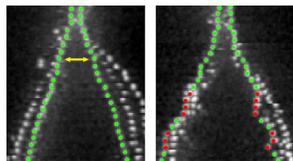


## Sds22 and Repo-Man keep anaphase chromosomes moving



Kymographs show that sister kinetochores (green dots) move apart steadily in control anaphase cells (left) but frequently pause (red dots) in the absence of Repo-Man (right).

**W**urzenberger et al. identify two phosphatase-targeting proteins that help chromosomes to segregate properly during anaphase.

In early mitosis, the kinase Aurora B localizes to centromeres, where it eliminates incorrect attachments to the mitotic spindle by phosphorylating microtubule-binding kinetochore proteins like Dsn1. When sister chromatids are attached to opposite spindle poles, tension pulls the kinetochore proteins away from Aurora B, stabilizing their interactions with spindle microtubules. This tension is lost when sister chromatids separate at the beginning of anaphase, however, so cells must somehow prevent Aurora B from weakening kinetochore–microtubule attachments during chromosome segregation. This is partly achieved by relocating Aurora B to

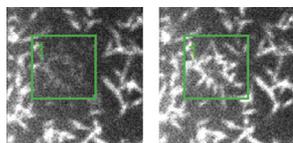
the central spindle, but Wurzenberger et al. suspected that phosphatases might also counteract Aurora B on anaphase chromosomes.

In an RNAi screen of phosphatase subunits, the researchers identified two proteins whose depletion enhanced the phosphorylation of Aurora B substrates on anaphase chromatin. Sds22 and Repo-Man both target the phosphatase PP1 to chromosomes. Though knocking down either protein had no effect on Aurora B's activity or relocation to the central spindle, Dsn1 was more phosphorylated in anaphase cells lacking Sds22 or Repo-Man. Segregating chromosomes often paused in these cells, suggesting that their attachments to the spindle were unstable. This frequently resulted in chromosome missegregation.

Senior author Daniel Gerlich now wants to investigate which Aurora B substrates, besides Dsn1, are hyperphosphorylated in the absence of Sds22 or Repo-Man and to determine which of these are responsible for the defects in kinetochore–microtubule attachment during anaphase.

Wurzenberger, C., et al. 2012. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201112112>.

## Microvillar scaffold is only temporary



Photobleached microvilli (green square, left) recover their GFP-EBP50 fluorescence within five seconds (right).

**A** scaffold protein required to hold membrane proteins inside microvilli is surprisingly dynamic, Garbett and Bretscher reveal.

Microvilli are actin-rich membrane protrusions on the apical surface of epithelial cells. Microvillar assembly requires both ezrin, a protein that links actin to the plasma

membrane, and EBP50, a scaffold protein that binds tightly to ezrin and couples it to a variety of membrane proteins like podocalyxin that interact with EBP50's PDZ domains. Individual microvilli last for 10–15 minutes before disassembling, so Garbett and Bretscher decided to compare the dynamics of different microvillar proteins.

Photobleaching experiments revealed that ezrin turned over fairly slowly in microvilli and that podocalyxin was even more stably associ-

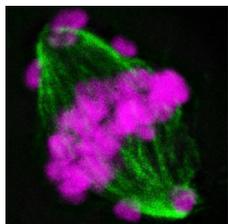
ated with the apical protrusions. But EBP50 was much more dynamic within microvilli, turning over rapidly despite its tight association with ezrin and its role in retaining podocalyxin in the microvillar membrane. Even more surprisingly, mutating EBP50's PDZ domains to inhibit their interactions with membrane proteins stabilized EBP50 inside microvilli instead of accelerating the protein's turnover still further.

The researchers recapitulated their findings *in vitro*. EBP50 attached firmly to ezrin-coated beads, but its grip was loosened in the presence of epithelial cell extract. An EBP50 mutant with nonfunctional PDZ domains maintained its hold on ezrin, however.

EBP50's scaffolding function may therefore be self-limiting, its association with ezrin weakening as it retains increasing numbers of membrane proteins in microvilli. The authors now want to identify the components of the epithelial cell extract that destabilize EBP50's interaction with ezrin.

Garbett, G., and A. Bretscher. 2012. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201204008>.

## BubR1 shows self-control



Several chromosomes (purple) fail to align on the mitotic spindle (green) of a cell expressing non-phosphorylatable BubR1.

**G**uo et al. describe how the mitotic kinase BubR1 phosphorylates itself in order to promote accurate chromosome segregation.

BubR1 localizes to kinetochores and has two important functions in mitosis: it helps chromosomes to align correctly on the mitotic spindle, and, by maintaining the mitotic checkpoint, it prevents cells from entering anaphase until every chromosome is properly attached to spindle microtubules. Whether BubR1's kinase activity is required

for these processes is unclear, however. No substrates have been identified in cells, and purified BubR1 shows little activity *in vitro*, though the enzyme will phosphorylate itself in the presence of its binding partner, the microtubule-based motor protein CENP-E.

Guo et al. found that CENP-E also stimulates BubR1's autophospho-

rylation *in vivo*. Cells expressing a nonphosphorylatable version of BubR1 frequently missegregated their chromosomes in mitosis. The cells' mitotic checkpoint was weakened because, unlike in wild-type cells, the checkpoint proteins Mad1 and Mad2 failed to accumulate strongly on kinetochores that weren't attached to the spindle. In addition, many kinetochores formed incorrect spindle attachments when BubR1 couldn't phosphorylate itself, preventing chromosomes from aligning properly in metaphase. Incorrect attachments are normally destabilized by the kinase Aurora B, which phosphorylates and inhibits microtubule-binding kinetochore proteins like Hec1. Guo et al. found that Aurora B's ability to phosphorylate Hec1 was diminished in the presence of nonphosphorylatable BubR1.

Hec1 phosphorylation was also decreased in CENP-E-deficient cells, but chromosome alignment was restored by a phosphomimetic BubR1 mutant. Senior author Yinghui Mao now wants to investigate how CENP-E-stimulated BubR1 autophosphorylation regulates the mitotic checkpoint and the ability of Aurora B to destabilize incorrect spindle attachments.

Guo, Y., et al. 2012. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201202152>.