In This Issue

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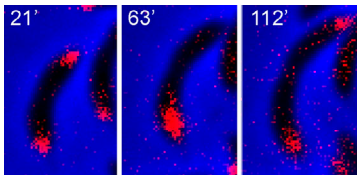
Chromosome search and rescue



A time-lapse series shows how two homologous loci (red) pair up for homologous recombination and then resegregate to opposite cell poles.

Badrinarayanan et al. describe how regions of bacterial chromosomes come together to repair DNA breaks and then move back to their original positions once the process is complete.

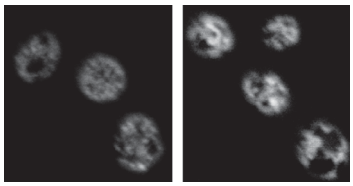
Just like eukaryotes, bacteria repair DNA double-strand breaks (DSBs) by homologous recombination, which requires a broken chromosomal locus to pair up with the equivalent, undamaged region of its sister chromosome. To investigate how homologous loci find each other, and how they separate once DSBs are repaired, Badrinarayanan et al. induced single DSBs at defined locations along the 4-Mb chromosome of *Caulobacter crescentus*. This chromosome is tethered to one pole of the bacterium via a specific locus, called *parS*, located near the chromosome's replication origin. During replication, one copy of *parS* is drawn to the opposite cell pole by the *parS*-binding protein ParB and the ATPase ParA.

When the researchers introduced a DSB near the replication origin of one of the chromosomes, ~250 kb of the surrounding DNA left the pole and "searched" around the cell before finding the homologous, undamaged region at the opposite end of the cell. After ~30 minutes, the homologous loci separated, and the repaired DNA was returned to its own pole by *parS*, ParB, and ParA. The rest of the chromosome retained its organization throughout this process.

When the researchers introduced DSBs further away from the replication origin, the damaged loci also sought out their intact copies. Their subsequent resegregation didn't depend on the ParABS system, however. Senior author Michael Laub thinks that, instead, physical forces may cause these regions to snap back into position once homologous recombination is complete.

Badrinarayanan, A., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201505019

SNAREs share atlastin's burden



Compared with wild-type cells (left), ER morphology is disrupted in yeast lacking both Sey1p and Sec22p (right).

SNARE proteins help the dynamin-like GTPase atlastin to fuse ER membranes together, Lee et al. reveal.

ER tubules fuse with each other to form an interconnected network that spreads throughout the cytoplasm. Homotypic ER fusion is mainly mediated by the atlastin family of dynamin-like GTPases, although, in the absence of the yeast atlastin Sey1p, SNARE proteins, which promote various membrane fusion events in the cell, can support a residual amount of ER membrane fusion. Whether SNARE proteins usually function in atlastin-mediated ER fusion is unclear, however.

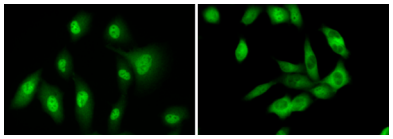
Lee et al. developed an in vitro assay to measure the Sey1p-dependent fusion of purified ER membranes and found that fusion was reduced when ER-localized SNAREs, such as Sec22p, were inhibited. Moreover, Sey1p bound to ER SNARE complexes, suggesting that the two protein families work together to promote ER membrane fusion.

Sey1p can stimulate the fusion of artificial liposomes on its own, but Lee et al. discovered that this was only true when Sey1p was present at a much higher level than its physiological concentration. In vivo, Sey1p may rely on SNAREs for efficient fusion, a requirement that might prevent ER tubules from mistakenly fusing with all the other organelles they contact in the cell.

Lee et al. found that, in vitro, Sey1p acts before SNAREs during ER fusion. Senior author Youngsoo Jun now wants to investigate whether Sey1p's main role is to tether ER membranes close enough together for SNAREs to drive their fusion.

Lee, M., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201501043

Integrin signaling tranquilizes Hippo



YAP (green) localizes to the nucleus of cells spread on fibronectin (left), but is mainly cytoplasmic in cells plated on laminin (right).

Kim and Gumbiner describe how adhesion to fibronectin can switch off the Hippo signaling pathway and stimulate cell growth.

The Hippo signaling pathway suppresses cell proliferation by activating a protein kinase called Lats that phosphorylates the transcription factors YAP and TAZ, promoting their retention in the cytoplasm. The pathway's activity is influenced by various factors, including intercellular adhesion and cytoskeletal tension. Mitogens, such as EGF and LPA, inhibit the Hippo pathway by activating PI3-kinase and its downstream target PDK1, allowing YAP/TAZ to enter the nucleus and induce the transcription of progrowth genes. Kim and Gumbiner noticed, however, that, even in the absence of mitogens, PI3-kinase and PDK1 still inhibited Lats and promoted YAP's entry into the nuclei of subconfluent epithelial cells.

The researchers discovered that, under these conditions, the Hippo pathway was suppressed by the cells' adhesion to the extracellular matrix protein fibronectin, which activated PI3-kinase via a signaling pathway involving the integrin-associated kinase FAK and its downstream target, Src. Inhibiting FAK or Src reactivated the Hippo pathway and induced YAP's relocalization to the cytoplasm.

Because integrins and FAK respond to mechanical forces, Kim and Gumbiner's findings might help explain how cytoskeletal tension modulates Hippo signaling, allowing cell growth to be regulated by the physical properties of the extracellular matrix. The authors now want to investigate how PI3-kinase and PDK1 inhibit the Hippo pathway in response to multiple upstream signals.

Kim, N.-G., and B.M. Gumbiner. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201501025