activation is (for unclear reasons) more ultrasensitive than Kss1 activation [1,16]. It is uncertain whether cytoplasmic Ste5 increases the ultrasensitivity of Fus3 activation or merely permits an intrinsically ultrasensitive step to occur [16].

One of the many interesting implications of the new work is that graded signaling is inducible. In unstimulated cells, Ste5 is found in the nucleus and cytoplasm, and thus MAPK activation has a relatively high threshold, which would minimize sensitivity to noise and to spurious crosstalk [14]. Pheromone stimulation would trigger Ste5 membrane recruitment, promoting graded signaling and, in effect, sensitizing the MAPK cascade.

The new work is consistent with the idea that the MAPK cascade has been conserved not because it performs a particular circuit function such as acting as a switch, but because it can be adapted to perform a variety of different functions depending on the performance objectives of the pathway in which it is embedded and the physiological requirements of the organism [17,18]. Versatility may be the most important design feature of the MAPK cascade.

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Mitosis: New Roles for Myosin-X and Actin at the Spindle

Roles for actin and myosin in positioning mitotic spindles in the cell are well established. A recent study of myosin-X function in early *Xenopus* embryo mitosis now reports that this unconventional myosin is required for pole integrity and normal spindle length by localizing to poles and exerting pulling forces on actin filaments within the spindle.

Martin Wühr, Timothy J. Mitchison and Christine M. Field*

The consensus view of the mechanism of cell division in animal cells holds that chromosome segregation is driven by microtubule dynamics in the mitotic spindle, whereas cytokinesis is driven by actin–myosin-II dynamics in the contractile furrow. During anaphase, the two cytoskeletal systems talk to each other when aster and midzone microtubules position the furrow through a complex RhoA-based signaling system [1]. Although useful as an overview, this neat partitioning of cytoskeleton function is surely oversimplified. Actin is also involved in positioning the spindle via an interaction with astral microtubules [2,3] and, in some systems, myosin-II is also involved in this process [4]. More speculatively, actin and myosin have been implicated in the organization and function of the spindle itself under certain conditions [4–7], but these roles are controversial because the spindle can assemble and function normally in the absence of polymerized actin in other systems [8]. Given this background, a recent report that myosin-X plays an important role in spindle assembly in *Xenopus* embryos [9] is exciting, and sure to generate further debate.

Myosin-X (also called myosin-10, gene name MYO10 in humans), seems to be specific to vertebrates [10]. Its heavy chain (Figure 1) comprises an amino-terminal motor domain with barbed-end-directed motor activity, a lever arm with three IQ-motif binding sites for calmodulin-like light chains, a long coiled-coil that presumably dimerizes the molecule, and a series of interesting motifs at the carboxyl terminus that include FERM, MyTH4 and three sequential pleckstrin homology (PH) domains. These carboxy-terminal domains are thought to mediate the interaction of myosin-X with cargo molecules, including phosphoinositide 3,4,5-trisphosphate (PIP_3) through the second PH domain and integrins through the FERM domain [10].

The MyTH4 domain is thought to promote an interaction with microtubules, suggesting a role in communication between cytoskeletal systems [11].

The first cellular studies of myosin-X found that it accumulated at the tips of filopodia at the leading edge of motile cells. It localized to filopodium tips by rapid outward movement, presumably using its motor activity to move along the oriented actin bundle in the core of the filopodium [12,13]. Subsequent studies broadened this view and now encompass possible microtubule interactions; myosin-X was implicated in positioning of the meiotic spindle at the cortex in unfertilized *Xenopus* eggs [11] and also in positioning the mitotic spindle parallel to the substrate in cultured human cancer cells [14]. Finding a role of myosin-X in spindle positioning was exciting, in that it provided a new sphere of action for the motor, but it was not controversial, as the actin cortex had already been implicated in spindle positioning in several systems. Also, a precedent existed for positioning of spindles by a transport myosin, given that myosin-V plays this role in budding yeast [2]. Extending their analysis of myosin-X function in *Xenopus* embryos, the Bement group now report that, in addition to positioning the spindle, the motor functions in spindle assembly and spindle-length control [9] — a more surprising discovery that questions the 'textbook' separation of microtubule and actin functions during cell division.

The authors started by localizing myosin-X in early *Xenopus laevis* embryos using indirect immunofluorescence. They showed that myosin-X localizes at the blastomere cortex, as expected for a function in spindle positioning, but also at the poles of mitotic spindles, where it co-localizes with the spindle-pole assembly factor TPX2 [15] and presumably other spindle-pole proteins.

To investigate myosin-X function, Woolner *et al.* [9] injected morpholinos (stable oligonucleotide analogs) that bind to the mRNA of myosin-X at the two-cell stage and scored phenotypes later in development, when the maternal pool of the protein has presumably been depleted. This methodology is standard for investigating protein function in early animal embryos, where RNA interference (RNAi) does not work well. The specificity of the depletion was verified by several controls, including rescue of the phenotype by co-injection of myosin-X mRNA. The major defects noted when myosin-X was depleted were various abnormalities of the mitotic spindle, including multiple, fragmented spindle poles, increased spindle length, and mispositioning of spindles within the dividing cell. These mitotic defects also extended the time blastomeres spent in mitosis, presumably due to activation of the spindle-assembly checkpoint.

Woolner *et al.* [9] found a physical interaction and also a co-localization between a MyTH4/FERM fragment of myosin-X and TPX2 and suggest that the spindle-pole function of myosin-X may depend on this interaction. TPX2 function is itself mysterious; this protein is regulated by the Ran–importin system, binds to microtubules, Aurora A kinase and other spindle-pole factors and is thought to promote microtubule assembly near chromosomes and kinetochores [16,17].

The discovery that a myosin is required for mitotic spindle assembly and length control immediately prompted the question of whether actin is also required. Woolner *et al.* [9] investigated this question in several ways: by depolymerizing actin in the embryo with latrunculin B; imaging F-actin; and exploring the role of myosin-X in spindle assembly in *Xenopus* egg extracts where spindles form in the absence of actin filaments. They conclude that myosin-X has multiple functions in spindle assembly, some of which depend on polymerized actin and others that do not. Treatment of embryos with latrunculin B rescued the spindle length, suggesting that F-actin acts with myosin-X to shorten spindles. Consistent with a role of actin filaments in spindle positioning and length control, live imaging with an actin probe comprising green fluorescent protein (GFP) fused to utrophin [18] revealed actin cables near and perhaps within embryo spindles. As reported in this issue of *Current Biology*, Azoury *et al.* [19] observe a similar localization of actin in meiotic spindles in mouse oocytes.

To further probe the actin-dependent and -independent aspects of myosin-X function, Woolner *et al.* [9] investigated the role of different domains of the molecule by co-injecting mRNAs encoding myosin-X fragments along with the morpholinos. An amino-terminal fragment that included the motor domain rescued the spindle-length phenotype most convincingly, while only the carboxy-terminal, cargo-binding fragment rescued the spindle-pole phenotype. These data are interpreted by the authors as showing that myosin-X has (at least) two functions — one that depends on actin filaments and is involved in positioning spindles and controlling their length and another that is actin-independent, may involve interaction with TPX2, and is involved in spindle-pole assembly.

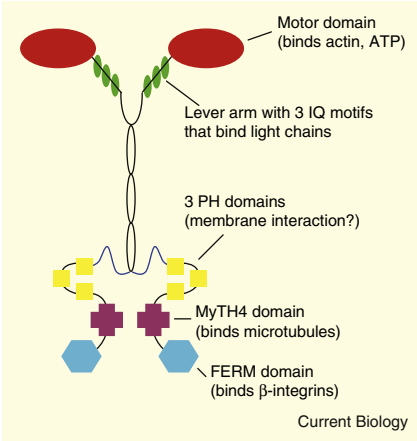

Figure 1. Model of the domain structures of myosin-X and their functional associations [10].

To account for their observations, the authors suggest two essentially independent models. For spindle length, they propose that myosin-X localizes to spindle poles and pulls on actin within the spindle, thereby shortening it (Figure 2). In this proposed role, myosin-X antagonizes dynein [20] and/or myosin-II [4], which transmit pulling forces from the cortex to the spindle pole via astral microtubules. This model rationalizes the observed increase of spindle length upon knockdown of myosin-X and its rescue by latrunculin B. For spindle-pole assembly, they propose that myosin-X binds to TPX2 and participates in the generation of cohesive forces between microtubule minus ends that focus the poles into two discrete entities. These models are a reasonable interpretation of the data, and assert an important new role for actin filaments within the spindle. When

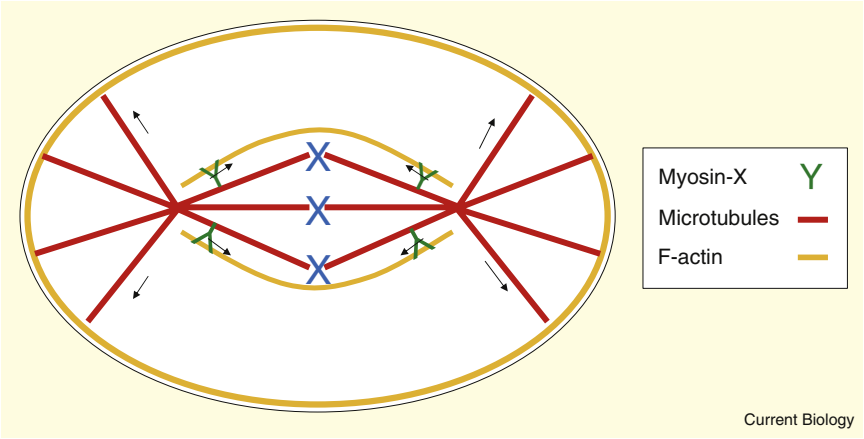

Figure 2. Model by Woolner *et al.* [9] for myosin-X's involvement in spindle-length regulation. Interaction of astral microtubules and cortical F-actin stretch the spindle while microtubule-bound myosin-X pulls on spindle-associated F-actin thereby shortening it.

the authors used an inhibitory antibody to inhibit myosin-X in *Xenopus* egg extract spindles that lack actin filaments, they observed both pole fragmentation and spindle-length increase, however, suggesting that these two functions are in fact coupled and that the length-regulating function does not depend on actin in a different system.

In our view, therefore, the data are subject to alternative explanations that do not require actin in the spindle. Inhibition of spindle-pole focusing by other means is known to increase spindle length [8]. Pole focusing is probably required for recruitment of minus-end-directed microtubule motors and/or microtubule-depolymerizing factors that shorten the spindle. Thus, it seems possible that myosin-X has a single, actin-independent function in pole assembly and that this same function also shortens spindles by actin-independent mechanisms. Differential rescue of pole assembly and spindle length in the fragment rescue experiments might be due to different dose dependencies of these two aspects of the phenotype. Furthermore, the latrunculin B experiment is complicated by the fact that this drug depolymerizes cortical actin and might release extra myosin-X into the cytoplasm, where it could be recruited to spindle poles, rescuing the spindle-length phenotype by a dosage effect. In this alternative interpretation, myosin-X has an important role within the spindle as a pole assembly factor, but not as a motor that acts on spindle actin. More speculatively, it is possible that myosin-X interacts with membranes in the mitotic spindle. Spindle poles contain abundant membrane systems, including parts of the endoplasmic reticulum (ER), whose role in pole organization and spindle length has been little studied.

In summary, the Bement group has opened an exciting new direction in mitosis research with their work on myosin-X. Much remains to be carried out to understand the role of this molecule in the spindle and to test whether this role is universal or specialized for some aspect of embryonic mitosis. These mitotic roles may inform us on the function of the motor in non-dividing cells — could it, for example, play a role in recruiting microtubules to the leading edge during cell migration, or in positioning the microtubule-organizing center during cell polarization? Myosin-X is clearly a molecule with a big future at the interface between the microtubule and actin cytoskeletons.

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