In This Issue

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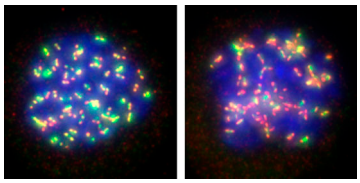
Enzyme helps fold the SAC



In these arrested mitotic cells, the spindle assembly checkpoint protein Bub1 (red) sticks to the kinetochores (green).

Espert et al. identify a phosphatase that helps shut down the spindle assembly checkpoint (SAC) when chromosomes are correctly attached to the spindle.

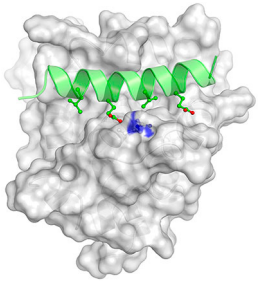
The SAC prevents cells from entering anaphase until they have verified the connections between spindle microtubules and the chromosomes. The checkpoint forms when the enzyme Mps1 phosphorylates the kinetochore protein Knl1, and this alteration attracts other SAC proteins such as Bub1 and BubR1 to kinetochores. Once all the chromosome–spindle links check out, cells remove the phosphates from Knl1, and the checkpoint shuts down as Bub1 and other components disperse. In yeast, the phosphatase PP1, a member of the phosphoprotein phosphatase (PPP) family, dephosphorylates Knl1, but researchers weren't sure which enzyme performs the task in mammalian cells.

When Espert et al. dosed mammalian cells with an inhibitor that blocks all members of the PPP family, they found that Bub1 and BubR1 remained on kinetochores even in the absence of Mps1 activity. But when they depleted the different catalytic subunits found in PPP proteins, they found that loss of the PP2A subunit prevented SAC disassembly, whereas loss of the PP1 subunit did not.

The researchers showed that one PPP family member, PP2A-B56, dephosphorylates Knl1 and that cells lacking this enzyme are slow to exit mitosis. PP2A-B56 arrives at the kinetochores with BubR1. Thus, even as the SAC assembles, it's preparing to disassemble. The results don't rule out a role for PP1, however. PP2A-B56 might initiate SAC shutdown but allow PP1 to remove the remaining phosphates from kinetochore proteins.

Espert, A., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201406109.

HIV's unkind cut



The activating domain of Casp8p41 (green coil) fits into a groove in Bak (white).

Sainski et al. reveal how an inactive caspase fragment spawned by HIV infection can still kill T cells.

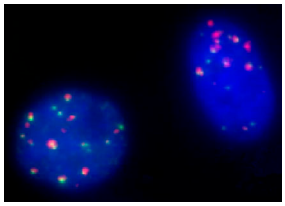
HIV triggers a precipitous decline in the number of CD4 T cells. One way that the virus slays these cells is by provoking apoptosis. But one aspect of the apoptotic mechanism remains puzzling. The HIV protease trims the apoptosis-promoting cellular enzyme caspase 8. The resulting fragment, known as Casp8p41, is enzymatically inert but nonetheless induces cell death.

Sainski et al. discovered that Casp8p41 binds to a proapoptotic protein called Bak. The researchers determined that caspase 8 carries a domain that fits into a groove on Bak and switches the protein on. However, the C terminus of caspase 8 normally overhangs this domain and prevents it from interacting with Bak. When HIV infects a CD4 T cell, the protease cuts away caspase 8's C terminus, exposing the Bak-activating domain. Bak molecules can then oligomerize and trigger cell death by spurring mitochondria to become leaky. Mutating two amino acids in the Bak-binding domain made cells less susceptible to viral infection.

The protease inhibitors that are staples of HIV treatment thwart this apoptosis-inducing mechanism by preventing the protease from trimming caspase 8. The study raises the possibility that other viruses or cell-killing pathways might also induce their effects by revealing hidden activator domains in proteins.

Sainski, A.M., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201405051.

DNA repair with a hefty price tag



Cells have begun resection (indicated by green) at DNA breaks induced by radiation (red).

Barton et al. identify a last-ditch repair mechanism that enables cells to enter S phase without DNA breaks.

Nonhomologous end joining (NHEJ) repairs DNA double-strand breaks throughout the cell cycle. In contrast, homologous recombination (HR) handles only breaks that occur during G2 or S phase. The enzyme CtIP triggers HR by stripping away the bases from one DNA strand to create a sticky overhang, a maneuver known as resection. CtIP also takes part in NHEJ during G1, although how the enzyme is activated during this phase of the cell cycle is unknown.

Barton et al. discovered that polo-like kinase 3 (Plk3) does the job. DNA damage during G1 spurs Plk3 to activate CtIP and stimulate resection. Cells that can't readily repair breaks by traditional NHEJ, which doesn't involve resection, can still restore their DNA during G1, the researchers determined. They use a mechanism involving Plk3 and CtIP that is a variant form of NHEJ. The process is necessary for healing complex breaks in which several DNA injuries are close to one other. Barton et al. found that depleting Plk3 or CtIP hampered repair of these breaks.

But there's a downside to DNA repair that relies on Plk3 and CtIP. It can induce translocations and genomic rearrangements, presumably because the sticky overhangs created by resection tangle with other DNA molecules. Despite the risk, cells might turn to this mechanism when they are pressed for time and are trying to avoid entering S phase with broken DNA. The study reveals that Plk3 initiates repair of damage caused by certain kinds of radiation, suggesting that inhibiting the kinase might increase the vulnerability of tumors to radiation treatment.

Barton, O., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201401146.