

Mitochondria
are doing
more than
just keeping
the cell's furnace
stoked.

The
Powerhouse
— and
Sentinel
— of
the Cell

by Karen F. Schmidt

— LOOK UP “MITOCHONDRIA” IN ANY SCIENCE TEXT AND INVARIABLY THESE TUBULAR BAGS OF ENZYMES THAT FLOAT IN THE CELL’S INTERIOR ARE CALLED “THE POWER HOUSES OF THE CELL.” OF COURSE, THE ROLE THEY PLAY AS CELLULAR FURNACES, converting nutrients and oxygen into energy, is immensely important. Every cell needs ATP—the chemical fuel generated by mitochondria—and some cells are particularly demanding: a muscle cell that pushes the leg into a sprint, a beta cell in the pancreas that synthesizes the hormone insulin, a brain cell that fires a signal to help create a thought. Each of these kinds of cells contains as many as 10,000 mitochondria, and it’s no secret that those tiny organelles keep the home fires burning. • However, recent studies suggest that mitochondria do much more than generate energy. They are intimately involved in cell signaling, raising a red flag during times of cellular stress, such as when viruses invade or oxygen levels drop.

photograph by Mark Hooper



It now appears that subtle abnormalities in mitochondria contribute not only to rare metabolic disorders but also to many common diseases, including chronic hepatitis, cancer, and certain aging-related diseases, such as type 2 diabetes. Says Gerald I. Shulman, an HHMI investigator at Yale University School of Medicine whose area of expertise is diabetes, “We’re moving into areas that affect large numbers of people—the 7 percent of the population with diabetes—and that gets a lot of attention.”

These days, mitochondria often take scientists by surprise. “Researchers keep stumbling into mitochondria,” says Gerald S. Shadel, a molecular biologist at Yale University School of Medicine who studies mitochondria and disease. “That probably reflects the fact that mitochondria are involved in many things besides what was historically assigned to them; people in many fields are now making important connections.”

Signaling Immunity

— RESEARCH ON MITOCHONDRIA HEATED UP IN THE 1990s, WHEN STUDIES REVEALED THAT THEY PLAY A KEY ROLE IN SIGNALING PROGRAMMED CELL DEATH. In 1996, Xiaodong Wang, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas, made the surprising discovery that mitochondria release a molecule called cytochrome *c*,

triggering a signaling cascade that leads to cell suicide, usually during embryonic development or in response to cellular stress or damage. Then, in 2001, the late HHMI investigator Stanley J. Korsmeyer reported that the activation of a pore in the mitochondrial membrane launches this process by enabling the cytochrome *c* signal to flow into the rest of the cell.

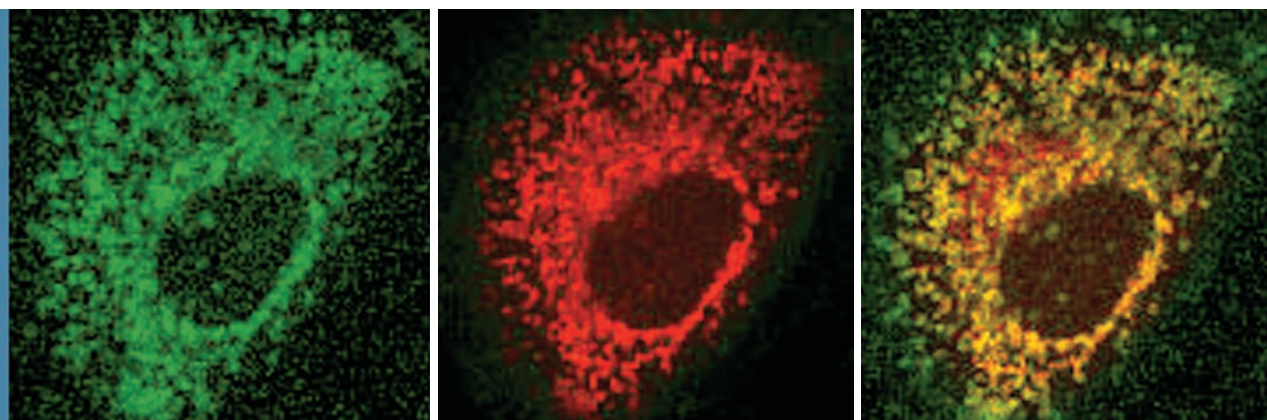
Now, another protein in the mitochondrial membrane has been discovered that for the first time links this organelle to the immune system. Zhijian “James” Chen, an HHMI investigator also at the University of Texas Southwestern Medical Center, found a protein in the mitochondrial membrane that contributes to viral defense. Chen wanted to know how cells detect and mount a response to infection by a virus—in particular, what activates the cell to produce important antiviral molecules called interferons (which are also used as medical therapies). His team searched for signaling proteins involved in antiviral immune responses and found a protein that appeared to activate two transcription factors known to trigger interferon production. They then engineered cells that express large amounts of this protein and grew them in culture with viruses.

Monitoring to see what would happen, they found that the cells had gained antiviral immunity. Conversely, when they silenced the protein’s expression, the resulting cells were swamped with replicating viruses.

The big surprise came when the group broke open the cells, spun them in a centrifuge, and found this protein not in the liquid extract but in the fatty membrane fraction. Using confocal microscopy, they pinpointed its location in the mitochondrial membrane and so decided to name it Mitochondrial Antiviral Signaling Protein, or MAVS. “It was quite surprising, but also very exciting,” says Chen, because this was the first time anyone had found a protein involved in immunity that was part of the mitochondrion. In fact, several other groups had encountered the same protein but didn’t figure out its cellular location, which, Chen’s team found, is essential for the signaling function of MAVS.

The group reported their findings in the September 9, 2005, issue of *Cell*; soon after, *Science STKE* named the discovery of MAVS one of the “signaling breakthroughs” of the year. Eric A. Shoubridge, a human geneticist and HHMI international research scholar at McGill University, in Montreal, Canada, says, “Chen’s work is pretty exciting stuff. Random bits of information had suggested that mitochondria might be involved in signaling and the immune system,

Confocal microscopy images show a cell stained with an antibody for the MAVS protein (left, green) and a mitochondria-specific dye (center, red). An overlay of the green and red images (right) indicates the mitochondrial localization of MAVS.



Courtesy of Rashu B. Seth and Zhijian “James” Chen

but this work is the clearest evidence yet—it's very convincing.”

Once he discovered MAVS, Chen investigated whether some viruses could specifically target the protein to cripple a host's defenses. “After we found MAVS, we suspected maybe it was the long-sought-after target for the hepatitis C virus,” he explains. Of the 170 million people in the world with hepatitis C, about 80 percent have persistent, chronic infections; their interferon production is suppressed. Sure enough, Chen's group discovered that the hepatitis C virus, using an enzyme called a protease, can clip MAVS off the mitochondrial membrane, effectively breaking the signaling pathway that triggers interferon production. The group reported these findings in the

December 6, 2005, issue of the *Proceedings of the National Academy of Sciences*.

Chen's team further observed that a change in just one letter of the MAVS genetic code—the kind of simple mutation that typically distinguishes the DNA of one individual from that of another—protects it from being clipped by the viral protease. This observation may explain why some people are better than others at fighting off hepatitis C infection and suggests an important target for drug treatments. “If we could come up with an inhibitor of the viral protease, we could prevent viral replication and also restore [interferon production in] the host immune system—like killing two birds with one stone,” says Chen.

A lot more remains to be learned about MAVS. Chen's group is exploring whether other viruses also target MAVS, whether other mechanisms can be used to cripple it, and whether MAVS serves any other functions in the cell. For instance, does MAVS ever talk

to neighboring membrane proteins and tell them to trigger cell suicide? Theoretically, it would make sense for cells to use suicide as an additional antiviral strategy; plant cells are known to use it to limit the spread of infection for the benefit of the whole organism. “Maybe if a mammalian cell can't produce enough interferons, then it will need to die,” Chen theorizes. However, any link between MAVS and cell suicide is still speculative, he says.

Low-Oxygen Alert

—IN MOST TISSUES, MITOCHONDRIA CONSUME 90 PERCENT OF THE OXYGEN THAT ENTERS THE BODY, SO IT MAKES SENSE THAT MITOCHONDRIA WOULD FUNCTION AS oxygen sensors as well. M. Celeste Simon, an HHMI investigator at the University of Pennsylvania (continued on page 32)

Numbers

game: Is more better?

—

SCIENTISTS HAVE known for more than two decades that type 2 diabetes begins its development as “insulin resistance,” in which tissues such as muscle respond poorly to the hormone insulin and, therefore, don't facilitate glucose transport out of the blood and into muscle cells where it is metabolized. It made sense to Gerald I. Shulman, an HHMI investigator at Yale University School of Medicine, that insulin resistance might be linked to mitochondrial function. After all, mitochondria convert glucose and fatty acids into energy—by a process called oxidation—and people with diabetes have too much unburned glucose in their

blood, and too much fat in their muscle and liver cells. • **SO HIS GROUP** developed a novel method to tell how well mitochondria are functioning, using NMR methods to noninvasively measure rates of oxidation and ATP production. In 2003, Shulman's team reported evidence in lean, healthy, elderly volunteers that an age-related decline in mitochondrial function may contribute to insulin resistance. They hypothesized that reduced mitochondrial function predisposed these older people to accumulate fat in muscle and liver cells, and that, in turn, led to defective insulin signaling and then insulin resistance. • **IN AN INTERESTING** twist, however, Shulman's most recent study suggests that reduced mitochondrial function might also be caused by low overall numbers of mitochondria—at least in young, lean adults whose parents have type 2 diabetes. The researchers had previously

studied this group and detected reduced rates of oxidation and ATP production in their muscle cells. Next, Shulman's team decided to take tissue samples and use an electron microscope to count the number of mitochondria. The samples—which already exhibited large amounts of intracellular fat, insulin resistance, and signs of impaired insulin signaling—had on average 38 percent fewer mitochondria than normal. The results of the study appear in the December 2005 issue of the *Journal of Clinical Investigation*. “Our data suggest that reduced mitochondrial function in this young group can be attributed to their low numbers of mitochondria,” Shulman says. Now, the team is trying to determine whether intracellular fat accumulation might cause

the low numbers, or vice versa. And how important are mitochondrial numbers? “Having more mitochondria might seem to be better, but it's probably not as simple as that,” says Shulman. • **SCIENTISTS** still have much to learn about how the cell senses that it should make more mitochondria or has enough already, according to David A. Clayton, HHMI's vice president and chief scientific officer. “The Holy Grail in this field at every seminar is: How does the cell regulate the number of mitochondria? It's a challenging question.” Researchers know that nuclear genes control the biogenesis of mitochondria (which have their own DNA), that tissues naturally have 100 to 10,000 mitochondria per cell, and that exercise increases the number of mitochondria in muscles. As scientists fill in the details, they expect to find many additional signaling pathways at the crossroads, Clayton notes.



CELESTE SIMON studies cellular responses to oxygen deprivation.

“Understanding how oxygen levels are sensed and adapted to is fundamentally important to dealing with pretty much all of the major diseases that we encounter — atherosclerosis, autoimmune disease, stroke, and cancer.”

CELESTE SIMON

School of Medicine, decided to explore this idea in 1997. “Understanding how oxygen levels are sensed and adapted to is fundamentally important to dealing with pretty much all of the major diseases that we encounter — atherosclerosis, autoimmune disease, stroke, and cancer,” she says. For instance, solid tumors begin to grow outside the body’s circulatory system, where oxygen levels are low, and they do so by turning on signals that tell tissues to sprout new blood vessels. Understanding how to disrupt this signaling might lead to new cancer treatments. Normal adult tissues, such as kidneys, can also experience low oxygen because of poor circulation and other dysfunctions. In that case, if doctors could enhance the signaling process, they might be able to promote blood vessel development and restore an organ’s function.

Research on how cells respond to low oxygen, or hypoxia, took off in 1995, when a transcription factor named hypoxia-inducible factor, or HIF, was isolated and later shown to activate blood vessel formation and make cancer more aggressive. When Simon began her studies, a key question needed answering: What signals cause HIF to accumulate when oxygen levels drop? Some researchers theorized that HIF directly sensed oxygen, but Simon and others decided to look to mitochondria for signals. The first clue came when her research team suppressed mitochondrial metabolism in a cell culture and found that HIF no longer accumulated during hypoxia.

Now, after a series of experiments, Simon’s group has strong evidence that metabolic by-products generated inside mitochondria called reactive oxygen species, or ROS, serve as important signals that stabilize HIF during hypoxia. The team reported its most recent findings in *Cell Metabolism* in June 2005.

For these studies, Simon’s team developed a tool to measure extremely small changes in ROS in real time under the microscope. With this probe, they showed that ROS are produced in larger amounts in mitochondria during hypoxia. Next, the team tinkered with the cells to suppress the amount of ROS they could produce — first by knocking out an important gene and then by adding an enzyme that specifically scavenges ROS. In both cases, without ROS the cells could not launch the

normal response to low oxygen. These results suggest that ROS is a necessary signal to cause HIF accumulation during hypoxia.

Simon’s working hypothesis is that ROS are released into the fluid portion of the cell’s interior, where they inhibit enzymes called HIF hydroxylases that lead to HIF degradation when oxygen levels are normal. Thus, HIF builds up and then sets in motion the cell’s hypoxic response — activating the myriad genes that lead to blood vessel development and cell motility, for example. Yale’s Shadel comments, “Simon has uncovered how this oxygen sensing has consequences beyond respiration — mitochondria are not just sensing oxygen for their own need but are telling the cell that it’s low in oxygen and that it needs to initiate a response.”

Some other labs have reported findings that support the theory that HIF hydroxylases (the enzymes leading to HIF degradation) serve as oxygen sensors. Simon reconciles the data by suggesting that two separate pathways may operate, one under extreme hypoxia (0.1 percent oxygen) and the other under modest hypoxia (1–3 percent oxygen). Her results indicate that when oxygen is nearly absent, mitochondria cease to be the dominant player and HIF hydroxylases become the oxygen sensor. However, she adds, cells in the body are more likely to encounter the conditions of modest hypoxia that she studies.

Understanding how mitochondria are involved in sensing and signaling may ultimately lead to new models for many diseases and their treatments. Researchers now know that mitochondrial dysfunction could affect more than just the cell’s ability to produce energy, says Simon. “Many metabolites produced in the mitochondria have an impact on the rest of the cell, and these will be really important to consider in disease.” As Shadel puts it, “The role of mitochondria in the cell is grossly underestimated, as is their role in human disease.” ■