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How the spindle keeps its focus

The microcephaly-associated protein Asp works with calmodulin to cross-link the minus ends of spindle microtubules.

During mitosis, the *Drosophila* protein Asp binds and focuses microtubule minus ends at the spindle poles, and maintains their attachment to the centrosomes. Two groups of researchers now provide insights into how the protein achieves these functions (1, 2).

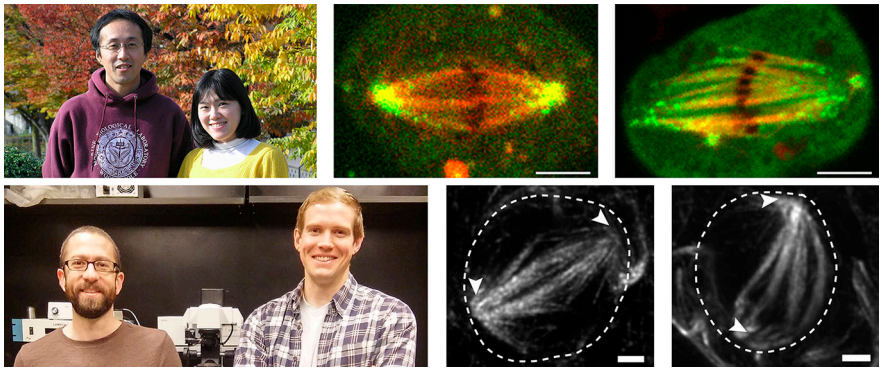
asp was one of the first genes to be identified as being required for correct assembly of the *Drosophila* mitotic spindle (3), and mutations in its human homologue, *ASPM*, cause microcephaly. Despite this, Asp's biochemical function has remained uncertain. "We wanted to know what the molecular activity of this protein was," explains Gohta Goshima, from Nagoya University in Japan.

One possibility was that Asp works with Ncd, a minus end–directed kinesin motor protein that can cross-link microtubules and promote spindle pole focusing (4). However, when Goshima and his graduate student Ami Ito closely compared the phenotypes of *Drosophila* S2 cells lacking Asp and/or Ncd, they realized that the two proteins act independently of each other (1). Asp was required to keep microtubule minus ends focused at the spindle poles from the onset of prometaphase onwards. Ncd, in contrast, wasn't required until mid-prometaphase, and it appeared to coalesce microtubules throughout the spindle, rather than just focusing their minus ends at the poles.

Ito and Goshima therefore wondered whether Asp itself was capable of cross-linking microtubules. Asp contains a microtubule-binding domain near its N terminus, but the researchers found that a central region of the protein was able to cross-link microtubules in vitro. This region was necessary, but not sufficient, for Asp's pole focusing activity in vivo.

Although Asp mainly accumulates at microtubule minus ends near the spindle poles, Ito and Goshima noticed that, in live cells, small puncta of GFP-tagged Asp localized throughout the spindle. These dots corresponded to the minus ends of intraspindle microtubules nucleated by the augmin complex, and the researchers found that Asp was required to cross-link these structures to the rest of the spindle so that they could be transported to the spindle poles by microtubule flux.

FOCAL POINT



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Two groups investigate how the microcephaly-associated protein Asp organizes mitotic spindles. (Top row, left to right) Gohta Goshima and Ami Ito reveal that Asp focuses spindle poles by cross-linking microtubule minus ends at the poles and within the spindle. Spindle microtubules (red) are focused at the poles in *Drosophila* S2 cells expressing full-length Asp (green, left) but unfocused in cells that instead express a C-terminally truncated version of the protein (green, right). Meanwhile (bottom row, left to right), Nasser Rusan, Todd Schoborg, and colleagues reveal that the association of calmodulin with Asp's C-terminal domain is crucial for the proteins' mutual function in pole focusing and centrosome attachment. In the neuroblasts of an *asp*-null fly, pole focusing is restored by full length Asp (left) but not by an Asp mutant unable to bind calmodulin (right). Calmodulin is not required for Asp's ability to promote brain growth, however.

Asp therefore focuses spindle poles by cross-linking microtubule minus ends within the spindle and at the poles themselves. Though this requires Asp's central region, it also depends on the protein's C-terminal domain, which is thought to bind to the calcium-sensing protein calmodulin (CaM). Nasser Rusan, from the NIH in Bethesda, Maryland, was interested in the interaction between these two proteins, because CaM has long been known to localize to spindle poles, and its knockdown induces unfocused poles and centrosome detachment, similar to the effects of depleting Asp (5). Rusan and colleagues, led by postdoc Todd Schoborg, confirmed that CaM binds to the C terminus of *Drosophila* Asp and found that the two proteins colocalize on the spindle, not only at the poles but also at intraspindle minus ends undergoing poleward flux (2). "Their movements are identical," Rusan says.

In keeping with *ASPM*'s links to microcephaly, flies carrying hypomorphic *asp* alleles develop abnormally small brains (6). To better understand Asp's role in neural development, Schoborg et al. used CRISPR to generate *asp* null flies. These animals also developed small brains, and their neuroblasts, which give rise to the nervous system through a series of asymmetric cell divisions, formed abnormal mitotic spindles with unfocused poles and detached centrosomes. These spindle defects could be rescued by full-length Asp, but not by mutant versions unable to bind CaM. "So the Asp-CaM interaction is important for pole focusing and centrosome attachment," Schoborg explains.

Surprisingly, however, these CaM-binding mutants were able to rescue the flies' brain size, suggesting that Asp has an additional, CaM-independent function, and that microcephaly doesn't arise from defects in the mitotic spindle. Schoborg and Rusan now want to investigate what this additional function might be and to determine how CaM assists Asp's role in spindle organization. Preliminary results suggest that CaM stabilizes Asp and might promote its oligomerization. Goshima and colleagues, meanwhile, plan to check whether Asp's function is conserved in human ASPM.

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5. Goshima, G., et al. 2007. *Science*. 316:417–421.
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