

Staying a step ahead of staph

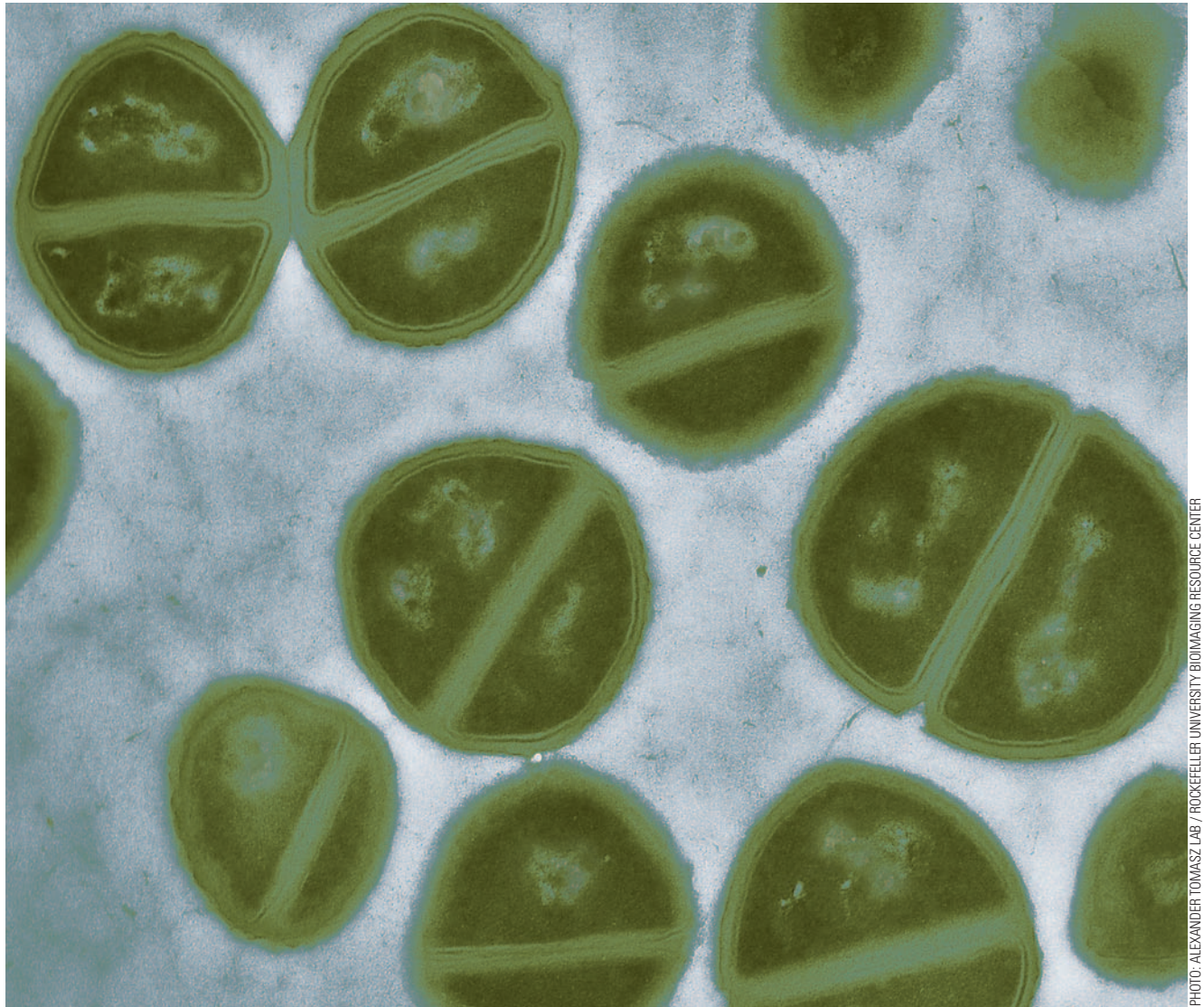
Antibiotic resistance is catching up with even the most powerful drugs. How Rockefeller scientists are fighting back.

BY JOSEPH BONNER

It started with a 40-year-old Michigan man with diabetes, heart disease and kidney failure.

He'd had a bad year. Because of vascular disease, he had sores on his feet that wouldn't heal, and two months earlier gangrene had developed on a toe, requiring amputation. He'd been on a cocktail of antibiotics including one called methicillin, a relative of penicillin, and vancomycin, which for 40 years has been the drug of last resort for fighting

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Resisting arrest. *Staphylococcus aureus* bacteria, photographed using an electron microscope.

PHOTO: ALEXANDER TOMASZ LAB / ROCKEFELLER UNIVERSITY BIOMAGING RESOURCE CENTER

A throttle for plant growth

Newly discovered protein tells seedlings when they've reached light

BY TIEN LEE

Take two genetically identical plants, grow them on two different windowsills, and they could look absolutely nothing like each other. "In order to optimize their growth, plants must continuously monitor the intensity, duration and direction of light," says Peter Hare, a research associate in **Nam-Hai Chua's** Laboratory of Plant Molecular Biology.

But since plants don't have brains — or muscles or eyes or gut feelings — the mechanics of that adaptation take place exclusively at a molecular level. More specifically, plants rely on phytochromes, a family of

receptors that monitor specific wavelengths of light. One such receptor, phytochrome A (phyA), is the key in sensing when a newly germinated seedling has emerged from underground and can begin devoting its resources to photosynthesis rather than upward growth.

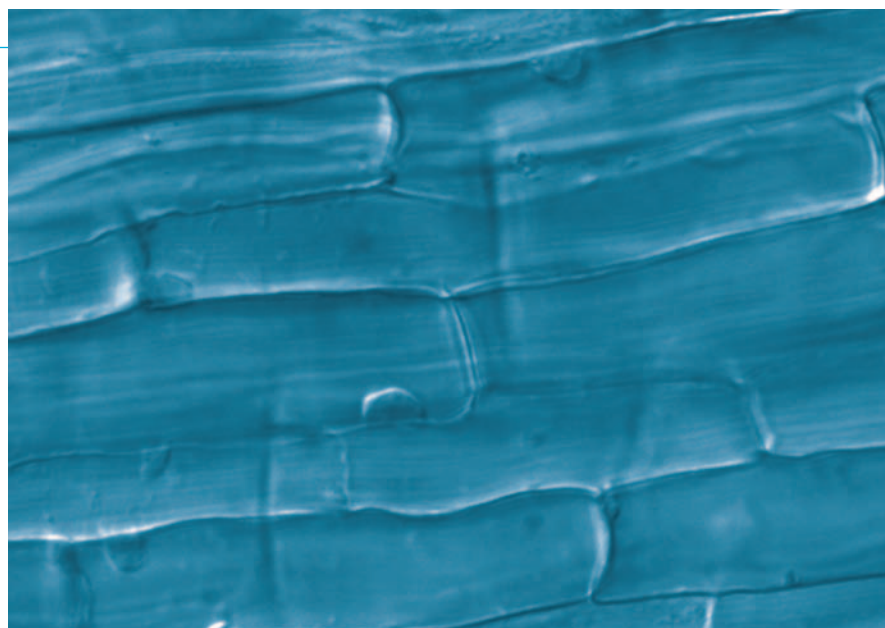
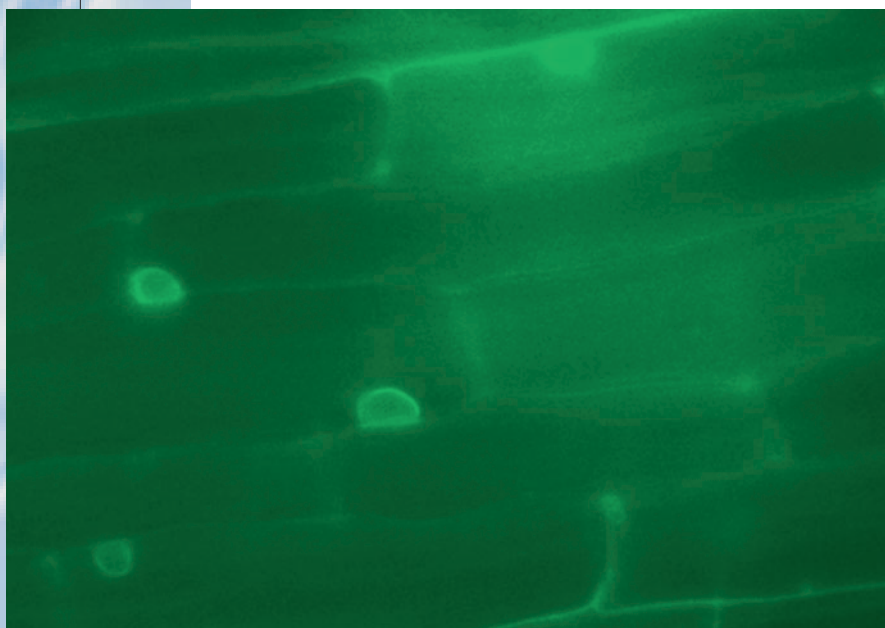
It's a critical transition, yet the specifics of how it occurs are still largely mysterious.

Now, experiments conducted by Chua, who is Rockefeller's Andrew W. Mellon Professor, and Hare, along with Simon Møller and Li-Fang Huang, show

that loss of a protein called LAF3 causes partial blindness — leading to plants that keep growing towards the light even when they have already reached it. Normal *Arabidopsis* plants grow about 4.2 millimeters during four days under conditions that specifically activate phyA. Mutants lacking LAF3 grew 9.7 millimeters during that time. Mutants lacking phyA, meanwhile, grew 12 millimeters.

Hare proposes that LAF3 is involved in the activation of only a few of the many genes that get switched

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Anatomy of a growth spurt. *Arabidopsis* seedling cells, stained with a fluorescent marker to highlight activity of the LAF3 protein (left), show that the protein accumulates around the periphery of the cells' nuclei.

PHOTOS: NAM-HAI CHUA LAB

SCIENCE BRIEFS

BY ZACH VEILLEUX

Family planning for mosquitoes. Smart females the world over try to avoid giving birth in places where predators lurk. From an evolutionary perspective, such logic would be likely rewarded within a species. Surprisingly, however, few studies have sought to test for such behavior in a natural habitat.

Rockefeller's **Joel Cohen**, in collaboration with scientists at the University of Haifa, Israel, and the University of California, Santa Cruz, designed an experiment in which mosquitoes and tiny flies called midges were released into artificial pools containing the predator *Notonecta maculata*, an insect commonly known as a



PHOTO: CENTERS FOR DISEASE CONTROL

Snack in a puddle. Mosquito larvae live just below the surface of water, where they are easy pickings for *Notonecta* predators.

backswimmer. They found that mosquitoes, which are *Notonecta* prey, avoided laying eggs in the pools, while midges, which are not *Notonecta* prey, did not. A second experiment in natural rock pools confirmed the results.

The researchers went on to demonstrate that the mosquitoes' cue came from a chemical released by *Notonecta* — mosquitoes refused to lay eggs in infested pools for eight days after the predators had been removed. The research could lead to new chemicals that repel mosquitoes.

Cohen is the Abby Rockefeller Mauzé Professor and head of the Laboratory of Populations.

Oecologia, January 2004.

The dendritic blame game. People with chronic hepatitis C virus (HCV) infections typically lack the CD8 T lymphocyte immune cells that fight off the virus. For this reason, many scientists who study HCV have concluded that immune system dendritic cells, which "train" CD8 T lymphocytes, must be defective in people unable to clear HCV.

Not so, say **Charles Rice**, the university's Maurice R. and Corinne P. Greenberg Professor and head of the Laboratory of Virology and Infectious Disease, and his colleagues, who point out that it's difficult to reconcile this theory with the fact that patients with chronic HCV have otherwise healthy immune systems. Rice, along with graduate fellow Randy Longman, former postdoc Matthew Albert, and Ira Jacobson and Andy Talal at Weill-Cornell, looked at several measures of dendritic function in 13 volunteers with chronic HCV infections. All 13 had fully functional dendritic cells.

"Our findings are consistent with clinical and immunologic data that show the deficit in the patient's immune repertoire is HCV-specific and suggests that refined models are required for understanding the role of dendritic cells in HCV pathogenesis," the authors say.

Blood, February 2004.

The shape of cell death. Scientists in **Milton Werner's** Laboratory of Molecular Biophysics have identified and described the surface at the heart of the death-inducing signaling complex (DISC) that initiates one type of programmed cell death. When cell death is triggered, several components come together to form the DISC at the cell's cytoplasmic membrane.

While several of the structures involved in this process have been studied, the mechanism by which the proteins recognize one another had not, until now, been defined. Detailed analysis of the "death domain" interaction now shows it consists of an expansive surface that is common to many other, unrelated proteins, and a secondary surface that may be responsible for stabilizing the other components of the DISC.

Journal of Biological Chemistry, January 2004.

Accounting for misclassified genes. Studies that seek to associate genes with specific traits rely on scientists correctly identifying which organisms display the trait being studied. But when misclassifications occur there is often no way to correct them. Derek Gordon, a research assistant professor in **Jürg Ott's** Laboratory of Statistical Genetics, has now figured out, statistically, how much damage such genotyping errors cause.

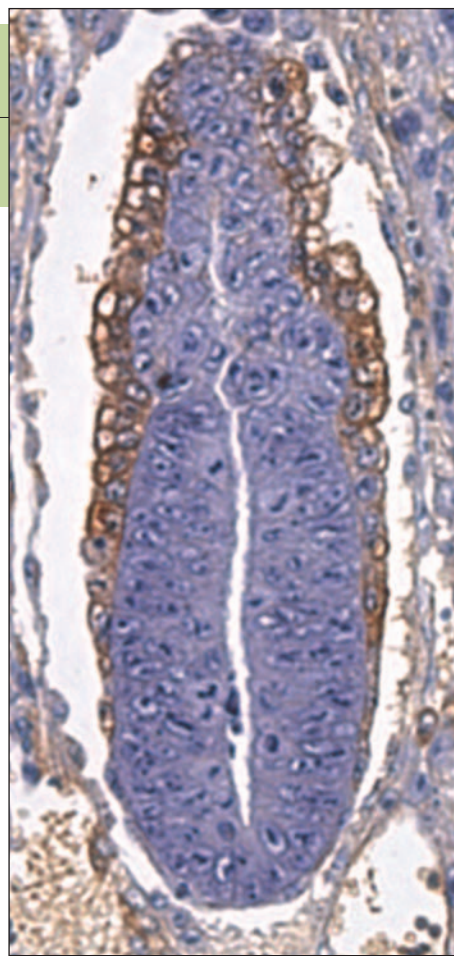
His findings: even relatively small error rates in some parameters can require large increases in sample size in order to maintain statistical significance. Gordon's research will help scientists better evaluate the accuracy of their results when genotyping errors are unavoidable. And it complements Gordon's previous work, which resulted in a method (and available computer program) to allow for errors in genetic association analyses, thereby avoiding inflated false positive results that occur when errors are ignored.

Genetic Epidemiology, January 2004.

New schizophrenia gene ID'd. A new cellular pathway, identified by **Maria Karayiorgou**, head of the Laboratory of Human Neurogenetics, may help explain how schizophrenia develops. Karayiorgou, working with colleagues at Columbia University and the University of Pennsylvania, found decreased levels of the protein Akt1 in the brains of deceased schizophrenia patients as well as in the blood of living individuals with schizophrenia — and they identified a particular form of the Akt1 gene that increases an individual's risk of developing the illness. The researchers also found an increase in the activation of a protein called GSK3beta, which forms a molecular pathway with Akt1.

Abnormalities in the Akt1-GSK3beta pathway have been linked with diabetes, stroke and Alzheimer's disease, and the pathway is also a target of lithium, one of the most established treatments for mood disorders.

Karayiorgou's new findings suggest that changes in the signaling of the Akt1-



Disappearing mice. Cells from mice embryos lacking the beclin 1 gene, required for development, begin to die after just six and a half days (left). By seven and a half days (right), little of the embryo remains.

When cells digest themselves. The beclin 1 gene, which plays a role in programmed cell death, is deleted in breast and ovarian cancers and is involved in a complex process called autophagy, in which cells that have been deprived of nutrients begin to break down and recycle their own organelles and cytoplasmic substances to provide metabolic precursors. Now, thanks to **Nat Heintz's** Laboratory of Molecular Biology, scientists know more.

When Heintz, Research Associate Zhenyu Yue, and their colleagues bred mutant mice lacking, or with reduced, beclin 1, they found the mice either died as embryos or suffered from a high incidence of spontaneous tumors. Additionally, mouse embryonic stem cells lacking beclin 1 do not have the normal autophagic response. The results demonstrate that beclin 1 plays a key role in early embryonic development and may be a natural tumor suppressor.

"This is the biological explanation for recent evidence implicating beclin 1 in human cancer, and it suggests that mutations in other genes operating in the autophagy pathway may contribute to tumor formation," says Heintz.

PNAS, December 2003.

GSK3beta pathway may contribute to the development of schizophrenia and that the antipsychotic drug haloperidol (Haldol), which increases activity of Akt1 in mice, may compensate for the pathway's impaired functioning.

Nature Genetics, February 2004.

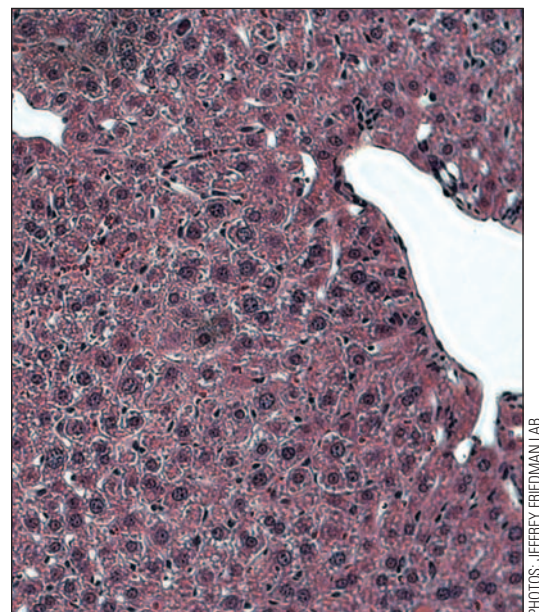
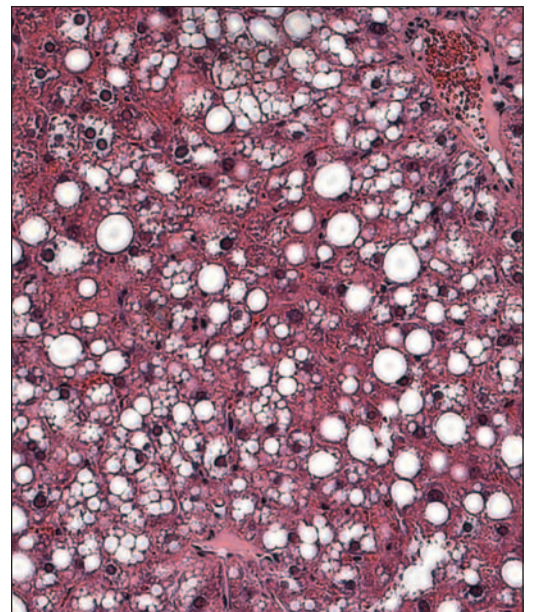
Controlling fat. Leptin, a hormone secreted by fat cells, has been highly successful in treating diabetes, insulin resistance and fatty liver degeneration associated with lipodystrophy in lab mice and humans. Lipodystrophy is a condition characterized by a disruption in the way the body produces, uses and distributes fat.

Now **Jeffrey Friedman's** lab has identified the central nervous system as the primary site where leptin's effects on the disease occur. Leptin is known to act on a variety of tissues including the brain, skeletal muscles, heart and pancreatic beta cells. Using a congenital mouse model of lipodystrophy, Friedman, who is head of the Laboratory of Molecular Genetics, graduate fellow Esra Asilmaz, and colleagues observed that subcutaneous administration of leptin was not as potent as a much lower dose administered directly to the central nervous system, which was able to correct the metabolic abnormalities associated with lipodystrophy.

Furthermore, using microarray ("gene chip") technology, the researchers identified repression of the enzyme SCD-1 as a mechanism of leptin action to improve fatty liver degeneration. The findings may have profound effects on the use of leptin treatment for metabolic diseases.

Friedman is the university's Marilyn M. Simpson Professor.

Journal of Clinical Investigation, February 2004.



Low-fat liver. Liver cells from mice show fatty deposits (top) that are eliminated (bottom) after leptin was administered to the central nervous system. Mice treated with leptin had one-seventh the liver triglycerides and one-third the liver mass of controls.

PHOTOS: NAT HEINTZ LAB

PHOTOS: JEFFREY FRIEDMAN LAB

DNA celebrates sixty years

Oswald Avery (left and below right) was recruited to Rockefeller in 1913 to find a way to treat pneumococcal pneumonia. But while attempting to understand how one strain of the deadly bacteria could transfer properties to another, Avery — along with young colleagues Colin MacLeod (below left) and Maclyn McCarty (below center) — stumbled onto something much bigger. Sixty years ago this month, they announced DNA was the chemical basis of heredity. Their studies, which have been likened in their revolutionary impact to the work of Gregor Mendel and Charles Darwin, laid the foundation for James Watson and Francis Crick's 1953 discovery of the double-helical structure of the DNA molecule. The anniversary celebration at Rockefeller included a cake presented to McCarty (right), the only one of the three still living.

PHOTOS: ROCKEFELLER UNIVERSITY ARCHIVE CENTER



PHOTO: ZACH VELLEUX

Understanding STAT

How a key cancer protein also plays a role in normal tissue development

BY JASON GORSS

Learning to read signals, whether they come from a boss or a spouse, can make all the difference in a relationship. The same goes for cells: understanding how they take their molecular cues could lead to new therapies for diseases that stem from miscommunication at the cellular level.

"When cells communicate with each other, there are some signals outside the cell, and somehow those signals need to be transduced into the cell to direct gene expression," says Yuhong Shen, a postdoc in James Darnell's lab.

Proteins call STATs — signal transducers and activators of transcription — which Darnell's lab discovered in 1992, play a critical role in this process. They help cells interpret and respond to a glut of incoming messages, and they travel to the nucleus to activate the proper genes. To better understand how STATs work, Darnell, the university's Vincent Astor Professor, Shen and colleagues engineered a mouse that produced less STAT3, one of the STAT proteins believed to play a key role in cancer.

In previous experiments, Darnell's Laboratory of Molecular Cell Biology showed that repeated activation of STAT3 can cause normal cells to behave like cancer cells, leading to tumors in mice. STAT3 has also been found to be active in leukemia, breast cancer and many head and neck cancers. Eliminate STAT3 completely, on the other hand, and cells die altogether — mice embryos without the protein can't survive past seven days.

This time, the researchers tested two types of mice: one that produced about 50 percent of the normal level of STAT3 and one that produced 25 percent. The mice with 50 percent STAT3 turned out normal; those with 25 percent did not. "With 25 percent STAT3, the mice survived through embryogenesis, but 70 percent died shortly after birth," Shen says. "Those that did not die were born smaller and grew at a reduced rate."

After exploring several potential connections, the researchers pinpointed one: a compound called insulin-like growth factor 1, or IGF-1, the main regulator of late-stage embryo development and early postnatal growth in mice. "We found that our STAT3 mutant animals had less IGF-1, probably about 50 percent when compared to control animals," Shen explains.

While the implications aren't yet clear, the bigger, more complicated picture of the STAT3 universe suggests that in addition to its role in tumor formation, STAT3 may be more important to normal, non-cancerous developmental processes than was previously believed.

Staying one step ahead of staph *continued*

stubborn bacterial infections that resist other antibiotics.

In June of 2002, the Michigan man developed an infection at the exit site of the catheter used for his kidney dialysis treatments. Cultures taken from the site showed that the responsible microbe was *Staphylococcus aureus*, the bacterium underlying as many as 2 million infections and 80,000 deaths each year in the U.S. — and that it was resistant to both methicillin and vancomycin.

It was the first time in medical history that staph had successfully fought off the antibiotics of last resort.

Less than four months later, bacteriologists' worst fears were confirmed: a culture from a separate patient in Pennsylvania also tested positive for vancomycin resistant *S. aureus*. In both the Michigan and Pennsylvania patients, the infections are believed to have been caused by staph bacteria that had acquired a gene complex called *vanA* from vancomycin-resistant *Enterococcus faecalis* bacteria that were also present at the sites of the infections.

Now, in Alexander Tomasz' Rockefeller University lab, scientists are beginning to piece together the molecular picture of how this strain of staph bacteria resists both drugs.

Both methicillin and vancomycin work

by destroying the bacterium's cell wall, but they do so in different ways. In the case of methicillin, the drug inactivates four different proteins — called penicillin-binding proteins — that the bacterium needs to construct its cell wall; to survive methicillin, staph acquired a gene called *mecA*, which produces a new penicillin-binding protein that takes over cell wall construction when the bacterium's apparatus becomes paralyzed. Vancomycin, on the other hand, physically traps the molecular building blocks that penicillin-binding proteins use to assemble the cell wall; to survive vancomycin, staph acquired a gene called *vanA*, which produces an abnormal cell wall precursor that the drug doesn't recognize.

"Both resistance mechanisms are targeted on the bacterial cell wall and each seems to be custom designed to match precisely the mode of action of the antibiotics," says Tomasz.

To search for new weaknesses, Tomasz and Anatoly Severin, senior research associate, working with colleagues at the U.S. Centers for Disease Control in Atlanta, examined a strain of *S. aureus* with resistance levels to vancomycin close to 1000 times higher than normal. They made two encouraging discoveries.

First, they noticed that using the two

drugs in combination was extremely effective, even at relatively low doses. The new penicillin-binding protein produced by staph resistant to methicillin, it turns out, is unable to utilize the abnormal cell wall precursor produced by staph resistant to vancomycin.

"The mechanism is antagonistic," says Tomasz, head of the Laboratory of Microbiology. "If you challenge this bacterium with either vancomycin or methicillin, it is very resistant. However, if you combine the two antibiotics, this resistance collapses." That finding — that both drugs together are still effective — is encouraging news for doctors who soon may find themselves without a single drug capable of killing staph infections.

Secondly, the Rockefeller scientists showed that the structure of the cell wall produced from the abnormal cell wall precursor has several abnormalities itself — abnormalities that may make the resistant bacteria less likely to spread, at least in patients with healthy immune systems.

"What we hope is that, at least for the time being, the vancomycin resistant *S. aureus* has a price to pay for its victory against vancomycin by surrendering some of the skills that make it such a dangerous pathogen," Tomasz says.

A throttle for plant growth *continued*

on or off when a seedling first encounters light. One of those genes is XTR7, which codes for an enzyme that breaks down cell walls to allow seedling stems to elongate. Like *phyA* mutants, LAF3 mutants express the XTR7 gene at a much higher level than normal seedlings.

But since LAF3 is just one of roughly a dozen proteins involved in the signaling process, the Rockefeller scientists' next step is to compile a scheme detailing how the individual components interact as a whole to control plant development. "At least for now, we've got enough bits and pieces in the puzzle. Our next challenge is try to fit these together and describe the relationships between them," says Hare.

"One of the most interesting things about LAF3 is its location around the rim of the cell's nucleus," says Hare. LAF3 is the first *phyA*-specific signaling protein known to accumulate in this area. Physical separation of molecules within cells is often key to regulating signaling pathways, and there is substantial evidence indicating that plants use this approach in responding to sunlight.

"We now want to expose the biochemical function of LAF3. The sequence of the protein offers us no compelling clues, but if

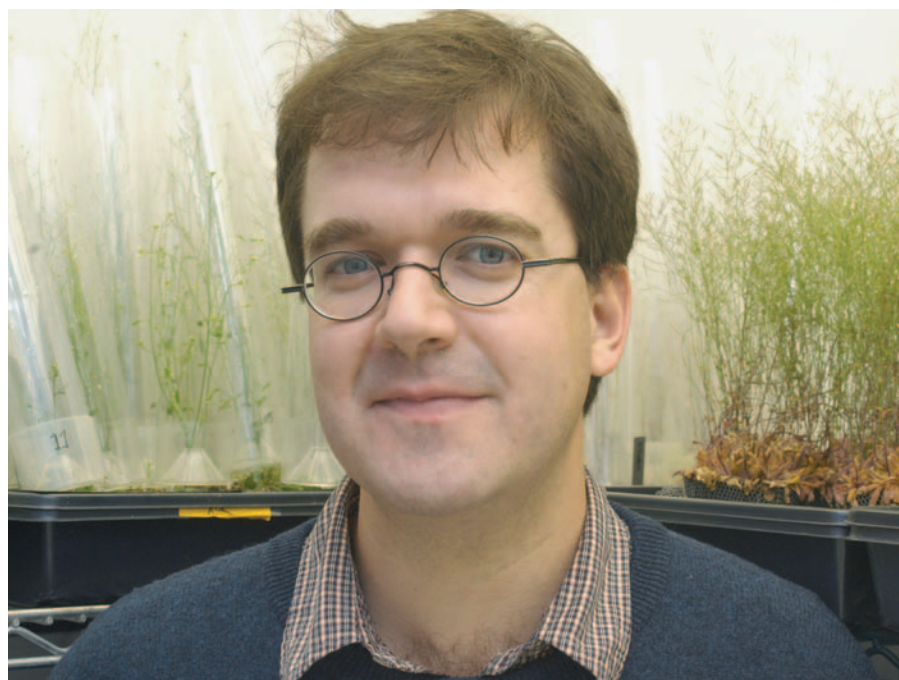


PHOTO: ZACH VELLEUX

Plant operations. "Plants can't do much to avoid situations, so they're constantly assessing the environment to prepare," says Peter Hare.

we mislocalize it, will it still execute its function? Although classical genetics is a great way to uncover the components of the light signaling pathway, it cannot fully reveal how the pathway works," says Hare.



Paul Nurse, President
Cathy Yarbrough, Vice President for Communications and Public Affairs

Editor: Zach Veilleux
Art Director: John Haubrich
Contributors: Joseph Bonner, Lynn Love

Address correspondence to:
Editor, RU Scientist, Box 68
1230 York Ave. | New York NY 10021

The corrections

For cells to replicate, chromosomes must segregate precisely. Sometimes, that doesn't happen.

BY JOSEPH BONNER

For nearly as long as scientists have been studying cells, they have been studying cell division. Yet despite over 150 years of research, plenty of details have yet to be understood.

In **Tarun Kapoor's** laboratory, which studies how dividing cells segregate their genetic material, a new element of the process has now emerged that demonstrates how one type of error in the progression of cell division is avoided.

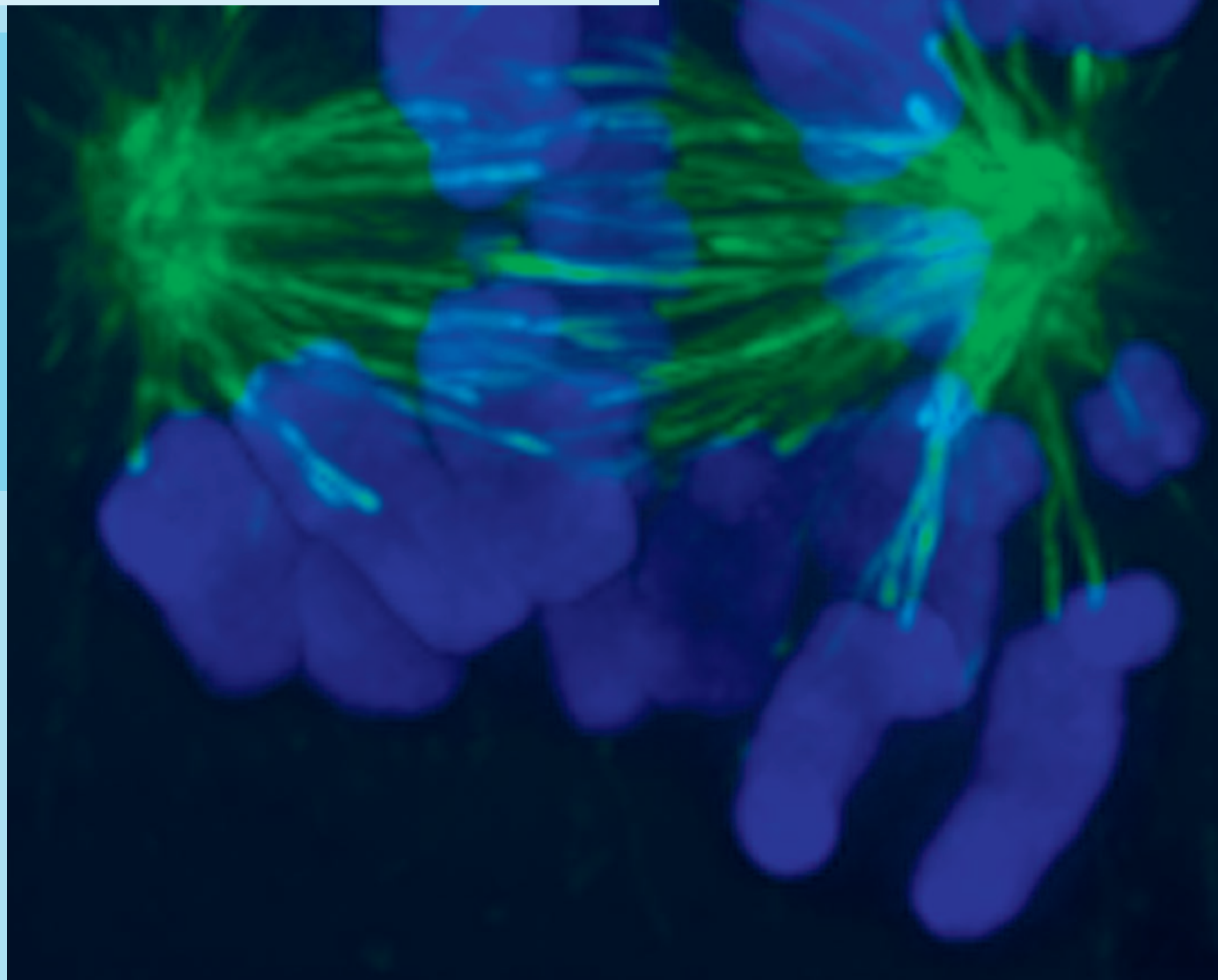
When cells divide, the parent cell must replicate and segregate — with exquisite precision — each of its 46 chromosomes so that two “daughter” cells inherit all of its genetic information. During normal cell division, each replicated chromosome pair attaches to a bipolar structure called the mitotic spindle, made up of protein polymers known as microtubules. One sister chromosome attaches to one pole of the spindle, and the other sister attaches to the opposite pole. As the cell divides, sister chromosomes split up and are pulled in opposite directions so that each daughter cell receives a copy of each chromosome.

When there are errors in this process, the daughter cells don't receive all their genes, leading to developmental defects and diseases such as cancer. One such error occurs when both sister chromosomes of a replicated pair attach to the same pole of the mitotic spindle in a dividing cell. The result is then one daughter cell with an extra copy of a chromosome and one daughter cell with one chromosome missing.

The question Kapoor and his colleagues wanted to answer was: How does the cell avoid this?

In general, researchers in Kapoor's lab use small organic molecules to interrupt the process of cell division. These inhibitors work by permeating the cell membrane and blocking the action of various proteins.

Enter an enzyme called Aurora kinase. Without Aurora kinase, chromosomes become very prone to improper attachments in dividing cells. But its role in correcting improper chromosome attachments was largely unknown. Michael Lampson, a



Division gone wrong. Chromosomes (*above, blue*) attach to microtubule structures (*green*) as a cell divides. When multiple copies of the same chromosome attach to just one pole of the dividing cell, the daughter cells will be defective.

postdoc in Kapoor's Laboratory of Chemistry and Cell Biology, used a small molecule called Aurora kinase inhibitor-1 (AKI-1), originally developed by AstraZeneca as a potential cancer drug. AKI-1, as its name implies, inhibits Aurora kinase activity.

According to Lampson, scientists at the Research Institute of Molecular Pathology in Vienna had proposed a model that suggested Aurora kinase activity would cause some of the incorrect attachments to break and lead to the formation of new, correct attachments. Lampson devised a strategy to test this hypothesis, incubating cells with the Aurora kinase inhibitor to accumulate attachment errors. The kinase could then be reactivated by simply removing the

inhibitor. Using real-time, high-resolution microscopy in Rockefeller's Bio-Imaging Resource Center, Lampson and his colleagues activated Aurora kinase and watched.

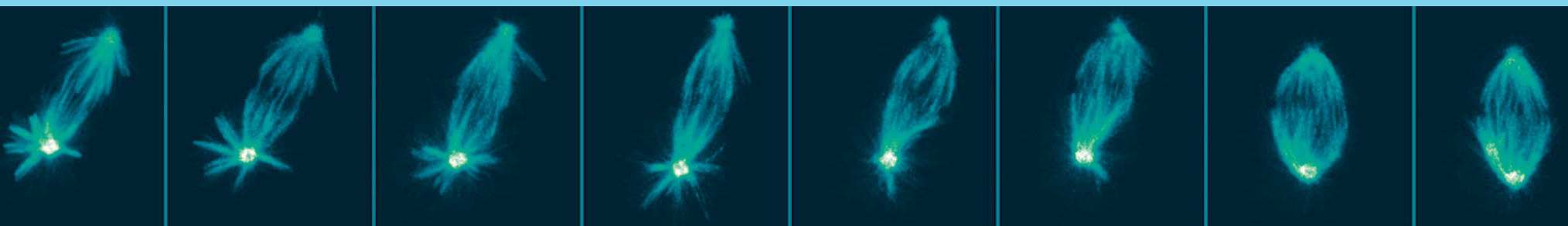
Within a few minutes, something unexpected happened. “Once we washed out the Aurora kinase inhibitor, the microtubule fibers shortened and the chromosomes moved directly to the pole,” says Lampson.

According to Lampson, Aurora kinase eliminates the microtubule fiber by causing it to disassemble. As it disassembles, it pulls the chromosomes into the pole, enabling them to also attach to a microtubule from the opposite pole. Once the chromosome has an attachment to both poles, it can

become correctly aligned.

“It's an unexpected mechanism. It shows how Aurora kinases are regulating the microtubules to correct errors,” says Lampson.

But what's even more exciting, the researchers showed that it's possible to use small organic molecules to switch proteins both off *and* on in living cells while observing the effects. “The technique has generally not been possible experimentally because once you turn a protein off you can't turn it back on,” says Lampson. “These experiments demonstrate how activation and deactivation of protein function, through chemical genetics, can be a valuable approach to understanding cellular mechanisms.”



Lost and found. When researchers activated an error-checking enzyme used by cells to prevent abnormal replication, incorrectly attached chromosomes relocated to their proper positions, as shown in this 40-minute time lapse series.

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