Cytoskeleton: **A catastrophic kinesin**

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The 'plus' ends of microtubules exhibit dynamic instability, switching stochastically from growth to shortening phases. The first endogenous regulator of such 'catastrophes' has been identified, and is a kinesin-related microtubule motor protein.

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Microtubule motor proteins are mechanochemical enzymes which are capable of using energy from ATP hydrolysis to translocate along a microtubule (reviewed in [1–3]). These proteins are classified according to the sequences of their motor domains and their direction of motion along the asymmetrical microtubule lattice (toward either the minus or plus end). They include axonemal and cytoplasmic dynein, and members of the kinesin superfamily. In most cases, different motor proteins produce different types of microtubule-based motility because their non-motor domains bind specific cargo. For example, a number of kinesins are specialized for membrane organelle motility, while the chromosome-binding kinesins have a DNA-binding region in their tail domains and other kinesin-related proteins form bipolar filaments and slide antiparallel microtubules apart.

Microtubule motors are generally thought to move by means of a conformational change that is driven by ATP hydrolysis and allows the motor protein to 'walk' along the microtubule polymer. Several microtubule motors, however, are capable of producing movement in a quite different manner — by holding onto the plus end of a depolymerizing microtubule (reviewed in [4]). *In vitro* reconstitution experiments by Lombillo *et al.* [5] have shown that kinesin bound to beads can stay attached to depolymerizing microtubule plus ends in the presence or absence of ATP. The same holds true for the kinesin-related protein CENP-E when it is associated with the kinetochores of isolated chromosomes [6]. In these situations, the energetics of plus-end microtubule depolymerization drive the motility, while motor proteins provide dynamic attachment to the shortening end.

Two studies have previously indicated that some motor proteins may also be important in regulating microtubule assembly. Endow *et al.* [7] reported that, in *in vitro* motility assays, the minus-end directed microtubule motor Kar3p induces minus-end, but not plus-end, depolymerization of taxol-stabilized microtubules. And Lombillo *et al.* [5] found that, in their *in vitro* studies, the rate of microtubule plus-end shortening induced by tubulin dilution was faster when the end was coupled to a bead by kinesin motor proteins.

The most telling evidence that a microtubule motor protein plays a major role in regulating microtubule assembly in cells has recently been reported by Walczak *et al.* [8]. They have identified a kinesin-related protein in *Xenopus* eggs, XKCM1, which is essential for normal mitotic-spindle formation and maintenance. XKCM1 is the first protein to be discovered that selectively promotes the switching of microtubule plus ends from the growth to the shortening phase of dynamic instability. This switch is called a catastrophe.

A kinesin-related motor protein necessary for mitotic spindle assembly and maintenance

To identify kinesins that play a role in cell division, Walczak *et al.* [8] used an antibody to a highly conserved region of the kinesin motor domain to screen a *Xenopus* ovary expression library. The most abundant clone identified in this way turned out to encode an 85 kDa member of the KIF2 kinesin family, dubbed XKCM1 for *Xenopus* kinesin central motor 1. KIF2 has been shown to be a plus-end directed motor, but the motile properties of XKCM1 have not yet been determined. XKCM1 is a homologue of the mammalian kinesin-related protein MCAK, which localizes to kinetochores, the microtubule plus-end attachment sites on the chromosomal centromere. From its sequence, XKCM1 has a globular amino-terminal domain, a central motor domain, and an α-helical carboxy-terminal tail. XKCM1 shows microtubule-binding properties similar to other kinesins; it sediments with microtubules when there in no ATP present, and is released from microtubules when ATP and salt are added.

The mitotic spindle is a bipolar array of microtubules oriented with their plus ends distal to the spindle poles. In their studies, Walczak *et al.* [8] used *Xenopus* egg extracts arrested in mitosis with cytostatic factor (CSF), which assemble *in vitro* mitotic spindles [9]. Polyclonal antibodies against the amino-terminus of XKCM1 were used to determine the localization of XKCM1 in both tissue culture cells and *in vitro* mitotic spindles. In XL177 *Xenopus* tissue culture cells, XKCM1 is diffuse throughout both the cytoplasm and the nucleus during interphase. During mitosis, this diffuse labeling persists, but XKCM1 also becomes concentrated at the centromeric region of the chromosome and the spindle poles. In mitotic spindles

assembled *in vitro*, XKCM1 is also found at the centromeres and the centrosomes, as well as along the spindle microtubules.

To investigate the function of XKCM1, the polyclonal antibody was used to deplete the protein from mitotic *Xenopus* extracts, essentially creating a 'null' phenotype [8]. In control cells, 70 % of added sperm nuclei formed normal mitotic spindles. When as little as 25 % or as much as >99 % of endogenous XKCM1 was depleted from the extract, spindle structure was grossly disturbed. The aberrant spindles had centrally located chromatin, from which long microtubules formed an abnormally large radial array. These long microtubule arrays were estimated to have ten times the polymer mass of a normal bipolar spindle. The same result was obtained whether the antibodies were added before or after spindle formation. Normal spindle assembly could be recovered by adding purified XKCM1 to immunodepleted extracts. XKCM1 is therefore essential for both normal formation and maintenance of mitotic spindles in *Xenopus* extracts.

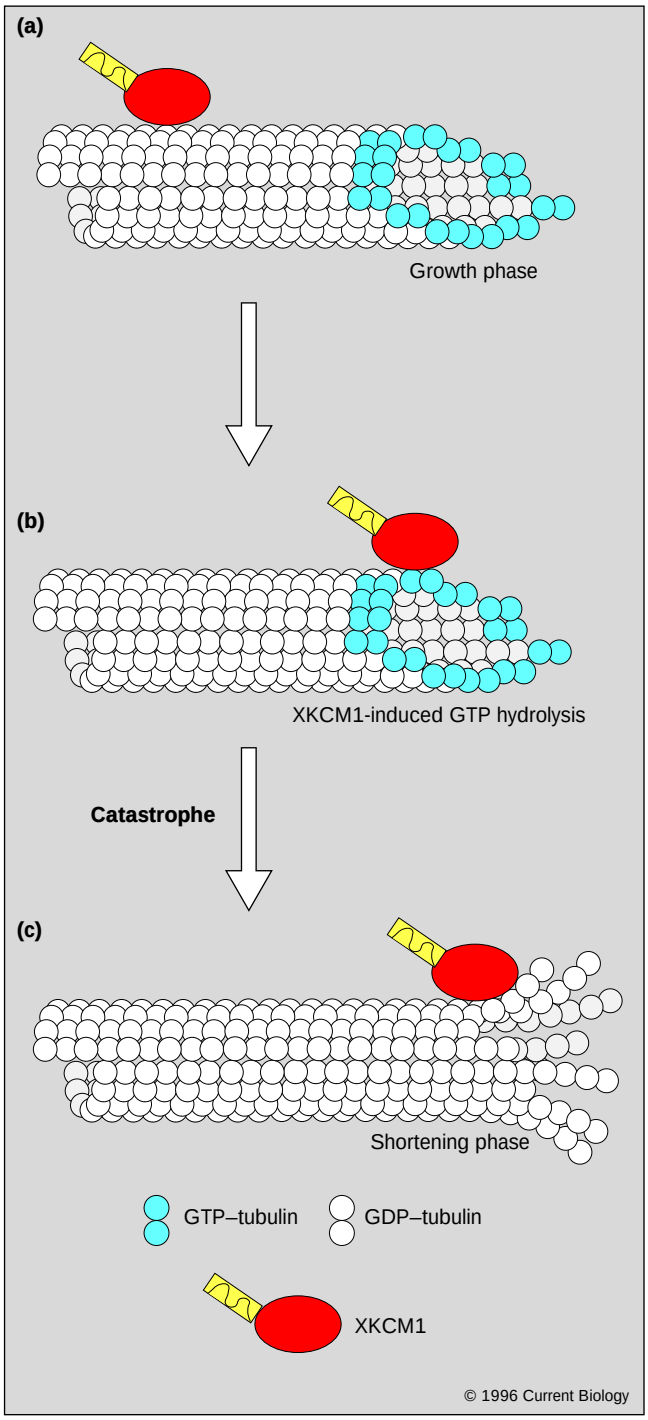
A component of the elusive mitotic catastrophe factor?

Microtubules grow and shorten primarily at their plus ends (reviewed in [10]). Walczak *et al.* [8] used video-enhanced differential interference contrast light microscopy to examine how the depletion of XKCM1 from clarified extracts alters microtubule plus-end dynamics. They found that depletion of XKCM1 from mitotic extracts did not affect the rates of microtubule growth or shortening, or the frequency of rescue events (switching from shortening back to growth). The frequency of catastrophes, however, was reduced four-fold following XKCM1 depletion. This reduction in catastrophe frequency explains the abnormally long spindle microtubule arrays seen in the XKCM1-depleted extracts.

The regulated changes in microtubule dynamics that occur during the cell cycle suggest that cells contain a factor that enhances catastrophe frequency during mitosis (reviewed in [11]). The transition from the long, persistent microtubules of the interphase cytoplasm to the much shorter and more dynamic microtubules of the mitotic spindle depends on activation of the mitotic kinase, p34^{cdc2}–cyclin B. Following activation of this kinase, the microtubule catastrophe frequency is increased and the rescue frequency decreased, producing the short, dynamic microtubules of the mitotic spindle. XKCM1 is the first endogenous protein to be discovered that promotes catastrophes — could it be a component of the long sought after catastrophe factor responsible for the change in microtubule dynamics from interphase to mitosis? Preliminary experiments by Walczak *et al.* [8] show that immunodepletion of XKCM1 from interphase extracts increases microtubule assembly, suggesting that XKCM1 is active during interphase. It will be interesting to see whether the catastrophe-promoting activity of XKCM1 is modified by phosphorylation by the p34^{cdc2}–cyclin B kinase.

Walczak *et al.* [8] also propose a role for XKCM1 in kinetochore motility. During mitosis in tissue culture cells,

Figure 1



Possible mechanism by which XKCM1 may facilitate plus-end catastrophe. See text for details.

kinetochores become tethered to spindle poles by attaching to the plus ends of polar microtubules. Attached kinetochores oscillate between movement towards and away from a pole throughout mitosis. These oscillations are coupled to the switching between growth and shortening of kinetochore microtubules at their plus-end attachment sites [12]. Walczak *et al.* [8] propose that XKCM1 bound to the kinetochore regulates the transition from growth to shortening, which in turn switches kinetochore movement from being away from a pole to being towards a pole.

A possible mechanism for XKCM1-induced catastrophes

How might a microtubule motor protein selectively facilitate a catastrophe? This is not known, but one possible model, based on the GTP-cap model of microtubule dynamic instability (reviewed in [13]), is illustrated in Figure 1. Growing microtubule ends are thought to be stabilized by a terminal cap of tubulin–GTP dimers. Both α and β subunits of the tubulin dimer bind GTP. After incorporation into the microtubule lattice, β-tubulin-bound GTP is hydrolyzed to GDP, so that the core of the microtubule consists of tubulin–GDP. A catastrophe is thought to be initiated by loss of the tubulin–GTP cap. This allows the terminal tubulin–GDP subunits to curl radially outward from one other and rapidly dissociate, producing the shortening phase of dynamic instability.

Catastrophes might be induced by the motor domain or a non-motor domain of XKCM1, or even by an accessory protein that needs to be targeted to the plus end of a growing microtubule by XKCM1. When XKCM1 is immunoprecipitated from cell extracts, however, it is not bound to any other protein [8], so it is likely that a domain within XKCM1 is capable of inducing catastrophes. In the model depicted in Figure 1 [8], the predicted plus-end directed motility of XKCM1 concentrates it at the growing plus end. A catastrophe is induced as XKCM1 causes a conformational change in the terminal GTP–tubulin subunits, inducing the hydrolysis or release of their bound GTP. Although only one motor protein is shown in Figure 1, several XKCM1 molecules may have to move to the growing end before a catastrophe is induced. As XKCM1 depletion does not appear to affect the growth rate, it seems unlikely that XKCM1 induces catastrophe by blocking the association of tubulin–GTP subunits with the plus end.

To date, XKCM1 is the only microtubule motor protein that has been reported to facilitate plus-end catastrophes. This suggests that catastrophe-promotion may be a unique property of this motor protein. Caution is needed, however, in drawing this conclusion, as few motor proteins have been assayed for their ability to affect microtubule dynamic instability and it is likely that there are other motor proteins that have not yet been identified.

A second endogenous inducer of catastrophe has very recently been identified by Belmont and Mitchison [13], who used a microtubule-polymerization-inhibition assay to purify a 17 kDa heat-stable protein from calf thymus. This protein increases the rate of catastrophe *in vitro* in purified tubulin assembly assays. Unlike XKCM1, which binds to microtubules, this protein binds preferentially to unpolymerized tubulin subunits. Sequence analysis showed that this castrophe-promoter is oncoprotein 18 (Op18/stathmin), a conserved protein known to be expressed at high levels in leukemia cells. Depletion of Op18/stathmin from metaphase *Xenopus* extracts caused an increase in the size of the mitotic asters, as a result of greater tubulin polymerization, while depletion of both XKCM1 and Op18/stathmin caused a greater increase in aster size than depletion of either protein alone. These two endogenous catastrophe factors thus have additive effects, and both are important in the regulation of spindle assembly.

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