Kinetochoore complexes need teamwork

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, suggesting that ATAD5 controls the lifespan of replication factories. Determined, PCNA adhered to DNA, forming extra-large clusters. This suggests that ATAD5 controls the lifespan of replication factories by dislodging PCNA from DNA.

In cells lacking ATAD5, replication factories lingered on the DNA, often into G2. The researchers found that 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.

Blame ATAD5 for factory closures

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.

Cdc42 helps cells take bigger bites

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, 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.