In This Issue

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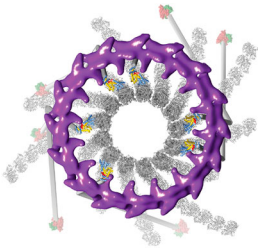
Kinetochore complexes need teamwork



This model shows that Dam1 (purple ring) encircles the microtubule (inner gray structures) and connects to Ndc80 (gray spokes).

Lampert et al. show how two kinetochore protein complexes work together to link up with microtubules.

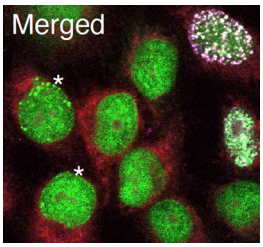
Kinetochores hitch spindle microtubules to the centromeres, helping ensure that chromosomes separate properly during mitosis. In yeast cells, two protein complexes—Ndc80 and Dam1—make the connection to microtubules. Ndc80 clings to the kinetochore, whereas Dam1 may form a ring around the microtubule. Although previous studies suggested that the two complexes cooperate, researchers didn't understand how they interact.

Lampert et al. addressed this question by testing the effects of different Ndc80 mutations on yeast cells. The researchers found that mutations in the portion of Ndc80 that attaches to Dam1 slowed cell growth and disrupted chromosome segregation, suggesting that Ndc80 and Dam1 need to make contact to work properly. The cells perished if the team combined one of these mutations with a different defect that shaves off part of Ndc80's N-terminal tail, indicating that the Dam1-binding site and N-terminal tail perform partly redundant functions.

The team also discovered that mutations in Ndc80's calponin homology domain prevented kinetochores from latching onto microtubules and were fatal for the cells. Previous studies have found that Ndc80 is crucial for kinetochore–microtubule attachment in human cells, and the new results confirm that this function is conserved in yeast.

Lampert, F., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201210091.

Blame ATAD5 for factory closures



Asterisks denote G2 cells lacking ATAD5 that have long-lasting replication factories containing PCNA (green).

Lee et al. show how a protein linked to cancer helps cells shut down DNA replication sites.

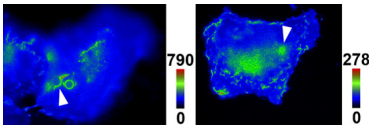
DNA duplication begins at multiple nearby sites, so-called replication factories that teem with enzymes. Cells eventually close the factories, but researchers don't know what determines their longevity. One key replication factory protein is proliferating cell nuclear antigen (PCNA), which clamps onto DNA and helps DNA polymerase move along the strand. Lee et al. previously demonstrated that ATAD5 removes ubiquitin from PCNA together with a deubiquitinating enzyme. However, mice defective in ATAD5 were cancer prone, whereas mice defective in the enzyme were not. Thus, Lee et al. investigated whether ATAD5 affects other aspects of PCNA function.

In cells lacking ATAD5, replication factories lingered on the DNA, often into G2, the researchers found. Many of these long-lived factories were inert during S phase. The absence of ATAD5 slowed DNA duplication and delayed the completion of S phase. Whereas most control cells had exited S phase within nine hours, more than 40% of cells missing ATAD5 had not moved on after that amount of time. In cells where ATAD5 was missing, Lee et al. determined, PCNA adhered to DNA, forming extra-large clusters. This suggests that ATAD5 controls the lifespan of replication factories by dislodging PCNA from DNA.

Just because a factory closes doesn't mean PCNA is out of a job. Cells have a limited supply of PCNA, and bumping the protein from DNA might allow it to transfer to new replication locations. Mutations in ATAD5 spur cancer in mice and humans. The researchers think that the mutations trigger cancer by disrupting removal of the replication factories and holding many replication or repair enzymes there.

Lee, K., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201206084.

Cdc42 helps cells take bigger bites



When cells are attempting to eat large beads, Rac1 activity (indicated by colored scale) is no different in control cells (left) and cells lacking Cdc42 (right).

Phagocytic cells can eat larger items by adding more material to their plasma membrane, Mohammadi and Isberg show.

The motivation for the study was a puzzling observation about a key participant in phagocytosis, the Rho GTPase Cdc42, which spurs actin polymerization that enables the plasma membrane to encircle its target. In certain cases, cells with inactive Cdc42 can engulf small objects such as bacteria, but they can't ingest larger items.

To find out why, Mohammadi and Isberg used RNAi to knock down Cdc42 in cultured cells. One possible explanation for the cells' inability to eat large beads is that the absence of Cdc42 diminishes the activity of another GTPase involved in phagocytosis, Rac1. However, the researchers found that Rac1 activity was normal in Cdc42-lacking cells.

When the researchers observed forming phagosomes in cells that were trying to consume large beads, they noticed that membrane of the phagosome churned, a sign that new material was arriving. However, this membrane flux ended early in cells lacking Cdc42, suggesting that less membrane traveled to the surface of these cells.

Cdc42 therefore directs material to the plasma membrane, enabling cells to eat larger items. Cdc42 exerts its effect by stimulating the exocyst, a protein complex that helps shepherd vesicles to the plasma membrane. When cells are trying to eat big beads, the researchers discovered, Cdc42 temporarily latches onto the exocyst component Sec8. Moreover, reducing the activity of the exocyst protein Exo70 also impaired cells' ability to engulf large objects. Still a mystery is how Cdc42 influences exocyst activity.

Mohammadi, S., and R.R. Isberg. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201204090.

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