In This Issue

Text by Mitch Leslie
mitchleslie@comcast.net

Ino80 tidies up after DNA repair

Like a surgeon stitching up an incision after an operation, cells restore their chromatin after repairing DNA damage. Sarkar et al. identify a protein that helps put the chromatin back in order.

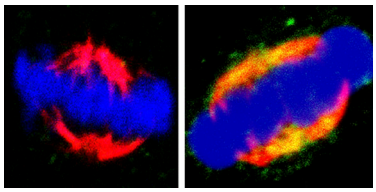
When UV light zaps DNA, the nucleotide excision repair apparatus swings into action, cutting out and replacing the damaged section. Before the work can begin, however, cells must rearrange their chromatin so that the repair enzymes can access the lesion. Scientists haven't determined whether cells remove nucleosomes, push them out of the way, or do both. A further question is how the cells return chromatin structure to normal after repairs are complete. The researchers tested whether the protein Ino80, which helps fix double-stranded DNA breaks, takes part in this process.

Sarkar et al. found that although yeast cells lacking Ino80 were able to mend UV damage, their death rate increased, suggesting a glitch in their post-repair recovery. To find out whether Ino80 helps spur restoration of chromatin structure, the researchers tracked chromatin changes in a gene packed with 14 nucleosomes. After a blast of UV radiation, H3 histones disappeared from the gene almost immediately, the team found. In wild-type cells, the histones returned gradually over the next three hours, matching the pace of DNA repair. But in cells lacking Ino80, the histones hadn't returned after three hours.

The results suggest that Ino80 helps restore nucleosomes after the DNA has been refurbished. A further question to answer, the researchers say, is whether Ino80 also helps patch up DNA damage.

Sarkar, S., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201006178.

Mad2 shifts a motor into idle



In control cells (left), scant MKlp2 (green) reaches the spindle (red), but the motor protein flocks to the spindle after knockdown of Mad2 (right).

Mad2 is all the rage in mitotic cells. The protein is a key part of the mitotic checkpoint, and now Lee et al. reveal that it also controls cytokinesis.

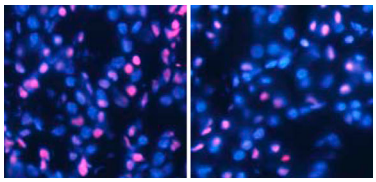
Mad2 detains cells in metaphase until the mitotic spindle connects to every chromosome. But cells often fail to divide if Mad2 goes awry. The reason for this, researchers have suggested, is that the chromosomes didn't separate properly. However, it's possible that Mad2 also helps manage cell division. Lee et al. had already discovered a possible link to cytokinesis—Mad2 latches onto MKlp2, a molecular motor. At the end of metaphase, MKlp2 lugs the chromosome passenger complex (CPC)—a cluster of proteins that includes the kinase Aurora B—from the centromeres to the central part of the mitotic spindle, a move necessary for cytokinesis.

In the new work, Lee et al. show that Mad2 hooks up with MKlp2 early in mitosis and blocks the motor from grabbing onto the mitotic spindle. By tying up MKlp2, Mad2 halts the CPC's relocation. The team found that in cells engineered to produce extra Mad2, the CPC was stuck on centromeres. Another regulator, Cdk1, also stops MKlp2 from transporting the CPC, but the researchers discovered that the two proteins act at different points in the process. Mad2 keeps MKlp2 off the mitotic spindle, whereas Cdk1 prevents the motor from moving after it has attached to microtubules.

For mitotic cells, the payoff from Mad2's double role might be more precise coordination between chromosome movements and cytokinesis.

Lee, S.H., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201003095.

Doubling up on cellular stress



Compared with controls (left), fewer stressed-out kidney cells commit suicide (red) in the absence of NADPH oxidase (right).

Cells have to cope with different sources of stress. Li et al. show how two potentially lethal kinds of cellular stress amplify each other.

Oxidative stress puts cells under pressure due to buildup of potentially damaging reactive oxygen species (ROS), whereas the accumulation of misshapen proteins in the endoplasmic reticulum (ER) can trigger ER stress, which cells try to alleviate through the unfolded protein response. Both kinds of stress can spur cell suicide and help drive several chronic diseases, including atherosclerosis and diabetes. Although researchers have long suspected a destructive synergy between oxidative and ER stress, the molecular connection between the two has remained elusive.

Li et al. teased out a link in stressed macrophages, which the researchers chose to study because they contribute to atherosclerosis. In these cells, ER stress induced ROS production through a pathway that included NADPH oxidase. To their surprise, the researchers found that activation of NADPH oxidase also revs up ER stress, indirectly boosting expression of a key apoptotic protein called CHOP. "There is this incredible tango between ER and oxidative stress that is integrated by NADPH oxidase," says senior author Ira Tabas.

ER stress often kills macrophages. But Li et al. found that the death rate was much lower in cells lacking the major version of NADPH oxidase. The authors then tested the enzyme's effects in vivo by tracking another cell type that is vulnerable to ER and oxidative stress during disease—kidney cells. After injections of a compound that triggers ER stress, mice missing NADPH oxidase showed less apopotosis in kidney tubule cells than did control animals. However, NADPH oxidase is essential for fighting pathogens, so shutting it down could be risky. But drugs that merely reduce its activity to normal levels might ease both kinds of stress without lowering immune defenses.

Li, G., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201006121.