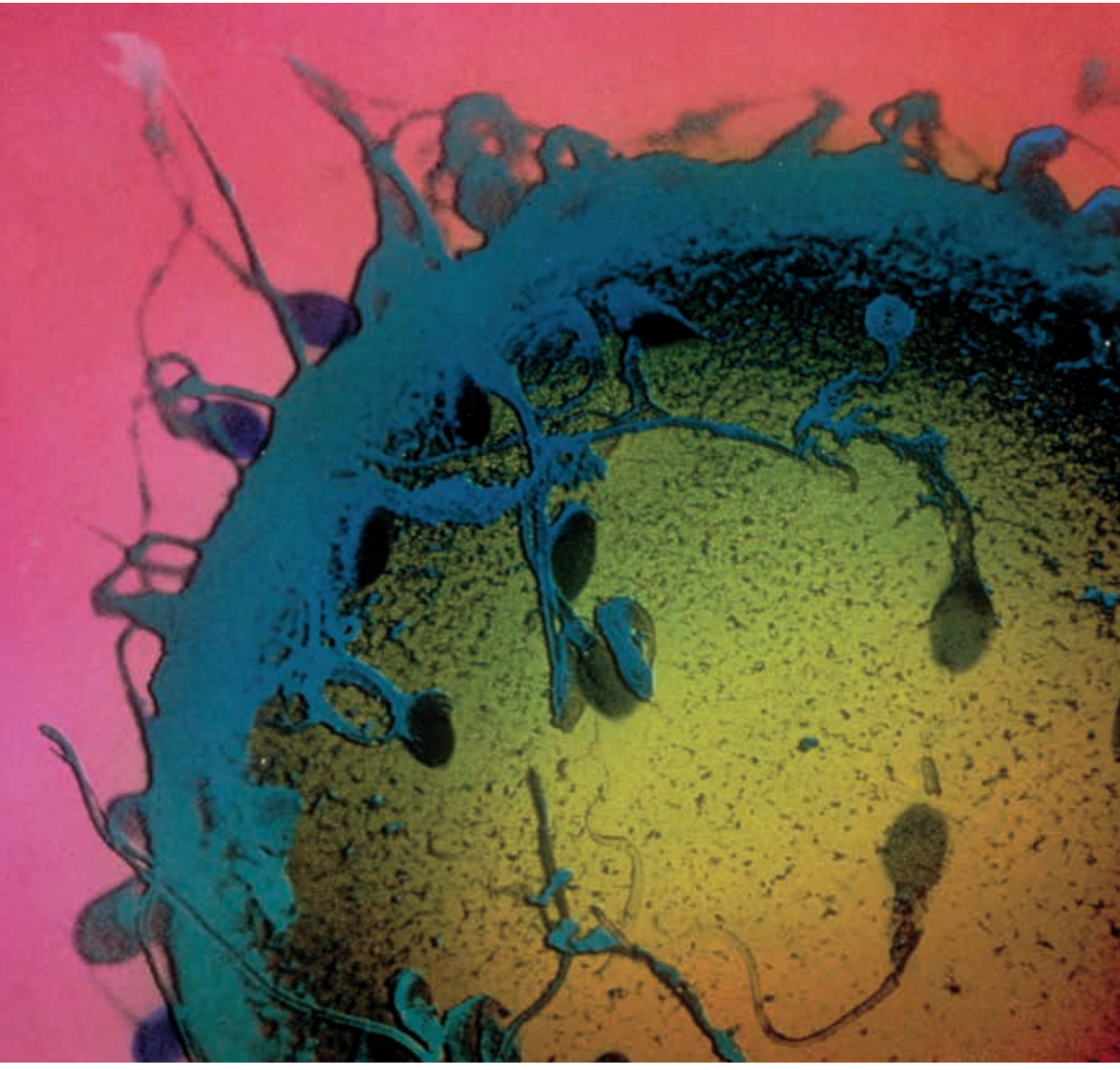
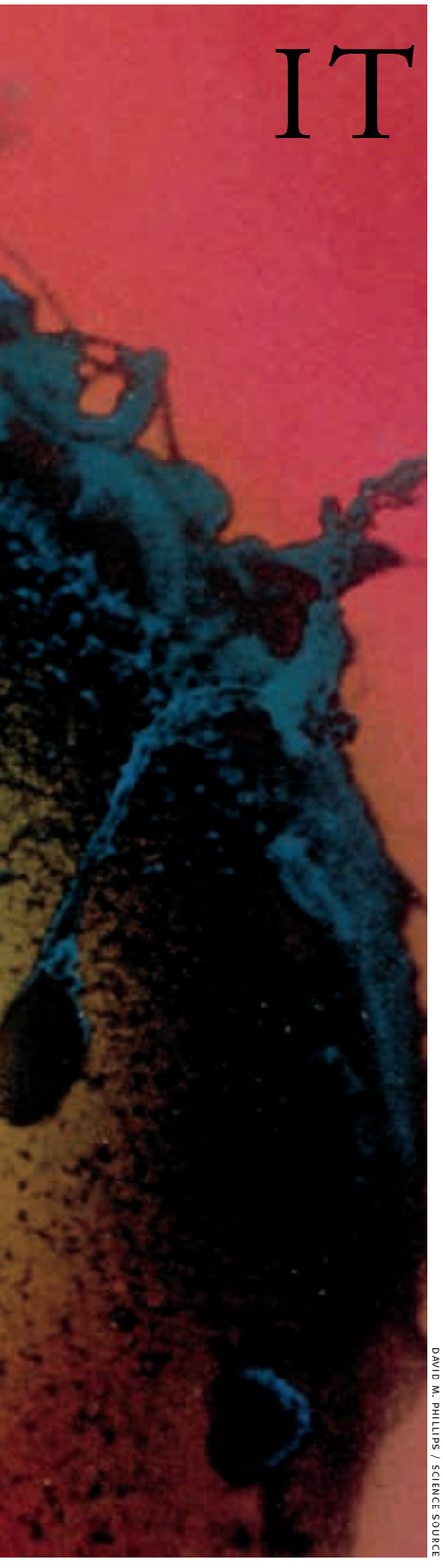
Explorations and revelations taking place in the medical school



Interacting reproductive proteins, from those of the eggs and sperm in humans (above) to those produced by certain sexually reproducing yeasts, evolve together. Nathan Clark is finding that the patterns of this process, called coevolution, could help scientists identify players in biochemical pathways much faster.



DAVID M. PHILLIPS / SCIENCE SOURCE

IT TAKES TWO TO COEVOLVE

LIFE LESSONS FROM THE BIRDS AND THE FLIES

BY ALLISON A. CURLEY

You might expect that millions of years of evolution would have perfected the process of fertilization. Actually, the interacting sperm and egg proteins are still works in progress, says Pitt's Nathan Clark, a PhD assistant professor of computational and systems biology. "As the egg protein changes, the sperm protein that binds to it has to keep up." This process, where one adaptively compensates for changes in the other, is known as coevolution.

Clark's recent insights on proteins and their dance partners could be a boon for biomedicine. He's showing that by studying coevolution patterns, it may be possible to sleuth out previously unknown players in a given biochemical pathway—or perhaps even predict gene function.

In a study published in February 2013 in *Genetics*, Clark compared the rates of evolution between 40 different species (18 yeasts and 22 mammals), concentrating on genes that regulate a process of cell division, called meiosis, which is involved in reproduction.

In some yeast species, reproductive methods have evolved so that meiosis is no longer essential for survival. Clark showed that the evolutionary rates of meiosis-related genes whose proteins had direct physical interactions coevolved, accelerating in a parallel, correlated fashion, leading to a loss of the now-unnecessary meiosis-related DNA sequences. The same was true of the genes for proteins that participated in the same biochemical pathway, even though they didn't directly interact.

Recently, Clark's methods came in handy in a collaboration with Cornell University's Mariana Wolfner, who studies reproductive processes in fruit fly models. Wolfner was interested in studying a protein called "the sex peptide," which gives male *Drosophila melanogaster* an advantage in fertilization. The protein, which is passed from the male to the female along with his sperm, makes her uninterested in mating with other flies for several days.

Seven genes had already been implicated in this behavior. To see whether the team might be able to expand on the list, Clark took a look at the 600 genes expressed in fly reproductive tissue and created a prioritized list of genes whose evolutionary rates changed in parallel with the original seven. A postdoc in the Wolfner lab, Geoff Findlay, then performed a series of experiments to confirm that, of the 18 candidate genes Clark identified, six were indeed part of the pathway. In just two years, the team nearly doubled the number of known genes involved in this behavior.

"A gene's rate of change over time, and who it's changing with, can tell you a lot about its function," says Clark. This approach can be used to discover the function of virtually any protein, so "it's a general recipe we want to try to repeat," he adds. In the future, Clark hopes to create a publicly accessible database into which researchers can input their proteins of interest and receive a list of potential other members of the same pathway.

A related branch of Clark's research examines the process of coevolution experimentally. He's altering the structure of NUP84, a protein that forms a specialized tunnel, or nuclear pore, that allows molecules to be transported in and out of a cell's nucleus. Clark transplanted the NUP84 gene from one yeast species (the donor) into a second, closely related yeast species (the recipient), replacing its native copy of the gene. Even though the change was very small—the protein sequence differs by only 5 percent—the cells containing the foreign NUP84 gene grew much slower than those with the gene from their own species. This stunted growth occurs because the donor NUP84 has not coevolved with the recipient partner proteins, Clark explains. "We've essentially broken this adaptive [coevolution] process," he says. In addition, because the donor and recipient species are each other's closest relatives, the slower growth rate "tells us that the process of coevolution is going on constantly," he adds. ■

SHOW AND TELOMERES

TYPOGRAPHY: ELENA GIALAMAS CERRI

ARTHRITIS IN KIDS LINKED TO PREMATURE IMMUNE AGING

BY DANA YATES

A swollen wrist, an inflamed knee, a stiff neck ... these sorts of playground injuries happen to youngsters all the time. But for children and adolescents with juvenile idiopathic arthritis (JIA), painful joints can point to a serious condition. A distinct form of arthritis that strikes people under age 17, JIA is a degenerative disease with no known cause. The most prevalent rheumatic condition in the world, affecting about 50,000 in the United States alone, JIA may involve one or more joints and can limit growth and lead to physical disfigurement.

Arthritis has commonly been seen as an old person's disease. But as one University of Pittsburgh scientist has found, there may be much more to that idea than researchers once thought—even in the case of JIA.

Abbe de Vallejo—associate professor of pediatrics and immunology, as well as a member of the Division of Pediatric Rheumatology at Children's Hospital of Pittsburgh of UPMC, the Cancer Immunology Program at the University of Pittsburgh Cancer Institute, and the Pitt–UPMC McGowan Institute for Regenerative Medicine—is senior author of the first study to show that premature aging is associated with the most common form of chronic inflammatory arthritis in children. In fact, the joints of children with JIA contain immune cells that look more like those of a 90-year-old than a 9-year-old. The findings, which were discovered by a team of researchers at Children's Hospital of Pittsburgh of UPMC, Pitt, and the Mayo Clinic, were published in the August issue of *Arthritis and Rheumatism.* Funding for the research was provided by the Nancy E. Taylor Foundation for Chronic Diseases, the Arthritis Foundation, and the National Institutes of Health.

Long considered an autoimmune disorder—a case of the body attacking its own tissues and cells—JIA is traditionally treated using broad-spectrum therapies that debilitate the entire immune system. But continued suppression of the body's natural defenses can lead to other health concerns, including infection, lymphoma, and reactivated tuberculosis. That cascade led de Vallejo to wonder whether the conventional, scorched-earth approach to treating JIA might not be optimal.

"The immune system is an army," he says. "Why paralyze the whole army when you may just need to maintain the good soldiers and retire the old ones?"

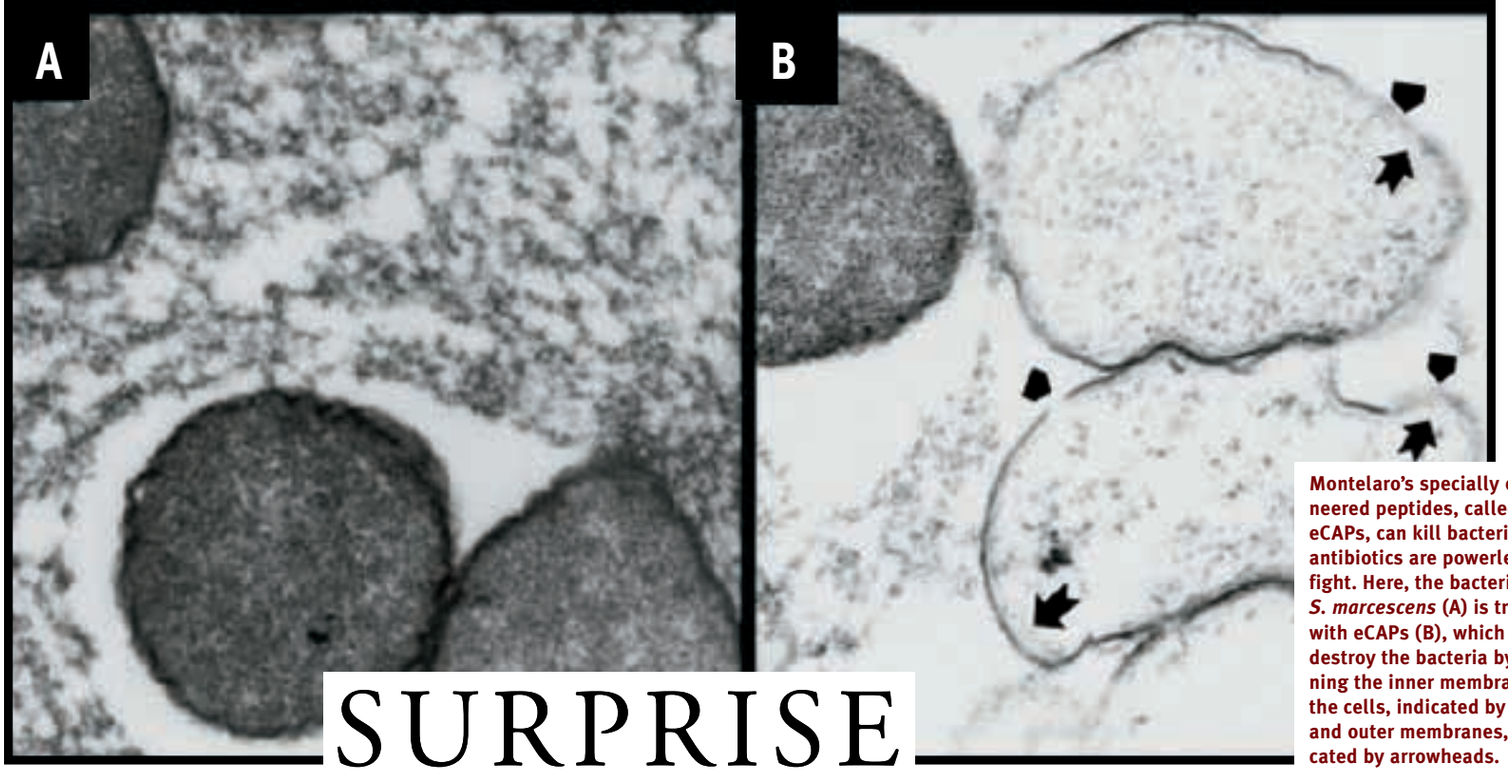
His previous studies involving young adults with rheumatoid arthritis have shown that some cells in the blood and joint synovial fluid appeared to be undergoing abnormal cell division and premature aging. In the latest project at Children's, de Vallejo was curious whether the earlier finding would hold true in pediatric arthritis.

The study focused on T cells, a type of immune cell that helps the body fight infection, disease, and other harmful agents. Specifically, the researchers investigated the T cells in the synovial fluid and blood from 98 children with JIA, as well as 46 blood samples from children who didn't have the disease.

One-third of the T cells of the children with JIA were found to be abnormal—and notably, their difference points to premature aging rather than autoimmune activity. Specifically, the T cells had shortened the protective caps on the ends of chromosomes, and thereby lost the ability to multiply. These chromosomal caps, called telomeres, prevent chromosomes from fraying. Every time a cell divides, the telomeres shorten. It's believed that aging happens when telomeres become too short to enable cell division.

Additionally, the T cells had begun to act in unusual ways, and their actions could be stimulated through atypical cell-surface receptors. While more studies are needed to understand why exactly that process occurs, one thing is certain right now, says de Vallejo.

"We need new ways of thinking about JIA. We also need to develop a new generation of selective drugs—ones that target and prevent premature aging of immune cells," he says. ■



COURTESY RONALD MONTELARO LAB

Montelaro's specially engineered peptides, called eCAPs, can kill bacteria that antibiotics are powerless to fight. Here, the bacterium *S. marcescens* (A) is treated with eCAPs (B), which rapidly destroy the bacteria by thinning the inner membranes of the cells, indicated by arrows, and outer membranes, indicated by arrowheads.

SURPRISE ATTACK

BACTERIA-BUSTER TAKES CUES FROM HIV

BY DANA YATES

Jeff Bezos, the founder of Amazon.com, was likely referring to business empires when he said, "There'll always be serendipity involved in discovery." And at the University of Pittsburgh, one researcher is proving that the world of science can still provide sudden and pleasant surprises, too.

Ronald Montelaro, a PhD professor in the Department of Microbiology and Molecular Genetics and codirector of Pitt's Center for Vaccine Research, is senior author of a study that has found a promising treatment possibility for drug-resistant bacterial infections, particularly those that afflict people with cystic fibrosis. The study, which was supported by the National Institutes of Health, was published in the June issue of *Antimicrobial Agents and Chemotherapy.*

Affecting about 30,000 children and adults in the United States, cystic fibrosis is a genetic disorder that leads to viscous secretions in the lungs and other organs. The continuous production and presence of that mucus creates a hotbed of chronic infection. In fact, after near-constant use of antibiotics, about 80 percent of cystic fibrosis patients have at least one antibiotic-resistant infection in their lungs by age 18.

Simply put, progressively resistant bacteria eventually kill patients by spurring infections that, in turn, block patients' airways and make breathing increasingly difficult. There is an urgent need to find a way to improve the efficacy of antibiotics.

Montelaro has found one potentially life-saving solution. It uses the same tactic to attack infections that the deadly human immunodeficiency virus (HIV) uses to infect cells. The secret weapons in both cases are peptides—compounds that contain two or more amino acids. While studying HIV, Montelaro and his colleagues discovered a sequence of amino acids on the tail of the virus that enabled it to bust through and infect cells. That unexpected discovery led Montelaro to wonder whether the punching action of peptides could instead be used for a life-supporting purpose.

While peptides handle many functions in the body, antimicrobial peptides are a natural defense system against bacteria. Indeed, more than 2,000 antimicrobial peptides have been identified in nature, found in everything from plants to the skin of frogs. However, researchers have long struggled to create effective antimicrobial peptides in the lab.

Montelaro had a realization: When it comes to peptides, one size does not fit all. In nature, each evolved to work only on certain bacteria and in particular environments. "They aren't generalists," says Montelaro. "I thought, *If I were Mother Nature, how would I design a peptide for a particular target?*"

After studying the unique qualities of various peptides, his team developed a synthetic antimicrobial peptide, using an algorithm. Designed as a more efficient version of the amino-acid sequence found on the tail of HIV, this sequence—dubbed engineered cationic antimicrobial peptides, or eCAPs—can quickly kill a broad range of bacteria that standard antibiotics are powerless to fight.

Made from the amino acids arginine and tryptophan, the patented eCAPs are specially manufactured to achieve a uniquely rapid antibiotic effect against diverse types of bacteria—even strains resistant to standard antibiotics. Whereas traditional antibiotics may take hours, or even days, to eradicate an infection, the eCAPs can destroy bacteria in seconds or minutes. They accomplish this by attaching to and disrupting bacterial membranes.

The eCAPs are also effective against biofilms, bacterial networks that are extremely resistant to antibiotics. The eCAPs blast through a biofilm's exterior to wipe out the whole bacterial community. As a result, says Montelaro, the eCAPs could have numerous important applications in the future.

"I'm passionate about improving cystic fibrosis patients' quality of life," he says. "And one day, eCAPs might also be used to help heal burn wounds, attack infections associated with venous catheters, and even save soldiers during a bioterrorism attack." ■