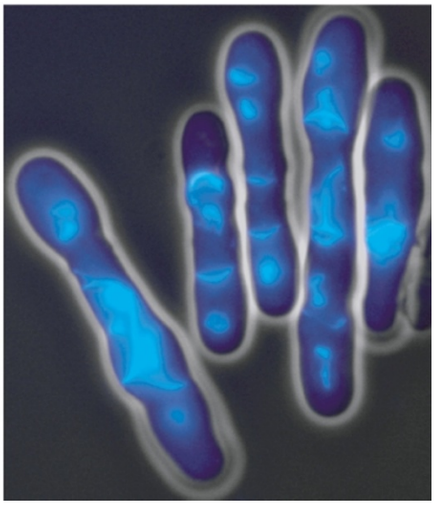

The authors conclude that the SAPK pathway is involved in controlling this actin-dependent mitotic checkpoint, which probably "ensures mitotic spindles are properly oriented before anaphase is allowed to take place". This checkpoint might be especially important during the development of multicellular organisms, when the establishment of asymmetric cell fates can depend on the orientation of the spindle during mitosis.

Alison Mitchell

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This serine lies in a consensus phosphorylation site for calcium–calmodulin-dependent protein kinase II (CaMKII), and purified brain CaMKII indeed phosphorylates myosin-V at this site. Moreover, inhibitors of CaMKII block myosin-V phosphorylation during mitosis, preventing the release of the motor from melanosomes.

So it seems that CaMKII is responsible for the specific release of myosin-V from melanosomes during mitosis. It will be equally interesting to next find out how these organelles recruit their motor again at the end of mitosis to resume their place in traffic.

Raluca Gagescu

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DNA REPAIR

The replication connection

The Mre11 complex — containing Mre11, Rad50 and Nbs1 — promotes the repair of double-strand DNA breaks (DSBs). One way to do this is by homologous recombination — a process that is intrinsically linked to DNA replication. Because of this connection, the Mre11 complex has been implicated in replication, and two new papers support this idea.

Jean Gautier and colleagues report in *Molecular Cell* the cloning and purification of the *Xenopus* Mre11 homologue. They show that X-Mre11 is phosphorylated in response to DSBs, and that this modification stimulates the 3′–5′ exonuclease activity of the X-Mre11 complex (the function of which is currently a mystery).

Gautier and colleagues asked whether X-Mre11 is phosphorylated in the absence of DSBs, using purified sperm nuclei that had been incubated in extracts to allow chromatin assembly and DNA replication. The authors detected phosphorylated X-Mre11 in the nuclear fraction during replication, indicating that active X-Mre11 can associate with chromatin during normal DNA synthesis. They then used several techniques to show that DSBs arise during replication, and that they accumulate when Mre11 is not present. The implication, they say, is that DSBs arise during normal replication and that the X-Mre11 complex is involved in their repair.

In *Molecular and Cellular Biology*, John Petrini and colleagues describe their investigations into the Nbs1 component of the Mre11 complex. Phosphorylation of Nbs1 is required for activation of the S-phase checkpoint, which stalls replication in response to DNA damage. However, cells containing an aberrant Nbs1 that lacks the amino terminus cannot activate the S-phase checkpoint, even when phosphorylated.

Given that the amino-terminal region of Nbs1 seems to be essential for checkpoint activation, Petrini and colleagues investigated it further using a two-hybrid screen and discovered that it interacts with the transcription factor E2F1. Within 400 base pairs of the Epstein–Barr virus latent origin of replication (*oriP*) are two E2F-binding sites, and the authors confirmed not only that E2F–Nbs1 binds near *oriP*, but also that binding increased as cells progressed through S phase. Finally they showed that the Mre11 complex co-localizes with DNA replication foci containing a replication protein — the proliferating cell nuclear antigen (PCNA) — throughout S phase. These results support the idea that the Mre11 complex influences the progression of S phase by acting not only at replication origins (through the interaction with E2F1) but also at the replication fork.

Alison Mitchell

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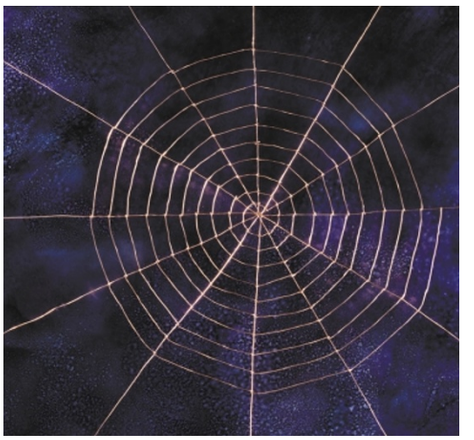
WEB SITE Gautier lab | Petrini lab



CELL POLARITY

Weaving a cellular web

The intricate web of protein–protein interactions in the cell is now revealing its secrets to us, due to the development of high-throughput two-hybrid screening. Reporting in the *Journal of Cell Biology*, Drees and colleagues now describe the results of their screen for cell polarity proteins.

To target this type of protein, Drees and colleagues used as bait 68 proteins known to mediate cell polarity, ranging from Rho-type GTPases to proteins involved in secretion. The result was 128 new protein–protein interactions, 44 of which involve proteins of unknown function. So what does this tell us about how the polarity factors are weaved together?

One important outcome is that new connections were made, both between signalling pathways — for example, the Rho1 and Cdc42 effector pathways — and between distinct processes, such as the assembly of actin and the morphogenesis checkpoint. To confirm some of these interactions, the authors fused factors to yellow fluorescent protein and looked at their subcellular localization.

Given that many of these factors have mammalian homologues, these interactions have implications for polarity in diverse cell types, but there is still much to learn. The next step will be to use genetic and biochemical tools to ask when and where these factors meet in the cell and how the information flows through this complex web.

Alison Schuldt,
Associate Editor, Nature Cell Biology

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