phenomenon, including tumor-like outgrowths.

Ryoo discovered that the unexpected growth occurred in the area next to the undead cells. Upon further exploration, he discovered that the undead cells actually produced signals that stimulate cell division, triggering the growth. These signals, controlled by genes such as wingless (wg) and decapentaplegic (dpp), were already known to be involved in cell proliferation. The scientists also showed that dying cells activate a cell signaling pathway known as the Jun-N-terminal Kinase (JNK) pathway, which is needed to induce wg signaling.

"This is a very unexpected finding, even to us," says Steller, who calls these findings one of his best research stories

in a decade. "When some of the first findings emerged, I was very excited, but also very careful because it was such a radically new concept. It was not received with universal enthusiasm."

If replicated in human cells, the findings could be revolutionary to cancer therapy. Cancer cells by definition are immortal — the mechanisms that lead to cell death have been turned off. Steller's results suggest that toxic treatments such as radiation and chemotherapy that stress cancer cells may actually stimulate some cancer growth. Specifically, the signals produced by these undead cells may trigger the relatively few omnipotent cancer stem cells found within tumors to create more cancer cells.

"If cells that are stressed through treatment are somewhat resistant to apoptosis, and so are not rapidly cleared, they could continuously send out signals that result in a significant

overgrowth of cells," says Steller. While such a theory has yet to be proven in cancer cells, in the least, the findings suggest that therapies that engage the apoptosis pathway more directly than blunter treatments may avoid this crucial stress response, he says.

The discovery also has implications for beneficial cell regeneration. "Sometimes you want to have cells growing back if there is an injury, so there may be a way to boost this response," says Steller. He is now looking at cells taken from the liver, an organ that can, to a limited degree, regenerate itself in response to viral infection or toxic stress, to see if they use this stress response pathway in a helpful manner.

"This is the start of something that needs further investigation, but it is a very testable model," says Steller. "If anything, our notion of compensatory proliferation is sure to stimulate a lot of debate."