FINDINGS

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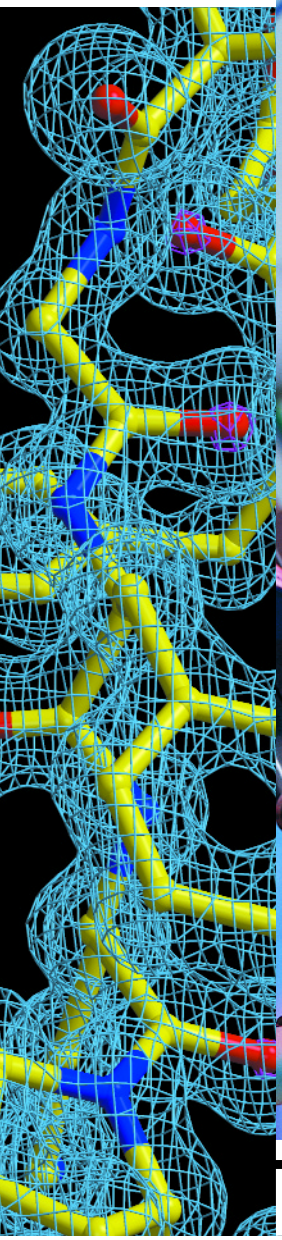

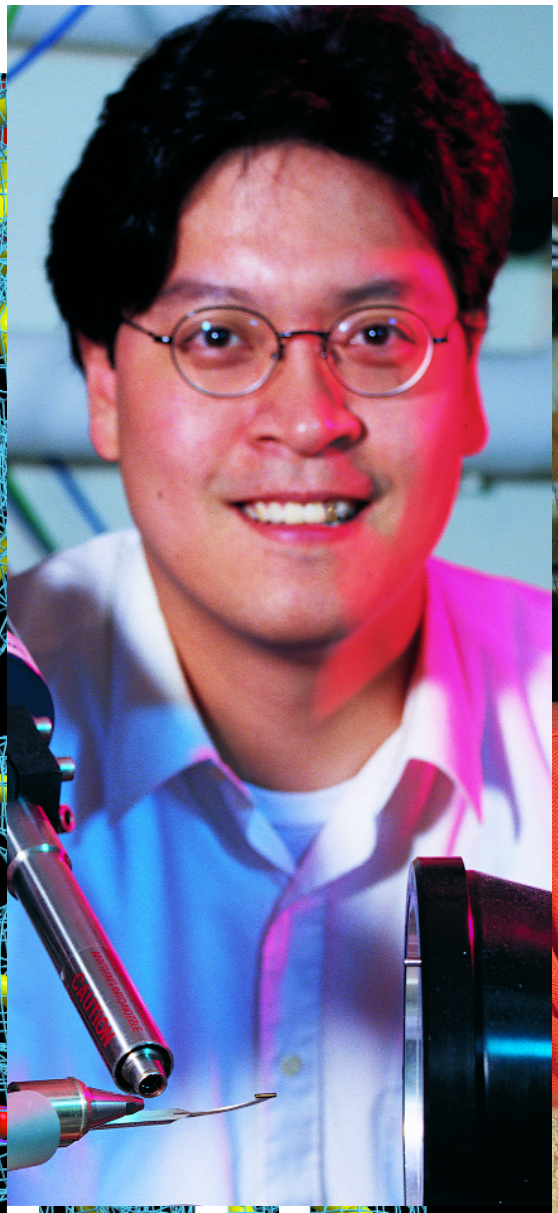

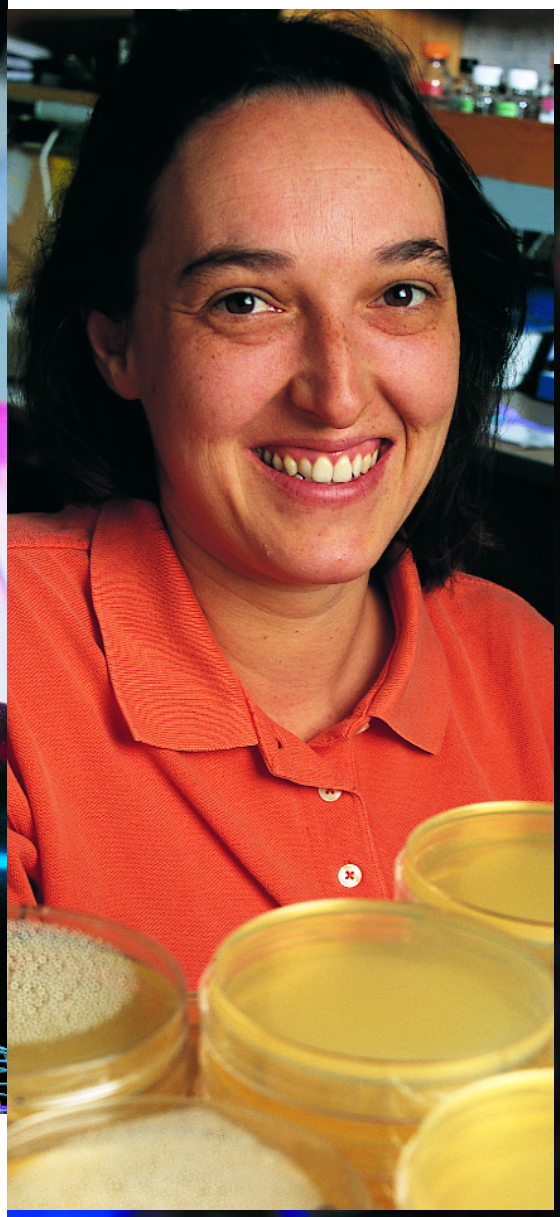

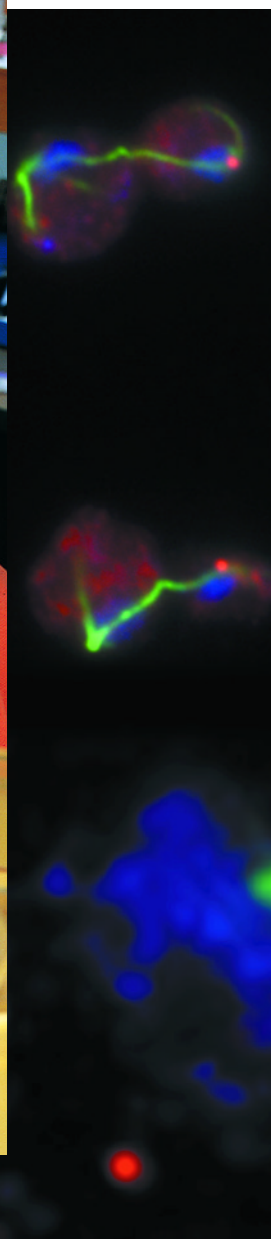

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On the Cover

Cover photo of Geoffrey Chang: *Alan McPhee*

Cover photo of Angelika Amon: *L. Barry Hetherington*

Editor's Note

Take a chance. Go ahead and try that harebrained idea of an experiment. Maybe it will work.

Two scientists featured in the "Bench to Bedside" section of this issue of *Findings* did just that. Researchers Jörg Goronzy and Douglas Kniss both had ideas they wanted to test that challenged the status quo. Both scientists knew that their experiments had a low probability of success. Yet Goronzy's basic curiosity into the behavior of immune cells called T-cells led him to uncover a novel cause for rheumatoid arthritis. And Kniss, an obstetrician and basic scientist, succeeded in developing a way to make an artificial placenta, so that researchers might soon be able to test drugs for safety during pregnancy.

The National Institute of General Medical Sciences, a component of the National Institutes of Health, understands that it can be risky to try to solve important scientific riddles. Scientists sometimes have to try an experiment that has a high chance of failure in order to reap the sweet rewards of success. NIGMS, through its "high-risk, high-impact" grants program, offers researchers a chance *to take a chance*. Both Kniss' and Goronzy's research funding came from this grants program, and both of these scientists' projects worked. Read about their successes on pages 14–15.

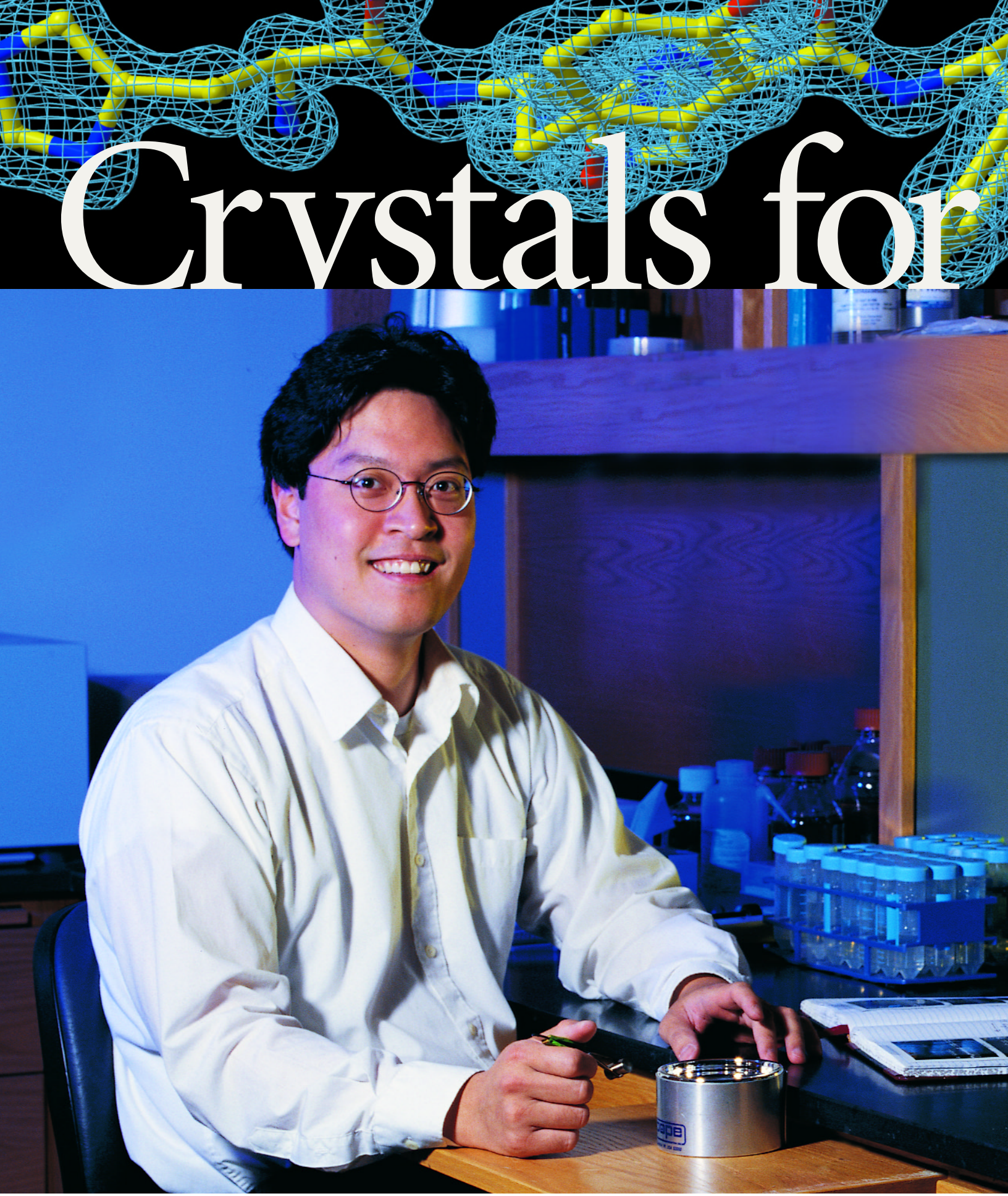
Many scientists will say that some degree of taking chances is critical to their success. But most researchers will also agree that working hard at something they love is the secret formula. The stories in this issue about young scientists Geoffrey Chang and Angelika Amon tell why.

Alison Davis

Alison Davis

Editor

Crystals for



a Cure

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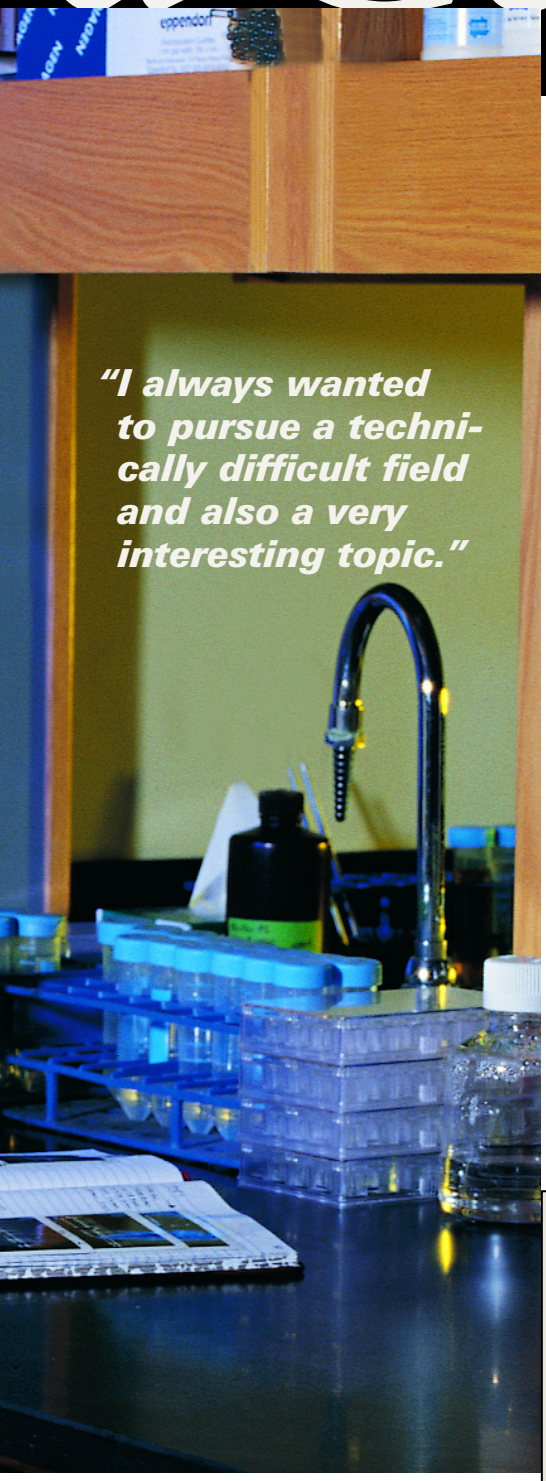


Alan McPhee

"I always wanted to pursue a technically difficult field and also a very interesting topic."

Geoffrey Chang (left) is a biophysicist at The Scripps Research Institute in La Jolla, California.

To Chang, an electron density map (top) reveals not only the atomic skeleton of a protein, but also a work of beauty and perfection.

By Alisa Zapp Machalek

These days, Geoffrey Chang, a biophysicist at The Scripps Research Institute in La Jolla, California, seeks to understand how some bacteria and cancer cells evade our most powerful killing potions. But not long ago, he seriously considered a career as a classical clarinetist.

At 13, he played in Carnegie Hall. During high school, he performed with the traveling student music group, America's Youth in Concert, in some of Europe's most renowned concert halls.

In college, Chang had to choose between science and music. Science won out, but he has not abandoned his love of music. Early on Saturday mornings, classical sounds from his clarinet—or the silkier jazz of his saxophone—echo through his lab in the Scripps molecular biology building.

Now 30, Chang is considered a world expert in a scientific field called membrane protein crystallography. Last year, he won a Presidential Early Career Award for Scientists and Engineers. Presented annually, it is the highest honor bestowed on promising young scientists by the U.S. Government.

Microbes, Molecules, and Medicines

Chang's research is aimed at combating an increasingly serious public health threat: drug resistance. He approaches the problem by trying to solve the detailed, three-dimensional structures of the "pump" proteins that in many cases are the molecular culprits. These proteins hail from a family called multidrug resistance (mdr) proteins and are called mdr-ABC proteins. These molecules shuttle medications—such as antibiotics or cancer chemotherapy drugs—out of living cells before the drugs have had a chance to do their jobs.

By kicking useful medicines out of cells, the mdr-ABC pump proteins foster the growth of drug-resistant bacterial and cancer cells. The first dose of an antibiotic or chemotherapy drug kills almost all of the diseased cells. But the remaining cells, many of which have a rich supply of pump proteins, survive and take over. Eventually, the cells resist treatment altogether by pumping out most drugs thrown at them.

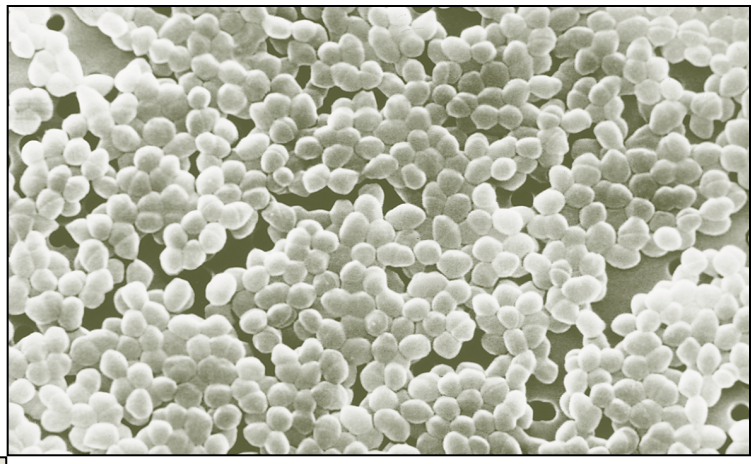
These pump proteins, also called "transporters," probably have some inherent function, but scientists haven't yet determined exactly what it is, Chang explains. "I believe they're defense mechanisms for bacteria to survive. In normal circumstances, these transporters pump out toxins. Our antibiotics are perceived as toxins," he says.

Chang's strategy is to figure out ways to jam the mdr-ABC pump proteins.

"The idea is that if we can solve the structure of this pump, we can begin to understand how it works," he says. And with this understanding, he predicts, researchers can design small molecules called inhibitors that sabotage the mdr-ABC pump proteins by clogging their works. With these pump proteins shut down, bacteria or cancer cells cannot expel—and once again become vulnerable to—medicines given to kill them.

"The great news for the pharmaceutical industry is that if we can block the pump, it can give new life to drugs that have lost their effectiveness," says Chang. Although such drugs may initially have worked well for a patient, as the drugs are pumped out, the patient must be given higher and higher doses, he says, which increases costs and possible side effects.

The escalating price of treating drug resistance is clear in the case of tuberculosis, a potentially fatal infection that remains a leading killer worldwide and that has re-emerged as a serious public health threat in the United States. A standard treatment costs as little as $20. But the cost to treat drug-resistant tuberculosis skyrockets to $2,000 or more per patient.



Janice Carr

Found normally in the digestive tract of humans and animals, enterococcal bacteria can sometimes cause lung, wound, and urinary tract infections. The bacteria shown [photo] have developed resistance to vancomycin, often called the antibiotic "of last resort."

Patterns of Light

To study the structures of mdr-ABC pump proteins, Chang harvests the proteins from specially designed, drug-resistant microorganisms that he grows in his lab. Chang creates these so-called "superbugs" by implanting into harmless bacteria genetic instructions from some of the most highly infectious microorganisms on Earth, such as those that cause pneumonia, tuberculosis, and cholera. Introducing these genes does not make the microbes capable of passing on these deadly diseases to humans.

Bathed in a rich nutrient soup, the bacteria grow rapidly inside the centerpiece of Chang's lab—a 20-gallon, stainless-steel fermenting vat.

Chang cracks open the bacterial cells, extracts their pump proteins, and with just the right mix of chemical ingredients, he coaxes the proteins to form precisely ordered crystals. He then blasts the crystals with a high-intensity X-ray beam. As the beam bounces off atoms within the crystal, it sprays out light like a ray of sunlight scattered by a crystal chandelier. Each protein creates its own signature pattern of scattered light, which biophysicists like Chang can use to piece together the protein's three-dimensional structure.

Such a structure reveals every detail of a protein's shape—its surface landscape, inner architecture, and the positions and chemical properties of all of its thousands of atoms. To scientists, a detailed structure offers tantalizing glimpses of a protein's biological function and helps define its role in health and disease.

Oil and Water Don't Mix

Chang's work is particularly difficult because pump proteins are embedded in the fatty outer membrane of cells. Scientists find such so-called membrane proteins notoriously difficult to crystallize.

Up to a third of all proteins in human cells are membrane proteins. But of the 4,000 or so unique protein crystal structures scientists have already solved, only 30 to 35 (fewer than 1 percent) are from membrane proteins. When the first membrane protein structure was solved in 1985, the feat was considered so remarkable that the researchers who accomplished it were awarded the Nobel Prize.

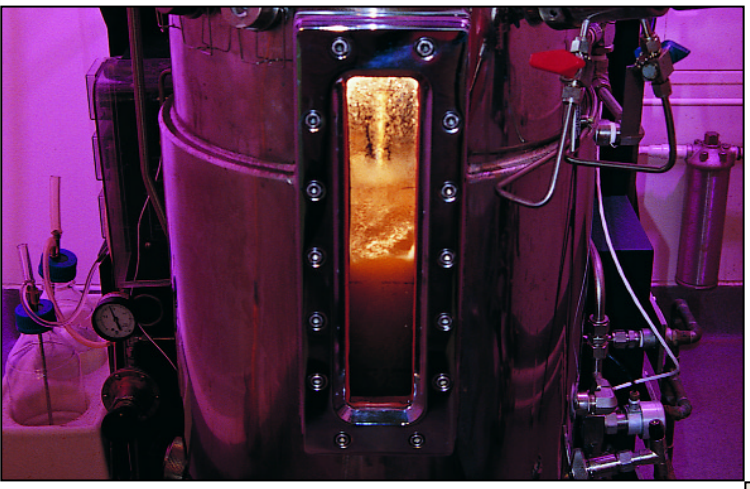
Membranes are essential cellular fixtures. They surround each living cell with a thin layer that helps protect the cell's chemical environment, seals in cellular structures, and keeps out foreign molecules and microbes. Membrane proteins, which poke through membranes and protrude from either one or both sides, serve as the primary way cells receive chemical, electrical, and mechanical messages.

Membrane proteins allow our bodies to transmit brain signals, regulate blood clotting, control certain immune system responses, and carry out a wide variety of other essential processes. Some membrane proteins serve as cellular security guards, others work like shipping companies

that accept and repel various molecules. Still others function as energy generation factories or as telecommunications hubs, sending and receiving cellular messages.

Membrane proteins are so difficult to crystallize because they must be wrestled out of their oily environment and forced to dissolve in a water-based crystallography solution. To do this, scientists like Chang use detergents that—like household soaps designed to dissolve grease—bridge the biochemical gap between oil and water.

For every membrane protein they study, Chang's group must first tease out the precise set of conditions—detergent type, temperature, acidity, salt concentration, and the like—that best favors crystallization. Sometimes it takes several thousand different combinations of factors and months of waiting to find one that works well.



Alan McPhee

Genetically engineered bacteria bubble and swirl as they grow in large batches in a pressurized, stainless-steel fermenting vat housed in the middle of Chang's lab.

Not only is Chang soaking the proteins in hundreds or thousands of different biochemical baths, but he and the members of his lab are doing this for dozens of proteins—each a slight genetic variation of the others. The goal is to coddle out at least one good crystal that they can use to examine the protein's innards in excruciating detail.

This systematic, multi-pronged approach isn't new, but many researchers don't implement it, Chang says. He considers the method a secret to his own success. "That's how in the year and a half I've been at Scripps, our lab has been able to come up with membrane protein crystals, while other labs go for years without crystals. It also makes life a lot busier, because 1 project becomes 30."

Chang thrives at this bustling pace. In fact, he purposefully chose such a challenging scientific area to study. "I always wanted to pursue a technically difficult field and also a very interesting topic," he says. "I chose membrane protein crystallography because there are not a lot of membrane protein structures. Also, I was fascinated with membranes. All of life's chemistry occurs within the bounds of membranes."

"I enjoy my work a lot," he says. "The day passes really quickly. I blink once and it's noon. I blink again and it's 6:00 p.m."

Chang often arrives in his lab before 8:00 a.m. and doesn't leave until 10 or 12 hours later. In addition to his long work hours and musical pursuits, Chang spends much of his remaining time at his local Chinese evangelical church. "I'm involved in children's church there, and I also help out with the junior high group on Friday nights," Chang says. "Church is a pretty big part of my life."

From Computers to Crystals

Chang's interests in membrane protein crystallography and drug resistance developed over time. He started out with a focus on computer science. He first got interested in X-ray crystallography as a sophomore in college when he worked in the University of Pennsylvania lab of crystallographer Mitchell Lewis. At Penn, Chang earned B.S., M.S., and Ph.D. degrees, all in biophysics.

"Geoff was interested initially in using computational methods to solve biological problems," Lewis says. "He made some major contributions to the field—a lot of the computational tools he developed are being used widely by crystallographers."

"When I first heard of crystallography, I had no idea what it was," Chang admits.

But before long, Chang became intrigued with the science of crystallography and worked with others in Lewis' lab to solve the structure of a protein involved in sugar metabolism in bacteria.

Lewis was so impressed with Chang that he recommended him to a good friend, Douglas Rees, a crystallographer at the California Institute of Technology in Pasadena, who specializes in membrane proteins. While still a Ph.D. student, Chang worked in Rees' lab for several months.

Heading west after being raised in Delaware, Chang hoped he might experience first-hand a California earthquake. One day a few weeks after he arrived, the ground began to shake and Chang's wish was granted.

"It didn't seem to faze him much, " says Rees, and Chang decided to stay.

He joined Rees' lab as a postdoctoral researcher. There, Chang completed his transformation from computer scientist into molecular biologist and biophysicist.

"What's impressive about Geoff is that once he started discovering molecular biology, he showed the same intensity and effort that he had with computers," Rees says. "He put in an amazing amount of work. I still don't know how he was able to get all the stuff done that he was able to get done."

According to Chang, working in a lab early on got him interested and excited about research, and he can offer some advice to others considering following a similar path.

"Get into a lab as soon as possible, preferably one that's not large, but one in which you can sit down with the advisor," he says.

Chang also stresses the importance of taking chances.

"Learn to take risks, go for challenging topics, and don't be afraid of them," he says.

Alan McPhee

It's cold even in southern California when you have to look for microscopic crystals in thousands of different solutions, all stored in a walk-in refrigerator.

A Brother's Inspiration

Despite his own hard work, accomplishments, and honors, Chang readily defers to his younger brother's achievements. At 20, Randy Chang is an accomplished pianist who plays in concerts and television fundraisers. Randy is also an inspiring public speaker, and he is about to graduate from high school at the top of his class.

"From a normal high school," stresses Geoffrey Chang, almost incredulously. Randy is legally blind and has Down syndrome, which can cause mental retardation and other health problems.

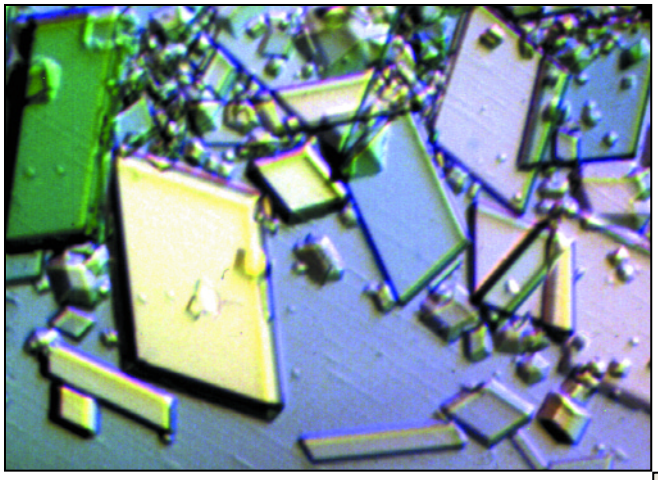
"My younger brother is one of my greatest inspirations," says Chang, who keeps a framed picture of Randy on his computer. "I look at what [he's been given], and most of us would perceive it as very little. If I think of what [I've been given] and where I should be if I extrapolate … It really makes me think."

Meanwhile, by focusing his expertise on solving the structure of membrane proteins related to drug resistance, Chang is not only blazing into new scientific territory, but he is also addressing a critical public health need.

He is excited by the multifaceted aspects of his work.

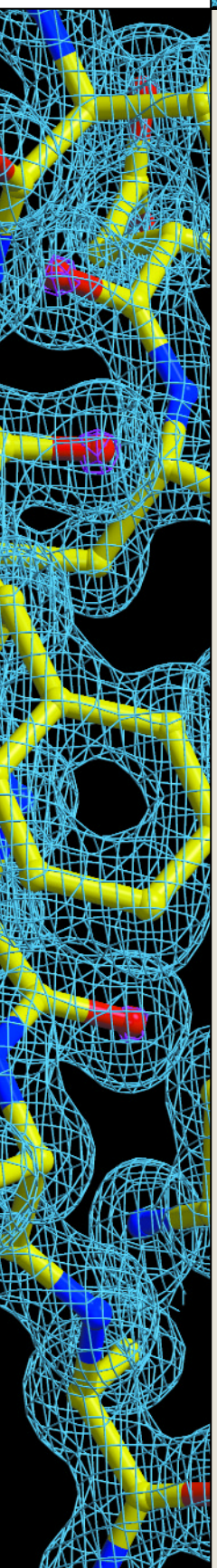
"It's a great problem to work on," Chang says. "On the one hand, you have an important medical problem: drug resistance. … On the other hand, you have a great scientific angle: membrane proteins. They're difficult to work with and challenging to crystallize."

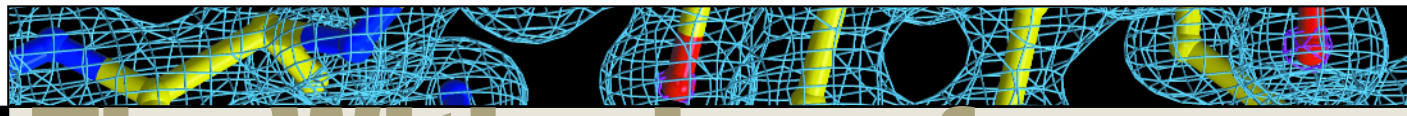
"It has a great medical aspect, a great scientific aspect, and [it will be] a great story in the end," he predicts.



Alex McPherson

Chemically coaxing membrane proteins to form precisely ordered crystals is one of the most difficult aspects of Chang's work.





The Withering of "Miracle Cures"

One of medicine's greatest triumphs—the development of antibiotics—is steadily growing into one of medicine's greatest fears: that the infectious diseases easily vanquished decades ago will be as deadly to our grandchildren as they were to our grandparents.

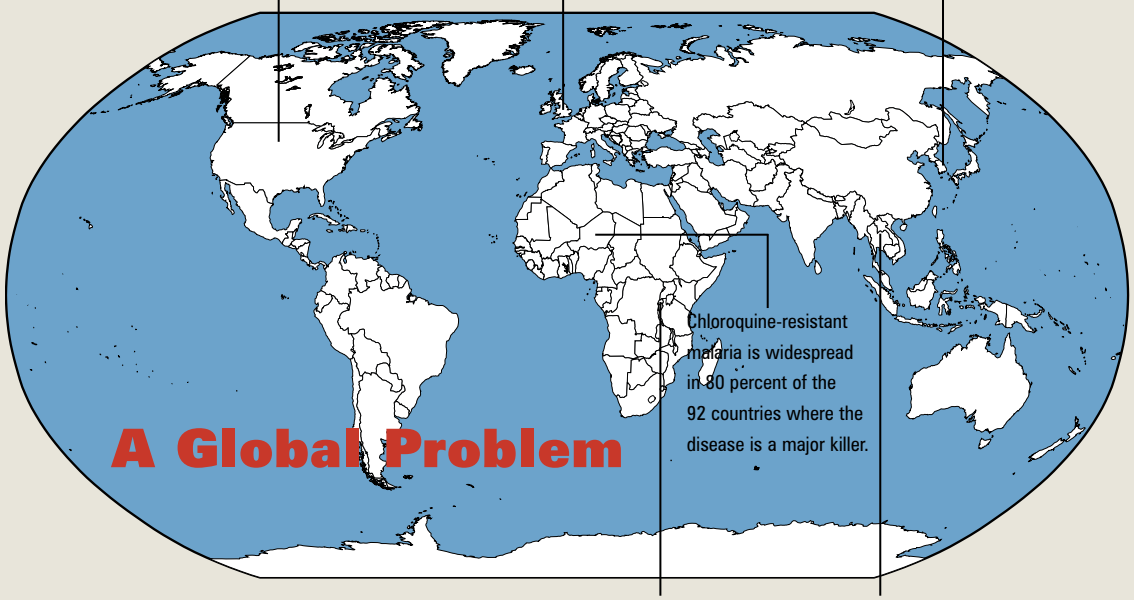
Once easily treated with antibiotics, major killers worldwide such as pneumonia, malaria, tuberculosis, cholera, and gonorrhea are progressively defying all treatment options. And with the ease of international travel, a drug-resistant microbe originating in another continent can arrive on U.S. shores within 24 hours.

In its June 2000 report "Overcoming Antimicrobial Resistance" (www.who.int/infectious-disease-report/2000), the World Health Organization states: "[Drug resistance] is a natural, unstoppable phenomenon exacerbated by the abuse, overuse, and misuse of antimicrobials. … Our challenge is to slow the rate at which resistance develops and spreads." —*A.Z.M.*

A few years ago, hospital workers in the U.S. detected strains of *Staphylococcus aureus*—"staph," the No. 1 cause of hospital-acquired infections—that are resistant to every known antibiotic medicine.

In hospitals in the United Kingdom, resistance of staph infections to the antibiotic methicillin rose from less than 2 percent in 1990 to more than 30 percent in 1997.

More than 70 percent of pneumonia cases in South Korean hospitals are resistant to the antibiotic penicillin.



Chloroquine-resistant malaria is widespread in 80 percent of the 92 countries where the disease is a major killer.

Even 5 years ago, 100 percent of cholera in Rwanda was resistant to two major antibiotics.

In most of Southeast Asia, 98 percent of all gonorrhea cases are multidrug resistant.

All data taken from the WHO Report "Overcoming Antimicrobial Resistance" (June 12, 2000).

A Great Div

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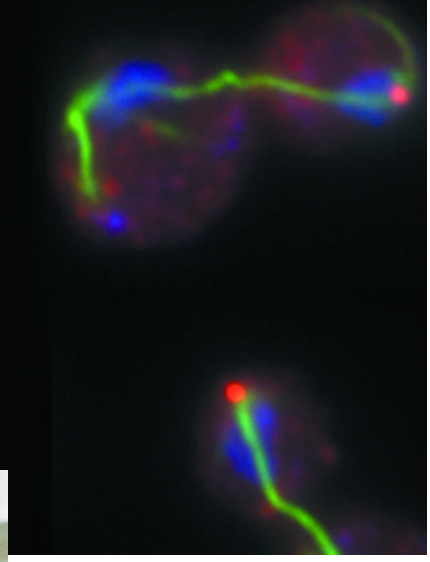


Allison Bardin and Anupama Seshan

L. Barry Hetherington

"Ever since I can remember, I wanted to study biology."

By Alison Davis

It was 1928, and a young Scottish scientist named Alexander Fleming had just returned to his London lab from a 2-week vacation. Fleming's nose pointed him straight to his lab bench, where he found a culture dish of bacteria he had forgotten to throw out before he left.

As luck would have it, the weather in London had been unexpectedly cool and damp, permitting a clump of blue-green mold to gain a foothold on the dish.

Just before pitching it, Fleming saw that a ring of bacteria encircling the mold had mysteriously vanished. He had a flash of insight, reasoning that something in the mold had probably killed off the bacteria surrounding it.

From this story, you may recognize that fuzz: a fungus that produces penicillin, the first antibiotic medicine.

"Fleming didn't set out to cure humankind, but by doing his own thing he helped humankind enormously," says Angelika Amon, 34, a biologist at the Massachusetts Institute of Technology in Cambridge.

Three-quarters of a century later, Amon is hopeful that her quest for fundamental knowledge about how cells work will also turn up medically useful insights.

"I sincerely believe that my own basic curiosity will someday help people," she says.

And while she acknowledges that luck is part of success in science, Amon knows that hard work is key.

Kim Nasmyth, Amon's advisor during her Ph.D. studies at the University of Vienna in Austria, remembers Amon's dogged persistence after her first project failed, "through no fault of her own," he says.

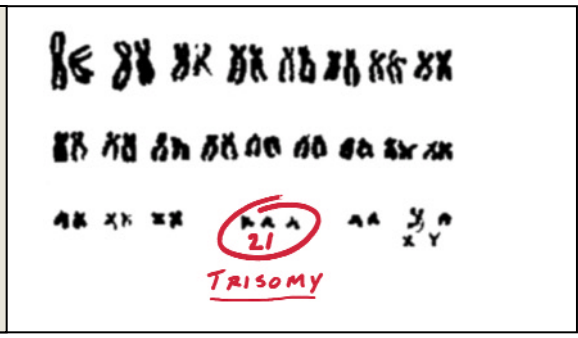
"I think Angelika learned very early on that one cannot count on being lucky, but that success only comes as a result of a commitment to asking the right questions," Nasmyth says. "She was fearless, ambitious, and brave—and above all, fun to talk to."

Beyond her genuine "need to know," as she calls it, Amon looks at statistics on the incidence of birth defects like Down syndrome and sees a social calling. She says that basic research into how cells grow and divide will make important inroads toward understanding the problem of birth defects.

Angelika Amon (left) is a molecular biologist at the Massachusetts Institute of Technology in Cambridge, Massachusetts.

Inside yeast cells (top), fluorescently tagged proteins glow when viewed under the lens of a special type of microscope.

For example, Amon explains that one reason it is harder for an older woman to get pregnant is not due to trouble conceiving, but rather to problems in distributing genetic material (DNA) to dividing fetal cells. This problem is called chromosomal missegregation. It underlies the high chance—1 in 63—that a 40-year old woman who becomes pregnant will carry a fetus with a birth defect called "trisomy."



Trisomy, the hallmark of Down syndrome, results when a baby is born with three copies of chromosome 21 instead of the usual two.

Trisomy, the hallmark of Down syndrome, is one outcome of having chromosomes in the wrong place. Just before a cell divides, it copies and then divvies up its DNA. The DNA is contained in packages called chromosomes, and each cell is supposed to have 23 pairs of them. The chromosomes are tucked away inside a compartment in the middle of the cell called the nucleus.

In trisomy, what happens is that too much DNA goes to one daughter cell, causing it to have three of a given chromosome instead of the usual two.

"How does this happen?" Amon wonders. She is also curious to know how chromosome segregation happens right almost all the time. Knowing that will help her and other scientists figure out what causes cells to divide wrong, leading to birth defects or diseases like cancer.

Simple Things

It's barely 6:00 a.m., but Amon is already busy at work, scanning e-mail messages, planning experiments and lectures, and poring over data. After some quiet time to herself, she walks around and talks to her students and postdoctoral researchers, catching up on what's happening in the lab. She is an outgoing person, and members of her lab say they love working with her.

"[Angelika] really likes the idea of supporting young scientists, especially women," says Susanne Prinz, a postdoctoral researcher in Amon's lab. "She is very open and down to earth, with a good sense of humor. She's full of energy and enthusiasm for science."

Amon loves what she does.

"Ever since I can remember, I wanted to study biology," she says. "I love being a scientist."

Growing up as one of four children in Vienna, Amon remembers how her parents were always pointing out ordinary, but interesting things.

"Really simple things," she says, "like when you're hiking up a mountain, you notice that the trees get smaller. Why is that?"

"It's not like my parents sat down and did experiments with us or anything," she says. "They just encouraged us to wonder."

Amon says wondering about nature prepared her mind for more complex inquiries about biology.

Scientists like her who study how cells work often turn to simple organisms like yeast or fruit flies. These organisms are easy and relatively inexpensive to breed for experiments. And fortunately, their chemistry is much like ours when it comes to fundamental processes like cell division.

For her studies, Amon uses a species of yeast called *Saccharomyces cerevisiae*, abbreviated *S. cerevisiae*. This is the kind of fungus that consumes sugar in dough and produces carbon dioxide as a byproduct, causing bread to rise into a fluffy loaf. Yeast cells are also responsible for fermenting sugar into ethanol, the alcohol in beer and wine.

According to Amon, the way human cells divide is very similar to how yeast cells divide. But key differences justify using a simple fungus model to study complicated puzzles of human biology. Take the fact that while the average human cell needs about a day to copy its DNA, ready itself for making two daughter cells, then finally split, *S. cerevisiae* cells do all of this in an hour and a half. Since researchers have to repeat experiments many times to check their results, waiting around for cells to divide can be very time-consuming.

The Cycle of Life

Amon's research on cell division hinges on the never-ending continuum that life scientists call the cell cycle. Your body produces 210 different types of new cells constantly throughout life—to regenerate skin, to make new blood, and to fight off infections. Your cells are not made from scratch, but rather they are generated from existing cells that split in two. This process is carefully orchestrated so that each daughter cell contains just the right amount of cell constituents.

A growing child and a healthy adult have this cycle in common. Most of the cells in your body divide constantly throughout life. This ongoing regeneration makes up for losses due to the normal wear and tear of living. In fact, just to keep things running smoothly, an adult human body manufactures millions of new cells every second of every day. Correctly copying DNA and dividing it up evenly into daughter cells is a critical feature of healthy cells.

A properly functioning cell cycle guarantees that this will be done right and that catastrophes like cancer won't happen.

The cell cycle consists of four phases, and passage through each phase is stringently controlled. This assures that everything is on track and no errors have been made.

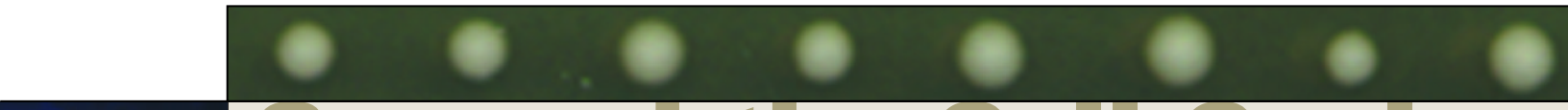
The cell completes a different task during each of the four phases of the cell cycle. These phases are called G1, S, G2, and M. Growth and cell maintenance take place during G1, S, and G2. Not surprisingly, much of a cell's life is spent here, and it is during this time that the cell doubles its contents. All cells except eggs and sperm (see sidebar) grow and divide this way.

During S phase, the cell copies its most precious cargo, DNA. The body's mechanism for copying DNA is remarkably accurate. An error occurs only once in duplicating 10 billion nucleotides, the chemical units of DNA. An elaborate repair system quickly fixes most errors that do occur.

Finally, during the M phase of the cycle (M stands for mitosis), a cell cuts itself in two, distributing roughly half of all its contents—and *exactly* half of its DNA—to two daughter cells.

This is what happens in human cells. The *S. cerevisiae* yeast cells Amon uses for her research bud off into a mother cell and a daughter cell, rather than splitting straight down the middle. However, according to Amon, most of the underlying molecular events are very similar. This allows her and thousands of other scientists around the world to study the cell cycle by using quick-dividing yeast cells.

Of course, scientists cannot take anything for granted. Once Amon has compiled enough convincing evidence from experiments with yeast cells, she will have to go on



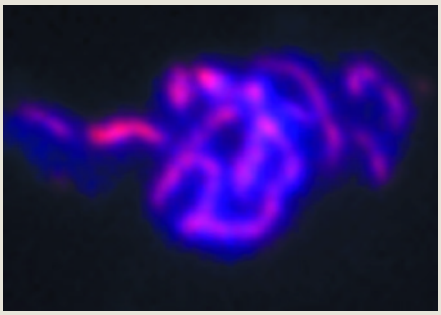
Sex and the Cell Cycle

After a fateful rendezvous between egg and sperm, the cells unite, then divide in two, and each progeny cell gets a set of marching orders. Each is told to divide in two again, passing on to its daughter cells a precise copy of the parent cell's genetic material. Throughout the lifespan of an organism, this cycle is repeated billions of times. Amazingly, every cell on the planet has an unbroken lineage that goes back three and a half billion years.

But egg and sperm cells are different from other cells in one very important way. These so-called sex cells have only half the amount of DNA as the other 208 cell types in the human body.

Why? It's a simple math problem if you think about it.

Human cells have 2 pairs of 23 chromosomes each, so the union of egg and sperm creates a cell with the right number of chromosomes (46). During fertilization, the DNA-containing chromosomes in a sperm cell mingle with the chromosomes in an egg cell, exchanging stretches of DNA called genes. This is the process that creates a unique individual, so that the offspring receives some genes from each parent.



Brian Lee

Eggs and sperm are the only cells to participate in a special cell cycle called meiosis. This type of cell cycle involves two successive sets of nuclear divisions to ensure that sex cells contain precisely half the amount of DNA present in other cell types—skin cells, immune cells, or the cells that line your stomach, for example.

These other body cells go through mitosis, which is the process of duplicating chromosomes in preparation for producing genetically identical daughter cells. —*A.D.*

to prove that her findings hold up in so-called "higher organisms" like fruit flies. Higher organisms are more similar to humans because they are constructed in an organized fashion from many different cell types.

Around and Around

How does any species of cell keep its cycle in tune? According to Amon, all cell types have an elaborate system of checks and balances.

"If a cell really cares about something, it has three or four ways to make sure it's done right," Amon says. "That tells you that it's really important."

For example, she says, if a cell mistakenly got to mitosis too soon, "You'd get one empty bag with no nucleus and two nuclei in the other." Since a cell's manufacturing manual—its DNA—is packaged inside its nucleus, such a situation would cause the daughter cells to die.

If a cell exerts all this energy to grow but it divides at the wrong time, then "everything's in vain," Amon says. "You simply can't build an organism this way."

Some of the most important regulators of the cell cycle are proteins called cyclins. These molecules work together like gears to propel the cycle forward through each of the four phases. A cell controls the levels of its cyclins through a cellular garbage disposal process called proteolysis, chewing the proteins up when they are no longer needed.

The wax and wane of cyclins and their partner molecules, called cyclin-dependent kinases (CDKs), keep the cycle going around and around.

Amon explains that the process of applying and removing tiny chemical tags called phosphates turns on or off both cyclins and CDKs, as well as many other molecules that keep the cycle running and in tune. Kinases put on phosphates, and phosphatases take them off.

In yeast cells, one such phosphatase molecule, a protein called Cdc14, is the "master regulator" that directs cells to get out of mitosis and get on with the business of splitting.

By releasing the brakes on the yeast cell cycle, Cdc14 kick-starts the cell to get past mitosis, so it can finally divide (a process called cytokinesis). Amon and other researchers figured out that Cdc14 is a key player in promoting this final step in the cell cycle. They discovered this because getting rid of the yeast gene that directs the cell to make Cdc14 makes those cells freeze in the late stages of mitosis, preventing them from finishing their job of dividing into two cells.

L. Barry Hetherington

Amon's lab members line up for a photo (left to right: Rosella Visintin, Anupama Seshan, Monica Boselli, Molly Saweikis, Susanne Prinz, Brian Lee, Adele Marston, Amon, Nicholas Hausman, and Frank Stegmeier).

But figuring out *how* Cdc14 accomplishes this was not so easy. Amon and her postdoctoral researcher Rosella Visintin performed a variety of test-tube experiments hoping to get a hint at how Cdc14 did its magic. Nothing was working, Amon recalls, and "we were getting desperate."

Sleuthing the Banana

Running out of options, Visintin suggested that they take a look at where Cdc14 lives inside the cell. Sometimes, where a protein hangs out in the cell can provide a clue to what it does, since cells have specialized compartments dedicated to carrying out their many different functions. Using glow-in-the-dark molecular tags and a microscope, the two researchers canvassed the yeast cells' interior landscape, hunting for signs of Cdc14. They found it in a banana-shaped object inside the nucleus.

As with much of science, the results were not immediately clear, and Amon and Visintin looked at each other asking the same question:

"What's the banana?"

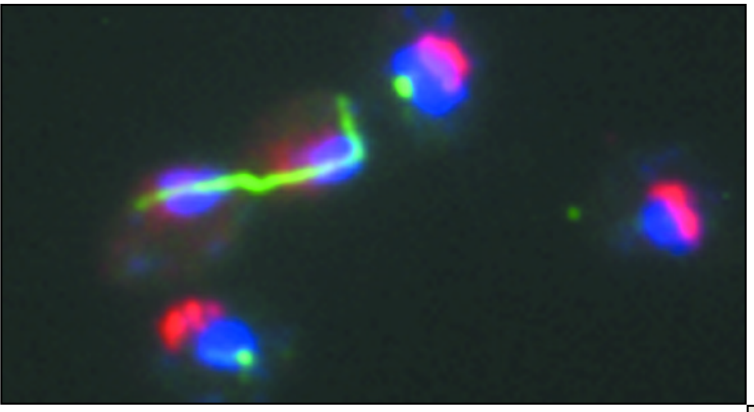
More studies revealed that the banana was a part of a tiny place inside the nucleus called the nucleolus.

The nucleolus is extremely important to a cell's livelihood. This cellular compartment plays a critical role in cell maintenance. It is the place where nearly all of the cell's ribosomes (the biological machines that manufacture proteins) are made.

Things began to get interesting when the yeast cells tipped Amon and Visintin off to an important clue. Amon noticed that Cdc14 hides inside the banana at all times during the cell cycle except for one time: just before the end of mitosis.

Then, they discovered, the molecule rushes out, flooding the entire cell. Amon determined that Cdc14 sends a signal to release the cell cycle gates, which allows the cell to proceed past mitosis and begin the process of splitting. This piece of news was encouraging, but Amon needed more convincing.

To find out whether Cdc14's trips into and out of the nucleolus really matter that much to the cell, Amon played a molecular biology trick. She forced the cell to produce gobs of the Cdc14 protein. In such a situation, called overexpression, normal cellular rules no longer apply. The excess protein leaks out of the nucleolus and goes virtually everywhere in the cell.



Rosella Visintin

During most of the cell cycle, the Cdc14 protein (pink) lives inside a banana-shaped compartment of a yeast cell's nucleus (blue). When the nucleus splits, Cdc14 floods the cell, pushing the cell cycle forward.

The consequences of overexpressing Cdc14 pointed to the extreme importance of having Cdc14 in the right place at the right time in the cell.

"The cells died," says Amon.

The Nucleolus Prison

Amon's studies are groundbreaking in that they bring to light an entirely new function for the nucleolus. Her studies, and those of an increasing number of other scientists, are beginning to paint a picture of the nucleolus as a cellular prison—a place to keep powerful cannonballs like Cdc14 out of the cellular battlefield where their actions, if gone unchecked, could wreak cellular havoc.

Already, scientists are finding other proteins important for cell cycle function to be prisoners of the nucleolus as well. Amon suspects that this role could be as important for the nucleolus as its "day job" of manufacturing proteins.

Amon's research detour into the nucleolus has landed her lots of interesting data and has provoked intrigue from other scientists. She can think of many more experiments to do.

But the cell cycle is calling her back, Amon says. She is eager to continue trying to crack the puzzle of how chromosomes "know how" to segregate correctly nearly every time. Eventually, Amon wants to move at least some of her research toward issues related to how embryos develop, an area she visited briefly while a postdoctoral researcher in fruit fly geneticist Ruth Lehmann's lab at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts.

Shortly after Amon arrived in Lehmann's lab, Lehmann accepted an offer to move to New York University. Recognizing Amon's talent, Lehmann says, Whitehead director Gerald Fink offered her a spot as a Whitehead Fellow, a coveted and privileged position offered to only a handful of graduate students. The position is unique in that it allows students to skip the postdoctoral training period and instead run a small, independent lab without the pressures of obtaining grant funding or teaching. In Amon's words, the fellowship was "the best thing that ever happened to me."

"She is extremely smart and so driven," says Lehmann, who adds that Amon quickly developed a strong research program in a very competitive environment.

Building on her past experiences with Lehmann, Amon is now starting some studies with fruit flies, which have body parts like people do. Fruit flies can be used to study developmental topics such as body symmetry, another process that hinges on precision cell cycling.

Amon glances at the clock, and it's time to go. Today, just like every other day at 3:30 p.m., she is out the door to spend the rest of the day with her 3-year-old daughter Theresa.

Some days, Amon is just another mom at the playground cleaning up Theresa after her sandbox excavations. But every day, Amon's intense drive as a scientist pushes her forward to ask new questions about biology and look for new ways to answer those important questions.

Says former advisor Nasmyth, "Science runs in her blood."

Old Drugs Learn New Tricks

Patients and doctors alike benefit when existing drugs find new uses. Such is the case for a group of medicines that are approved to treat osteoporosis and other bone ailments. NIGMS grantee **Eric Oldfield** of the University of Illinois discovered that the drugs may also be useful for treating malaria, sleeping sickness, and an AIDS-related infection called toxoplasmosis. This is welcome news because scientists have not yet succeeded in developing vaccines against these parasitic infections, which collectively affect 3 billion people worldwide. According to the World Health Organization, malaria alone kills more than 1 million people per year across the globe, and a child dies of malaria every 30 seconds. Oldfield and his colleagues investigated alternate uses for several so-called "bisphosphonate" drugs including Fosamax®, Actonel®, and Aredia®. Previous research by these scientists had hinted that the active ingredient in these medicines blocks a key step in parasite metabolism. To see whether this was true, the researchers gave the medicines to parasites cultured in plastic lab dishes. The researchers found that fairly low concentrations of the drugs killed the parasites, while sparing human cells.

WHO/TDR/Stammers

Post-Trauma Infection is More Common in Men

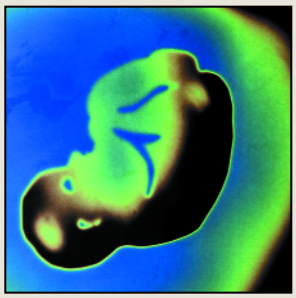
NIGMS grantee **Patrick Offner** of the University of Colorado has discovered that men are much more likely than women to develop infections after trauma. Offner and his colleagues tracked 545 patients who had suffered serious trauma and had been admitted during a 5-year period to the Denver Health Medical Center intensive care unit. The researchers followed the patients—135 women and 410 men—from hospital admission to discharge or death, recording major infections such as pneumonia, abdominal or pelvic abscesses, and wound infections. Overall, their study revealed that men were 58 percent more likely to develop a major infection after trauma. The scientists suspect that the disparity might be caused by hormones, possibly testosterone. Further studies are ongoing to test this notion.

Exhausted T-Cells Spell Trouble in Arthritis

Rheumatoid arthritis is a debilitating condition affecting nearly 1 percent of the U.S. population—approximately 2.1 million people. The chronic condition causes inflammation of joint linings, usually of the hands and feet. In addition to swelling, the inflammation leads to pain, stiffness, and deformity. With time, rheumatoid arthritis can irreversibly destroy the tendons, cartilage, and bone that make up a joint. Doctors have long believed that the inflammation was due to an attack by the patient's own immune system on his or her joints. So the standard treatment for the disease involves knocking back the patient's immune system, even though the medicines used for this purpose can put patients at greater risk for infections and heart disease, the two leading causes of death in people with rheumatoid arthritis. These treatment practices may need to be revisited, according to a recent study by NIGMS grantee **Jörg J. Goronzy** of the Mayo Clinic in Rochester, Minnesota. Goronzy and his coworkers found that instead of having overactive immune systems, patients with rheumatoid arthritis appear to have exhausted, prematurely aged immune systems. The scientists examined immune cells called T-cells in 51 patients with rheumatoid arthritis and 47 people of a similar age with no arthritis. The researchers found that the 20- to 30-year-old arthritis patients' T-cells looked like the T-cells of people without arthritis who were 50 to 60 years old. The DNA-containing chromosomes in these patients' T-cells had worn-out ends, the researchers found, and the extent of the frayed ends correlated with the severity of the patients' disease.

Artificial Placenta May Help Test Drugs During Pregnancy

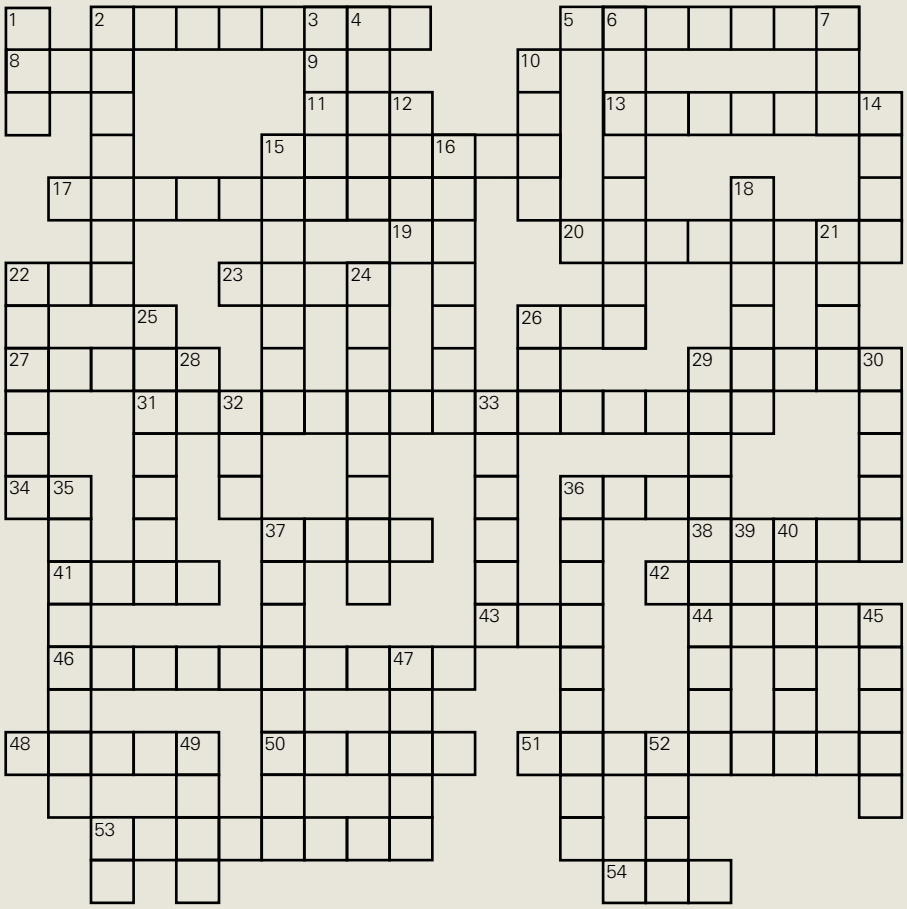
Scientists have figured out a way to grow an artificial placenta, which may help researchers learn which drugs can be given safely during pregnancy. Inside a woman's uterus, the placenta is a complicated matrix of finger-like cell structures that nourishes and protects a developing baby. NIGMS grantee **Douglas Kniss**, an obstetrician and researcher at the Ohio State University Medical Center, worked with a team of physicians, biologists, and engineers to get "trophoblast" cells from a real placenta to grow in a lab chamber called a bioreactor. To create the placenta, the researchers first designed a non-woven, polyethylene (Dacron™) fabric on which the placental cells could survive and get the proper mix of nutrients from the circulating culture fluid. Kniss and his coworkers discovered that by heating the fabric under pressure, they could create a material with just the right number of correctly sized holes, or pores, through which the placental cells could grow and spread into a three-dimensional placenta-like structure. The artificial placenta is expected to help scientists sort out which drugs are safe in pregnancy, since currently there is no good way to test medicines for safety and effectiveness in pregnant women. Kniss, whose two sons were born prematurely, hopes the research will also help scientists understand what causes pre-term births.



New Test Uncovers Lupus

Extreme fatigue, unexplained fever, painful and swollen joints, and skin rashes may sound like a laundry list of ill-defined symptoms. But these are the hallmark signs of lupus, a disease that affects approximately 1.4 million Americans. These symptoms can range from mild to severe and come and go with time, making lupus very difficult to diagnose. Like rheumatoid arthritis, lupus is an "autoimmune" disease in which a person's immune system attacks his or her own cells. To help diagnose lupus, doctors sometimes order a set of blood tests that detect "autoantibodies"—immune system fighter molecules that react against genetic material (DNA or RNA) and other molecules in the cell's command center, the nucleus. However, about 20 percent of patients who are eventually diagnosed with lupus don't test positive using these tests. NIGMS grantee **Mark Roth** of the Fred Hutchinson Cancer Research Center in Seattle has devised a new lupus diagnostic tool that may help this group of patients. Roth, a cell biologist, had been investigating how RNA gets sliced up in the cell during the process of protein manufacturing by a group of proteins called SR splicing proteins. Roth noticed that antibodies to SR proteins were prevalent in cells of the majority of lupus patients, and he went on to develop a color-coded test that screens for the presence of these antibodies in a patient's blood sample. Underscoring the value of the test, of 36 lupus patients testing positive in Roth's test, only 31 percent would have been picked up by the previous blood test that detects other autoantibodies.

The Last Word



ACROSS

2. fetus nourisher
5. Geoff Chang's lab location
8. not him
9. either
11. ___teria
13. boy deer have them
15. sex cells do it
17. fungus wonder drug
19. toddler's favorite word
20. fast African animal
22. 2,000 pounds
23. basketball net
26. Angelika Amon's univ.
27. 20th-century music holder
29. cuts
31. determining protein structure from X-rays
34. opposite of 53 DOWN
36. seen on a farm
37. first step in eating
38. 3.3 feet
41. the majority
42. luxurious
43. ostrich-like bird
44. job for a hose
46. bacteria develop it over time
48. over opposite
50. get hold of
51. Cdc14 home
53. can be carried
54. snakelike fish

Puzzle answers can be found at
www.nigms.nih.gov/news/findings

DOWN

1. global health org.
2. worker molecule made by ribosomes
3. big science prize
4. path
6. Geoff Chang uses one to make music
7. after dear in a letter
10. have to
12. penny or dime
14. a few
15. most body cells do it before they split
16. underwater breathing tube
18. not so quickly
21. protein that repels antibiotics
22. serious injury
24. causes malaria or tuberculosis
25. cell compartment where DNA lives
26. coffee holder
28. spouse to Mrs.
29. DNA-containing package
30. go the right direction
32. me's companion
33. Baltimore's bird
35. oily cell envelope
36. up-close view of a protein
37. like viruses, they cause infections
39. ex. sens. percep.
40. delight
45. science tool; also makes dough rise
47. cells repeat it over and over
49. uncommon
52. where to find an earring
53. eve. time

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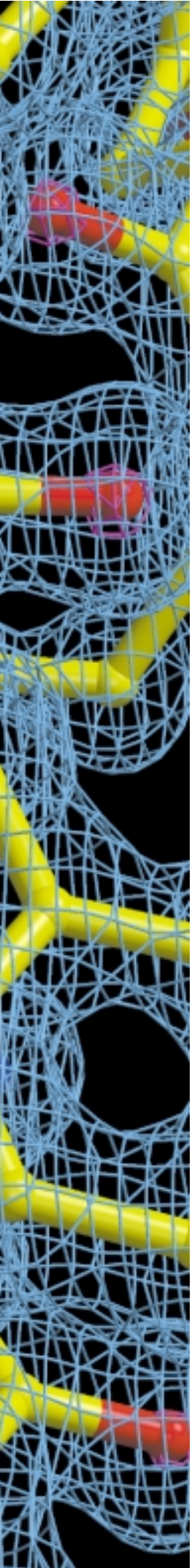

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