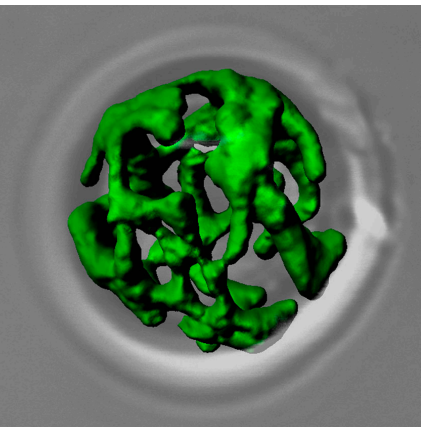
In This Issue



Without cardiolipin—made with the help of Tam41—transport of proteins into mitochondria like these falters.

Cardio(lipin) workout beefs up mitochondrial imports

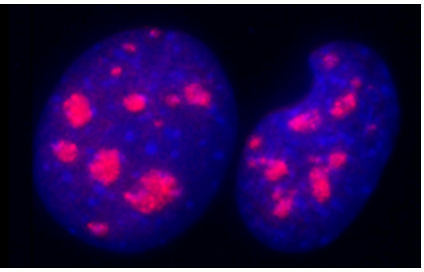
Mitochondria depend on imported proteins. Kutik et al. show how a protein keeps this molecular traffic flowing by helping manufacture a key membrane lipid.

Almost all of the 1,000 or so different proteins mitochondria need to operate are made elsewhere in the cell. After crossing a mitochondrion's outer membrane, proteins follow one of two routes through the organelle's inner membrane. In the carrier pathway, the protein complex TIM22 shepherds molecules into the inner membrane. The presequence pathway, by contrast, relies on the TIM23 complex. Researchers recently discovered that a protein called Tam41 was required for TIM23 to efficiently import presequence molecules. Whether Tam41 also helped out in the carrier pathway was controversial.

Kutik et al. used yeast devoid of Tam41 to answer the question. The researchers measured whether carrier-transported proteins combined into complexes, a sign that they'd arrived at their destination. The team found that in the mutant cells, the proteins often reached the inner membrane but didn't assemble into mature complexes. And the electrical potential that usually exists across the inner membrane dwindled. Those results suggest that not only is the presequence pathway faulty when Tam41 is missing, so is the carrier pathway.

The mitochondrial defects resembled those caused by a scarcity of cardiolipin, an inner membrane lipid. Cardiolipin performs many jobs in mitochondria, including stabilizing the protein complexes involved in electron transport. Cardiolipin was almost absent from the mutant cells. Tam41 does not synthesize cardiolipin directly, but helps to fashion one of its precursors, the team found. Next task, the researchers say, is to identify that step.

Kutik, S., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806048.



Glowing clusters of UBF mark rRNA genes.

DNA, just chill

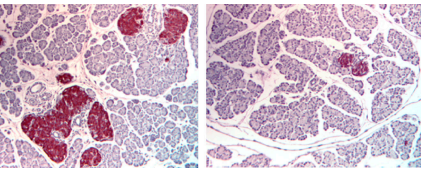
To keep transcribing, the genes that encode ribosomal RNA (rRNA) need to remain relaxed. Sanij et al. reveal how a transcription-promoting protein prevents these genes from getting uptight.

Human cells have rRNA genes aplenty—roughly 200 of them, which are arranged in clusters. Tuning their output is crucial because too little rRNA can stifle cell growth, whereas too much might lead to cancer. A factor that controls rRNA production is the protein UBF. It maintains rRNA genes in a loose conformation that allows transcription, rather than a condensed and inactive state. How UBF achieved this feat was an unanswered question.

To find out more, Sanij et al. used RNAi to trim UBF levels in human cells by 80%. The researchers' analysis ruled out one method that cells often employ to shut down genes permanently—affixing methyl groups. In fact, the team showed, rRNA silencing is reversible, unlike methylation. The researchers think that UBF works by obstructing the H1 linker histone, which helps stabilize nucleosome particles and allows them to condense into higher-order structures. The linker histone's effect is to compact the DNA, thereby silencing genes. But when UBF is present, the histone can't gain access to the DNA.

The study's big surprise came when the researchers measured rRNA output in cells with scant UBF. Instead of plunging, rRNA levels remained constant. The cells compensated by cranking up the activity of the genes that remained turned on. Cells could muster sufficient rRNA even with 90% of the genes shut down. This result indicates that UBF's actions serve another purpose besides adjusting rRNA levels, such as stabilizing DNA.

Sanij, E., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200805146.



More insulin-positive cells (brown) occur in mice lacking leptin-making fat cells (left) than in control mice (right).

Leptin's long-distance call to the pancreas

Rube Goldberg—the cartoonist who devised complex machines for simple tasks—would have smiled at one of leptin's mechanisms for curbing insulin release. As Hinoi et al. show, the fat-derived hormone enlists the sympathetic nervous system to prevent bone-making cells from releasing a molecule that prods the pancreas to discharge insulin.

In the fight against obesity and diabetes, leptin is one of the good guys. Mice lacking the hormone become corpulent, and their sky-high insulin levels eventually lead to diabetes. Leptin can curtail insulin release directly. But there's also a back-door route that researchers are still trying to piece together. Scientists knew that leptin nudges osteoblasts, cells known for building bone but that also manufacture osteocalcin, a protein that stimulates insulin release. Hinoi et al. tested whether leptin acts on the pancreas through osteocalcin.

Leptin channels many of its effects through the nervous system. The team thus tested whether

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the hormone relied on the sympathetic nervous system, the branch that pumps out adrenaline during emergencies but at other times emits a hormonal trickle to maintain a low level of stimulation. This baseline activity was lower in mice lacking the leptin receptor, indicating a connection between leptin and the sympathetic nervous system. When that connection was broken by deleting the adrenaline receptor from mice osteoblasts, insulin levels shot up.

The researchers then dosed mice with a sympathetic stimulator. Rodents lacking leptin or the adrenaline receptor on their osteoblasts turned out normal amounts of osteocalcin, but much of it was in an inert form. That result suggests leptin affects insulin release by indirectly inactivating osteocalcin. The work boosts researchers' hopes of using osteocalcin to treat diabetes—a possibility some drug companies have already started to investigate.

Hinoi, E., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200809113.

Matrix fragments trigger fatal excitement

Shredded extracellular matrix (ECM) is toxic to neurons. Chen et al. reveal a new mechanism for how ECM demolition causes brain damage.

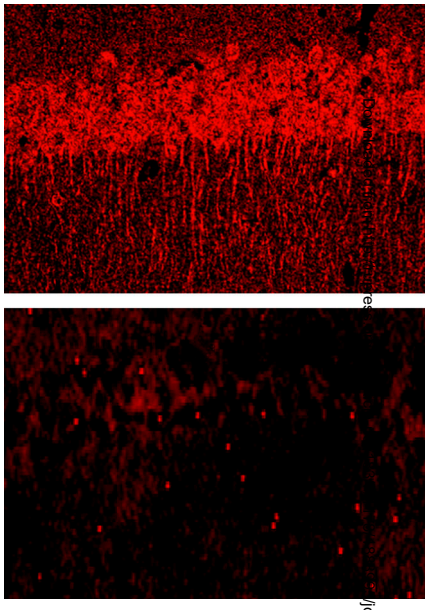
A stroke or head injury kills large numbers of neurons through a process called excitotoxicity. A surge of the neurotransmitter glutamate jolts receptors such as the kainate receptor and stimulates cell death. Enzymes add to the death toll by chopping up ECM near the injury site. How ECM breakdown takes out neurons was mysterious. The standard view was that neurons perished because they got separated from the ECM as it dissolved.

Chen et al. found otherwise when they engineered mice to lack the ECM component laminin in the hippocampus, a brain region often damaged by stroke or injury. If cells languished after parting from the ECM, the researchers reasoned that mice missing laminin would suffer more damage from excitotoxicity. But when excitotoxicity was spurred with an injection of kainate—a molecule that, like glutamate, activates the kainate receptor—the laminin-lacking mice showed less brain damage. After a dose of diced laminin, however, the mutant mice were vulnerable to kainate, indicating that the fragments are the culprit in cell death.

The researchers discovered that chopped-up ECM kills cells by ramping up production of one subunit of the kainate receptor, known as KA1. They speculate that hiking the amount of KA1 subunits might make the receptor more sensitive and thus more likely to trigger an overreaction by the cell.

Although drugs that obstruct the glutamate receptor slow brain cell death, they can lead to serious cognitive impairment and even coma. The study suggests that drugs that block KA1 might provide an alternative way to save brain cells after stroke or head trauma.

Chen, Z.-L., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200803107.



The extracellular matrix thins in mice lacking laminin in the hippocampus (bottom).

Slow down, enzymes at work

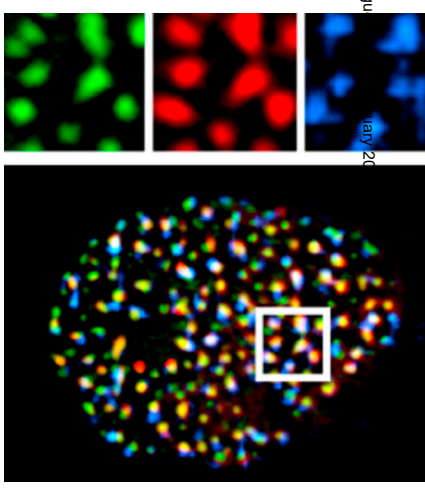
DNA replication lags when the molecule is undergoing repairs. Sugimura et al. reveal that one DNA-fixing protein helps create this delay by fending off another DNA repair enzyme.

Damage that severs both strands of a DNA molecule creates what's called a double-stranded break, or DSB. When the cell is copying its DNA, DSBs can cause trouble for replication forks, the spots where the double helix unzips so enzymes can copy the two strands. If a replication fork runs into a double-stranded break, researchers think that DNA duplication usually slows or stops. Previous work has revealed that the protein PARP-1 helps correct single-stranded DNA breaks. But whether it helps heal the DSBs that arise during replication was unclear.

Sugimura et al. tested whether PARP-1 inhibits the movement of replication forks along the DNA molecule. In vitro studies suggested that it did, but in vivo evidence was lacking. In human cells, replication fork speed doubled after addition of RNAi that targeted PARP-1, the scientists found. A PARP-1 inhibitor had a similar effect.

Cells deploy two mechanisms for mending DSBs—non-homologous end-joining (NHEJ) and homologous recombination (HR). The team found that the replication forks decelerate in cells that can't perform NHEJ, but not in cells where HR is defective. That result suggests that replication fork slowing occurs because HR is at work repairing the break. PARP-1 helps delay the fork's progress, the researchers determined, by allowing HR proteins access to the DNA but obstructing the protein Ku70, which is necessary for NHEJ and can block HR.

Sugimura, K., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806068.



PARP-1 (green) associates with replicating regions of the genome (red and blue) and will slow fork progression during double-strand break repair.