CELL CYCLE

Split timing

The microtubular central spindle is a molecular assembly that participates in the regulation of cytokinesis. And, the centralspindlin complex — which consists of the kinesin-like protein ZEN-4 and the Rho-family GTPase-activating protein CYK-4 in *Caenorhabditis elegans* (and their orthologues MKLP1 and MgcRacGAP in mammalian cells) — bundles the individual microtubules together and so is essential for the assembly of this machine at anaphase. But how is the timing of this assembly regulated?

Reporting in *Nature*, Mishima *et al.* started to dissect this problem by identifying the mechanism that regulates centralspindlin activity and, therefore, central-spindle assembly. They showed, using *in vitro* assays, that the amino-terminal motor domains of ZEN-4 (ZEN-4^{MOT}) and MKLP1 are phosphorylated by the cyclin-B–CDK1 complex, which is active during metaphase and becomes inactivated at the metaphase–anaphase transition. Deletion of an amino-terminal basic extension, which contained one of the cyclin-B–CDK1 phosphorylation sites of ZEN-4^{MOT}, also reduced its ATPase activity and its ability to cause microtubule movement — both of which are vital functions of the kinesin.

Phosphorylation of a conserved threonine residue in the isolated ZEN-4^{MOT} reduced the ATPase and microtubule-mobility functions of the domain, which indicates that this protein might be controlled by phosphoregulation. In agreement with this, when a mutated, unphosphorylatable form of MKLP1 (MKLP1-AA) was expressed in cultured mammalian cells, the protein localized inappropriately to the spindle and the genomic material failed to segregate properly. Furthermore, by using a phospho-threonine-proline antibody and comparing chromosome-segregation events in normal and MKLP1-AA-expressing cells, Mishima *et al.* showed that MKLP1 is phosphorylated during metaphase — which prevents its association with the spindle — and is dephosphorylated during anaphase when it becomes localized to the central spindle.

Finally, the CDC14 phosphatase was shown to dephosphorylate both MKLP1 and ZEN-4 *in vitro*. And in *C. elegans*, depletion of CDC-14 prevented the localization of a tagged form of ZEN-4, but not that of an unphosphorylatable mutant of ZEN-4, to the central spindle in anaphase. So, it seems that cell-cycle kinase and phosphatase activities control the timing of central-spindle formation and, consequently, cell division.

Lesley Cunliffe

References and links

ORIGINAL RESEARCH PAPER Mishima, M. *et al.* Cell cycle regulation of central spindle assembly. *Nature* **430**, 908–913 (2004)

WEB SITE

Michael Glotzer's laboratory: http://www.imp.univie.ac.at/glotzer/glo_hp.html

CELL POLARITY

Territorial proteins

We look symmetrical from the outside, but it's well known that our organs are distributed asymmetrically within our bodies. Cells also hide a distinct asymmetry, generating, for example, an apicobasal polarity by unevenly organizing cellular components. Two well-studied proteins that are implicated in apicobasal polarity in mammalian epithelial cells are atypical protein kinase C (aPKC) and PAR1, and Suzuki *et al.* now show that aPKC functions upstream of PAR1 in establishing and maintaining this polarity.

aPKC and PAR1 segregate along the apicobasal axis of Madin–Darby canine kidney (MDCK) cells — PAR1 localizes at the basolateral membrane, whereas aPKC is distributed in the apical membrane. Could one affect the localization of the other? Using RNA interference, the authors showed that PAR1b (one of four PAR1 homologues) was required for membrane domains to develop asymmetrically in MDCK cells. But depleting PAR1b didn't affect the development of the cell–cell junctions known as tight junctions (TJs) or the asymmetric localization of aPKC. By contrast, depleting aPKC inhibited TJ formation and prevented PAR1b accumulation.

Suzuki *et al.* next showed that aPKC could phosphorylate PAR1b on threonine 595 and that PAR1b was phosphorylated more in depolarized than in polarized MDCK cells. Late during the cell-polarization process, T595 seemed to become dephosphorylated. These data, with results from cell-fractionation studies and immunocytochemistry, are consistent with the need for PAR1b T595 to be dephosphorylated for its stable lateral localization — T595-phosphorylated PAR1b was mainly found in the soluble fraction.

In *Drosophila melanogaster*, the scaffold protein 14-3-3 binds to PAR1, and Suzuki *et al.* found not only that these proteins also interacted in MDCK cells, but that T595 phosphorylation enhanced this. Because 14-3-3 is thought to alter the intracellular localization of its binding partners, and because it interacted mainly with soluble, phosphorylated PAR1, the authors suggest the following model: aPKC at the membrane phosphorylates PAR1b, which then dissociates from the membrane into a soluble fraction by the actions of 14-3-3. In this way, PAR1b is excluded from the apical membrane of TJs, thereby maintaining appropriate asymmetric membrane domains. Supporting this model, a non-phosphorylatable mutant of PAR1b invaded the apical membrane of MDCK cells and caused asymmetric membrane domains to develop abnormally.

Katrin Bussell

References and links

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WEB SITE

Shigeo Ohno's laboratory: http://www-user.yokohama-cu.ac.jp/~ohnos/index.html