MITOSIS

Formin' an attachment

The mammalian diaphanous-related formins (mDIAs), originally characterized for their ability to nucleate unbranched actin, can also regulate microtubule stabilization during cell polarization and migration. Cheng *et al.* now extend the remit of formins to the control of microtubules during mitosis by showing that mDIA3 (also known as DIAPH2) ensures stable attachment of microtubules to kinetochores.

mDIA3 has previously been shown to affect the alignment of chromosomes during metaphase. Cheng *et al.* set out to pin down the basis of this effect: using small interfering RNA, they confirmed that depletion of human mDIA3 disrupted the alignment of metaphase chromosomes, and they used timelapse analysis to show that anaphase onset was delayed. This phenotype was not affected by loss of actin binding or nucleation by mDIA3, suggesting that it might reflect a microtubule-related role of mDIA3.

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A key step in driving chromosome alignment is the formation of stable attachments between microtubules and kinetochores, and indeed, cells depleted of mDIA3 had an impaired ability to stabilize kinetochore–microtubule bundles in response to cold treatment. In other contexts, formins can regulate the capture of microtubules by associating with the microtubule tip-binding protein EB1. Consistent with this, Cheng *et al.* found that cells expressing an mDIA3 mutant that cannot bind EB1 showed increased chromosome misalignment.

How is this activity of mDIA3 controlled? The authors found that mDIA3 localizes to the kinetochore from prometaphase onwards, although it does not seem to be important for the structural integrity of the kinetochore itself. The Aurora B kinase regulates several kinetochore proteins to ensure proper microtubule attachments, and the authors show that Aurora B also phosphorylates mDIA3 *in vitro*. They mapped this activity to four potential residues,

two of which were in the formin-homology domains of mDIA3. They then confirmed that at least one of these, Ser196, is phosphorylated in an Aurora B-dependent manner *in vivo*. Moreover, mDIA3 mutants that could not be phosphorylated at either one or all four of these sites showed defects in chromosome alignment. Closer inspection using live-cell microscopy analysis revealed that, in cells expressing a phosphomimetic mDIA3 mutant, initial attachments could form between microtubules and kinetochores, but these were unstable. In addition, phosphomimetic mDIA3 could not stabilize microtubules upon cold treatment *in vitro*.

This suggests that formins can regulate microtubules, independently of their effects on actin, in both interphase and mitotic cells. During mitosis, mDIA3 stabilizes the attachment of microtubules to kinetochores, and this is carefully controlled by Aurora B-mediated phosphorylation. This role seems to require EB1 binding by mDIA3, and it will be important to understand how the actions of mDIA3 are coordinated with the other kinetochore complexes at this intricate interface.

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ORIGINAL RESEARCH PAPER Cheng, L. *et al.* Aurora B regulates formin mDia3 in achieving metaphase chromosome alignment. *Dev. Cell* **20**, 342–352 (2011).