Lucky 13 – microtubule depolymerisation by kinesin-13 motors

Carolyn A. Moores[1] and Ronald A. Milligan[2]

[1]School of Crystallography, Birkbeck College, Malet Street, London, WC1E 7HX, UK
[2]Department of Cell Biology, CB227, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA
e-mail: c.moores@mail.cryst.bbk.ac.uk; milligan@scripps.edu

Accepted 21 August 2006
Journal of Cell Science 119, 3905-3913 Published by The Company of Biologists 2006
doi:10.1242/jcs.03224

Summary

The kinesin-13 class of motors catalyses microtubule depolymerisation by bending tubulins at microtubule ends. Depolymerisation activity is intrinsic to the kinesin-13 motor core but the activity of the core alone is very low compared with that of constructs that also contain a conserved neck sequence. The full-length dimeric motor is an efficient depolymeriser and also diffuses along the microtubule lattice, which helps it to find microtubule ends. Current evidence supports the idea of a generic mechanism for kinesin-13-catalysed depolymerisation. However, the activity of kinesin-13 motors is precisely localised and regulated in vivo to enable a wide range of cellular roles. The proteins are involved in global control of microtubule dynamics. They also localise to mitotic and meiotic spindles, where they contribute to formation and maintenance of spindle bipolarity, chromosomal congression, attachment correction and chromatid separation. In interphase cells, intricate and subtle mechanisms appear to allow kinesin-13 motors to act on specific populations of microtubules. Such carefully controlled localisation and regulation makes these kinesins efficient, multi-tasking molecular motors.

Key words: Kinesin-13, Microtubule, Depolymerisation, MCAK, Cell division

Introduction

Microtubules (MTs) are polar polymers built from αβ-tubulin heterodimers that polymerise in a head-to-tail fashion to form protofilaments; these associate laterally to form the wall of the hollow MT polymer (Desai and Mitchison, 1997) (Fig. 1). Although MTs may be viewed as static structures around which cell biology moves, their dynamics are important for cellular function. MTs exhibit dynamic instability and undergo cycles of random growth and shrinkage. The transition from growth to shrinkage is called catastrophe and that from shrinkage to growth is called rescue. αβ-tubulin binds GTP and hydrolyses it to GDP. Whether GTP or GDP is bound controls MT dynamics and it is convenient to think of this in terms of heterodimer structure. GTP-tubulin favours MT growth because it has a straight conformation that enables incorporation into the lattice (Wang and Nogales, 2005), whereas GDP-tubulin favours catastrophe and depolymerisation because of its curved conformation (Ravelli et al., 2004). However, tubulin dimers are held within the MT lattice even when GDP is bound. Consequently, MTs only exhibit dynamics from their ends and it is here that cellular regulators exert their control.

Members of the kinesin superfamily of ATP- and MT-dependent motor proteins not only move cargo along MTs but also regulate MT dynamics and their best-described function in this capacity is MT depolymerisation. Kinesin-8, kinesin-14 and kinesin-13 all have depolymerisation activity, but kinesin-13 motors are the best characterised. Kinesin-13 class members were initially identified in screens for motors involved in spindle function (Wordeman and Mitchison, 1995; Walczak et al., 1996) and in neurons (Aizawa et al., 1992). Depletion/overexpression experiments revealed the importance of these motors as catastrophe factors (Walczak et al., 1996; Maney et al., 1998) and a seminal paper in 1999 characterised their MT-depolymerising function (Desai et al., 1999). Kinesin-13 activity can be required either simply to deconstruct MTs at specific cellular sites or to enable coupling of MT depolymerisation to movement of the cellular cargo to which the motor is attached (e.g. a chromosome). Here, we discuss recent work that has shed light on the depolymerisation mechanism and the roles of kinesin-13 motors in various cellular processes.

Kinesin-13 domain structure

Kinesin-13 proteins are homodimeric catastrophe factors in which the kinesin motor domain, which contains the MT- and ATP- binding sites, lies in the middle of the amino acid sequence (Fig. 2A). Consequently, this class was originally termed Kin I (I for internal) or M-type (M for middle) kinesins (Vale and Fletterick, 1997; Miki et al., 2001; Lawrence et al., 2004). Sequence analysis of the kinesin-13 motor core shows it has high sequence similarity to all kinesins, and elements such as the nucleotide-binding motifs are well conserved (Fig. 2B). High-resolution structure determination confirms that this sequence conservation is reflected in the 3D structure of the motor core (Sablin et al., 1996; Ogawa et al., 2004; Shipley et al., 2004) (Fig. 3A) and electron microscopy (EM) studies demonstrate that there is a conserved interaction between the MT lattice and kinesin motors (Moores et al., 2003). N-terminal to the motor core is a kinesin-13 class-specific stretch

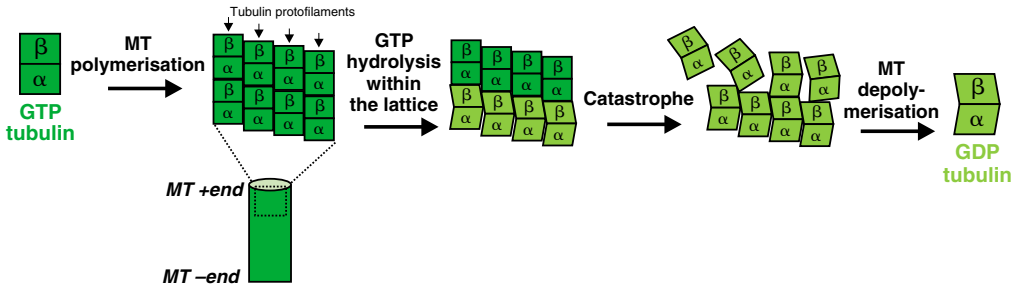

Fig. 1. MT dynamic instability. (Left to right) GTP-tubulin (dark green) adopts a straight conformation that favours MT polymerisation. MT polymers are polar with β-tubulin at the plus end and α-tubulin at the minus end. Dynamic instability occurs at both ends but only the plus end is illustrated for clarity. GTP is hydrolysed within the MT lattice to GDP (light green) but GDP-tubulin is held in place by the lattice until hydrolysis occurs at the MT end, catastrophe occurs and MTs depolymerise.

of ~60 highly charged residues known as the neck. N-terminal to the neck region is a 25-30 kD domain involved in subcellular localisation (Maney et al., 1998; Wordeman et al., 1999; Walczak et al., 2002; Kline-Smith et al., 2004). Constructs comprising this domain alone appropriately localise to the site of kinesin-13 function in vivo, independently of MTs. The C-terminus contains a predicted coiled-coil region but data indicate that both N- and C-termini are required for dimerisation (Maney et al., 2001).

The depolymerisation mechanism

The basis of kinesin-13 motor depolymerisation activity is the production of the bent, catastrophe-favouring conformation of tubulin at MT ends (Desai et al., 1999). Theoretically, this could occur either by selectively stabilising a bent tubulin conformation or by actively catalysing a straight-to-bent tubulin transition. MTs stabilised by paclitaxel or the non-hydrolysable GTP analogue GMPCPP have been used in many kinesin-13 studies because unstabilised (and therefore dynamic) MTs are difficult to work with (e.g. Desai et al., 1999; Maney et al., 2001; Moores et al., 2002; Hunter et al., 2003; Hertzer et al., 2006). That kinesin-13 motors can readily depolymerise these stabilised substrates supports the argument that kinesin-13 motors actively deconstruct their MT substrate rather than passively relying on inherent conformational fluctuations in the dimers found at MT ends (the 'terminal tubulins'). However, whether this mechanistic conclusion can be applied to the dynamic MTs that are the in vivo substrates for these motors remains an open question.

The key step in the kinesin-13 MT-depolymerisation mechanism occurs as ATP binds, demonstrated by the unpeeling of stabilised MTs in the presence (and only in the presence) of the non-hydrolysable ATP analogue, AMPPNP (Desai et al., 1999; Moores et al., 2002; Shipley et al., 2004; Moores et al., 2006a). These bent tubulin structures form because the kinesin-13 motor is trapped in a pre-hydrolysis state that actively bends the underlying terminal tubulin. This supports the idea of direct coupling between ATP binding and terminal tubulin bending (Desai et al., 1999; Moores et al., 2002; Moores et al., 2006a). ATP hydrolysis is not required for tubulin release (Desai et al., 1999; Moores et al., 2002; Hunter et al., 2003) but catalytic depolymerisation requires that the ATPase cycle be completed for the motor to be released.

Because of the high degree of sequence conservation in the motor core, the location of the kinesin-13 motor within the middle of the protein sequence was initially thought to be responsible for depolymerisation activity. However, more recent experiments have shown that this property is intrinsic to the kinesin-13 motor core (Maney et al., 2001; Moores et al., 2002; Ovechkina et al., 2002). The kinesin-13 motor core, like all kinesin core structures, is shaped like an arrowhead (Sack et al., 1999; Shipley et al., 2004) (Fig. 3A). The motor core is built around a central eight-stranded β-sheet with three α-helices on each face. As the kinesin motor sits on the MT surface, the arrowhead tip points towards the MT plus end while the nucleotide-binding pocket near the base of the arrowhead faces towards the MT minus end (Fig. 3B).

Examination of the structure of the kinesin-13–MT interface suggests that the α4 helix of the motor – its principal energy-transducing element – is directly involved in depolymerisation because it sits directly over the tubulin intra-dimer interface and could deform it (Niederstrasser et al., 2002; Moores et al., 2003; Ogawa et al., 2004) (Fig. 3A,C). Mutation of kinesin-13-specific residues on or near the α4 helix simply weakens the motor-MT interaction. Mutation of conserved residues in loop 2 of the motor, by contrast, does not diminish MT binding, but these mutants are unable to perform the ATP-dependent tubulin straight-to-bent step of the depolymerisation cycle (Ogawa et al., 2004; Shipley et al., 2004) (Fig. 3C). It is probably the cooperation of kinesin-13-specific residues throughout the motor core that leads to depolymerisation activity.

Although the motor core defines the depolymerisation ability of the kinesin-13 class, it is a poor depolymeriser in comparison with larger constructs and does not appear to work at all in vivo (Ovechkina et al., 2002; Ogawa et al., 2004; Hertzer et al., 2006). By contrast, a monomeric neck+motor construct has depolymerisation activity similar to that of the full-length dimeric motor both in vitro and in vivo (Maney et al., 2001; Ovechkina et al., 2002; Hertzer et al., 2006). The neck region has a high positive charge, and point mutations that maintain this retain depolymerisation function (Ovechkina et al., 2002). The motor-proximal third of the neck sequence has been visualised at atomic resolution and forms an α-helix along the side of the motor domain, pointing towards the MT surface (Ogawa et al., 2004) (Fig. 3C). Recent work has demonstrated that the neck is likely to be important during the tubulin deformation step. We observed that neck+motor constructs do

not release tubulin from MT ends on ATP binding – as is seen for the motor core (Moores et al., 2002) – but that tubulin protofilaments, nevertheless, form bent curls around the motor (Moores et al., 2006a). Our current hypothesis is that the neck acts as an additional tether to the MT wall but its exact contribution to depolymerisation efficiency in the context of the full-length dimer remains to be determined.

Recognising MT ends

All kinesin-13 constructs from every species examined depolymerise MTs at both plus and minus ends, which implies an inherent apolarity in their mechanism. Given their depolymerisation activity, these motors must discriminate against binding sites on the MT lattice and preferentially associate with MT ends, their true substrates (Desai et al., 1999; Hunter et al., 2003). The mechanism of end recognition is unknown but, presumably, terminal tubulins have increased flexibility and distinct binding surfaces that facilitate binding (Fig. 4). In vitro experiments have shown that the full-length motor quickly finds MT ends (see below) but the kinesin-13 motor core is not efficient at this and readily decorates the MT lattice (Moores et al., 2003). The experiments that characterise lattice binding by the core are instructive, however, since they demonstrate that (at least for the core) the ATPase activity of the kinesin-13 motor is largely inhibited when the motor is bound to the lattice. Indeed, ADP release is induced on binding of the motor core to the lattice (Moores et al., 2003) but ATP cannot then bind to the motor. Inhibition of ATP binding has been observed kinetically (Hekmat-Nejad and Sakowicz, 2004) and is consistent with the absence of conformational change in the motor-MT complex on binding to AMPPNP (Moores et al., 2003). This supports the idea that ATP binding is coupled to bending of the terminal tubulin. End-specific ATPase activity might be attributable to the class-specific tertiary structure of the motor core, in which the convex kinesin-13 MT-binding surface seems to complement the presumed flexibility of terminal tubulins (Ogawa et al., 2004; Shipley et al., 2004) (Fig. 3C). Neck+motor constructs target MT ends but they do so less efficiently than the full-length motor (Hertzer et al., 2006), which again suggests that additional elements in either the N- or C-terminal domains enhance end recognition.

The ATPase activity of kinesin-13 motors is stimulated by individual αβ-tubulin heterodimers (Hunter et al., 2003; Moores et al., 2003; Hertzer et al., 2006). Dimeric tubulin is unlikely to be a true substrate for kinesin-13, so this probably reflects the importance of a flexible tubulin conformation within the ATPase cycle of the motors. In fact, the kinesin-13 motor core does not discriminate at all between MT ends and dimeric tubulin and its ATPase activity is stimulated equally by both (Moores et al., 2003). By contrast, the ATPase activity of both neck+motor and full-length constructs is stimulated approximately ten

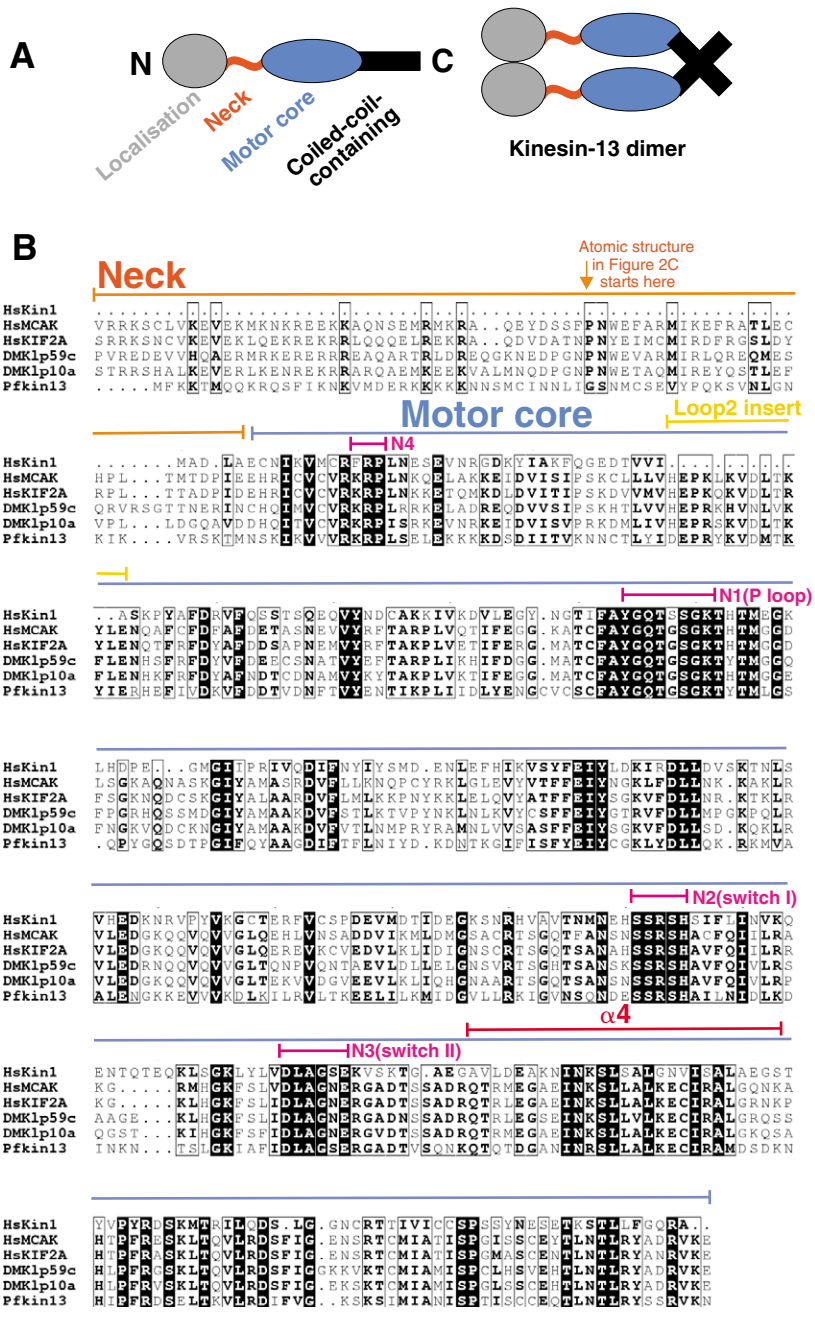

Fig. 2. Kinesin-13 molecular architecture. (A) Kinesin-13 domain organisation. The same colour scheme of the neck+motor region is used throughout the figures. (B) Sequence alignment of neck+motor constructs of select kinesin-13 motors with the motor core from human kinesin-1. The nucleotide-binding motifs, N1-N4, are as described by Sablin et al. (Sablin et al., 1996). The alignment was performed using T-Coffee (Notredame et al., 2000) and visualised using ESPript (http://espript.ibcp.fr/ESPript/ESPript/index.php). Sequences used: *Homo sapiens* kinesin-1, X65873; *Homo sapiens* MCAK, Q99661; *Homo sapiens* KIF2A, O00139; *Drosophila melanogaster* KLP59C, AE003459; *Drosophila melanogaster* KLP10A, AE003485; *Plasmodium falciparum* PFL2165w, AE014851.

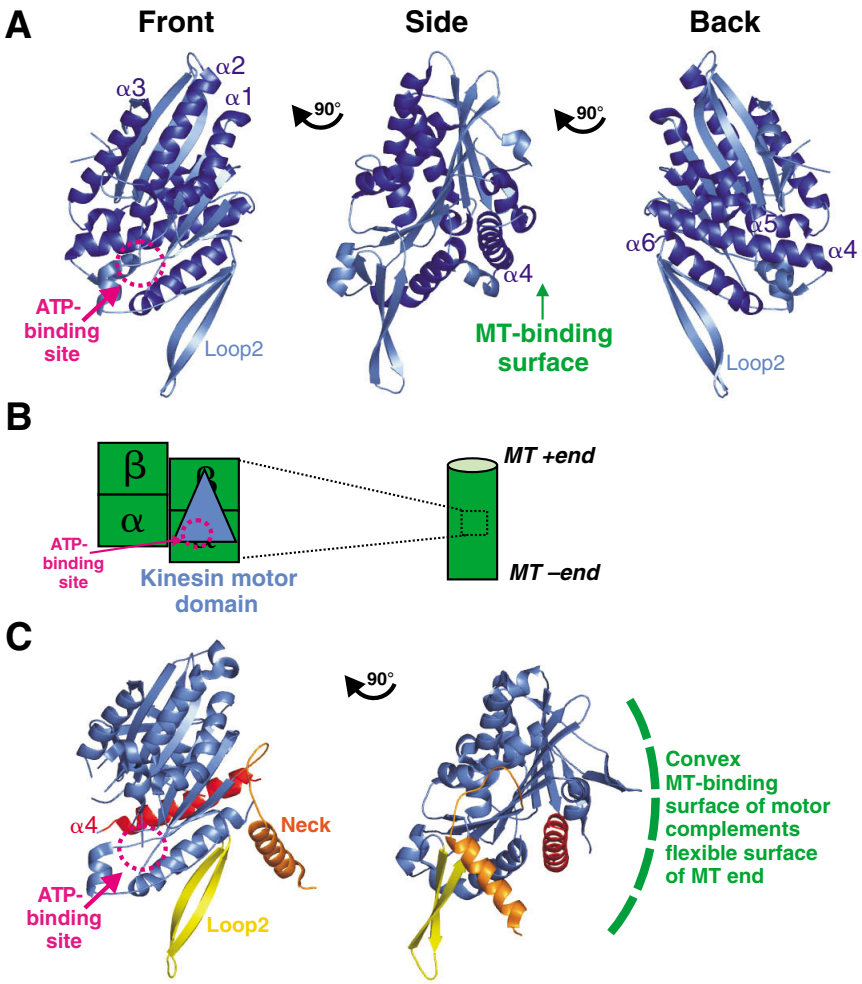

Fig. 3. Kinesin-13 motor domain structure. (A) 3D ribbon structure of the *Plasmodium falciparum* kinesin-13 motor core (Shipley et al., 2004). Orthogonal views are shown in which the 'front' view is as if the MT surface were behind the motor domain; the top of the motor domain would point towards the MT plus end. The α-helices are shown in dark blue and are individually labelled and the β-sheets are shown in light blue. The pdb code for this structure is 1RY6. (B) Schematic of the interaction between the MT lattice and a kinesin motor domain. (C) 3D ribbon structure of the *Homo sapiens* MCAK neck+motor construct (Ogawa et al., 2004). The visible portion of the neck region is shown in orange but only represents about a third of the total neck sequence (see alignment in Fig. 2B). The left-hand view shows the front of the motor and the right-hand view shows a 90° rotation, as if viewing the motor-MT interaction from the side. The green line indicates where the MT surface would be and shows that the curvature of the motor domain would match the more curved surface of flexible tubulin dimers at MT ends. The pdb code for this structure is 1V8K. Atomic structures were displayed using PyMOL (http://www.pymol.org).

times more effectively by MT ends than by tubulin heterodimers (Hunter et al., 2003; Hertzer et al., 2006). This once again reinforces the idea that components that contribute to depolymerisation efficiency lie outside the motor core, residues at the extreme C-terminus of the full-length motor being strongly implicated in end discrimination (Moore and Wordeman, 2004a).

Kinesin-13 dimers move rapidly along the MT lattice by 1D diffusion (Hunter et al., 2003; Helenius et al., 2006) (Fig. 4). This movement occurs in the post-hydrolysis, ADP.Pi state of the motor. It does not require additional rounds of ATP binding and hydrolysis and is, of course, more efficient than 3D diffusion in allowing the motor to get to MT ends. Importantly, this 1D diffusion is not directional and therefore does not favour one end of the polar MT. However, because of this non-directionality and because the motor can change direction during a single diffusive interaction, the motor will not necessarily ever find the end of longer MTs. The kinesin-13 N-terminal domain targets the protein to specific sites in the absence of MTs in vivo, so localisation to MT ends could occur independently of such lattice-based diffusion (Maney et al., 1998; Walczak et al., 2002). However, kinesin-13 molecules have been observed along MTs in cells and cell extracts (Walczak et al., 1996; Wordeman et al., 1999; Moore and Wordeman, 2004a), which suggests that transient interaction with the lattice is important in vivo.

The dimeric motor

Why are these motors dimeric? Dimer formation allows motile kinesin-1 motors to step processively along MT tracks, enabling long-range cargo transport (Vale and Milligan, 2000). By contrast, why kinesin-13 needs to be dimeric is unclear. A recent study showed that the affinity for tubulin heterodimers of a monomeric neck+motor construct is higher than that of the dimeric full-length motor (Hertzer et al., 2006). Dimeric kinesin-13 motors might therefore be optimally efficient depolymerisers because they can more readily detach from the products of depolymerisation (tubulin dimers). However, it is also plausible that the availability of two heads enables processive kinesin-13-catalysed depolymerisation – i.e. removal of successive tubulin dimers by a single kinesin-13 molecule (Hunter et al., 2003; Helenius et al., 2006). Processive dimeric kinesin-13-catalysed depolymerisation of GMPCPP- and paclitaxel-stabilised MTs has been observed, although the number of ATP molecules required per terminal tubulin depends on the means of stabilisation (Hunter et al., 2003). These observations again speak to the question of whether artificially stabilised MTs reflect these enzymes' true, dynamic substrate – for example, in a dynamic MT there might be a stabilising cap of only one to two GTP-tubulins per protofilament and so only these would need to be removed by kinesin-13 to initiate catastrophe. Processivity may also vary between different kinesin-13 motors. However, processivity would certainly have

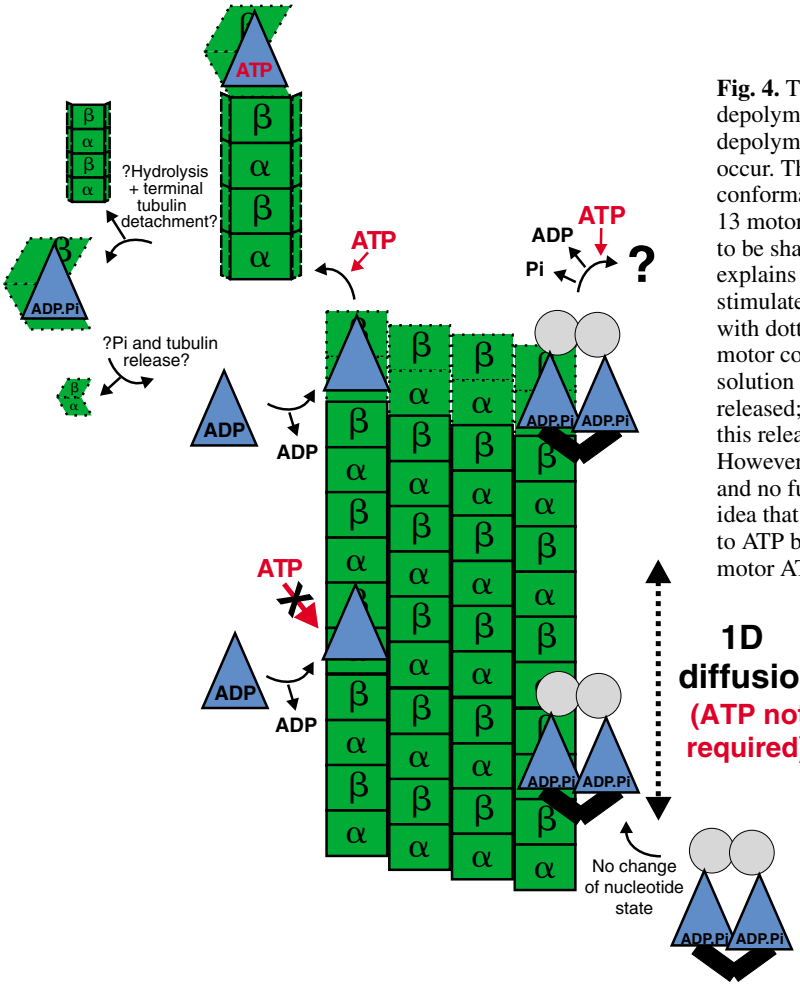

Fig. 4. The kinesin-13 ATPase cycle. Kinesin-13 motors use ATP to depolymerise MTs and their ATPase cycle is controlled by whether depolymerisation can (at MT ends) or cannot (on the MT lattice) occur. The MT end is thought to have distinct structural and/or conformational properties that allow it to be recognised by kinesin-13 motors and +TIPs. (It is these distinct properties that are believed to be shared, to some extent, by individual tubulin heterodimers and explains why the ATPase activity of kinesin-13 motors can be stimulated by them.) In the diagram, terminal tubulins are depicted with dotted outlines. On the left side, the behaviour of a kinesin-13 motor core is illustrated. The nucleotide state of this motor core in solution is not known but when it binds the MT lattice, ADP is released; a conformational change that presumably corresponds to this release step is observed in cryo-EM maps (Moores et al., 2003). However, the ATPase activity of the motor is inhibited by the lattice and no further conformational changes are seen, which supports the idea that the ATPase cycle of the motor is blocked on the lattice prior to ATP binding (Moores et al., 2003). At the MT end, however, the motor ATPase activity is stimulated, which suggests that ATP can bind. The ATP binding step (mimicked by AMPPNP) is the only point at which bent tubulin intermediates, representing the active deformation of the terminal tubulins by the kinesin motor, are observed (Moores et al., 2002). By contrast, dimeric kinesin-13 (on the right) in solution contains ADP.Pi and undergoes 1D diffusion along the lattice in this nucleotide state; ATP is not required for this process (Helenius et al., 2006). Once at the MT end, because ATP is required for depolymerisation, presumably ADP and Pi must be lost from at least one motor domain before ATP can bind and depolymerisation can occur. Presumably, there is a similar coupling between ATP binding by the dimer and bending of the terminal tubulin (Desai et al., 1999).

the mechanistic advantage that the motor remains attached to its substrate and presumably allows any cellular cargo (e.g. chromosomes) to maintain contact with the MT too.

The tubulin tail

Aspects of the MT substrate have also been examined. The acidic tubulin C-terminal tails (CTT) are important for processivity of motile kinesins and for dynein (Wang and Sheetz, 2000) and they are also important for various aspects of kinesin-13-catalysed depolymerisation. The β-tubulin CTT is required for tubulin bending by the motor core but removal of these residues does not affect lattice binding (Moores et al., 2002). CTTs are also involved in the depolymerisation mechanism of full-length kinesin-13 but their importance for the full-length motor is less clear. 1D diffusion and, consequently, depolymerisation is slowed when the CTTs (α and/or β) are removed (Helenius et al., 2006), whereas other work has shown that the CTTS are essential for depolymerisation by the dimeric motor (Niederstrasser et al., 2002). Whether the requirements for the CTTs by different constructs reflect related aspects of kinesin-13 function is uncertain.

The effects of other MT-associated proteins

In many cases, the reported effects of overexpression and/or depletion of a kinesin-13 on other MT regulators (and vice versa) are indirect and occur because the finely balanced forces present in vivo are disrupted (see below). However, several direct effectors/antagonists of kinesin-13 function have been characterised in simpler in vitro systems. The MCAK activator ICIS (inner centromere Kin I stimulator) localises to the centromere in a kinesin-13-dependent fashion (Ohi et al., 2003). ICIS itself binds to MTs and stimulates the already potent depolymerising activity of kinesin-13 in vitro through an unknown mechanism. In vivo, its function may be linked to the specific localisation and regulation of depolymerising activity (see below). By contrast, the proposed MT-stabilising activity of XMAP215, a well-conserved MT-associated protein (MAP), has been shown to counter kinesin-13 depolymerisation, and the combined activities of XMAP215 and kinesin-13 alone can generate MT dynamics similar to those seen in vivo (Tournebize et al., 2000; Kinoshita et al., 2001; Holmfeldt et al., 2004; Noetzel et al., 2005). The nature of the XMAP215-MT interaction is complex and this may be reflected in its ability to balance rather than completely block kinesin-13 activity.

The classical neuronal MAP tau inhibits kinesin-13 (Noetzel et al., 2005). Tau is thought to stabilise MTs by binding along protofilament crests (Al-Bassam et al., 2003; Santarella et al., 2005) and therefore tau and its relatives probably competitively block the kinesin-13-binding site. Whether this occurs at MT

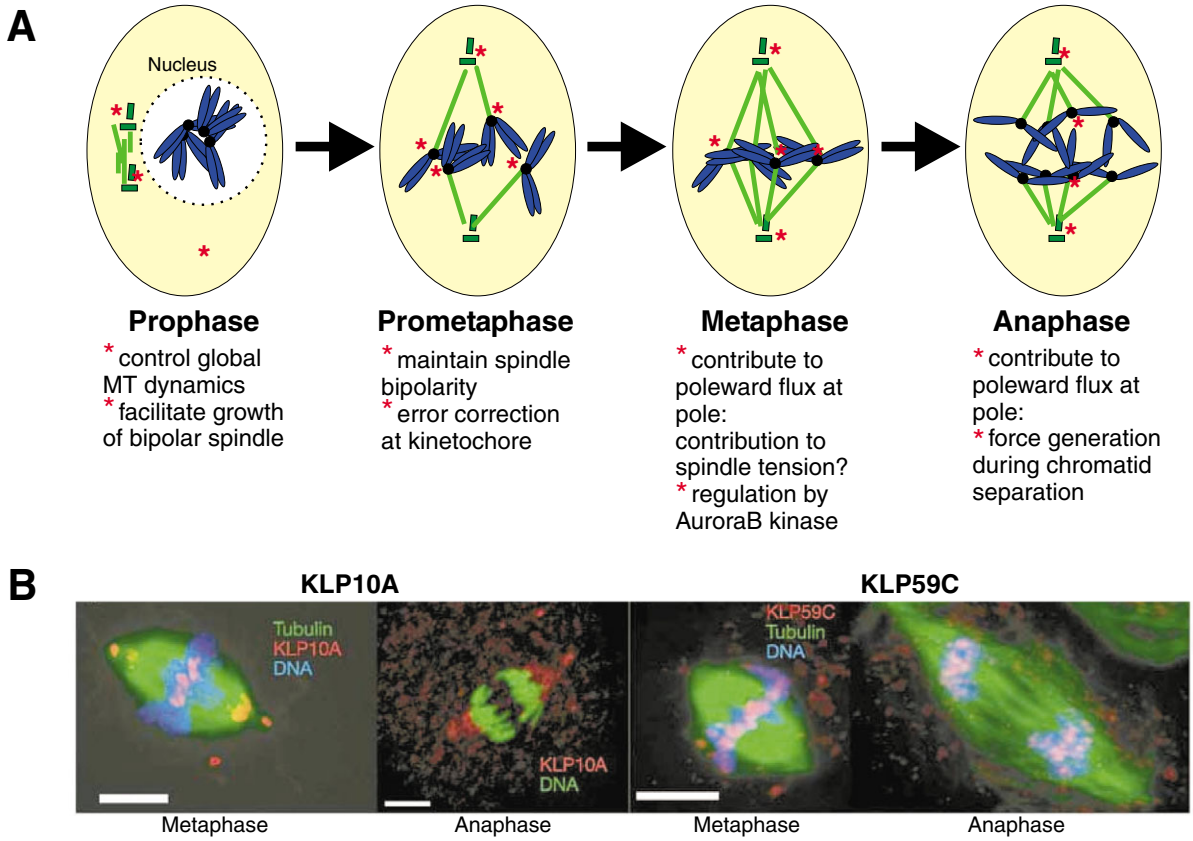

Fig. 5. Roles of kinesin-13 motors during mitosis. (A) Schematic of the stages of mitosis when kinesin-13 motors are known to be active. Chromosomes are shown in blue with a black kinetochore, spindle MTs are in green, centriole pairs are in dark green and the location of active kinesin-13 motors is indicated by red asterisks. (B) Localisation of KLP10A (mainly at the spindle poles) and KLP59C (mainly at the kinetochore) during cell division in *Drosophila* S2 tissue culture cells. Bar, 5 mm. Reprinted from Rogers et al. (Rogers et al., 2004) with permission from Macmillan Publishers Ltd.

ends, where the effect on kinesin-13 depolymerisation will presumably be direct, or tau just inhibits the motor's diffusive search for MT ends along the lattice is not yet known. Tau requires tubulin CTTs for a strong interaction with MTs (Santarella et al., 2004), which further reinforces the overlap of binding requirements with kinesin-13. By contrast, the neuronal MT-stabilising protein doublecortin does not block kinesin-13 activity, probably because it binds in between the MT protofilaments and so does not impede the MT–kinesin-13 interaction (Moores et al., 2004; Moores et al., 2006b).

Cellular roles of kinesin-13 motors

The generic depolymerisation mechanism discussed above is thought to be broadly applicable to all kinesin-13 motors, but more detailed comparisons will probably reveal mechanistic refinements for individual class members (Ogawa et al., 2004; Mennella et al., 2005; Moore et al., 2005). Indeed, when one considers the cellular roles of these kinesins, the picture becomes very complicated. Roughly equivalent class members can be identified in different organisms (Table 1) but the functional parallels frequently break down because the experimental systems and the roles of the individual kinesin-13 motors, even among vertebrates, are not identical. Nevertheless, the evidence for the involvement of kinesin-13 motors in both global and local control of MT dynamics is clear, and we outline this, in general terms, below.

Kinesin-13 in cell division

The MT cytoskeleton undergoes a dramatic transformation when cell division begins. The characteristic interphase radial MT array completely disappears and the highly dynamic bipolar mitotic/meiotic spindle forms (Fig. 5). Kinesin-13 motors are found, in part, as a soluble pool in cells. Perturbation of this pool disrupts the spindle, which is consistent with a role for kinesin-13 in global regulation of MT dynamics (Walczak et al., 1996; Maney et al., 1998; Kline-Smith and Walczak, 2002; Rogers et al., 2004). The spindle is maintained by the balance of many forces. Its complexity makes functional dissection difficult, because many compensating mechanisms prevent failure of this important cellular structure. Nevertheless, key and, in some cases, conserved functions have been assigned to kinesin-13 class members.

Probably the most clear-cut role is at spindle poles and reflects the importance of these in initiation of spindle bipolarity. Depletion of kinesin-13 motors in multiple organisms results in formation of monopolar spindles or unusually long, MT-dense bipolar spindles (Walczak et al.,

Table 1. Kinesin-13 nomenclature

Organism	Principal cellular location: Cell division: Pole	Cell division: Kinetochore	Other	Unknown	Selected references
Mammals (*H. sapiens*, *C. griseus*, *M. musculus*, *R. norvegicus*)	KIF2A (formerly KIF2)	KIF2C/MCAK (mitotic centromere associated protein)		KIF2B	Aizawa et al., 1992; Wordeman and Mitchison, 1995; Maney et al., 1998
X. laevis	XKFI2	XKCM1 (*Xenopus* kinesin central motor 1), now XMCAK – also strongly localises at poles			Walczak et al., 1996; Desai et al., 1999
D. melanogaster	KLP10A	KLP59C		KLP59D	Goshima and Vale, 2003; Rogers et al., 2004
C. elegans	CeMCAK	CeMCAK			Oegema et al., 2001
P. falciparum				PFL2165w (motor core=pKinI)	Moores et al., 2002
A. thaliana				At3g16630 At3g16060	
C. fusiformis (diatom)			DSK1 (diatom spindle kinesin 1)		Wein et al., 1996

Classification by location during cell division is arbitrary and, at best, approximate because of the multiple locations at which these motors are found, but is helpful for functional differentiation. For more detailed phylogenetic analysis, please consult the Kinesin home page (http://www.proweb.org/kinesin/).

1996; Goshima and Vale, 2003; Gaetz and Kapoor, 2004; Ganem and Compton, 2004; Rogers et al., 2004; Goshima et al., 2005; Zhu et al., 2005; Laycock et al., 2006). These phenotypes are consistent with the need for regulated MT growth at spindle poles. Pole-based kinesin-13 motors may also act in anaphase to depolymerise the minus ends of kinetochore MTs, contributing to poleward flux and chromatid separation (Rogers et al., 2004; Rogers et al., 2005). However, the role of poleward flux in anaphase is very system-dependent and, consequently, the generality of this aspect of kinesin-13 function is unclear (Ganem et al., 2005).

At the other end of spindle MTs, kinesin-13 motors are also found at the kinetochore and several roles are ascribed to them at this location. The kinetochore consists of distinct domains – the centromeric, DNA-proximal region, the inner kinetochore and the outer kinetochore plate, where spindle MTs attach (reviewed in Maiato et al., 2004; Moore and Wordeman, 2004b; Chan et al., 2005; Parra et al., 2006). Kinesin-13 motors are believed to be active at all these sites. Depletion of centromeric kinesin-13 (by overexpression of a construct containing only the N-terminal localisation domain) results in several distinct phenotypes. In mammalian cells (hamster), MCAK depletion causes a trailing-chromatid defect in anaphase, which supports a model in which MCAK progressively depolymerises spindle MTs from their plus ends and drags the chromatid to which they are attached towards the spindle poles during anaphase (the 'Pac-Man' model) (Maney et al., 1998; Sharp and Rogers, 2004). Rogers et al. have proposed a similar function for KLP59c on the basis of the effects of its depletion in *Drosophila* (Rogers et al., 2004), in which the rate of chromatid separation during anaphase is reduced.

A role in correction of inappropriately attached pre-metaphase chromosomes has also been assigned to centromeric kinesin-13 motors (Kline-Smith et al., 2004). Depletion of kinesin-13 from centromeres generates lagging chromatids, despite normal rates of chromatid movement (rat kangaroo MCAK in PtK2 cells). In these depleted cells, correlated light and EM studies showed that incorrectly attached MTs ran laterally along the surface of kinetochores on unaligned chromatids. This suggests that kinesin-13 at the centromere prunes away inappropriately attached MTs (merotelic and syntelic) and allows correct chromosome congression and subsequent accurate anaphase. Some aberrant MT attachments do not significantly perturb the tension across the sister chromatids and, therefore, are not detected by the spindle checkpoint. Anaphase proceeds even when such incorrect attachments are present so this error correction by kinesin-13 is particularly important.

Regulation of kinesin-13 motors (specifically MCAK) at the kinetochore by the aurora B kinase is also a focus of attention (Andrews et al., 2004; Lan et al., 2004; Ohi et al., 2004; Parra et al., 2006). Aurora B has a general role promoting chromatid biorientation and it phosphorylates MCAK on several serine/threonine residues, including within the neck, thereby inactivating it. Aurora B activity is required for localisation of MCAK to the kinetochore and controls its movement between kinetochore subdomains through prophase and metaphase. Unaligned chromatids that are not under tension have a rapidly exchanging population of phosphorylated and dephosphorylated MCAK at their centromeres, whereas dephosphorylated MCAK is located towards the outer kinetochore in aligned chromatid pairs under tension. Thus, MCAK activity is dynamically controlled and localised. These MCAK sub-populations presumably perform specific activities, such as MT attachment error correction at the centromere during prometaphase and force generation during anaphase at the kinetochore. Such subtleties of regulation may also be controlled by ICIS (Ohi et al., 2003). All that is certain is that the known regulatory mechanisms controlling kinesin-13 location and activity represent the tip of the iceberg.

Within the spindle, the different kinesin-13 activities do not operate independently; indeed, different compensatory effects have been observed when DNA- or pole-based kinesin-13

activities are perturbed. For example, in *Drosophila*, KLP10A and KLP59C work co-ordinately at either end of kinetochore MTs to bring about accurate chromosome segregation during anaphase (Rogers et al., 2004). If both Kif2a and MCAK are removed in vertebrate cells, by contrast, poleward flux is eliminated but mitotic progression is unperturbed. However, lagging chromatids are observed in anaphase, presumably because the centromere-based, error correction activity of MCAK has also been eliminated from these spindles (Ganem and Compton, 2004; Ganem et al., 2005). Thus although the individual roles of kinesin-13 motors are gradually being revealed, when we consider the combination of force-generators within the spindle, the picture is still very complex and varies from organism to organism.

Kinesin-13 in interphase cells

Attention has recently turned to the activities of kinesin-13 motors during interphase. Their role in control of global MT turnover has already been mentioned, but there are also more subtle regulatory mechanisms at work and this is a fast-developing area. A recent study, for example, has revealed that a kinesin-13 (KIF2A) plays an essential role in brain development in vivo by controlling neurite outgrowth (Homma et al., 2003). Additionally, recent studies have highlighted the association of kinesin-13 motors with the plus ends of growing MTs as they approach and interact with the cell periphery (Menella et al., 2005; Moore et al., 2005). This apparent paradox seems to occur through recruitment of kinesin-13 by MT plus-end tip tracking proteins (+TIPs) (Banks and Heald, 2004; Akhmanova and Hoogenraad, 2005; Menella et al., 2005; Moore et al., 2005). Presumably, kinesin-13 motors ride growing MT plus ends in an inactive state until the appropriate cellular cue activates them. Individual kinesin-13 motors appear to be regulated in different ways, and it will be interesting to discover whether their different roles reflect genuine differences in their enzymatic activity or are the consequence of modulation by other MT-binding proteins.

Other kinesin depolymerisers

As mentioned above, MT depolymerisation activity has also been observed in the case of kinesin-8 and kinesin-14 classes (reviewed in Ovechkina and Wordeman, 2003). Depolymerisation by kinesin-14 motors, traditionally seen as minus-end-directed motors, has been described from one or other MT end but is inhibited on stabilised MTs (Endow et al., 1994; Chu et al., 2005; Sproul et al., 2005). The depolymerisation activity of kinesin-8 (shown to have plus-end-directed motility) (Pereira et al., 1997) was proposed on the basis of the effect of knockout and knockdown experiments on cell division in vivo. Here, the reduction of kinesin-8 activity has a deleterious effect on spindle bipolarity (Goshima and Vale, 2003; Zhu et al., 2005) and chromosome congression and separation (West et al., 2001; Garcia et al., 2002; West et al., 2002). Detailed studies, such as those published recently (Gupta et al., 2006; Varga et al., 2006), are required if we are to understand the role of depolymerisation by these motors and, in particular, how they coordinate directional motility and depolymerisation. Data concerning kinesin-13 motors provide a framework for functional and mechanistic comparison. What is particularly tantalising about depolymerising kinesins is that yeast genomes (*S. cerevisiae* and *S. pombe*) encode both kinesin-8 and kinesin-14 motors but not kinesin-13. Do kinesin-8 and kinesin-14 motors perform the functions in yeasts that kinesin-13 motors do in higher eukaryotes, or are the MT cytoskeletons of higher eukaryotes sufficiently different that kinesin-13 motors have evolved specifically to perform the necessary roles? Future studies will address these important questions.

Conclusion and perspectives

Ultimately, the key to defining the roles of kinesin-13 in vivo will hinge on understanding the context in which they operate and the impact of other MT-associated factors on their activity. In the meantime, there is plenty still to uncover about their molecular mechanism, particularly the role of the dimer in the depolymerisation mechanism. While it is now more than 20 years since the activity of kinesin motors was first defined (Vale et al., 1985), it is less than 10 years since the depolymerising kinesin-13 motors were characterised (Desai et al., 1999) and it is encouraging to see how far we have come.

We thank our kinesin-13 collaborators in Linda Wordeman's lab (University of Washington, Seattle, WA) and at Cytokinetics Inc. (San Francisco, CA) for exciting experiments and stimulating discussions. C.A.M. is a BBSRC David Phillips Fellow and R.A.M. is supported by an N.I.H. grant (GM52468).

References

Aizawa, H., Sekine, Y., Takenura, R., Zhang, Z., Nangaku, M. and Hirokawa, N. (1992). Kinesin family in murine central nervous system. *J. Cell Biol.* **119**, 1287-1296.

Akhmanova, A. and Hoogenraad, C. C. (2005). Microtubule plus-end-tracking proteins: mechanisms and functions. *Curr. Opin. Cell Biol.* **17**, 47-54.

Al-Bassam, J., Ozer, R. S., Safer, D., Halpain, S. and Milligan, R. A. (2002). MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments. *J. Cell Biol.* **157**, 1187-1196.

Andrews, P. D., Ovechkina, Y., Morrice, N., Wagenbach, M., Duncan, K., Wordeman, L. and Swedlow, J. R. (2004). Aurora B regulates MCAK at the mitotic centromere. *Dev. Cell* **6**, 253-268.

Banks, J. D. and Heald, R. (2004). Adenomatous polyposis coli associates with the microtubule-destabilising protein XMCAK. *Curr. Biol.* **14**, 2033-2038.

Chan, G. K., Liu, S. T. and Yen, T. J. (2005). Kinetochore structure and function. *Trends Cell Biol.* **15**, 589-598.

Chu, H. M., Yun, M., Anderson, D. E., Sage, H., Park, H. W. and Endow, S. A. (2005). Kar3 interaction with Cik1 alters motor structure and function. *EMBO J.* **24**, 3214-3223.

Desai, A. and Mitchison, T. J. (1997). Microtubule depolymerisation dynamics. *Annu. Rev. Cell Dev. Biol.* **13**, 83-117.

Desai, A., Verma, S., Mitchison, T. J. and Walczak, C. E. (1999). Kin I kinesins are microtubule-destabilising enzymes. *Cell* **96**, 69-78.

Endow, S. A., Kang, S. J., Satterwhite, L. L., Rose, M. D., Skeen, V. P. and Salmon, E. D. (1994). Yeast Kar3 is a minus-end microtubule motor protein that destabilised microtubule preferentially at the minus ends. *EMBO J.* **13**, 2708-2713.

Gaetz, J. and Kapoor, T. M. (2004). Dynein/dynactin regulate metaphase spindle length by targeting depolymerising activities to spindle poles. *J. Cell Biol.* **166**, 465-471.

Ganem, N. J. and Compton, D. A. (2004). The KinI kinesin Kif2a is required for bipolar spindle assembly through a functional relationship with MCAK. *J. Cell Biol.* **166**, 473-478.

Ganem, N. J., Upton, K. and Compton, D. A. (2005). Efficient mitosis in human cells lacking poleward microtubule flux. *Curr. Biol.* **15**, 1827-1832.

Garcia, M. A., Koonrugsa, N. and Toda, T. (2002). Two kinesin-like Kin I family proteins in fission yeast regulate the establishment of metaphase and the onset of anaphase A. *Curr. Biol.* **12**, 610-621.

Goshima, G. and Vale, R. D. (2003). The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the Drosophila S2 cell line. *J. Cell Biol.* **162**, 1003-1016.

Goshima, G., Wollman, R., Stuurman, N., Scholey, J. M. and Vale, R. D. (2005). Length control of the metaphase spindle. *Curr. Biol.* **15**, 1979-1988.

Gupta, M. L., Carvalho, P., Roof, D. M. and Pellman, D. (2006). Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle. *Nat. Cell Biol.* doi: 10.1038/ncb1457.

Hekmat-Nejad, M. and Sakowicz, R. (2004). Regulation of ATPase activity of internal motor kinesin, pKinI: binding to microtubule lattice is non-productive. *Am. Soc. Cell Biol*. December 2004, http://www.cytokinetics.com/cyto/research_publications#other.

Helenius, J., Brouhard, G., Kalaidzidis, Y., Diez, S. and Howard, J. (2006). The

depolymerising kinesin MCAK uses lattice diffusion to rapidly target microtubule ends. *Nature* **441**, 115-119.
Hertzer, K. M., Ems-McClung, S. C., Kline-Smith, S. L., Lipkin, T. G., Gilbert, S. P. and Walczak, C. E. (2006). Full-length dimeric MCAK is a more efficient microtubule depolymerase than minimal domain monomeric MCAK. *Mol. Biol. Cell* **17**, 700-710.
Holmfeldt, P., Stenmark, S. and Gullberg, M. (2004). Differential functional interplay of TOGp/XMAP215 and the KinI kinesin MCAK during interphase and mitosis. *EMBO J.* **23**, 627-637.
Homma, N., Takei, Y., Tanaka, Y., Nakata, T., Terada, S., Kikkawa, M., Noda, Y. and Hirokawa, N. (2003). Kinesin superfamily protein 2A (KIF2A) functions in suppression of collateral branch extension. *Cell* **114**, 229-239.
Hunter, A. W., Caplow, M., Coy, D. L., Hancock, W. O., Diez, S., Wordeman, L. and Howard, J. (2003). The kinesin-related protein MCAK is a microtubule depolymerase that forms an ATP-hydrolyzing complex at microtubule ends. *Mol. Cell* **11**, 445-457.
Kinoshita, K., Arnal, I., Desai, A., Dreschel, D. N. and Hyman, A. A. (2001). Reconstitution of physiological microtubule dynamics using purified components. *Science* **294**, 1340-1343.
Kline-Smith, S. L. and Walczak, C. E. (2002). The microtubule-destabilising kinesin XKCM1 regulates microtubule dynamic instability in cells. *Mol. Biol. Cell* **13**, 2718-2731.
Kline-Smith, S. L., Khodjakov, A., Hergert, P. and Walczak, C. E. (2004). Depletion of centromeric MCAK leads to chromosome congression and segregation defects due to improper kinetochore attachments. *Mol. Biol. Cell* **15**, 1146-1159.
Lan, W., Zhang, X., Kline-Smith, S. L., Rosasco, S. E., Barrett-Wilt, G. A., Shabanowitz, J., Hunt, D. F., Walczak, C. E. and Stukenberg, P. T. (2004). Aurora B phosphorylates centromeric MCAK and regulates its localisation and microtubule depolymerisation activity. *Curr. Biol.* **14**, 273-286.
Lawrence, C. J., Dawe, R. K., Christie, K. R., Cleveland, D. W., Dawson, S. C., Endow, S. A., Goldstein, L. S., Goodson, H. V., Hirokawa, N., Malmberg, R. L. et al. (2004). A standardized kinesin nomenclature. *J. Cell Biol.* **167**, 19-22.
Laycock, J. E., Savoian, M. S. and Glover, D. M. (2006). Antagonistic activities of Klp10A and Orbit regulate spindle length, bipolarity and function in vivo. *J. Cell Sci.* **119**, 2354-2361.
Maiato, H., DeLuca, J., Salmon, E. D. and Earnshaw, W. C. (2004). The dynamic kinetochore-microtubule interface. *J. Cell Sci.* **117**, 5461-5477.
Maney, T., Hunter, A. W., Wagenbach, M. and Wordeman, L. (1998). Mitotic centromere-associated kinesin is important for anaphase chromosome segregation. *J. Cell Biol.* **142**, 787-801.
Maney, T., Wagenbach, M. and Wordeman, L. (2001). Molecular dissection of the microtubule depolymerising activity of mitotic centromere-associated kinesin. *J. Biol. Chem.* **276**, 34753-34758.
Mennella, V., Rogers, G. C., Rogers, S. L., Buster, D. W., Vale, R. D. and Sharp, D. J. (2005). Functionally distinct kinesin-13 family members cooperate to regulate microtubule dynamics during interphase. *Nat. Cell Biol.* **7**, 235-245.
Miki, H., Setou, M., Kaneshiro, K. and Hirokawa, N. (2001). All kinesin superfamily protein, KIF, genes in mouse and human. *Proc. Natl. Acad. Sci. USA* **98**, 7004-7011.
Moore, A. and Wordeman, L. (2004a). C-terminus of mitotic-centromere-associated kinesin (MCAK) inhibits lattice-stimulated ATPase activity. *Biochem. J.* **383**, 227-235.
Moore, A. and Wordeman, L. (2004b). The mechanism, function and regulation of depolymerizing kinesins during mitosis. *Trends Cell Biol.* **14**, 537-546.
Moore, A. T., Rankin, K. E., von Dassow, G., Peris, L., Wagenbach, M., Ovechkina, Y., Andrieux, A., Job, D. and Wordeman, L. (2005). MCAK associates with the tips of polymerising microtubules. *J. Cell Biol.* **169**, 391-397.
Moores, C. A., Yu, M., Guo, J., Beraud, C., Sakowicz, R. and Milligan, R. A. (2002). A mechanism for microtubule depolymerisation by KinI kinesins. *Mol. Cell* **9**, 903-909.
Moores, C. A., Hekmat-Nejad, M., Sakowicz, R. and Milligan, R. A. (2003). Regulation of the KinI kinesin ATPase activity by binding to the microtubule lattice. *J. Cell Biol.* **163**, 963-971.
Moores, C. A., Perderiset, M., Francis, F., Chelly, J., Houdusse, A. and Milligan, R. A. (2004). Mechanism of microtubule stabilisation by doublecortin. *Mol. Cell* **14**, 833-839.
Moores, C. A., Cooper, J. R., Wagenbach, M., Ovechkina, Y., Wordeman, L. and Milligan, R. A. (2006a). The role of the kinesin-13 neck in microtubule depolymerization. *Cell Cycle* **5**, 1812-1815.
Moores, C. A., Perderiset, M., Kappeler, C., Kain, S., Drummond, D., Perkins, S. J., Chelly, J., Cross, R., Houdusse, A. and Francis, F. (2006b). Distinct roles of doublecortin modulating the microtubule cytoskeleton. *EMBO J.* In press.
Niederstrasser, H., Salehi-Had, H., Gan, E. C., Walczak, C. and Nogales, E. (2002). XKCM1 acts on a single protofilament and requires the C terminus of tubulin. *J. Mol. Biol.* **316**, 817-828.
Noetzel, T. L., Dreschel, D. N., Hyman, A. A. and Kinoshita, K. (2005). A comparison of the ability of XMAP215 and tau to inhibit the microtubule destabilising activity of XKCM1. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **360**, 591-594.
Notredame, C., Higgins, D. and Heringa, J. (2000). T-Coffee: a novel method for multiple sequence alignments. *J. Mol. Biol.* **302**, 205-217.
Oegema, K., Desai, A., Rybina, S., Kirkham, M. and Hyman, A. A. (2001). Functional analysis of kinetochore assembly in Caenorhabditis elegans. *J. Cell Biol.* **153**, 1209-1226.
Ogawa, T., Nitta, R., Okada, Y. and Hirokawa, N. (2004). A common mechanism for microtubule destabilisers – M type kinesins stabilise curling of the protofilament using the class-specific neck and loops. *Cell* **116**, 591-602.
Ohi, R., Coughlin, M. L., Lane, W. S. and Mitchison, T. J. (2003). An inner centromere protein that stimulates the microtubule depolymerising activity of a KinI kinesin. *Dev. Cell* **5**, 309-321.
Ohi, R., Sapra, T., Howard, J. and Mitchison, T. J. (2004). Differentiation of cytoplasmic and meiotic spindle assembly MCAK functions by Aurora B-dependent phosphorylation. *Mol. Biol. Cell* **15**, 2895-2906.
Ovechkina, Y. and Wordeman, L. (2003). Unconventional motoring: an overview of the Kin C and Kin I kinesins. *Traffic* **4**, 367-375.
Ovechkina, Y., Wagenbach, M. and Wordeman, L. (2002). K-loop insertion restores microtubule depolymerizing activity of a "neckless" MCAK mutant. *J. Cell Biol.* **159**, 557-562.
Parra, M. T., Gomez, R., Viera, A., Page, J., Calvente, A., Wordeman, L., Rufas, J. S. and Suja, J. A. (2006). A perikinetochoric ring defined by MCAK and Aurora-B as a novel centromere domain. *PloS Genet.* **2**, e84.
Pereira, A. J., Dalby, B., Stewart, R. J., Doxsey, S. J. and Goldstein, L. S. (1997). Mitochondrial association of a plus end-directed microtubule motor expressed during mitosis in Drosophila. *J. Cell Biol.* **136**, 1081-1090.
Ravelli, R. B. G., Gigant, B., Curmi, P. A., Jourdain, I., Lachkar, S., Sobel, A. and Knossow, M. (2004). Insight into tubulin regulation from a complex with colchicines and a stathmin-like domain. *Nature* **428**, 198-202.
Rogers, G. C., Rogers, S. L., Schwimmer, T. A., Ems-McClung, S. C., Walczak, C. E., Vale, R. D., Scholey, J. M. and Sharp, D. J. (2004). Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase. *Nature* **427**, 364-370.
Rogers, G. C., Rogers, S. L. and Sharp, D. J. (2005). Spindle microtubules in flux. *J. Cell Sci.* **118**, 1105-1116.
Sablin, E. P., Kull, F. J., Cooke, R., Vale, R. D. and Fletterick, R. J. (1996). Crystal structure of the motor domain of the kinesin-related motor ncd. *Nature* **380**, 555-559.
Sack, S., Kull, F. J. and Mandelkow, E. (1999). Motor proteins of the kinesin family. *Eur. J. Biochem.* **262**, 1-11.
Santarella, R. A., Skiniotis, G., Goldie, K. N., Tittmann, P., Gross, H., Mandelkow, E. M., Mandelkow, E. and Hoenger, A. (2004). Surface-decoration of microtubules by human tau. *J. Mol. Biol.* **339**, 539-553.
Sharp, D. J. and Rogers, G. C. (2004). A Kin I-dependent pacman-flux mechanism for anaphase A. *Cell Cycle* **6**, e108-e111.
Shipley, K., Hekmat-Nejad, M., Turner, J., Moores, C., Anderson, R., Milligan, R., Sakowicz, R. and Fletterick, R. (2004). Structure of a kinesin microtubule depolymerisation machine. *EMBO J.* **23**, 1422-1432.
Sproul, L. R., Anderson, D. J., Mackey, A. T., Saunders, W. S. and Gilbert, S. P. (2005). Cik1 targets the minus-end kinesin depolymerase kar3 to microtubule plus ends. *Curr. Biol.* **15**, 1420-1427.
Tournebize, R., Popov, A., Kinoshita, K., Ashford, A. J., Rybina, S., Pozniakovsky, A., Mayer, T. U., Walczak, C. E., Karsenti, E. and Hyman, A. A. (2000). Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in Xenopus egg extracts. *Nat. Cell Biol.* **2**, 13-19.
Vale, R. D. and Fletterick, R. J. (1997). The design plan of kinesin motors. *Annu. Rev. Cell Dev. Biol.* **13**, 745-777.
Vale, R. D. and Milligan, R. A. (2000). The way things move: looking under the hood of molecular motor proteins. *Science* **288**, 88-95.
Vale, R. D., Reese, T. S. and Sheetz, M. P. (1985). Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* **42**, 39-50.
Varga, V., Helenius, J., Tanaka, K., Hyman, A. A., Tanaka, T. and Howard, J. (2006). Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. *Nat. Cell Biol.* doi: 10.1038/ncb1462.
Walczak, C. E., Mitchison, T. J. and Desai, A. (1996). XKCM1: a Xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell* **84**, 37-47.
Walczak, C. E., Gan, E. C., Desai, A., Mitchison, T. J. and Kline-Smith, S. L. (2002). The microtubule-destabilising kinesin XKCM1 is required for chromosome positioning during spindle assembly. *Curr. Biol.* **29**, 1885-1889.
Wang, H.-W. and Nogales, E. (2005). Nucleotide-dependent bending flexibility of tubulin regulates microtubule assembly. *Nature* **435**, 911-915.
Wang, Z. and Sheetz, M. P. (2000). The C-terminus of tubulin increases cytoplasmic dynein and kinesin processivity. *Biophys. J.* **78**, 1955-1964.
Wein, H., Foss, M., Brady, B. and Cande, W. Z. (1996). DSK1, a novel kinesin-related protein from the diatom Cylindrotheca fusiformis that is involved in anaphase spindle elongation. *J. Cell Biol.* **133**, 595-604.
West, R. R., Malmstrom, T., Troxell, C. L. and McIntosh, J. R. (2001). Two related kinesins, klp5+ and klp6+, foster microtubule disassembly and are required for meiosis in fission yeast. *Mol. Biol. Cell* **12**, 3919-3932.
West, R. R., Malstrom, T. and McIntosh, J. R. (2002). Kinesins klp5(+) and klp6 (+) are required for normal chromosome movement in mitosis. *J. Cell Sci.* **115**, 931-940.
Wordeman, L. and Mitchison, T. J. (1995). Identification and partial characterisation of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *J. Cell Biol.* **128**, 95-104.
Wordeman, L., Wagenbach, M. and Maney, T. (1999). Mutations in the ATP-binding domain affect the subcellular distribution of mitotic centromere-associated kinesin (MCAK). *Cell Biol. Int.* **23**, 275-286.
Zhu, C., Zhao, J., Bibikova, M., Leverson, J. D., Bossy-Wetzel, E., Fan, J.-B., Abraham, R. T. and Jiang, W. (2005). Functional analysis of human microtubule-based motor proteins, the kinesins and dyneins, in mitosis/cytokinesis using RNA interference (RNAi). *Mol. Biol. Cell* **16**, 3187-3199.