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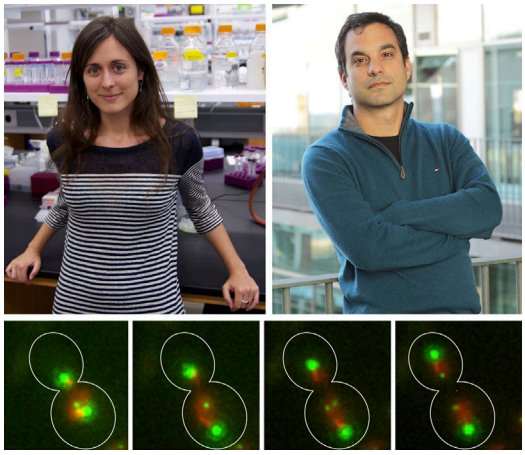
Topoisomerase II has to work late

Chromatid-untangling enzyme takes longer than expected to complete job.

Focal Point



Iris Titos (top left), Manuel Mendoza (top right), and Tsvetomira Ivanova (not pictured) followed the activity of topoisomerase II to figure out how long it takes the enzyme to disentangle sister chromatids. They found that the enzyme doesn't complete its task until anaphase. A time series (bottom) shows that sister chromatids separate properly when topoisomerase II functions normally in yeast cells. Bright green dots mark the spindle pole bodies on either end of the spindle, and the red and faint green dots mark two genes on chromosome IV. As mitosis progresses, the two copies of the genes move apart.

TITOS PHOTO COURTESY OF BENJAMIN SANCHEZ; MENDOZA PHOTO COURTESY OF THE CENTRE FOR GENOMIC REGULATION/IVAN MARTI; YEAST CELL IMAGE COURTESY OF GABRIEL NEUROHR

Without the enzyme topoisomerase II (topo II), sister chromatids can't separate during mitosis. Contrary to conventional wisdom, however, the enzyme is still unraveling tangled DNA molecules during anaphase in budding yeast, Titos et al. reveal (1).

When Watson and Crick described the structure of DNA, researchers immediately foresaw a problem during replication. The two DNA molecules resulting from replication would tangle, and, unless they could be separated, sister chromatids wouldn't be able to part during mitosis. But topo II comes to the rescue, cutting one of the DNA molecules so that the other can slip free, a process known as decatenation (2).

Topo II starts removing the so-called sister chromatid intertwines (SCIs) during S phase, and most researchers think that it completes the job before anaphase. Studies in yeast support that conclusion, showing that the enzyme releases entwined plasmids before anaphase (3). But another yeast study found that topo II waited until anaphase to resolve entanglements in ribosomal DNA (4). However, this chromosome region is unusual in that it consists of a long stretch of repeated sequences and may therefore take more time to unravel than the rest of the genome. Deleting topo II from animal cells also suggests that the enzyme is still at work during anaphase (5). Animal chromosomes are typically longer than their yeast counterparts, though, and the impact of length on SCI resolution is unclear.

> "We have discovered a role for microtubule dynamics in decatenation."

Titos et al. wanted to determine how chromosome length affects the timing of SCI removal in yeast, and they wanted to avoid the potential complication of ribosomal DNA sequences. The researchers therefore fused chromosome IV to several small chromosomes to produce giant chromosomes that carry no ribosomal DNA. Cells harboring these oversized chromosomes grew and reproduced normally, and the plus-sized chromosomes didn't change the rate of elongation of the mitotic spindle or the duration of anaphase.

To track the progress of topo II, the scientists fluorescently labeled two genes, *TRP1* and *LYS4*, that reside on chromosome IV and on the elongated chromosome. *TRP1* is close to the centromere, whereas *LYS4* sits in the middle of the long arm. The two copies of each gene initially appeared as one glowing dot because the sister chromatids were so close. But in early anaphase the individual dots split into two as topo II disentangled the sister chromatids and allowed them to move apart.

Titos et al. then tested mutant yeast cells that have normal chromosomes but carry a version of topo II that stops working at high temperatures, allowing the team to switch the enzyme on and off. If Titos et al. turned up the heat shortly after S phase, they found that the two copies of *TRP1* separated normally, but only ~40% of the *LYS4* dots had parted by the end of anaphase, presumably because topo II usually hadn't progressed that far along chromosome IV before being inactivated.

The researchers then evaluated mutants with normal or long chromosomes at moderate temperatures, where some topo II remains active. They found that the *LYS4* dots separated in cells that had normal chromosomes but failed to diverge in most cells with the elongated chromosomes. The findings suggest that the enzyme requires more time to disentangle longer chromosomes.

Another factor besides chromosome length slows the resolution of DNA entanglements: chromosomes are attached to the nuclear envelope during interphase. Titos et al. showed that cutting the chromosomes loose increased the efficiency of separation when topo II activity was compromised.

Topo II gets help during anaphase from a surprising source: microtubules. The researchers found that a mutation that impairs the protein Stu2, which promotes microtubule dynamics, hindered growth of cells with large chromosomes, a sign that their chromosomes aren't separating properly.

Thus, in yeast, topo II continues working into anaphase to disentangle sister chromatids. It's not just unraveling the ribosomal DNA region; it's also finishing the longer chromosomes. However, the enzyme doesn't have to do the job alone. "We have discovered a role for microtubule dynamics in decatenation," says senior author Manuel Mendoza. Increased turnover of microtubules, spurred by Stu2, might alternatively tighten and release DNA molecules and help them disengage.

1. Titos, I., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201404039.
2. Wang, J.C. 2002. *Nat. Rev. Mol. Cell Biol.* 3:430–440.
3. Baxter, J., et al. 2011. *Science*. 331:1328–1332.
4. D'Ambrosio, C., et al. 2008. *Curr. Biol.* 18:1084–1089.
5. Oliveira, R.A., et al. 2010. *Nat. Cell Biol.* 12:185–192.