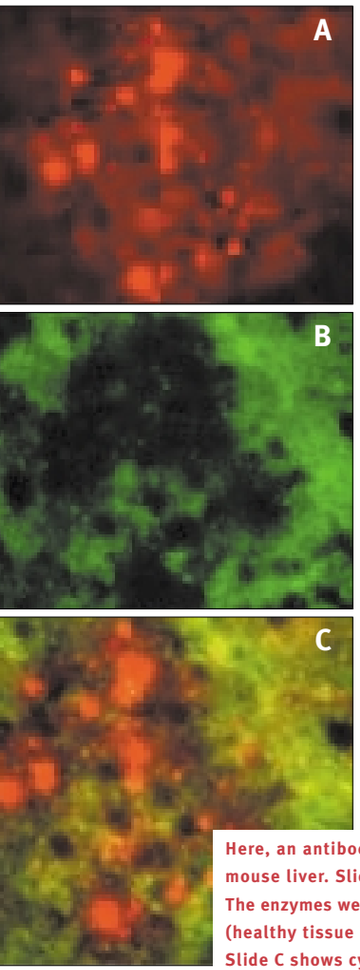
LEAVES: PHOTODISC; CELLS COURTESY YIN

# THE CELL'S EXECUTIONER

**KEYS TO APOPTOSIS REVEALED** | **BY EMILY TIPPING**

Researchers in 1972 chose the Greek word suggesting falling leaves, apoptosis, to describe a newly characterized morphology of programmed cell death. It's a fitting name on many different levels, says Xiao-Ming Yin, School of Medicine assistant professor of pathology and an MD/PhD. Leaves die and fall intact, and cells killed by apoptosis shrink with nuclei intact. This is not a violent death compared with what often happens in cells killed by inflammation or injury—they are more likely to explode. And both apoptosis and a tree's autumnal blush mean development as well as death. Leaf death makes room for spring buds; cellular suicide allows fetal fingers and toes to separate and helps tadpoles shed their tails.




Here, an antibody whose effects mimic viral hepatitis destroys a mouse liver. Slide A shows caspase 3 enzymes (red) killing the tissue. The enzymes were activated by cytochrome c, shown in black in Slide B (healthy tissue is green) and initially set loose by the Bid molecule. Slide C shows cytochrome c and caspase 3 working in the same cells.

Death is as important as growth, says Yin, whose lab is one of several at the University of Pittsburgh seeking to uncover the mysteries of apoptosis. His research into a molecule that triggers apoptosis might translate into, for example, a more effective means of killing tumors or preventing certain autoimmune diseases and liver cell death due to disease.

Apoptosis, Yin notes, appears to follow one of two pathways, both of which can be associated with the pathogenesis of human disease.

A well understood pathway begins with the death receptor Fas/TNF-R1, which is tied to a host of diseases. When Fas/TNF-R1 is activated on a cell, it sets off a chain reaction that ends in the cell's destroying itself. A more mysterious pathway involves mitochondria that can be activated by radiation or other stimuli to release cytochrome c, a substance that causes a flood of caspase enzymes that hack apart DNA and kill the cell.

No one knows exactly how mitochondria go about releasing cytochrome c. Many, however, believe that studying a molecule called "Bid"—which was first cloned by Yin—could unlock those hidden mechanisms and pave the way for more detailed study of the role apoptosis plays in disease. Yin is trying to figure out, for example, whether Bid enters the mitochondria and then creates a special pore that releases cytochrome c or whether it helps release it some other way.

He cloned the killer molecule four years ago. Then, in 1998, researchers at Harvard University and the University of Texas cloned Bid again, revealing more of its characteristics. This helped Yin and his colleagues pinpoint where Bid stepped in to facilitate the apoptotic chain reaction.

The molecule continues to intrigue those studying the intricate biochemistry of a range of diseases. Although it is one of four molecules—family members are Bax, Bak, and Noxa—believed to play similar apoptotic roles, Bid is the only molecule with a defined trigger.

Yin's earlier research helped scientists focus on Bid. His work determined that Bid links the two cell death pathways, with the activated death receptor Fas/TNF-R1 releasing a caspase enzyme that triggers Bid to move to the mitochondria and complete its work.

Using knock-out, or genetically altered, mice, Yin also determined that Bid is important in hepatic diseases. Mice without Bid molecules proved resistant to apoptosis when injected with an antibody directed against Fas whose effects mimic viral hepatitis. Wild type mice—i.e., mice that were not genetically altered and that, presumably, still had Bid molecules—died. It appears the antibody was ineffective without Bid, implicating the molecule in the executioner's role.

In addition to unveiling its hidden mechanisms, Yin wants to find out why Bid is important to apoptotic events in hepatocytes and in neuronal cell death after stroke.

This sort of investigation could help scientists elucidate the apoptotic chain reactions in other disease models, too, he believes.

It looks like understanding how this executioner works may save lives down the road. ■

**FOR MORE INFORMATION: http://path.upmc.edu/people/faculty/yin.html**

# ARRESTING SUDDEN CARDIAC DEATH

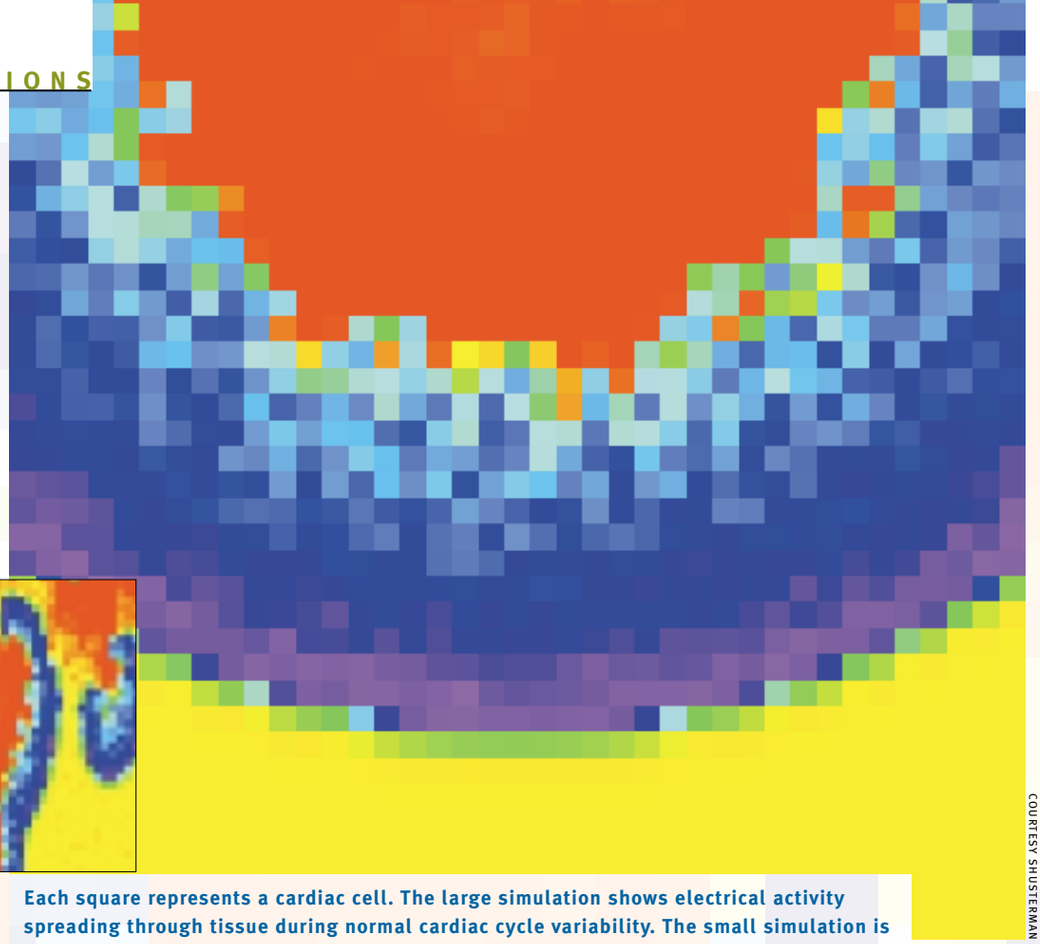
**SHUSTERMAN CAN PREDICT ARRHYTHMIA | BY ERICA LLOYD**

Sudden cardiac death is not as sudden as everyone thought. It is not random. We can predict it."

Vladimir Shusterman's claim is likely to sound bold to cardiologists and others grappling to contain the damage inflicted by arrhythmias—the irregular heart rhythms that often seem to come out of nowhere and kill 300,000 in the United States each year. Yet Shusterman, a research instructor of medicine and director of the Noninvasive Cardiac Electrophysiology Laboratories at the University of Pittsburgh's Cardiovascular Institute, has reason to be confident. He just patented a computerized method that accurately predicts atrial arrhythmias and ventricular tachyarrhythmias (VTAs) 80 to 90 percent of the time. Atrial arrhythmias are common, but not usually life threatening.

VTAs, though rarer, are the typical path to ventricular fibrillation leading to sudden death.

Shusterman didn't always speak with such conviction. He remembers a day not so long ago, somewhere in the blur of 1996, when he was mired in data and, he thought, getting nowhere. In 1995, Shusterman, who is from Novosibirsk, Russia, had accepted a postdoctoral fellowship with the Cardiovascular Institute's Kelley Anderson. Anderson was helping to wrap up a huge multi-year, multicenter clinical trial led by the University of Utah in Salt Lake City. The electrophysiological study tracked 487 patients who had experienced VTAs, monitoring them in periodic 24-hour intervals using a device that continuously records ECG data. Shusterman, who has a PhD in artificial intelligence as well as an MD, was eager to mine the data. He had developed mathematical methods for analyzing how the nervous system controls heart and blood vessel functions; and both he and Anderson thought it would be fruitful for him to apply those methods to the Utah study.



COURTESY SHUSTERMAN

**Each square represents a cardiac cell. The large simulation shows electrical activity spreading through tissue during normal cardiac cycle variability. The small simulation is a snapshot of disorganized activation after conduction abnormalities accumulate during several cardiac cycles. Multiple rotating spiral waves of disorganized activity portend ventricular fibrillation—which leads to a cardiac arrest.**

"Everyone understood that the data were unique," says Shusterman.

Once he got settled in Pittsburgh, Shusterman used spectral analysis to examine frequency patterns from electrocardiograph signals, applied various analytic techniques among patient groups, and gleaned a few interesting things from the data, but nothing that allowed him to predict arrhythmia. *This is a mess,* he began to think.

"I doubted it was possible to predict arrhythmia accurately—others had tried and were not successful," he says.

"Finally I said, 'Maybe I'm missing something. Let's forget everything that I learned or used before.'"

Not so long after Shusterman shifted his mind-set, something occurred to him. He had seen enough data to realize that everyone's heartbeat has a unique pattern, just as everyone's voice has a unique pitch. So all this analyzing of patient groups would never allow him to see which electrophysiological events might portend arrhythmia. However, if he could identify individual patterns of heart rate, then his methods would allow him to pinpoint abnormalities and, perhaps, *predict* when those abnormalities might take place.

Bingo. He was finally on the right track. He and his colleagues went back to the Utah study and decoded individual heart patterns—using 24-hour ECG recordings from 60 patients when they experienced VTAs and the control recordings from the same patients when they did not have VTAs. Applying pattern recognition techniques adapted from AI methods, he was able to see that persistent abnormalities in the heart-beat pattern occurred in the hours before an arrhythmia. This could facilitate an accumulation of disturbances in waves of electrical activation spreading throughout the heart—which appeared to trigger the arrhythmia.

From there, Shusterman was able to develop an algorithm to predict arrhythmia up to eight hours before its onset. Together with Pitt's Barry London, he tested the process on arrhythmia-prone mouse models that Arthur Feldman of the Cardiovascular Institute had developed.

Just as he'd hoped, Shusterman was able to predict arrhythmias in the mice.

So far, Shusterman's technique has been applied by taking ECGs and running his software to analyze them. He is collaborating now with Guidant Corporation outside Minneapolis, Minnesota, to insert the software and a miniature computer into defibrillators to allow real-time analysis and detection. The technology could be applied inexpensively to many cardiac monitoring situations. It could be used in devices such as pacemakers and in hospital monitors connected to patients at their bedsides; or physicians could use it to analyze periodic ECGs.

All this doesn't exactly mean a sudden death for sudden cardiac death, though its downfall seems to be looming.

Once Shusterman gets past what he calls the "organizational" challenges of large clinical trials and puts his invention to broad use, physicians who use this technology will be alerted to danger signs in their patients with histories of arrhythmia, enabling them to intervene as necessary.

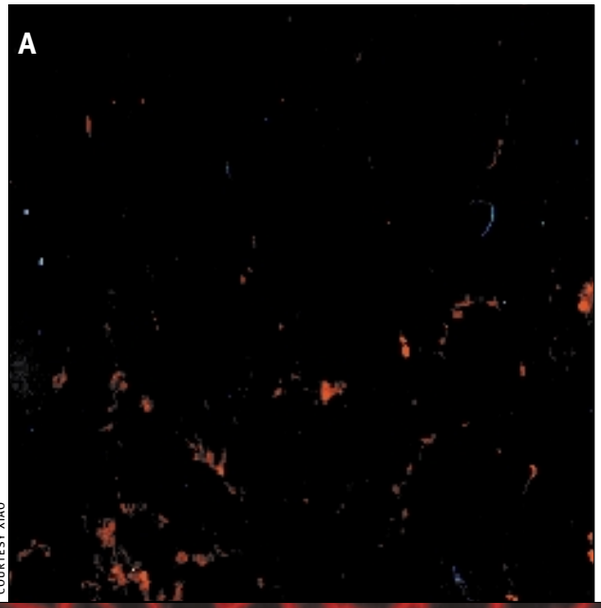
Looking back at those days when he felt he was treading in a morass of data and getting nowhere, Shusterman notes, "Sometimes you have to disregard prior knowledge and concepts that blur your vision. It's useful to look at the problem without prejudice." ■

**FOR MORE INFORMATION:**
**http://www.uspto.gov/ Search under Patent Application No. 067586**

# LABORED STEPS

## A GENE THERAPY SOLUTION TO MUSCULAR DYSTROPHY

BY REBECCA SKLOOT

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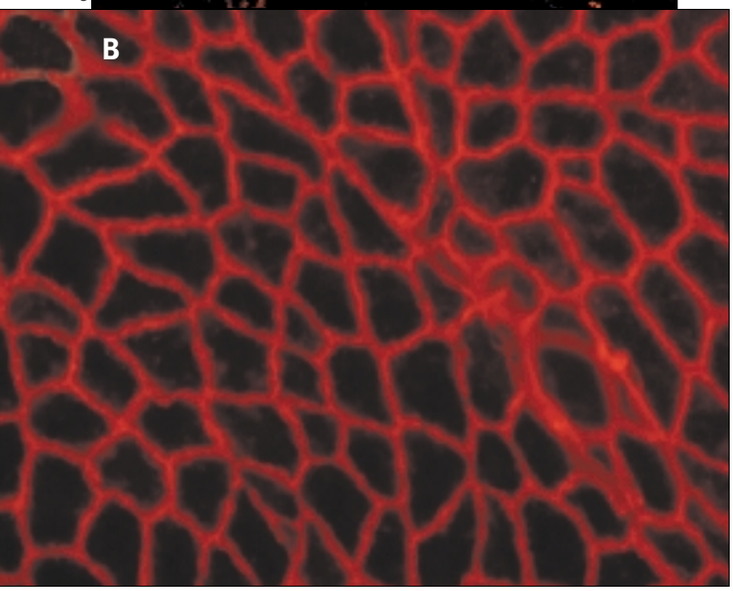


**The untreated hamster muscle afflicted with muscular dystrophy (A) shows a lack of the delta-sarcoglycan protein. Injection of an adeno-associated viral vector carrying the delta-sarcoglycan gene restored the missing protein to the muscle cells (B).**

For every 3,000 baby boys, one is born with Duchenne muscular dystrophy (DMD). He looks and acts like other children: he cries, nurses, and smiles; learns to support his body, first head, then frame. But sometime between his third and seventh year, his muscles begin to deteriorate. If he can walk, each step becomes a forced, concentrated effort. If he runs, he propels himself forward with labored, waddling strides, his back arched and belly thrown forward to balance against his weakening pelvic muscles. He falls. Accidents grow more frequent. As early as his ninth birthday, braces may be his only hope for walking; and by about age 12, he will be confined to a wheelchair. Soon after, the muscles in his hands and arms will weaken, as will those that allow him to breathe. He will probably not live through his teens.

Muscular dystrophy is a family of disorders marked by slow muscular degeneration. The most common form, DMD, is caused by a mutation in the gene for dystrophin, a protein essential for muscle-cell membranes. Those with limb-girdle muscular dystrophy (LGMD) are born with a mutated gene for sarcoglycan, another muscle-cell membrane protein. (LGMD is much less common than DMD.) These mutations leave the body unable to produce a protein vital for muscle function. The price is something Xiao Xiao, assistant professor of molecular genetics and biochemistry, calls "leaky muscle cells." Eventually muscles cease functioning as the body slowly replaces them with firm connective tissue. There is no approved treatment. But in Xiao's lab, when he injects viral vectors with functional copies of these genes into animal models, protein levels rise to near normal, and the disease state vanishes.

Xiao works with the adeno-associated viral (AAV) vector, which offers the first hint at a treatment for patients with muscular dystrophy. It's considered the safest vector—even in its natural state, AAV doesn't cause human disease and is incapable of replicating without assistance from another virus. And Xiao is intimately familiar with it. AAV was developed by his Pitt PhD advisor, Jude Samulski, who isolated the virus and removed its viral genome to allow insertion of therapeutic genes.

The sarcoglycan gene, which Xiao hopes to use for treating LGMD, fits nicely into AAV, but the dystrophin gene is about 600 times too big. So Xiao created a minidystrophin gene, one with all the functional DNA it needs, and made it fit into an AAV vector. Then, in two studies, he injected these genes into the leg muscles of animal models. Both the LGMD and the DMD groups began producing normal levels of their missing protein and showed verifiable improvement. The LGMD study is furthest along. That collaboration with John Watchko, a Pitt professor of pediatrics, showed muscle force, which was 50 percent of normal prior to treatment, recovered to 97 percent.

Xiao is preparing for an LGMD clinical trial. He's not sure how the turmoil over gene therapy will affect his plans, but he believes the AAV vector is a highly promising therapy and will also help safeguard his subjects' health. He is determined to help those with MD—even if, along the way, each step becomes more labored as time progresses. ■