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Sending sisters their separate ways

Repair protein helps resolve entanglements between sister chromatids.

Sister chromatids are often reluctant to separate during mitosis. Germann et al. show that a protein involved in DNA replication and repair helps eliminate lingering connections that can hold sister chromatids together (1).

Sister chromatids tangle almost every time a cell enters mitosis. Unless the cell releases these links, the structures can break or fail to separate properly. Two kinds of attachments can persist even as late as anaphase (2). Chromatin bridges are the larger, better known variety. As their name indicates, they contain chromatin and result when one sister chromatid adheres to its sibling. In 2007 scientists discerned a second type of anaphase DNA entanglement in mammalian cells: ultrafine bridges, or UFBs (3). These thin filaments extend between sister chromatids and don't appear to contain chromatin. Some UFBs sport replication protein A, which latches onto single DNA strands, suggesting that the bridges are also single stranded (4). Scientists have proposed four different ways in which anaphase bridges might arise. Germann et al. tested these ideas and how cells deal with the entanglements.

The researchers started by identifying UFBs in budding yeast cells. Because the nuclear envelope doesn't break down during yeast mitosis, the structures extend through a narrow tube that links the mother and daughter nuclei. Like some UFBs in mammalian cells, many yeast bridges appear to be single-stranded DNA. The team found that UFBs are typically decorated with the protein Dpb11, which has several jobs, including detection and repair of DNA damage, as well as DNA replication.

One hypothesis holds that anaphase bridges form at locations in the genome that haven't yet been replicated or where DNA duplication has halted. To evaluate this idea, Germann et al. dosed yeast cells with methyl methanesulfonate, which stops the advance of replication forks. The result was a surge in UFBs, suggesting that replication stress, a disruption in DNA synthesis, triggers production of UFBs.

Another possible source of bridges is catenated DNA, in which both strands of one sister chromatid interweave with both strands of the other sister. Topoisomerase II normally smoothes out these snarls, and the number of UFBs increased in cells lacking this enzyme, the team found. The team also discovered that, in normal cells, topoisomerase II clusters on UFBs, indicating that the enzyme is hard at work to resolve these interconnections.

Some sisters aren't that close, with only a single strand from each chromatid snarled. This hemicatenated DNA might also generate UFBs. Germann et al. disabled the cell's mechanism for dealing with hemicatenated DNA by deleting the gene for Sgs1, sparking an increase in UFBs.

> "What we show is that [Dpb11] binds both kinds of anaphase bridges."

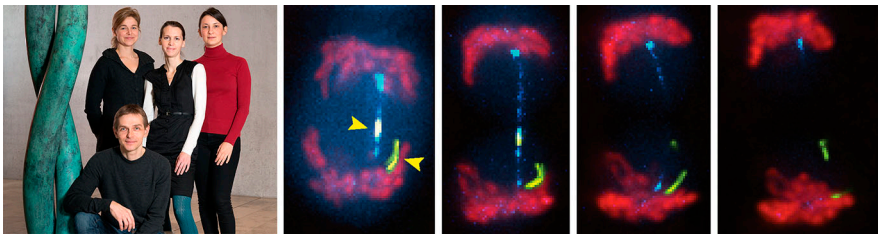
Thus the study supports three of the potential origins for UFBs—catenated DNA, hemicatenated DNA, and unreplicated sections of the genome. Germann et al.'s results discounted the fourth potential source, the DNA repair mechanism homologous recombination. The team discovered that homologous recombination spurs the formation of chromatin bridges but not UFBs.

The researchers also determined the roles of Dpb11 during bridge formation and breakdown. "What we show is that this protein binds both kinds of anaphase bridges," says senior author Michael Lisby. But the protein has opposite effects on the two types of structures. Dpb11 inhibits chromatin bridges by curbing homologous recombination. By contrast, the protein strengthens UFBs and enables them to elongate. Using chick cells, the researchers showed that the vertebrate equivalent of Dpb11, TopBP1, also performs dual functions, thwarting chromatin bridges and stabilizing UFBs.

Those results raise a question: If TopBP1 and Dpb11 stabilize UFBs, how do they spur chromosome separation? The researchers speculate that stretching out UFBs might promote their eventual resolution by providing access for topoisomerase II, which can disentangle the strands.

A question for further research is what happens after the bridges come down. Although anaphase bridges usually disappear before the end of mitosis, some DNA damage persists into the next round of the cell cycle. "It's not clear how this damage is repaired," says Lisby.

Focal Point



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(Clockwise from top left) Vibe Oestergaard, Susanne Germann, Irene Gallina, Michael Lisby, and colleagues (not pictured) investigated four possible sources for the bridges between sister chromatids. They found that one mechanism promotes the formation of large chromatin bridges. Three mechanisms spur formation of the thinner ultrafine bridges. This time series shows an ultrafine bridge (blue) as a chick cell advances through anaphase and telophase. Red marks the chromosomes, and yellow marks TopBP1 attached to the bridge.

1. Germann, S., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201305157.
2. Kaulich, M., F. Cubizolles, and E.A. Nigg. 2012. *Chromosoma.* 121:395–408.
3. Chan, K.L., P.S. North, and I.D. Hickson. 2007. *EMBO J.* 26:3397–3409.
4. Chan, K.L., and I.D. Hickson. 2009. *Cell Cycle.* 8:3065–3066.