

Kin I Kinesins Are Microtubule-Destabilizing Enzymes

Arshad Desai,*§ Suzie Verma,† Timothy J. Mitchison,‡ and Claire E. Walczak†||

*Department of Biochemistry and Biophysics

†Department of Cellular and Molecular Pharmacology
University of California

San Francisco, California 94143

‡Department of Cell Biology

Harvard Medical School

Boston, Massachusetts 02115

Summary

Using *in vitro* assays with purified proteins, we show that XKCM1 and XKIF2, two distinct members of the internal catalytic domain (Kin I) kinesin subfamily, catalytically destabilize microtubules using a novel mechanism. Both XKCM1 and XKIF2 influence microtubule stability by targeting directly to microtubule ends where they induce a destabilizing conformational change. ATP hydrolysis recycles XKCM1/XKIF2 for multiple rounds of action by dissociating a XKCM1/XKIF2-tubulin dimer complex released upon microtubule depolymerization. These results establish Kin I kinesins as microtubule-destabilizing enzymes, distinguish them mechanistically from kinesin superfamily members that use ATP hydrolysis to translocate along microtubules, and have important implications for the regulation of microtubule dynamics and for the intracellular functions and evolution of the kinesin superfamily.

Introduction

Microtubules (MTs) are noncovalent polar polymers of $\alpha\beta$ -tubulin heterodimers found in all eukaryotic cells that play an essential role in cell division, cytoplasmic organization, generation and maintenance of cell polarity, and many types of cell movements. MTs are inherently dynamic polymers that transduce energy derived from nucleotide hydrolysis into polymer dynamics. In addition, the surface of the MT polymer serves as a track on which motor proteins transport cargoes throughout the cell. These two distinct facets of the MT polymer are central to the many biological functions of the MT cytoskeleton.

MTs are 25 nm diameter, 12–15 protofilament polymers that utilize polymerization-induced GTP hydrolysis on β -tubulin to generate dynamic instability—a behavior where polymerizing and depolymerizing MTs coexist in the same population, infrequently interconverting between these two states (Mitchison and Kirschner, 1984;

Erickson and O'Brien, 1992; Desai and Mitchison, 1997). Polymerizing MT ends are thought to maintain a stabilizing “cap” of GTP/GDP.P_i-tubulin, the loss of which results in exposure of an unstable GDP-tubulin core and rapid depolymerization. Thus, tubulin has a built-in lattice destabilization mechanism driven by GTP hydrolysis on β -tubulin. Kinetically, hydrolysis and phosphate release result in a GDP-tubulin lattice that has a >1000-fold higher subunit off rate from an MT end than GTP-tubulin (Walker et al., 1988). Structurally, GDP-tubulin protofilaments are thought to prefer a conformation with increased outward curvature relative to GTP-tubulin protofilaments (Melki et al., 1989; Mandelkow et al., 1991; Müller-Reichert et al., 1998). In the lattice of a polymerizing MT, GDP-tubulin protofilaments are constrained to being straight, presumably by lattice interactions, but during depolymerization they can relax into the preferred curved conformation. The free energy released during this relaxation is thought to drive the rapid depolymerization phase of dynamic instability. Consistent with these ideas, tubulin polymerized with GMPCPP, a slowly hydrolyzable GTP analog, forms stable MTs that do not undergo dynamic instability (Hyman et al., 1992; Caplow et al., 1994). Taxol, a drug isolated from the bark of the yew tree that binds tubulin, also stabilizes MTs by suppressing dynamic instability (Horwitz, 1994).

MT polymerization dynamics are fundamentally important to the intracellular functions of the MT cytoskeleton, as best illustrated by analysis of chromosome movement (Inoué and Salmon, 1995). *In vivo*, the intrinsic dynamic instability of tubulin is extensively regulated (Cassimeris, 1993; McNally, 1996; Desai and Mitchison, 1997). MTs *in vivo* turn over much more rapidly than MTs assembled from pure tubulin *in vitro*, in large part because of an increase in the frequency of transitions from the polymerization phase to the depolymerization phase, called the frequency of catastrophe (Belmont et al., 1990; Verde et al., 1992). This finding is consistent with modeling studies showing that regulation of catastrophe frequency is an extremely efficient way to rapidly modulate MT dynamics (Verde et al., 1992). The observed high frequency of catastrophe has been suspected to result from the action of cellular proteins that destabilize the stabilizing caps at polymerizing MT ends.

In addition to being dynamic polymers, MTs also serve as tracks for motor proteins. Conventional kinesin, the founding member of the kinesin superfamily, was identified on the basis of its ability to use energy derived from ATP hydrolysis to translocate along the MT lattice (Vale et al., 1985). In the last decade, ~100 eukaryotic proteins have been identified that contain a domain homologous to the ~300 amino acid catalytic ATPase domain of conventional kinesin (Vale and Fletterick, 1997; Hirokawa et al., 1998). Based solely on sequence alignments of their catalytic domains, the majority of kinesins have been classified into eight subfamilies with a minority being “orphans” (Vale and Fletterick, 1997). These subfamily categorizations are supported to varying extents by location of the catalytic domain within the polypeptide chain of the protein, by analysis of the quaternary

§ To whom correspondence should be addressed at the following present address: European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany (e-mail: arshad.desai@embl-heidelberg.de).

|| Present address: Indiana University, Medical Sciences, Bloomington, Indiana 47405.

structures of the native proteins, and by the similarity of the biological processes in which members of a subfamily derived from different organisms are implicated. Approximately 20% of identified kinesins have been reported to have motor activity in *in vitro* motility assays. Among the kinesins characterized *in vitro* is at least one member of each of the eight subfamilies, strongly supporting the assertion that kinesins are mechanochemical ATPases that translocate along the MT lattice.

Previously, we described a kinesin, XKCM1, that is involved in regulating MT dynamics in frog egg extracts (Walczak et al., 1996). Depletion of XKCM1 resulted in a dramatic increase in MT polymerization, suggesting that XKCM1 promotes MT destabilization in egg cytoplasm. Analysis of MT dynamics showed that depletion of XKCM1 did not influence the rates at which MTs polymerized or depolymerized in extracts but did result in a 4-fold reduction in the catastrophe frequency. These results indicate that XKCM1 acts, either directly or indirectly, to destabilize the ends of polymerizing MTs in frog egg extracts. The dramatic effect of XKCM1 depletion indicated that XKCM1 is an important regulator of MT polymerization in egg cytoplasm, prompting us to investigate how XKCM1, a kinesin, has such a striking effect on MT dynamics.

XKCM1 is one of eight kinesins in the internal catalytic domain subfamily (Kin I subfamily for Kinesin Internal; Vale and Fletterick, 1997). This subfamily also includes MCAK, a hamster kinesin implicated in cell division (Wordeman and Mitchison, 1995; Maney et al., 1998), and mouse KIF2 (mKIF2), implicated in nervous system function (Noda et al., 1995; Morfini et al., 1997). Kin I kinesins were expected to be motor proteins on the basis of their sequence homology to kinesins. Consistent with this expectation, fast plus end-directed motility was reported for mKIF2 (Noda et al., 1995), suggesting that motility was likely to be central to the mechanism of MT destabilization by XKCM1. Therefore, at the conclusion of our previous study, we proposed that XKCM1 used plus end motility to target itself to MT ends where it either directly destabilized the MT end or indirectly aided destabilization by delivering a catastrophe-inducing factor (Walczak et al., 1996).

In this paper we show that pure XKCM1 and XKIF2 (*Xenopus* KIF2; 87% overall identity to mKIF2) directly destabilize MTs in a catalytic, ATP-dependent manner. More significantly, we find no involvement of motor activity in MT destabilization. Instead, we find that both XKCM1 and XKIF2 target directly to MT ends where they trigger a destabilizing conformational change. Catalytic domain ATP hydrolysis, used to power motility in kinesins that translocate along the MT lattice, is instead used to dissociate a complex of XKCM1/XKIF2 with tubulin dimer released upon MT depolymerization. The distinction of the molecular mechanism for MT destabilization from that of motility, and the similar *in vitro* behavior of XKCM1 and XKIF2, two functionally distinct Kin I kinesins, lead us to conclude that Kin I kinesins are MT-destabilizing enzymes and not motor proteins. These results represent not only a characterization of the mechanism of a MT-destabilizing enzyme but also reveal the existence of a novel molecular mechanism inherent to the kinesin superfamily that is distinct from the mechanism of motility.

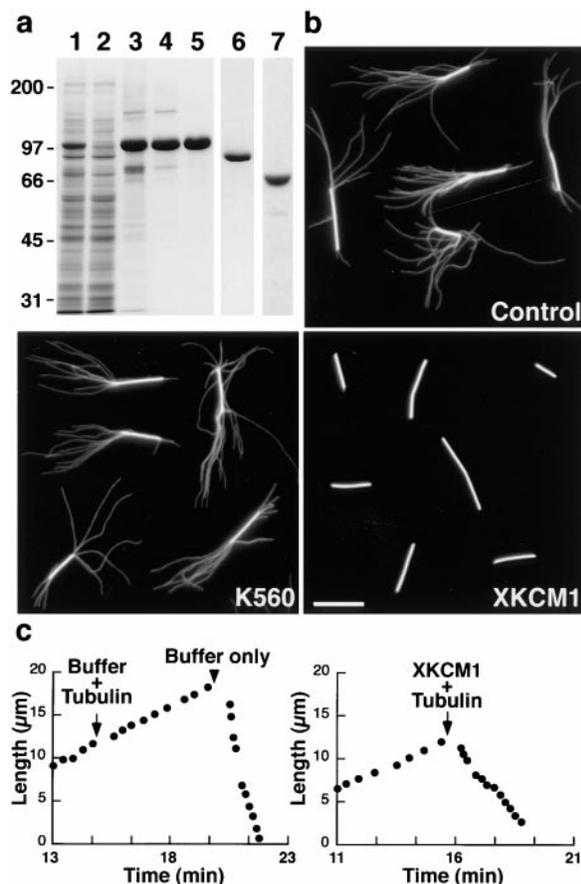


Figure 1. Purified XKCM1 Inhibits MT Assembly and Induces Catastrophes

(a) Baculovirus expression and purification of XKCM1 and XKIF2. Clarified Sf-9 cell lysate (1), SP Sepharose flowthrough (2), SP Sepharose elution (3), Superose 6 elution (4), and Mono S elution (5) from an XKIF2 prep. Lane 6 shows XKCM1 prepared similarly. Lane 7 shows K560 purified from bacteria.

(b) XKCM1 inhibits MT polymerization off axonemes. Bovine brain tubulin (15 μ M) was polymerized off axonemes in the presence of a control buffer, 80 nM K560, or 80 nM XKCM1 in BRB80 + 70 mM KCl + 0.5 mM GTP + 1.5 mM MgATP. After 7 min at 37°C, the reactions were fixed, pelleted onto coverslips, and processed for tubulin immunofluorescence. Bar, 10 μ m.

(c) XKCM1 induces catastrophes. Life history traces of MT plus ends polymerizing off axonemes with 7 μ M frog egg tubulin in assembly buffer (BRB80 + 1 mM GTP + 1.5 mM MgATP). Time 0 is when the tubulin was introduced into the flow cell. At times indicated by the arrows, the solution in the flow cell was exchanged by a mixture of 7 μ M frog egg tubulin and either a control buffer (left panel) or 80 nM XKCM1 (right panel) in assembly buffer. The control buffer trace also shows rapid depolymerization following isothermal dilution with BRB80 buffer alone (arrowhead).

Results

XKCM1 Inhibits Microtubule Assembly and Induces Catastrophes

Our previous study established that XKCM1, a Kin I kinesin, promoted MT destabilization in frog egg extracts (Walczak et al., 1996). To determine whether purified Kin I kinesins could directly destabilize MTs, we expressed full-length, untagged XKCM1 and XKIF2 in

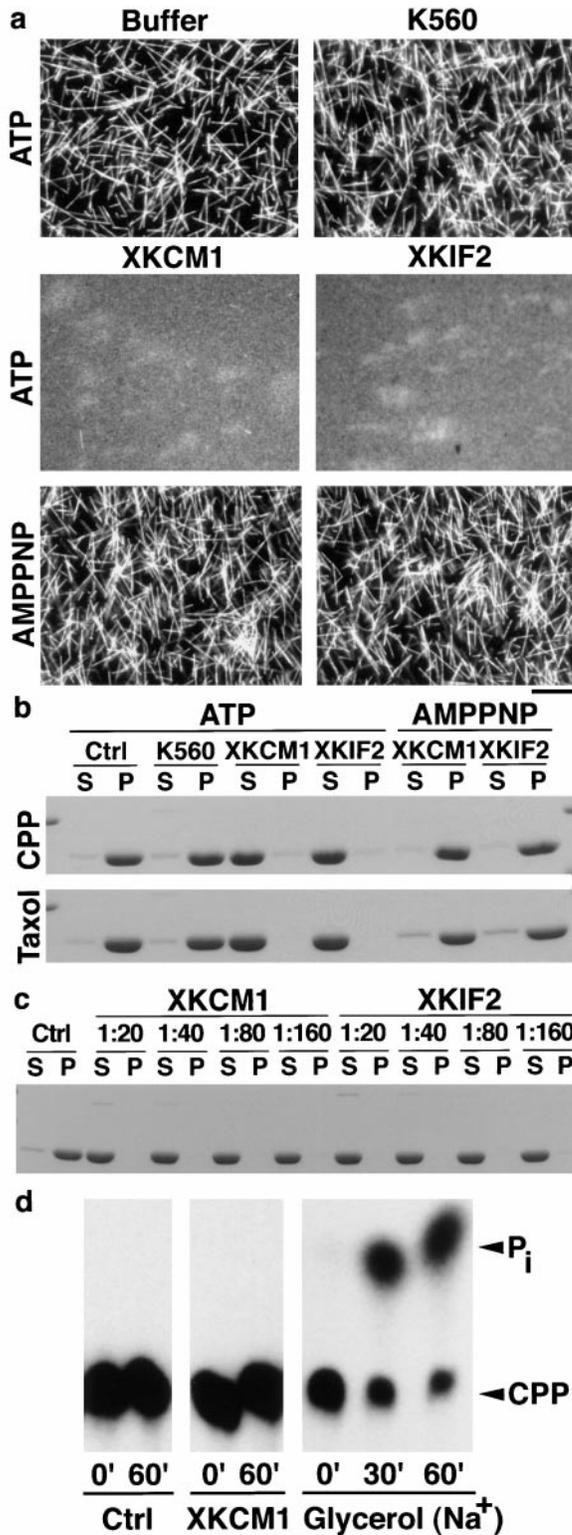


Figure 2. XKCM1 and XKIF2 Depolymerize Stabilized MT Substrates

(a) Visual assay of depolymerization of GMPCPP MTs. GMPCPP MTs (1.5 μM) were mixed with K560/XKCM1/XKIF2 (10 nM) or a control buffer in ATP or AMPPNP, as indicated. Panels show microscope fields after 15 min of incubation at RT. Similar results were obtained with taxol-stabilized MTs. Bar, 10 μm .

baculovirus and purified them by conventional chromatography (Figure 1a). As a control we used K560, a plus end-directed motor protein that is the 560 aa N-terminal fragment of human conventional kinesin (Woehlke et al., 1997). We tested the effect of XKCM1 and K560 on MT polymerization nucleated off axonemes. Pure XKCM1, in the presence of ATP and at concentrations highly substoichiometric to tubulin, completely inhibited MT assembly off axonemes, whereas K560 had no effect (Figure 1b). A similar activity was exhibited by native XKCM1 purified by immunoaffinity from frog egg extracts (data not shown).

To determine what parameter of MT dynamic instability was being affected, we used video microscopy to analyze the fate of prepolymerized MTs after exposure to XKCM1. Purified frog egg tubulin was used for this assay because it is the physiological substrate of XKCM1, it assembles robustly at room temperature, and it has an extremely low intrinsic frequency of catastrophe ($<0.00011\text{ s}^{-1}$ for plus ends and $<0.0003\text{ s}^{-1}$ for minus ends at 7 μM). MTs were polymerized off axonemes adhered to the surface of a flow cell. The solution in the flow cell was then replaced with a mixture of tubulin and control buffer or tubulin and XKCM1. Without XKCM1, all MT ends observed remained in the polymerization phase ($n = 25$ plus ends; 18 minus ends; Figure 1c). In contrast, in the presence of XKCM1, nearly all MT ends had transited to the depolymerization phase ($n = 35/36$ plus ends; 11/13 minus ends; Figure 1c), indicating that the MTs had undergone a catastrophe. The introduced XKCM1 rapidly adsorbed to the flow cell surfaces, resulting in binding of the MTs to the coverslip surface and significant reduction in their depolymerization rate relative to MTs depolymerized by dilution in buffer without tubulin (Figure 1c). This adsorption prevented us from determining whether XKCM1 influenced the rate of depolymerization of dynamic MTs in solution. These results demonstrate that pure XKCM1 directly inhibits MT polymerization and is a potent catastrophe factor that can destabilize polymerizing MT ends.

XKCM1/XKIF2 Catalytically Depolymerize Stabilized Microtubules

To characterize how XKCM1 destabilizes MTs, we analyzed its effect on MTs stabilized by the drug taxol or

(b) Sedimentation analysis of GMPCPP (CPP) and taxol MT depolymerization. Reactions, identical to those in (a), were sedimented after 30 min at RT, and supernatants (S) and pellets (P) were analyzed by SDS-PAGE.

(c) XKCM1/XKIF2 act catalytically. Taxol MTs (1.5 μM) were incubated for 30 min at RT with the indicated substoichiometric molar amounts of XKCM1/XKIF2 and analyzed as in (b). Depolymerization is complete even at 1:160 molar ratio of XKCM1/XKIF2:tubulin (control reactions indicated that 85%–90% of the tubulin in the reaction is polymer; thus, at 1:160 XKCM1/XKIF2:total tubulin molar ratio, the relevant molar ratio of XKCM1/XKIF2:tubulin polymer is $\sim 1:140$). XKCM1/XKIF2 are visible in the supernatant at the two highest concentrations used (1:20 and 1:40).

(d) Analysis of GMPCPP hydrolysis during GMPCPP MT depolymerization. GMPCPP MTs (1.5 μM) containing $[\gamma\text{-}^{32}\text{P}]\text{GMPCPP}$ were mixed with a control buffer (Ctrl) or 40 nM XKCM1 (XKCM1) in BRB80 + 1.5 mM MgATP or adjusted to 60% glycerol in Na-BRB80 [Glycerol (Na⁺)]. At the indicated time, the reaction was stopped and hydrolysis assayed by thin layer chromatography.

by polymerization with GMPCPP, a GTP analog that is essentially nonhydrolyzable by tubulin over the time course of most experiments (Hyman et al., 1992; Caplow et al., 1994). Because taxol and GMPCPP eliminate the intrinsic GTP hydrolysis-driven destabilization mechanism of tubulin, depolymerization of stabilized MTs requires input of free energy and multiple rounds of action at an MT end. We used fluorescent stabilized MTs to qualitatively assay the effect of XKCM1/XKIF2/K560 on their stability. In the presence of ATP, substoichiometric amounts of XKCM1 depolymerized both GMPCPP- and taxol-stabilized MTs; purified XKIF2 had an identical activity, whereas K560 had no depolymerizing activity (Figure 2a). The activity of both XKCM1 and XKIF2 required ATP and was inhibited by the nonhydrolyzable ATP analog, AMPPNP (Figure 2a). Addition of an anti-XKCM1 antibody inhibited the activity of XKCM1 but not of XKIF2 (data not shown), suggesting that the observed depolymerization activity is not due to a cofractionating contaminant.

We used sedimentation and SDS-PAGE analysis to analyze the extent of stabilized MT depolymerization in our reactions. This analysis confirmed the conclusions derived from the qualitative visual assays (Figure 2b). To demonstrate that XKCM1/XKIF2 catalytically depolymerize stabilized MTs in the presence of ATP, we titrated XKCM1/XKIF2 while keeping the concentration of the substrate MTs constant. This analysis demonstrated that each XKCM1/XKIF2 dimer can release at least 140 tubulin dimers (Figure 2c), suggesting a catalytic mechanism. Gel filtration analysis indicated that the end product of GMPCPP MT destabilization by XKCM1/XKIF2 is 6S tubulin dimer; in addition, 85% \pm 5% (n = 2 experiments) of the tubulin released into the supernatant was capable of repolymerizing into MTs (data not shown).

If GMPCPP MTs are treated with glycerol in the presence of sodium ions, the normal β - γ phosphate bond of GMPCPP bound to β -tubulin is hydrolyzed, and the MT lattice is destabilized (Caplow et al., 1994). To test whether XKCM1/XKIF2 destabilize GMPCPP MTs by a similar mechanism, we analyzed XKCM1-induced depolymerization of fluorescent GMPCPP-stabilized MTs containing [γ - 32 P] GMPCPP. Depolymerization was monitored using fluorescence microscopy, and GMPCPP hydrolysis was monitored using thin layer chromatography. Treatment with glycerol in the presence of sodium ions was used as a positive control (Figure 2d). This assay showed that XKCM1 depolymerizes GMPCPP MTs without inducing hydrolysis of the β -tubulin-bound GMPCPP (Figure 2d). This result demonstrates that catalytic depolymerization of stabilized MTs by XKCM1/XKIF2 occurs independently of the intrinsic lattice destabilization mechanism of tubulin and suggests that XKCM1/XKIF2 do not induce catastrophes by stimulating GTP hydrolysis at polymerizing MT ends. In addition, this result implicates ATP hydrolysis by these kinesins as the free energy source in the catalytic depolymerization of stabilized MTs.

XKCM1/XKIF2 Depolymerize Stabilized Microtubules Equivalently from Both Ends and Do Not Exhibit Motor Activity

A previous report that mKIF2 is a fast plus end-directed MT motor (Noda et al., 1995) suggested that XKCM1/

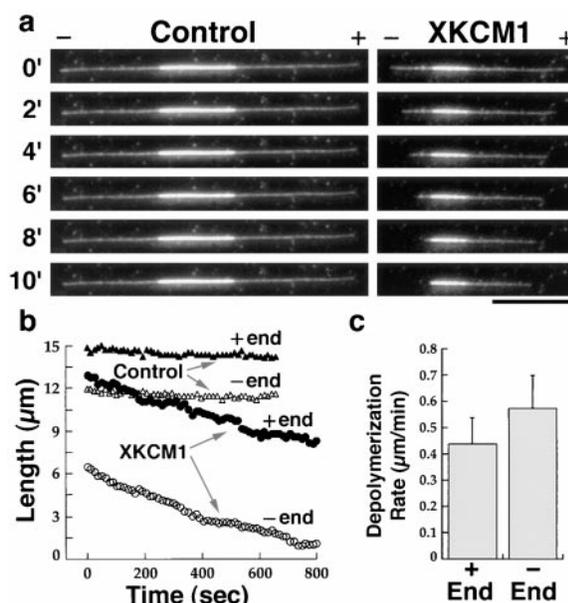


Figure 3. Real Time Analysis of GMPCPP MT Depolymerization

(a) Panels show fate of segmented GMPCPP MTs in a control versus XKCM1 reaction. Plus and minus signs indicate MT polarity. Both reactions contained 20 nM XKCM1; the control was preincubated with an anti-XKCM1 antibody (30-fold molar excess), while the XKCM1 reaction was preincubated with an equivalent amount of an irrelevant rabbit IgG.

(b) Length versus time traces of plus and minus end segments of the MTs shown in (a).

(c) Summary of analysis of 14 MTs showing the depolymerization rate at both MT ends is nearly equivalent.

XKIF2 use ATP-dependent motility to target to MT plus ends, where they induce end destabilization (Walczak et al., 1996; Waters and Salmon, 1996). A prediction of this hypothesis is that depolymerization would occur preferentially from the plus end of the MT. To test this hypothesis, we monitored XKCM1-induced depolymerization of GMPCPP MTs in real time using an assay that unambiguously assigns polarity to the substrate MTs. GMPCPP MTs with dimly labeled plus and minus end segments polymerized off brightly labeled GMPCPP MT seeds (Figure 3a) were adhered to the surface of a flow cell, exposed to either XKCM1 inactivated by preincubation with an anti-XKCM1 antibody (control) or XKCM1 preincubated with an irrelevant antibody (XKCM1), and monitored by time lapse fluorescence microscopy. After \sim 10 min, the plus end-directed K560 motor was introduced into the flow cell, and the resulting motility of the MTs was recorded to retroactively and unambiguously assign their polarity. Using this assay, we found that XKCM1 depolymerized GMPCPP MTs from both ends at nearly equivalent rates (Figures 3a, 3b, and 3c). A similar result was obtained using taxol-stabilized MTs as substrates (data not shown). These results suggest that XKCM1 does not use directed motility to reach an MT end. They also demonstrate that stabilized MTs are depolymerized endwise and not by an internal severing mechanism as described for katanin, an MT-severing ATPase (McNally and Vale, 1993; Hartman et al., 1998).

In attempts to observe MT motor activity, we performed standard motility assays that have been used

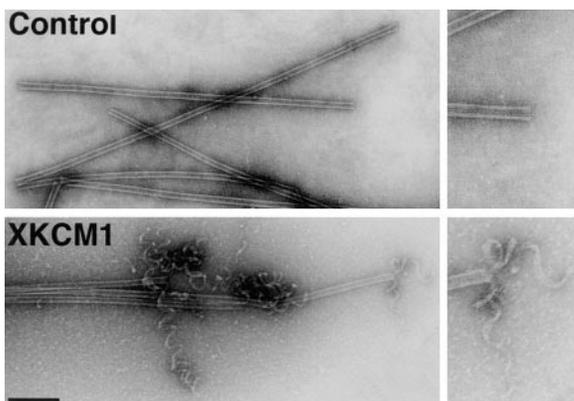


Figure 4. Ultrastructural Analysis of GMPCPP MTs Being Depolymerized by XKCM1

Protofilament peeling is evident at GMPCPP MT ends undergoing XKCM1-induced depolymerization. Reactions containing 1.5 μ M GMPCPP MTs and 40 nM XKCM1 in BRB80 + 1 mM DTT + 1.5 mM MgATP were analyzed by negative stain electron microscopy after incubation at RT for 3 min. Right panels show a higher magnification view. Similar structures were observed in reactions containing XKIF2. Bar, 200 nm for left panels.

to demonstrate motility of other kinesins (Cohn et al., 1993). When XKCM1/XKIF2 were adsorbed to a coverslip surface and taxol MTs added, the MTs bound to the surface and exhibited slow bipolar depolymerization. However, motility of the bound MTs was never observed (data not shown). In parallel assays, K560 exhibited robust motility without any MT depolymerization. Preliminary ATPase analysis of XKCM1 and XKIF2 showed that both proteins have very weak MT-stimulated ATPase, inconsistent with their being fast plus end-directed motors (data not shown). Cumulatively, these results suggest that XKCM1/XKIF2 directly target to MT ends, prompting us to investigate how this targeting occurs and what happens at ends to cause destabilization.

XKCM1/XKIF2 Induce a Destabilizing Conformational Change at Microtubule Ends

One attractive possibility for the mechanism of end destabilization is that XKCM1/XKIF2 physically disrupt end structure. Consistent with this idea, negative stain electron microscopy of GMPCPP MTs during depolymerization induced by either XKCM1 or XKIF2 revealed the presence of peeling protofilaments at their ends (Figure 4). Such peeled protofilaments were found on most of the residual GMPCPP MTs in the process of being depolymerized by XKCM1/XKIF2, whereas the ends of control buffer-treated GMPCPP MTs were blunt (Figure 4; $n > 200$ MTs). Where clearly visible, we observed protofilament peels at both MT ends, consistent with our finding that XKCM1 depolymerizes GMPCPP MTs from both ends (also see Figure 5d). These protofilament peels are reminiscent of the structure of rapidly depolymerizing dynamic MT ends, where relaxation of the exposed GDP-tubulin subunits to their preferred "curved" state can generate protofilament bulbs (Mandelkowitz et al., 1991; Tran et al., 1997). Because XKCM1/

XKIF2 do not induce GMPCPP hydrolysis (Figure 2d), these kinesins must have the ability to change tubulin protofilament structure, independent of the nucleotide state of the tubulin. Extension of these results to dynamic MTs suggests that these kinesins induce catastrophe by physically disrupting end structure and not by stimulating GTP hydrolysis on tubulin subunits at polymerizing MT ends.

XKCM1/XKIF2 Target to and Induce a Destabilizing Conformational Change at Microtubule Ends in AMPPNP

To analyze the role of catalytic domain ATP hydrolysis in MT destabilization by XKCM1/XKIF2, we attempted to determine which of the following three steps of the destabilization reaction cycle could occur in the presence of AMPPNP: (1) targeting to MT ends; (2) induction of the destabilizing conformational change; and (3) recycling for multiple depolymerization cycles.

To test whether XKCM1 targeted to MT ends in AMPPNP, GMPCPP MTs were incubated in the presence of AMPPNP and XKCM1, fixed and sedimented onto coverslips, and stained with an anti-XKCM1 antibody to visualize the XKCM1 protein. Under these conditions, XKCM1 targeted to and accumulated at both ends of GMPCPP MTs (Figures 5a, 5b, and 5c; similar results were obtained with XKIF2). XKCM1 was present at both ends of 87% of the MTs, one end of 8%, and neither end of 4% ($n = 303$ MTs). The observed end localization represents an intermediate in the destabilization reaction cycle because it was also observed in GMPCPP MTs undergoing depolymerization in the presence of ATP (data not shown; in this case XKCM1 was present at both ends of 73% of the MTs, at one end of 11%, and neither end of 16%; $n = 153$ MTs). Green fluorescent protein (GFP)-K560 (Pierce et al., 1997) used in a similar assay did not show end targeting and accumulation but localized all along the MTs (data not shown). The unique ability of XKCM1/XKIF2 to target to both MT ends in the presence of AMPPNP strongly argues against a role for ATP-dependent motility in end targeting and distinguishes them further from motile kinesins.

To test whether ATP hydrolysis by Kin I kinesins was necessary to induce the conformational change seen at GMPCPP MT ends, we analyzed GMPCPP MTs by negative stain electron microscopy in the presence of AMPPNP and XKCM1/XKIF2. Under these conditions there is no significant depolymerization of the GMPCPP MTs (Figures 2a and 2b). Protofilament peeling was clearly evident in the presence of AMPPNP and XKCM1/XKIF2, resulting in large protofilament bulbs at both MT ends (Figure 5d). These results suggest that the lattice-destabilizing conformational change is derived from the binding energy of XKCM1/XKIF2 at MT ends and not from ATP hydrolysis.

Unlike GMPCPP MTs, taxol MTs do not exhibit end accumulation of XKCM1/XKIF2 or protofilament peels even though they undergo bipolar depolymerization (data not shown). We suspect this reflects differences in the interactions of tubulin dimers within the lattices of the two different stabilized MT substrates (Mickey and Howard, 1995). The strong immunofluorescence

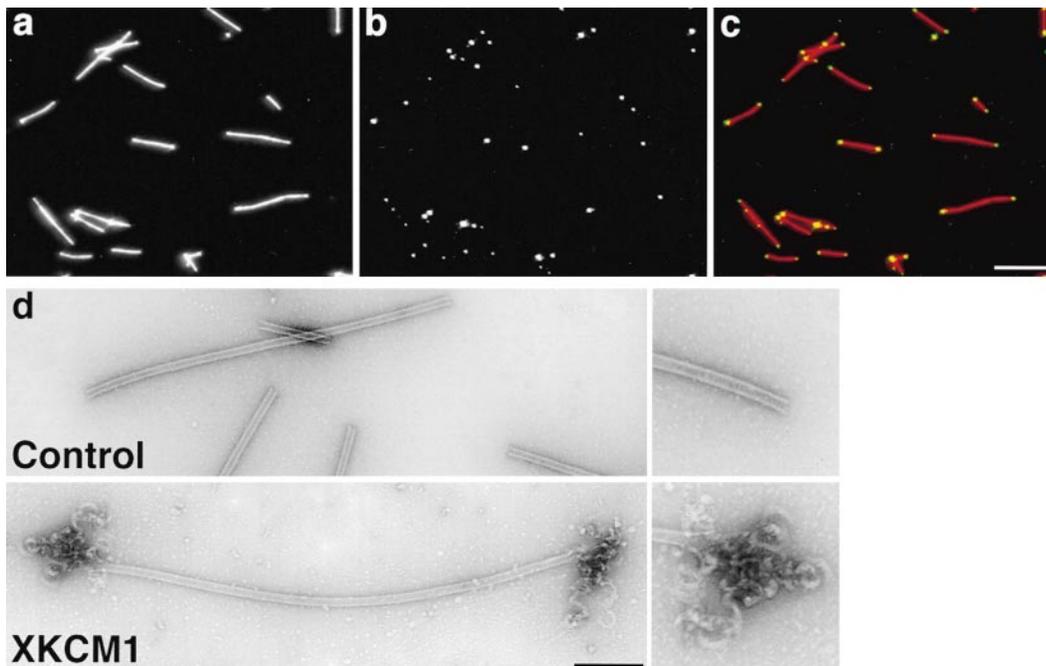


Figure 5. Targeting and Accumulation of XKCM1 at GMPCPP MT Ends in 5 mM MgAMPPNP

Panels show (a) GMPCPP MTs, (b) anti-XKCM1 immunofluorescence, and (c) a color overlay of a microscope field demonstrating that targeting and accumulation to both MT ends can occur in AMPPNP. Bar, 10 μ m. (d) Negative stain analysis under similar conditions revealed the presence of protofilament bulbs at both MT ends in reactions containing XKCM1. Right panels show a higher magnification view. Similar results are obtained with XKIF2 in both assays and in reactions performed in the presence of apyrase and AMPPNP. Bar, 200 nm for left panels.

signal at GMPCPP MT ends presumably reflects accumulation of XKCM1/XKIF2 along protofilament peels generated as a consequence of strong longitudinal tubulin dimer interactions. In taxol-stabilized MTs, weaker longitudinal interactions would inhibit formation of the peels and instead result in release of XKCM1/XKIF2-bound tubulin dimers from the ends. This hypothesis is supported by sedimentation analysis in AMPPNP that indicates a greater soluble fraction of tubulin dimer and XKCM1/XKIF2 in reactions containing taxol MTs versus GMPCPP MTs (data not shown).

XKIF2 Forms a Nucleotide-Sensitive Complex with Tubulin Dimer

Because ATP hydrolysis is not required for MT end targeting or for induction of a conformational change at the MT end, we hypothesized that it is used to recycle XKCM1/XKIF2 by dissociating them from tubulin dimer. With dynamic MT substrates, disruption of end structure would presumably induce a catastrophe, releasing a small number of XKCM1/XKIF2-tubulin dimer complexes and a much larger number of free tubulin dimers as the unstable GDP-tubulin core of the MT depolymerizes. The XKCM1/XKIF2-tubulin dimer complex would need to dissociate to allow the XKCM1/XKIF2 to act again at a new MT end. This hypothesis predicts that an XKCM1/XKIF2-tubulin dimer complex should persist in the presence of AMPPNP but not ATP. To test this, we used gel filtration chromatography to analyze the interaction of either XKIF2 or K560 with tubulin dimer in the presence

of ATP or AMPPNP (Figure 6). Consistent with our hypothesis, XKIF2 formed a complex with tubulin dimer in AMPPNP but not in ATP. K560 used at identical concentrations did not interact with tubulin dimer in either AMPPNP or ATP, further distinguishing XKCM1/XKIF2 from motile kinesins. This result is also consistent with XKCM1/XKIF2 having an affinity for MT ends that may have binding sites similar to those present in tubulin dimers/oligomers but buried by subunit interactions in the MT lattice.

Discussion

Kin I Kinesins Are Microtubule-Destabilizing Enzymes

Seven of the ten Kin I subfamily kinesins identified to date fall into two categories based on alignment of catalytic domain sequences (The Kinesin Home Page; <http://www.blocks.fhcrc.org/~kinesin/>). The first category is exemplified by MCAK/XKCM1, kinesins implicated in cell division (Wordeman and Mitchison, 1995; Walczak et al., 1996; Maney et al., 1998). The second category is exemplified by mKIF2, a kinesin implicated in neuronal function (Noda et al., 1995; Morfini et al., 1997). XKCM1 and X/mKIF2 share a high degree of homology in their catalytic domains (75% identity) but are divergent in their N- and C-terminal domains (20%–25% identity in each domain). Despite differences in primary sequence and biological function, our results show that both XKCM1 and XKIF2 destabilize MTs *in vitro*, leading us to conclude that members of both major categories of

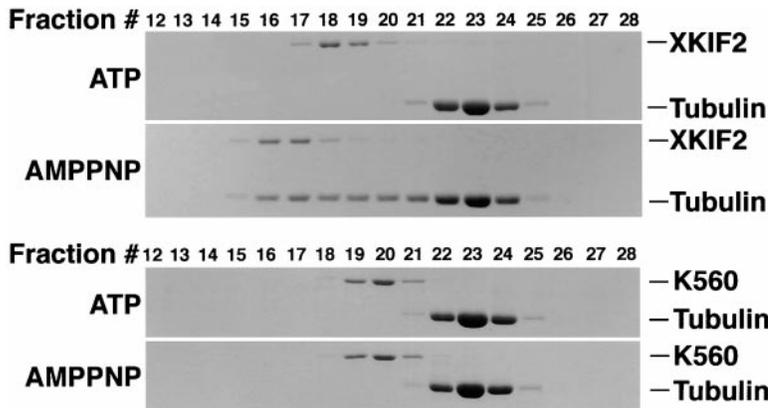


Figure 6. XKIF2 Forms a Nucleotide-Sensitive Complex with Tubulin Dimer

Mixtures of 2 μM XKIF2 or K560 and 10 μM tubulin dimer in buffer containing either ATP or AMPPNP were analyzed by gel filtration on a column equilibrated in buffer containing the same adenine nucleotide as the reaction. The elution volume of each protein of the two-protein mixture in ATP is identical to the elution volume of each protein analyzed individually in ATP. The observed shift for XKIF2 in the presence of tubulin dimer and AMPPNP represents a change in Stokes radius of 9 Å (from 69 Å to 78 Å).

Kin I kinesins are MT-destabilizing enzymes. The three more divergent members of the Kin I subfamily are DSK1, a diatom kinesin implicated in spindle elongation (Wein et al., 1996), and two kinesins identified by genomics, *C. elegans* 11d9 and *Leishmania* MCAK. It will be interesting to test whether these more divergent Kin I kinesins are also MT-destabilizing enzymes.

A Model for the Mechanism of Microtubule Destabilization by Kin I Kinesins

We propose a preliminary molecular mechanism for MT destabilization by Kin I kinesins (Figure 7). The key features of this mechanism are direct targeting of Kin I kinesins to MT ends, physical disruption of MT end structure, and utilization of catalytic domain ATP hydrolysis to dissociate a released complex of Kin I kinesins with tubulin dimer. Below we describe how our results support the key features of this model.

The targeting of Kin I kinesins directly to MT ends is supported by the equivalent depolymerization of GMPCPP MTs from both ends in the presence of ATP and by targeting and accumulation of XKCM1/XKIF2 at both ends of GMPCPP MTs in the presence of AMPPNP. These results suggest that Kin I kinesins have a special affinity for MT end structures, and they argue against models that invoke motility as a mechanism for targeting them to MT ends. The end structures recognized by Kin I kinesins are probably exposed protofilament edges at

polymerizing MT ends (Chretien et al., 1995; Desai and Mitchison, 1997). This idea is supported by our finding that Kin I kinesins can interact with tubulin dimer, which may share sites with exposed protofilament edges that are buried within the lattice of the MT.

Our model proposes that binding of Kin I kinesins to MT ends induces a destabilizing conformational change that results in a catastrophe. Physical disruption of end structure is supported by negative stain analysis of GMPCPP MTs undergoing XKCM1 catalyzed depolymerization. Biochemical analysis showed that this disruption occurs without GMPCPP hydrolysis. These results highlight the remarkable ability of Kin I kinesins to destabilize tubulin conformation without triggering nucleotide hydrolysis on β -tubulin. Because XKCM1/XKIF2 induce a destabilizing conformational change at MT ends in the presence of AMPPNP, the lattice-destabilizing conformational change must be derived from the binding energy of these kinesins at MT ends and not from ATP hydrolysis.

In the presence of AMPPNP, XKCM1/XKIF2 do not significantly depolymerize GMPCPP MTs despite targeting to their ends and inducing a destabilizing conformational change. Thus, we propose that the role of ATP hydrolysis by Kin I kinesins is to make their action catalytic by dissociating a released complex with tubulin dimer. Consistent with this idea, gel filtration analysis provided evidence for a complex of XKIF2 with tubulin

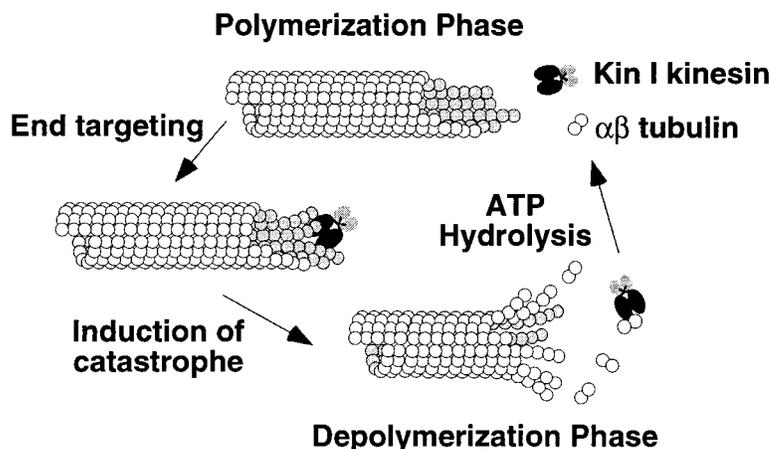


Figure 7. A Model for the Induction of MT Catastrophes by Kin I Kinesins

For simplicity, only one end of an MT is shown. Kin I kinesins target to MT ends during polymerization and disrupt end structure. A catastrophe occurs that induces MT depolymerization and release of a Kin I-tubulin dimer complex. ATP hydrolysis-dependent dissociation of a Kin I-tubulin dimer complex allows for repeated action. For simplicity, a 1:1 Kin I kinesin:tubulin dimer complex is depicted, and the tubulin GTPase cycle, nucleotide exchange on tubulin dimers and Kin I kinesins, and reverse reactions are not shown.

dimer in AMPPNP but not in ATP; such a complex was not observed with the motile K560 kinesin.

Relationship of Microtubule Destabilization by Kin I Kinesins to Potential Motor Activity

The key features of Kin I kinesins—unique affinity for MT ends, physical disruption of end structure, and use of catalytic domain ATPase activity to dissociate a complex with tubulin dimer—are also the features that strongly distinguish them from motile kinesins. Motile kinesins, such as K560 used in our assays, target to the outside surface along the length of the MT lattice, do not induce a destabilizing conformational change in the MT lattice, and use catalytic domain ATPase activity to power motility along the MT lattice. Our results also conclusively disprove the hypothesis that plus end motility targets XKCM1 to MT plus ends, where either XKCM1 itself or its cargo induces MT destabilization (Walczak et al., 1996; Waters and Salmon, 1996). In addition, the assays described here suggest that XKIF2 is not a plus end-directed MT motor. In contrast, fast plus end-directed motility was reported for mKIF2 (Noda et al., 1995). Because XKIF2 and mKIF2 are very similar in primary sequence (96% identity in the catalytic domain; 87% overall identity), we think it unlikely that the difference between our analysis of XKIF2 and the previously reported plus end motility of mKIF2 is species based. Resolving this discrepancy will require a reexamination of the reported motile activity of mKIF2.

Functional and Evolutionary Implications of Kin I Kinesins Being Microtubule-Destabilizing Enzymes

The robust MT-destabilizing activity of Kin I kinesins *in vitro* leads us to suspect that these kinesins are major regulators of MT dynamics inside cells. Consistent with this expectation, our previous characterization of XKCM1 in *Xenopus* extracts demonstrated it to be a major negative regulator of MT dynamics in egg cytoplasm (Walczak et al., 1996). A small fraction of XKCM1/MCAK also localizes to the centromere/kinetochore region where it presumably plays a role in chromosome movement. Deciphering this role will be aided by considering that XKCM1/MCAK may act exclusively as an MT-destabilizing enzyme and not as a motor protein (Maney et al., 1998). Similarly, analysis of the role of KIF2 in neuronal development will be aided by knowledge of its *in vitro* activity, and previous studies interpreting its function primarily as a motor protein may need to be reevaluated (Morfini et al., 1997).

Our findings also provide strong support for the hypothesis that regulation of MT dynamics is a fundamental intracellular activity of kinesins. Interestingly, KAR3 and KIP3, two of the six kinesins in the genome of the budding yeast *Saccharomyces cerevisiae*, have been genetically implicated in MT depolymerization (Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Saunders et al., 1997; Huyett et al., 1998), and a bacterially expressed fragment of KAR3 that exhibits minus end motility also slowly depolymerizes MT minus ends *in vitro* (Endow et al., 1994). Neither of these two kinesins belongs to the Kin I subfamily, indicating that MT destabilization may

be an activity selected for in other kinesins. The Kin I subfamily may represent an eventual specialization for exclusively performing the function of MT destabilization.

The ability of members of the kinesin superfamily to move toward either end of an MT (Vale and Fletterick, 1997; Hirokawa et al., 1998), to couple to the dynamic behaviour of MTs (Lombillo et al., 1995), and, as described here, to actively promote MT destabilization by a mechanism distinct from motility highlights the remarkable flexibility inherent in the design of kinesins. A chimera analysis of the relationship between the mechanisms of motility and destabilization, similar to that employed for opposite polarity kinesins (Case et al., 1997; Henningsen and Schliwa, 1997), will be important for exploring this remarkably flexible design. We speculate that coupling to or regulation of polymer dynamics may be evolutionary precedents to motor activity. Dynamic polymers most likely preceded the motor proteins that utilized these polymers as substrates, and coupling to or regulation of the dynamics of the polymers may be the function initially selected for that later evolved into motility.

Future Directions

Future insight into the reaction mechanism proposed here will come from many avenues, including determination of whether Kin I dimerization is required for the destabilization activity, identification of the minimal domain capable of carrying out the destabilization reaction, characterization of the ATPase activity of the catalytic domain, and further analysis of the Kin I-tubulin dimer complex. Elucidation of the detailed coupling of the ATPase cycle to MT destabilization, determination of the strength of coupling of binding to an MT end to the induction of catastrophe, and analysis of the relationship between mechanisms of motility and destabilization are more distant future challenges. The assays developed here will also allow a systematic dissection of other Kin I kinesins, as well as kinesins not belonging to this subfamily but implicated in MT destabilization, such as KIP3 and KAR3 from budding yeast. The potency of Kin I kinesins as MT destabilizers *in vitro* suggests their activity will be regulated *in vivo* either directly and/or by regulation of the susceptibility of the MT substrate to their action. Thus, *in vitro* analysis of the effect of Kin I kinesins on complex substrates, such as MTs coated with single or different MT associated proteins (MAPs) will also be important. Perhaps the most interesting future endeavor will be analysis of the biological functions of Kin I kinesins in light of their remarkable *in vitro* activity.

Experimental Procedures

Expression and Purification of Recombinant Proteins

Baculovirus expression was performed as described (Walczak et al., 1997) using either a full-length XKCM1 clone inserted into the BamHI to KpnI site of pVL1393N or a full-length XKIF2 clone inserted into the NotI to KpnI site of pVL1392N. Sf-9 cells (250 ml) expressing either XKCM1 or XKIF2 were pelleted, frozen in liquid nitrogen, and stored at -80°C . Frozen cell pellets were resuspended by vigorous pipeting in 25 ml of ice-cold lysis buffer (BRB80 + 125 mM KCl + 2 mM EGTA + 1 mM DTT + 0.5 mM MgATP + 1 mM PMSF +

10 $\mu\text{g/ml}$ leupeptin/pepstatin/chymostatin [LPC]; BRB80: 80 mM PIPES, 1 mM MgCl_2 , 1 mM EGTA [pH 6.8] with KOH). The lysate was cleared by centrifugation, loaded onto a HiTrap SP Sepharose column (Pharmacia), and eluted with an increasing KCl gradient. The peak of XKCM1/XKIF2 from the HiTrap SP column (assayed by SDS-PAGE) was loaded onto a Superose 6 gel filtration column (Pharmacia) equilibrated in BRB80 + 75 mM KCl + 1 mM DTT + 10 μM MgATP + 1 $\mu\text{g/ml}$ LPC. The peak of full-length XKCM1/XKIF2 was loaded onto a 1 ml Mono S column (Pharmacia) equilibrated in BRB80 + 75 mM KCl + 1 mM DTT + 10 μM MgATP + 1 $\mu\text{g/ml}$ LPC and eluted with an increasing KCl gradient. K560 (Woehlke et al., 1997) was purified from bacteria by nickel affinity followed by Superose 6 gel filtration in BRB80 + 200 mM KCl + 1 mM DTT + 10 μM MgATP + 1 $\mu\text{g/ml}$ LPC. The final XKCM1/XKIF2/K560 fractions were supplemented with solid sucrose to 10% (w/v), aliquoted, frozen in liquid nitrogen, and stored at -80°C . Control buffers of ionic strength and composition identical to the purified protein fractions were used in control reactions, unless indicated otherwise. Protein concentration was determined colorimetrically using BSA as a standard (Bradford, 1976). All three proteins (XKCM1, XKIF2, and K560) are dimers as judged by sucrose density gradient and gel filtration analysis, and all reported concentrations are of the molar concentration of dimer in the reaction. For unknown reasons, X/mKIF2 migrate anomalously large on denaturing SDS-PAGE gels (apparent MW ~ 20 kDa larger than MW calculated from primary sequence).

General Microtubule Methods and Assays for Dynamic Microtubules

Purification of bovine brain/frog egg tubulin, labeling of bovine tubulin with fluorescent dyes, and polymerization of taxol/GMPCPP MTs from bovine tubulin was performed using standard procedures (Hyman et al., 1991). Preparation of [$\gamma\text{-}^{32}\text{P}$] GMPCPP and analysis of its hydrolysis were performed as described (Hyman et al., 1992). Fixed and real time assays on dynamic MTs nucleated off axonemes were performed as described (Mitchison and Kirschner, 1984; Walker et al., 1988) except that in the latter frog egg tubulin and double-stick tape flow cells were used, and the flow cell surface was blocked with 5 mg/ml BSA in BRB80 after adsorption of axonemes. For GMPCPP and taxol MT reactions, MT concentration (i.e., concentration of tubulin dimer in MT polymer) was determined from the A_{280} of an aliquot of sedimented and resuspended MTs diluted in BRB80 + 5 mM CaCl_2 and incubated at 0°C for 10 min to induce depolymerization (using ϵ -tubulin, 280 nm = 115,000 $\text{M}^{-1}\text{cm}^{-1}$).

Visual and Sedimentation Analysis of Stabilized Microtubule Depolymerization

For qualitative visual analysis of depolymerization of stabilized MTs, 40 nM XKCM1/XKIF2/K560 was mixed with 1.5 μM fluorescent GMPCPP/taxol MTs (1:9 rhodamine-labeled:unlabeled tubulin; $\sim 1:40$ molar ratio of XKCM1/XKIF2/K560:tubulin) in BRB80 + 70 mM KCl + 1 mM DTT + 1.5 mM MgATP/5 mM MgAMPPNP (+ 15 μM taxol for taxol MT reactions) and incubated at RT. At various intervals 1 μl of the reaction mix was squashed under an 18 \times 18 mm coverslip and viewed by fluorescence microscopy using a 60X, 1.4 NA Olympus objective mounted on a Nikon Optiphot-2 microscope. Sedimentation analysis of depolymerization of stabilized MTs was performed using SDS-PAGE of supernatants and pellets generated by centrifuging 80 μl reactions at 100,000 rpm for 5 min in a TLA100 rotor at 23°C . The pellet was resuspended in 80 μl BRB80 + 70 mM KCl + 5 mM CaCl_2 on ice for 10 min with vigorous pipeting. Equivalent amounts (20–25 μl) of the supernatant and pellet were analyzed by 10% SDS-PAGE followed by Coomassie staining.

Real Time Analysis of GMPCPP Microtubule Depolymerization

Segmented GMPCPP MTs were prepared by diluting bright GMPCPP seeds (1:2 rhodamine-labeled:unlabeled tubulin) into 1.5 μM dim GMPCPP-tubulin mix (1:11 rhodamine-labeled:unlabeled tubulin) and incubating at 37°C for 1–2 hr. Double-stick tape flow cell surfaces were coated with a very dilute partially pure fraction of XKIF2 and blocked with 5 mg/ml BSA in BRB80. This preparation binds MTs to the coverslip surface without significantly releasing,

moving, or depolymerizing them in the presence of ATP. Segmented GMPCPP MTs were allowed to bind to the flow cell surface and rinsed extensively with BRB80 + 1 mM DTT + 1.5 mM MgATP + 1X Oxygen Scavenging Mix (1X OSM: 200 $\mu\text{g/ml}$ glucose oxidase, 35 $\mu\text{g/ml}$ catalase, 4.5 mg/ml glucose, 0.5% 2-mercaptoethanol). XKCM1 (20 nM), preincubated for 10 min on ice with 30-fold molar excess of either anti-N-terminal XKCM1 antibody (Walczak et al., 1996) or an irrelevant rabbit IgG, was adjusted to BRB80 + 1 mM DTT + 1.5 mM MgATP + 1 mg/ml BSA + 1X OSM and introduced into the flow cell. The reaction was monitored using time lapse fluorescence microscopy with a 60X, 1.4 NA Nikon objective and a Princeton Instruments cooled CCD camera. After 10–15 min, ~ 200 nM K560 was introduced into the flow cell and the motility of the observed MTs recorded to retroactively and unambiguously determine their polarity.

Immunofluorescence Analysis and Negative Stain

Electron Microscopy

Reactions of GMPCPP MTs and XKCM1/XKIF2 identical to those described above were fixed using 10 vol of 1% glutaraldehyde in BRB80, sedimented onto coverslips as described (Evans et al., 1985), postfixed in methanol, rehydrated, blocked with BSA, and processed for immunofluorescence using an affinity-purified anti-C-terminal XKCM1 antibody raised against the carboxy-terminal 138 amino acids of XKCM1. An affinity-purified anti-N-terminal XKIF2 antibody was used to detect XKIF2. To obtain antibodies that would recognize their antigens after fixation with glutaraldehyde, the immunogens were briefly fixed with glutaraldehyde prior to injection into rabbits. AMPPNP reactions were fixed after incubation at RT for 15 min: ATP reactions were fixed at the midpoint of depolymerization gauged by qualitative visual analysis. Negative stain electron microscopy was performed by adsorbing 1–2 μl of a reaction mix to a glow-discharged, formvar-coated, carbon-coated 200-mesh copper EM grid for ~ 10 s and applying 20 μl of 0.5% aqueous uranyl acetate while simultaneously absorbing it using a Whatman #1 filter paper strip. The grid was thoroughly dried prior to viewing with a Philips EM400 electron microscope.

Analysis of Tubulin Dimer Binding

XKIF2/K560 (2 μM) and tubulin dimer (10 μM) were incubated in BRB80 + 75 mM KCl + 1 mM DTT + 50 μM GDP + 3 mM MgATP/3 mM MgAMPPNP for 15 min at RT and 20 μl was analyzed by gel filtration using a Superose 6 column on a Pharmacia Smart system. The column was equilibrated in BRB80 + 75 mM KCl + 20 μM GDP + 200 μM MgATP/MgAMPPNP. Column fractions were analyzed by 10% SDS-PAGE followed by Coomassie staining. The column was calibrated using the following standards (Stokes radii are indicated in brackets): chymotrypsinogen (20.9 Å), ovalbumin (30.5 Å), aldolase (48.1 Å), catalase (52.2 Å), ferritin (61.0 Å), and thyroglobulin (85.0 Å). Stokes radii were estimated from a Porath correlation generated using the elution volumes of the standards (Siegel and Monty, 1966).

Acknowledgments

A. D. dedicates this work to Christine Mirzayan. We thank Peg Coughlin and Mei Lie Wong for assistance with electron microscopy, D. Pierce for GFP-kinesin, J. Hartman for advice on ATPase assays, and Matt Welch, Karen Oegema, Aaron Straight, Tarun Kapoor, Bruce Schnapp, and Ted Salmon for comments on the manuscript. C. E. W. was supported by the USAMRMC Breast Cancer Research Program, T. J. M. by grants from the National Institutes of Health and the Human Frontier Science Project, and A. D. by a Howard Hughes Medical Institute predoctoral fellowship.

Received October 14, 1998; November 17, 1998.

References

Belmont, L.D., Hyman, A.A., Sawin, K.E., and Mitchison, T.J. (1990). Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. *Cell* 62, 579–589.

- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.
- Caplow, M., Ruhlen, R.L., and Shanks, J. (1994). The free energy for hydrolysis of a microtubule-bound nucleotide triphosphate is near zero: all of the free energy for hydrolysis is stored in the microtubule lattice. *J. Cell Biol.* **127**, 779–788.
- Case, R.B., Pierce, D.W., Hom-Booher, N., Hart, C.L., and Vale, R.D. (1997). The directional preference of kinesin motors is specified by an element outside of the motor catalytic domain. *Cell* **90**, 959–966.
- Cassimeris, L. (1993). Regulation of microtubule dynamic instability. *Cell Motil. Cytoskel.* **26**, 275–281.
- Chretien, D., Fuller, S.D., and Karsenti, E. (1995). Structure of growing microtubule ends: two-dimensional sheets close into tubes at variable rates. *J. Cell Biol.* **129**, 1311–1328.
- Cohn, S.A., Saxton, W.M., Lye, R.J., and Scholey, J.M. (1993). Analyzing microtubule motors in real time. *Methods Cell Biol.* **39**, 75–88.
- Cottingham, F.R., and Hoyt, M.A. (1997). Mitotic spindle positioning in *Saccharomyces cerevisiae* is accomplished by antagonistically acting microtubule motor proteins. *J. Cell Biol.* **138**, 1041–1053.
- Desai, A., and Mitchison, T.J. (1997). Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* **13**, 83–117.
- DeZwaan, T.M., Ellingson, E., Pellman, D., and Roof, D.M. (1997). Kinesin-related KIP3 of *Saccharomyces cerevisiae* is required for a distinct step in nuclear migration. *J. Cell Biol.* **138**, 1023–1040.
- 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 destabilizes microtubules preferentially at the minus ends. *EMBO J.* **13**, 2708–2713.
- Erickson, H.P., and O'Brien, E.T. (1992). Microtubule dynamic instability and GTP hydrolysis. *Annu. Rev. Biophys. Biomol. Struct.* **21**, 145–166.
- Evans, L., Mitchison, T., and Kirschner, M. (1985). Influence of the centrosome on the structure of nucleated microtubules. *J. Cell Biol.* **100**, 1185–1191.
- Hartman, J.J., Mahr, J., McNally, K., Okawa, K., Shimizu, T., Vale, R.D., and McNally, F. (1998). Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit. *Cell* **93**, 277–287.
- Henningsen, U., and Schliwa, M. (1997). Reversal in the direction of movement of a molecular motor. *Nature* **389**, 93–96.
- Hirokawa, N., Noda, Y., and Okada, Y. (1998). Kinesin and dynein superfamily proteins in organelle transport and cell division. *Curr. Opin. Cell Biol.* **10**, 60–73.
- Horwitz, S.B. (1994). Taxol (paclitaxel): mechanisms of action. *Ann. Oncol.* **5** (Suppl 6), S3–6.
- Huyett, A., Kahana, J., Silver, P., Zeng, X., and Saunders, W.S. (1998). The Kar3p and Kip2p motors function antagonistically at the spindle poles to influence cytoplasmic microtubule numbers. *J. Cell Sci.* **111**, 295–301.
- Hyman, A., Drechsel, D., Kellogg, D., Salser, S., Sawin, K., Steffen, P., Wordemann, L., and Mitchison, T. (1991). Preparation of modified tubulins. *Methods Enzymol.* **196**, 478–486.
- Hyman, A.A., Salser, S., Drechsel, D.N., Unwin, N., and Mitchison, T.J. (1992). Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMPCPP. *Mol. Biol. Cell* **3**, 1155–1167.
- Inoué, S., and Salmon, E.D. (1995). Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol. Biol. Cell* **6**, 1619–1640.
- Lombillo, V.A., Stewart, R.J., and McIntosh, J.R. (1995). Minus-end-directed motion of kinesin-coated microspheres driven by microtubule depolymerization. *Nature* **373**, 161–164.
- Mandelkow, E.M., Mandelkow, E., and Milligan, R.A. (1991). Microtubule dynamics and microtubule caps: a time-resolved cryo-electron microscopy study. *J. Cell Biol.* **114**, 977–991.
- 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.
- McNally, F.J. (1996). Modulation of microtubule dynamics during the cell cycle. *Curr. Opin. Cell Biol.* **8**, 23–29.
- McNally, F.J., and Vale, R.D. (1993). Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **75**, 419–429.
- Melki, R., Carlier, M.F., Pantaloni, D., and Timasheff, S.N. (1989). Cold depolymerization of microtubules to double rings: geometric stabilization of assemblies. *Biochemistry* **28**, 9143–9152.
- Mickey, B., and Howard, J. (1995). Rigidity of microtubules is increased by stabilizing agents. *J. Cell Biol.* **130**, 909–917.
- Mitchison, T., and Kirschner, M. (1984). Dynamic instability of microtubule growth. *Nature* **312**, 237–242.
- Morfini, G., Quiroga, S., Rosa, A., Kosik, K., and Caceres, A. (1997). Suppression of KIF2 in PC12 cells alters the distribution of a growth cone nonsynaptic membrane receptor and inhibits neurite extension. *J. Cell Biol.* **138**, 657–669.
- Müller-Reichert, T., Chretien, D., Severin, F., and Hyman, A.A. (1998). Structural changes at microtubule ends accompanying GTP hydrolysis: information from a slowly hydrolyzable analogue of GTP, guanlyl (a,b) methylenediphosphonate. *Proc. Nat. Acad. Sci. USA* **95**, 3661–3666.
- Noda, Y., Sato-Yoshitake, R., Kondo, S., Nangaku, M., and Hirokawa, N. (1995). KIF2 is a new microtubule-based anterograde motor that transports membranous organelles distinct from those carried by kinesin heavy chain or KIF3A/B. *J. Cell Biol.* **129**, 157–167.
- Pierce, D.W., Hom-Booher, N., and Vale, R.D. (1997). Imaging individual green fluorescent proteins. *Nature* **388**, 338.
- Saunders, W., Hornack, D., Lengyel, V., and Deng, C. (1997). The *Saccharomyces cerevisiae* kinesin-related motor Kar3p acts at pre-anaphase spindle poles to limit the number and length of cytoplasmic microtubules. *J. Cell Biol.* **137**, 417–431.
- Siegel, L.M., and Monty, K.J. (1966). Determination of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation. *Biochem. Biophys. Acta* **112**, 346–362.
- Tran, P.T., Joshi, P., and Salmon, E.D. (1997). How tubulin subunits are lost from the shortening ends of microtubules. *J. Struct. Biol.* **118**, 107–118.
- 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., 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.
- Verde, F., Dogterom, M., Stelzer, E., Karsenti, E., and Leibler, S. (1992). Control of microtubule dynamics and length by cyclin A- and cyclin B-dependent kinases in *Xenopus* egg extracts. *J. Cell Biol.* **118**, 1097–1108.
- 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., Verma, S., and Mitchison, T.J. (1997). XCTK2: a kinesin-related protein that promotes mitotic spindle assembly in *Xenopus laevis* egg extracts. *J. Cell Biol.* **136**, 859–870.
- Walker, R.A., O'Brien, E.T., Pryer, N.K., Soboeiro, M.F., Voter, W.A., Erickson, H.P., and Salmon, E.D. (1988). Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J. Cell Biol.* **107**, 1437–1448.
- Waters, J.C., and Salmon, E.D. (1996). Cytoskeleton: a catastrophic kinesin. *Curr. Biol.* **6**, 361–363.
- 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.
- Woehlke, G., Ruby, A.K., Hart, C.L., Ly, B., Hom-Booher, N., and Vale, R.D. (1997). Microtubule interaction site of the kinesin motor. *Cell* **90**, 207–216.
- Wordeman, L., and Mitchison, T.J. (1995). Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *J. Cell Biol.* **128**, 95–104.