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Kin I Kinesins: Insights into the Mechanism of Depolymerization

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ABSTRACT: Kin I kinesins are members of the diverse kinesin superfamily of molecular motors. Whereas most kinesins use ATP to move along microtubules, Kin I kinesins depolymerize microtubules rather than walk along them. Functionally, this distinct subfamily of kinesins is important in regulating cellular microtubule dynamics and plays a crucial role in spindle assembly and chromosome segregation. The molecular mechanism of Kin I-induced microtubule destabilization is as yet unclear. It is generally believed that Kin Is induce a structural change on the microtubule that leads to microtubule destabilization. Recently, much progress has been made towards understanding how Kin Is may cause this structural change, and how ATPase activity is employed in the catalytic cycle.

KEYWORDS: Microtubule dynamics, mitosis, molecular motor proteins

INTRODUCTION

Motor proteins encompass a large variety of proteins that use the hydrolysis of ATP to produce force. These proteins are responsible for a number of cellular functions. In particular, the kinesin superfamily is a class of motor proteins that use ATP to walk along microtubules (MTs) or to alter the MT structure. Kinesins are involved in diverse functions such as intracellular transport of organelles and mRNA, and the assembly of a bipolar spindle for proper chromosome segregation.

Members of the kinesin superfamily share homology within their catalytic cores, which contain the MT and nucleotide binding sites. Kinesin-related proteins can be divided into three main subfamilies (Kin N, Kin I, and Kin C) based on the placement of the catalytic core (N-terminus, Internal, or C-terminus) (Figure 1a). In the simplest analysis, the location of the catalytic domain has proven important for understanding the actions of kinesin motors. Many Kin N and Kin C kinesins demonstrate MT-based motility (Vale and Fletterick, 1997), whereas the Kin I kinesins depolymerize MTs and influence MT polymer dynamics (Walczak *et al.*, 1996; Desai *et al.*, 1999; Maney *et al.*, 2001; Kline-Smith and Walczak, 2002; Moores *et al.*, 2002; Niederstrasser *et al.*, 2002; Ovechkina *et al.* 2002; Homma *et al.*, 2003; Hunter *et al.*, 2003).

In addition to the catalytic core, several other domains have been identified that are important for the cellular functions of kinesins. All kinesin-related proteins contain a class-specific neck (Vale and Fletterick, 1997) that has been implicated in either the direction of motility on the MT (Case *et al.*, 1997; Henningsen and Schliwa, 1997; Endow and Waligora, 1998) or efficient MT depolymerization (Ovechkina *et al.*, 2002). In the case of Kin N kinesins such as conventional kinesin, this neck lies toward the C-terminal side of the catalytic core. In contrast, the placement of this neck in Kin C and Kin I kinesins is N-terminal to the catalytic

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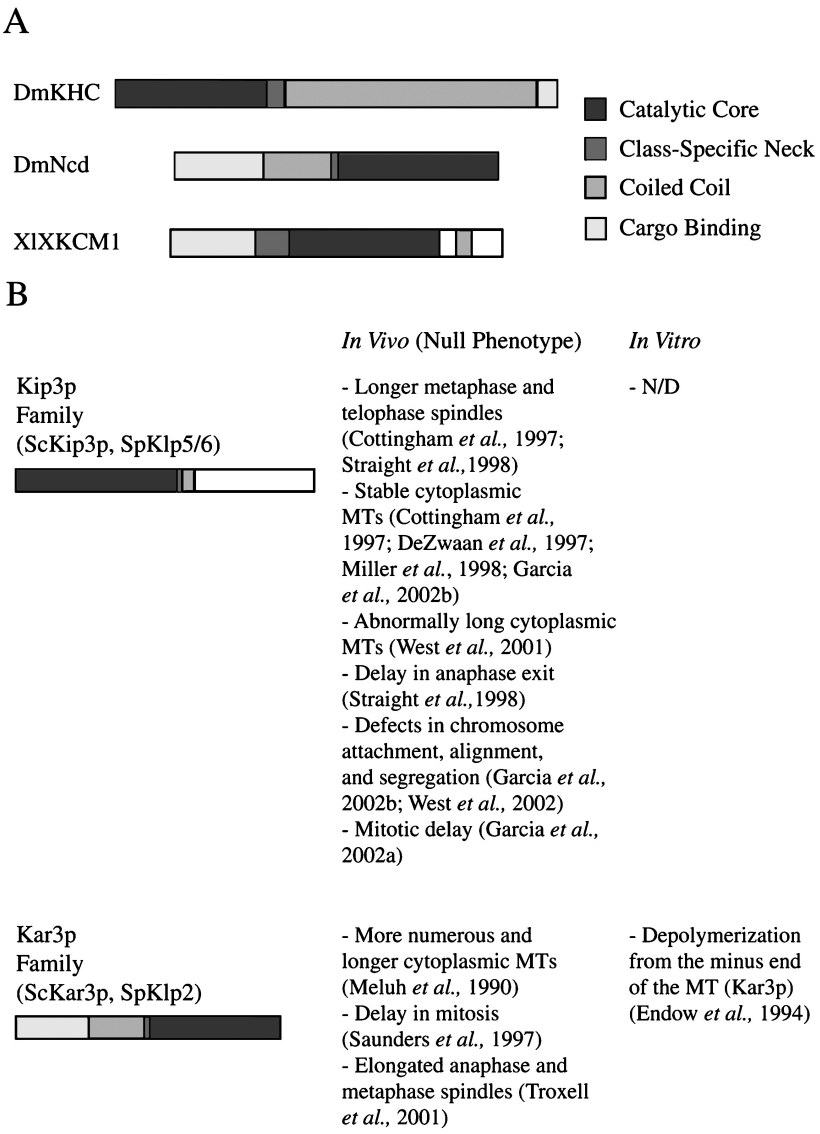


FIGURE 1. Kinesin superfamily members. (a). The kinesin superfamily can be divided into three main subfamilies that are related through the homology found in the catalytic core: Kin N, Kin I, and Kin C. This classification is based on the position of the kinesin-like catalytic domain. In general, Kin N motors (DmKHC) walk towards the plus end of the MT, Kin C kinesins (DmNcd) walk towards the minus end, and Kin I kinesins (XlXKCM1) depolymerize MTs. Other important domains include the class-specific neck which is positioned either N-terminally or C-terminally to the catalytic domain, and a region which mediates binding accessory proteins, cargo, or other interacting proteins. In addition, many kinesins contain coiled coil regions that are responsible for oligomerization. The first two letters of the protein name refer to the species name: Dm, *Drosophila melanogaster*; Xl, *Xenopus laevis*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*. (b) Several Kin N and Kin C motors have been identified that are proposed to destabilize MTs. Although there is very little biochemical data, null mutant phenotypes in *S. cerevisiae* and *S. pombe* indicate that these motors affect MT polymer levels, dynamics, and spindle morphology. (Figure appears in color online at www.crbmb.com)

domain (Figure 1a). The length and sequence of the class-specific neck regions vary, but it is generally longer in Kin I kinesins than in Kin N or Kin C kinesins. In addition to the class-specific neck, coiled coil structures outside of the catalytic domain are responsible for the dimerization of conventional kinesin (Yang *et al.*, 1989; de Cuevas *et al.*, 1992). Similar coiled coil regions are found in many

other kinesin superfamily members and mediate dimerization as well, but within the superfamily there is a wide variation of predicted or known quaternary structures. Furthermore, most kinesins also contain a cargo-binding domain responsible for such tasks as mediating interactions with cellular freight, additional MT binding to allow MT crosslinking, or targeting to subcellular structures (Vale and Fletterick, 1997) (Figure 1a).

MTs are inherently dynamic polymers consisting of α- and β-tubulin heterodimers that serve as tracks for many important cellular motors, form the mitotic spindle, and are important for the integrity and function of cilia and flagella. These polymers are often described as being polar in nature due to the orientation of the heterodimer subunit. The minus end of MTs refers to the exposure of the α-tubulin, whereas at the plus end, the β-tubulin is exposed. In addition, the polarity of the polymer indicates a difference in polymerization dynamics. The growth rate at the plus end of the MT is faster than that of the minus end. *In vivo,* MTs are oriented such that the minus ends are embedded within the centrosome and the plus ends extend towards the periphery of the cell (Desai and Mitchison, 1997). Pure tubulin can polymerize *in vitro*, but MTs are much more dynamic *in vivo*, where the polymer dynamics are influenced by many cellular factors (Andersen, 2000; Hunter and Wordeman, 2000), including Kin I kinesins such as mammalian MCAK and *Xenopus* XKCM1 (Maney *et al.*, 1998; Kline-Smith and Walczak, 2002; Homma *et al.*, 2003).

Traditionally, kinesins were thought to be molecular motors that used the energy of ATP hydrolysis to translocate along MTs, but the discovery of the Kin I kinesins as MT-destabilizing enzymes has added another dimension to the kinesin superfamily. The identification of Kin I kinesins as potential regulators of MT dynamics came from early studies in *Xenopus* egg extracts in which the depletion of XKCM1 resulted in long MTs and consequently the inability to form spindles (Walczak *et al.*, 1996). Since its initial functional discovery in extracts, several members of the Kin I family have been shown to influence cytoplasmic MT dynamics and polymer levels *in vitro* with purified proteins, in extracts and in cells (Walczak *et al.*, 1996; Maney *et al.*, 1998; Tournebize *et al.*, 2000; Kinoshita *et al.*, 2001; Kline-Smith and Walczak, 2002; Homma *et al.*, 2003). In addition, MCAK/XKCM1 functions specifically at centromeres, where it is required for proper chromosome congression and segregation (Wordeman and Mitchison, 1995; Maney *et al.*, 1998; Wordeman *et al.*, 1999; Walczak *et al.*, 2002).

Several other MT dynamics regulators known to destabilize MTs have been well characterized *in vitro* as well as *in vivo.* Op18 is a bifunctional protein that promotes catastrophes specifically from the plus end of the MT as well as sequesters tubulin heterodimers (Andersen, 2000). XMAP215/Stu2p, previously considered MT stabilizers, have also exhibited MT destabilization activity (Andersen, 2000; Shirasu-Hiza *et al.*, 2003; van Breugel *et al.*, 2003). Other proteins that appear to influence MT dynamics, such as the yeast Kip3p, Klp2, Klp5, Klp6, and Kar3p, have shown probable MT destabilizing activity *in vivo*, but the biochemical mechanisms are unknown (Meluh and Rose, 1990; Cottingham and Hoyt, 1997; DeZwaan *et al.*, 1997; Saunders *et al.*, 1997; Miller *et al.*, 1998; Straight *et al.*, 1998; Troxell *et al.*, 2001; West *et al.*, 2001, 2002; Garcia 2002a, 2002b). These proteins are kinesin superfamily members, but they are not Kin I kinesins. With the exception of Kar3p and Klp2 (which are Kar3p subfamily members), the other MT destabilizing kinesins belong to the Kip3p subfamily of Kin N kinesins (Figure 1b). It should be noted that the Kip3p subfamily members cluster nearest to the Kin I subfamily on the kinesin tree (Lawrence *et al.*, 2002). There is good evidence that these enzymes influence MT polymer levels *in vivo*, but they have not been shown to exhibit MT destabilization activity *in vitro*. Kar3p belongs to the Kin C subfamily of kinesins that walk toward the minus end of MTs (Figure 1b). The Kin C subfamily members are generally believed to be MT cross-linkers that are important for the assembly of the mitotic spindle (Chandra *et al.*, 1993b; Walczak *et al.*, 1997; Matuliene *et al.*, 1999; Mountain *et al.*, 1999; Ovechkina and Wordeman, 2003). A truncated Kar3p construct is the only member of this family shown to have destabilization activity *in vitro*, and this activity was seen in the context of minus-end directed motility in the presence of weakly stabilized MTs (Endow *et al.*, 1994). Of the MT destabilizing enzymes that have been studied, the most thoroughly characterized are the Kin I kinesins. In general, it is believed that members of the Kin I family bind to the end of a MT and cause a conformational change within the MT lattice that leads to a catastrophe event, which destabilizes the MT. The focus of this review will highlight the progress made recently towards understanding how Kin I kinesins depolymerize MTs rather than walk along them.

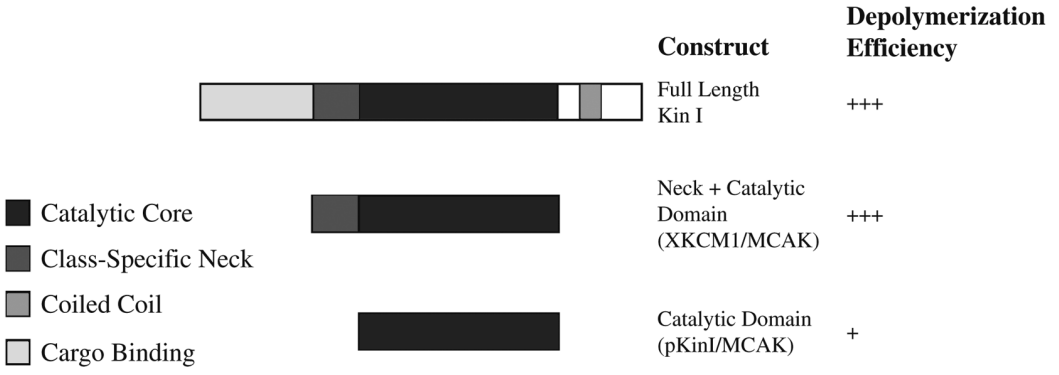
WHAT STRUCTURAL FEATURES OF KIN I KINESINS CORRELATE WITH DESTABILIZATION ACTIVITY?

Alignments of the amino acid sequences of the known Kin I kinesins reinforce the presence of conserved class-specific Kin I sequences within the N-terminal domain and the catalytic core. Interestingly, the proposed yeast MT destabilizers (Kip3p, Klp5, Klp6, Klp2, and Kar3p) do not share these conserved domains, whereas the newly identified *Drosophila* Klp10A and Klp59C and *Plasmodium* pKinI homologues have these regions in common with the founding members of the Kin I subfamily, MCAK and XKCM1. This suggests that the biochemical mechanism of Kin I kinesin MT destabilization is likely different from that of the Kip3p/Kar3p MT destabilization activity. Though some Kip3 proteins have been reported to be Kin I kinesins, our analysis suggests that they are not truly members of the Kin I family as judged by overall domain organization and by conservation of the class-specific Kin I sequences. However, given that the yeasts do not contain any kinesins that share these unique Kin I conserved domains, it is likely that the Kip3p, and perhaps Kar3p family play a functionally orthologous role to the Kin I kinesins.

The N-Terminal Neck

The class-specific neck plays a key role in the motility properties of Kin N and Kin C kinesins (Case *et al.*, 1997; Henningsen and Schliwa, 1997; Endow and Waligora, 1998). Similarly, the Kin I neck seems to be important for Kin I function. In cellular and *in vitro* studies of MCAK and XKCM1, the neck region N-terminal to the catalytic core was shown to be important for efficient MT depolymerization (Figure 2a). This is illustrated by the finding that full-length XKCM1, at enzyme concentrations substoichiometric to tubulin heterodimer concentration (1 to 200) (Desai *et al.*, 1999), destabilizes MTs much more efficiently than the catalytic core

A



B

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XlXKCM1    189 NQGRRKSNIVKEMEKMKNKREEQRAQNYERRMKRAQ--DYDTSVPNWEFGKMIKEFRATMDCHR
CgMCAK     186 NPARRKSCIVKEMEKMKNKREEKRAQNSEIRIKRAQ--EYDSSFPNWEFARMIKEFRVTLECHP
MmKIF2     123 PPSRRKSNCVKEVEKLQEKREKRRLQQQELREKRAQ--DVDATNPNYEIMCMIRDFRGSLDYRP
PfKinI       6 MQQKRQSFIKN---KVMDERKKKKKNNS-----------------------------------
DmKLP10A   202 GASTRRSHALKEVERLKENREKRRARQAEMKEEKVALMNQDPGNPNWETAQMIREYQSTLEFVP
DmKLP59C   110 APPVREDEVVHQAERMRKERERRREAQARTRLDREQGKNEDPGNPNWEVARMIRLQREQMESQR
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FIGURE 2. The Kin I kinesin neck is important for depolymerization. (a) Studies using truncations of Kin I kinesins have shown that although the catalytic core can destabilize MTs, Kin Is depolymerize MTs more efficiently when the positively charged neck is present. The C-terminal tail of Kin Is, which is responsible for the dimerization of the molecule, is not necessary for efficient depolymerization. (b) Class-specific neck sequences within the Kin I N-terminal domains. The N-terminal domains of the Kin I kinesins, yeast Kip3p family members and yeast Kar3p were aligned using the T-Coffee sequence alignment server (http://www.ch.embnet.org/software/Tcoffee.html) and then adjusted manually for maximum homology. Only the Kin I kinesins showed sequence homology. Regions with high sequence homology within the aligned sequences are noted in purple. This region represents the class-specific neck domain of Kin I kinesins. (Figure appears in color online at www.crbmb.com)

of pKinI at enzyme to tubulin ratios of 1 to 10 (Moores *et al.*, 2002). Similarly, in studies of the catalytic core of MCAK, the efficiency of depolymerization activity correlates with the presence of the lysine/arginine-rich region of the neck linker (Figure 2b) (Ovechkina *et al.*, 2002). The action of the neck may simply be a reflection of the charged nature of this region, as recent studies showed that the replacement of the neck of MCAK with either the K-loop of KIF1A or merely a lysine-rich sequence restores the depolymerizing activity of a neckless MCAK mutant (Ovechkina *et al.*, 2002). The positively charged K-loop of KIF1A interacts with the C-terminus of tubulin, and this electrostatic interaction helps KIF1A diffuse along MTs (Okada and Hirokawa, 2000). Interestingly, the neck of pKinI merely consists of a lysine-rich stretch of amino acids (Figure 2b), but the activity of a pKinI protein containing this neck or of the full-length protein has not been reported. The importance of the positively charged nature of the Kin I neck is further supported by the observation that reducing the salt concentration from 60 mM to 7 mM in MT depolymerization experiments caused a two-fold increase in the MT depolymerization activity of the MCAK catalytic core (Ovechkina *et al.*, 2002). These low salt conditions mimic those that were used to demonstrate that the catalytic core of pKinI depolymerizes MTs. It would be of interest to compare the MT destabilization activity of the catalytic core of pKinI with that of the catalytic core plus its positively charged neck. All of these experiments suggest that the catalytic core alone is sufficient for depolymerization, but that the neck region is required for the depolymerization activity to be similar to the full-length molecule.

Sequences Within the Catalytic Core

The finding that the catalytic core alone is sufficient for MT depolymerization suggests that there are unique sequences or sequence modifications within this region that mediate depolymerization activity. We identified three class-specific sequences within the catalytic core of Kin I kinesins, two of which were previously identified (Hunter and Wordeman, 2000; Niederstrasser *et al.*, 2002). The Kin I loop, a 13 amino acid, highly basic stretch within loop 2 of the catalytic core, is conserved and unique among the Kin I kinesins (Figure 3) (Niederstrasser *et al.*, 2002). To determine the three-dimensional location of this sequence, it was modeled onto the crystal structure of KIF1A interacting with a tubulin heterodimer (Kikkawa *et al.*, 2000; Niederstrasser *et al.*, 2002). One can see that the Kin I loop (Figure 4) could be in a position to interact with the C-terminus of tubulin (orange asterisk), and perhaps serve as an important structural element required to induce a conformational change at the end of the microtubule.

Other sequence changes of interest are those that occur in two of the MT binding regions. These changes lie within the analogous MT1 and MT3 regions of the catalytic core of KIF1A (Kikkawa *et al.*, 2000). Within the MT1 binding region there are several residues that are changed from the conventional kinesin hydrophilic residues to basic residues. This region, termed the Lys/Arg loop (Niederstrasser *et al.*, 2002), is the least well conserved sequence among the Kin I class-specific sequences within the catalytic core (Figures 3 and 4). In fact, the putative yeast destabilizers and KIF1A have weak sequence similarity in this region as well. These positively charged residues may also affect the interaction of Kin I kinesins with tubulin (Figure 4).

In contrast, the sequence changes within the MT3-binding region are much more strictly conserved. The MT3 binding region in KIF1A consists of helix 4, the K-loop, and helix 5 (Kikkawa *et al.*, 2000). Similar to the MT1 binding region, there is a change in the distribution of charged residues in helix 4 of MT3 when compared to conventional kinesin (Figures 3 and 4). Specifically, kinesin-heavy chain (KHC) residues G260, N261, V262, and S264 are changed to lysine, glutamic acid, cysteine, and arginine, respectively, in Kin I kinesins. This is of particular importance because helix 4 of KIF1A lies in the cleft between the tubulin monomers (Niederstrasser *et al.*, 2002) (Figure 4b), and upon ATP binding this helix moves in closer to the MT surface (Kikkawa *et al.*, 2001; Kull and Endow, 2002). The corresponding KIF1A residues that are modified and highly conserved in Kin I kinesins project toward this cleft at the intradimer interface (Figure 4b). Helix 4 may interact with residues of the tubulin dimer within this cleft and may be responsible for causing strain within the MT lattice.

In the future it will be interesting to determine if these unique Kin I sequences play an important role in destabilization activity. It will also be interesting to determine how the Kin I neck interacts with the catalytic core, or if it has some contact with the acidic C-terminus of tubulin.

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                      -4-                                 Kin I Loop                                                                                    -ATP 1-
XlXKCM1    263  ICVCVRKRP-LNKQELSKKEIDIISVPSKNIVLVHEPKLKVDLTKYL----ENQAFRFDFSFDET--------ATNEVVYRFTARPLVQSIFEGG-KATCFAYGQTGSGKTHTMGGDFSGKSQNVSKGVYAFAS
CgMCAK     253  ICVCVRKRP-LNKQELAKKEIDVISVPSKCLLFVHEPKLKVDLTKYL----ENQAFCFDFAFDET--------ASNEVVYRFTARPLVQTIFEGG-KATCFAYGQTGSGKTHTMGGDLSGKSQNTSKGIYAMAS
MmKif2     196  ICVCVRKRP-LNKKETQMKDLDVITIPSKDVVMVHEPKQKVDLTRYL----ENQTFRFDYAFDDS--------APNEMVYRFTARPLVETIFERG-MATCFAYGQTGSGKTHTMGGDFSGKNQDCSKGIYALAA
PfpKinI     68  IKVVVRKRP-LSELEKKKKDSDIITVKNNCTLYIDEPRYKVDMTKYI----ERHEFIVDKVFDDT--------VDNFTVYENTIKPLIIDLYENGCVCSCFAYGQTGSGKTYTMLGS-QPYGQSDTPGIFQYAA
DmKlp10A   277  ITVCVRKRP-ISRKEVNRKEIDVISVPRKDMLIVHEPRSKVDLTKFL----ENHKFRFDYAFNDT--------CDNAMVYKYTAKPLVKTIFEGG-MATCFAYGQTGSGKTHTMGGEFNGKVQDCKNGIYAMAA
DmKlp59C   188  IMVCVRKRP-LRRKELADREQDVVSIPSKHTLVVHEPRKHVNLVKFL----ENHSFRFDYVFDEE--------CSNATVYEFTARPLIKHIFDGG-MATCFAYGQTGSGKTYTMGGQFPGRHQSSMDGIYAMAA
ScKip3p     99  IFDPADRNP-LNKVSDQVLNSMRARATKATASSINNSNATNKFSSQRRRHGGEIKFVFDKLFDET--------SSQARVYKETTSPLLDSVLDGF-NSTVFAYGATGCGKTYTVSGTPS------QPGIIFLAM
SpoKlp5     51  VVKVLDGRV-LVFDPPDETTATLSATNRRLSTSQQSLARLSRKSNNSAGFGRDLRYAFDRVFDET--------ATQQQVYERTARPLLDNILDGF-NATIFAYGATGCGKTHTISGTM-----QDPGLIYLTL-
SpoKlp6     57  IVRVLDDNV-LIFDPPE---------------ENPLAKVQKSLLPAGKRFRDVRYAFDRLFGEE--------ASQEDVYKGTTEPLLDSVLQGY-NATVFAYGATGCGKTHTISGRP-----DDPGIIFLTMR
SpoKlp2    474  IRVFCRVRPPLGDGESAQIAFPDQNSEASTIEIVAQAPGSSLTGNGI----KQYAFNFDRVFSPE--------TTNEDVFNELSQ-LIQSAMDGY-NVCIFAYGQTGSGKTHTMSS---------NTGMIPSSV
MmKIF1A      6  VKVAVRVRP-FNSREMSRDSKCIIQMSGSTTTI-VNPKQPKETPK---------SFSFDYSYWSHTSPEDINYASQKQVYRDIGEEMLQHAFEGY-NVCIFAYGQTGAGKSYTMMGK----QEKDQQGIIPQLC
ScKar3p    387  IRVYCRIRPALKNLENSDTSLI--NVNEFDDNSGVQSMEVTKIQNTA----QVHEFKFDKIFDQQ--------DTNVDVFKEVGQ-LVQSSLDGY-NVCIFAYGQTGSGKTFTMLN--------PGDGIIPSTI
DmNcd      349  IRVFCRIRPPLESEENRMCCTWTYH----DESTVELQSIDAQAKSKM----GQQIFSFDQVFHPL--------SSQSDIFEMVSP-LIQSALDGY-NICIFAYGQTGSGKTYTMDGV------PESVGVIPRTV
HsKHC        9  IKVMCRFRP-LNESEVNRGDKYIAKFQGEDTVVIAS-----------------KPYAFDRVFQSS--------TSQEQVYNDCAKKIVKDVLEGY-NGTIFAYGQTSSGKTHTMEGKL---HDPEGMGIIPRIV

                                                                                     Lys/Arg Loop
                                                                                   -------MT 1-----------                          ---ATP 2--
XlXKCM1    383  RDVFLLLDQPRYKHLDLDVFVTFFEIYNGKVFDLLN----------KKTKLRVLEDAKQ-EVQVVGLLEKQVISADDVFKMIEIGSACRTSGQTFANTSSSRSHACLQIILRRGS------------KLHGKFS
CgMCAK     373  RDVFLLKSQPRYRNLNLEVYVTFFEIYNGKVFDLLN----------KKAKLRVLEDSKQ-QVQVVGLQEYLVNCADDVIKMLNMGSACRTSGQTFANSNSSRSHACFQILLRAKG------------RLHGKFS
MmKif2     316  RDVFLMLKKPNYKKLELQVYATFFEIYSGKVFDLLN----------RKTKLRVLEDGKQ-QVQVVGLQEREVKCVEDVLKLIDIGNSCRTSGQTSANAHSSRSHAVFQIILRRKG------------KLHGKFS
PfpKinI    187  GDIFTFLNI-YDKDNTKGIFISFYEIYCGKLYDLLQ----------KRKMVAALENGKK-EVVVKDLKILRVLTKEELILKMIDGVLLRKIGVNSQNDESSRSHAILNIDLKDINK----------NTSLGKIA
DmKlp10A   397  KDVFVTLNMPRYRAMNLVVSASFFEIYSGKVFDLLS----------DKQKLRVLEDGKQ-QVQVVGLTEKVVDGVEEVLKLIQHGNAARTSGQTSANSNSSRSHAVFQIVLRPQGS----------TKIHGKFS
DmKlp59C   308  KDVFSTLKTVPYNKLNLKVYCSFFEIYGTRVFDLLMP---------GKPQLRVLEDRNQ-QVQVVGLTQNPVQNTAEVLDLLELGNSVRTSGHTSANSKSSRSHAVFQIVLRSAAG----------EKLHGKFS
ScKip3p    216  EELFNKITDLK-DEKDFEISLSYLEIYNERIRDLLKP-------ETPSKRLVIREDTQN-HIKVANLSYHHPNTVEDVMDLVVQGNINRTTSPTEANEVSSRSHAVLQIHIMQTNKL----VDLTSQHTFATLS
SpoKlp5    168  KELFERMDHLR-DEKIFDLRLSYLEIYNETIRDLLVS-----PTPNQAKPLNLREDADR-RITVPGLTSLSPESLEEIIDIIMKGNANRTMSPTEANAASSRSHAVLQVTLIQKPRTA---G-INEDHTLATLS
SpoKlp6    158  ALLDRVEGLKR--TMNVDISVSYLEIYNEKIRDLLVQ---DPLSMEKPKSLNICEDAEQ-NVSVPGLSYFTPTNLEEVMEIIIRGNSNRTMSPTEANAVSSRSHAVLQIYITQTPKSGEESESQNSHKVRSVFS
SpoKlp2    585  RMIYNRSTSLKERGWEYRMEGQFLEIYNETIIDLLASGNEEE---KGKKKLEIYHDTKAGRTTITNITSEPLDTPEQVTWLLDQASKNRSVAATNANEHSSRSHSVFMLHLNGSNSTT---GE----TCRSTLN
MmKIF1A    124  EDLFSRINDTTNDNMSYSVEVSYMEIYCERVRDLLNP--------KNKGNLRVREHPLL-GPYVEDLSKLAVTSYNDIQDLMDSGNKPRTVAATNMNETSSRSHAVFNIIFTQKRHDA---ETNITTEKVSKIS
ScKar3p    497  SHIFNWINKLKTKGWDYKVNCEFIEIYNENIVDLLRSDNNNKEDTSIGLKHEIRHDQETKTTTITNVTSCKLESEEMVEIILKKANKLRSTASTASNEHSSRSHSIFIIHLSGSNAKT---GA----HSYGTLN
DmNcd      459  DLLFDSIRGYRNLGWEYEIKATFLEIYNEVLYDLLSNE-------QKDMEIRMAKNNKN-DIYVSNITEETVLDPNHLRHLMHTAKMNRATASTAGNERSSRSHAVTKLELIGRHAEK-----Q--EISVGSIN
HsKHC      112  QDIFNYIYSMD-ENLEFHIKVSYFEIYLDKIRDLLDV---------SKTNLSVHEDKNR-VPYVKGCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNEHSSRSHSIFLINVKQENTQT--EQ-----KLSGKLY

                                                                  Helix 4
                  -ATP 3-       ---MT 2--    -------------------------MT 3---------------------------------
XlXKCM1    494  LVDLAGNERGVDTASADRITRMEGAEINRSLL----------ALKECIRAL-----GQNKS----------HTPFRESKLTQILRDSFIGENSRTCMIAMLSPGFNSCEYTLNTLRY   Q91636
CgMCAK     484  LVDLAGNERGADTSSADRQTRMEGAEINKSLL----------ALKECIRAL-----GQNKA----------HTPFRESKLTQVLRDSFIGENSRTCMIAMISPGISSCEYTLNTLRY   AAB17358
MmKif2     427  LIDLAGNERGADTSSADRQTRLEGAEINKSLL----------RLKECIRAL-----GRNKP----------HTPFRASKLTQVLRDSFIGENSRTCMIATISPGMASCENTLNTLRY   P28740
PfpKinI    299  FIDLAGSERGADTVSQNKQTQTDGANINRSLL----------ALKECIRAM-----DSDKN----------HIPFRDSELTKVLRDIFVGK-SKSIMIANISPTISCCEQTLNTLRY   NP_701793
DmKlp10A   509  FIDLAGNERGVDTSSADRQTRMEGAEINKSLL----------ALKECIRAL-----GKQSA----------HLPFRVSKLTQVLRDSFIGEKSKTCMIAMISPGLSSCEHTLNTLRY   AAF48003
DmKlp59C   421  LIDLAGNERGADNSSADRQTRLEGSEINKSLL----------VLKECIRAL-----GRQSS----------HLPFRGSKLTQVLRDSFIGKKVKTCMIAMISPCLHSVEHTLNTLRY   AAF46959
ScKip3p    332  IIDLAGSERAAATRNRGIR-LHEGANINRSLL----------ALGNCINALCLND-GSRSC----------HIPYRDSKLTRLLKFSLGGN-CKTVMIVCISPSSSHYDETLNTLKY   P53086
SpoKlp5    287  IIDLAGSERATATKLRGSR-LFEGANINKSLL----------ALGNCINALCDP--HRRA-----------HVPYRDSKLTRLLKFSLGGN-CRTVMIVCVSPSSVHYEETHNTLKY   CAB10160
SpoKlp6    283  FIDLAGSERASATKNRGKR-LVEGANINRSLL----------ALGNCINSLCEP--RRRQ-----------HVPYRDSKLTRLLKFSLGGN-CRTCMIVCISPSSEHYDETHNTLKY   CAA19043
SpoKlp2    707  LIDLAGSERLSSSQSVGEP-FEGTQAINKVVLGDVYMLGGLSCLGDVIHAL-----GSGKEGT--------YIPYRNSKLTNLLQYS-LGGNSKTLIFVNISPLKQHVPETLCSLRF   CAB65811
MmKIF1A    240  LVDLAGSER-ADSTGAKGTRLKEGANINKSLT----------TLGKVISALAEMDSGPNKNKKKKKTDKTDFIPYRDSVLTWLLREN-LGGNSRTAMVAALSPADINYDETLSTLRY   P33173
ScKar3p    622  LVDLAGSERINVSQVVGDR-LRETQNINKSLS----------CLGDVIHAL-----GQPDSTKR-------HIPFRNSKLTYLLQYS-LTGDSKTLMFVNISPSSSHINETLNSLRF   P17119
DmNcd      576  LVDLAGSES-PKTS----TRMTETKNINRSLS----------ELTNVILAL-----LQKQD----------HIPYRNSKLTHLLMPS-LGGNSKTLMFINVSPFQDCFQESVKSLRF   P20480
HsKHC      227  LVDLAGSEK-VSKTGAEGAVLDEAKNINKSLS----------ALGNVISALAE---GST------------YVPYRDSKMTRILQDS-LGGNCRTTIVICCSPSSYNESETKSTLLF   P33176
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FIGURE 3. Sequence alignment of the catalytic core of the known and putative kinesin MT destabilizers. The conserved kinesin superfamily motifs are represented per the Kinesin Home Page (http://www.proweb.org/kinesin/KinesinAlign.html) and are depicted in blue text. The four ATP binding sites and the three MT binding sites of KIF1A are highlighted above the amino acid sequences (Kikkawa *et al.*, 2000). The unique KIF1A K-loop is represented in purple text. The highly conserved Kin I loop and helix 4 residues are highlighted in red and green text, respectively. The lysine and arginine residues of the lysine/arginine loop (Niederstrasser *et al.*, 2002) are highlighted in yellow text. The GenBank protein accession numbers are indicated to the right of the sequences. (Figure appears in color online at www.crbmb.com)

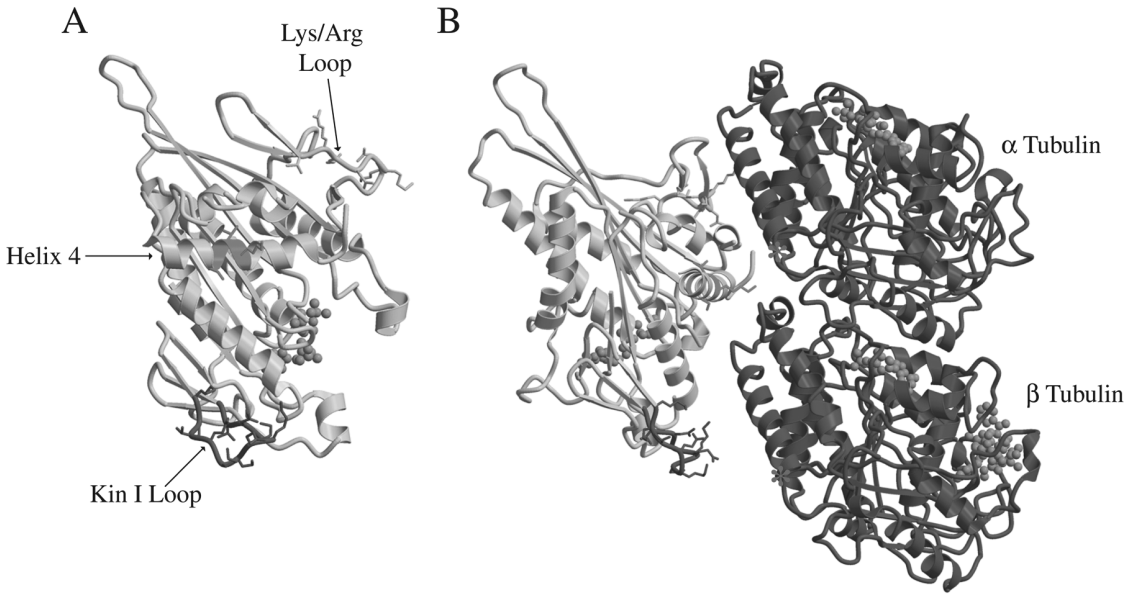

FIGURE 4. Three-dimensional-model of conserved Kin I class-specific sequences. The relative positions of the conserved amino acids found in Kin I kinesins are indicated on the crystal structure of KIF1A (Kikkawa *et al.*, 2000) using MolScript. (a) Ribbon view of the KIF1A 3D structure. The nucleotide is shown in gray, modeled as a ball and stick figure. (b) Ribbon diagram of KIF1A docked onto the tubulin heterodimer (gray). KIF1A is turned 90° with respect to KIF1A in (a). The nucleotides and paclitaxel molecule are represented as gray ball and stick figures. The MT axis runs vertically, with the polarity as indicated. The placement of KIF1A with respect to the interdimer or intradimer interface is arbitrary. (Figure appears in color online at www.crbmb.com)

Domains Responsible for Dimerization

Physiologically, Kin I kinesins are homodimeric proteins (Walczak *et al.*, 1996; Maney *et al.*, 2001). It is clear that constructs of MCAK and XKCM1, which lack the C-terminus, are monomeric, suggesting that the C-terminus is important in dimerization (Maney *et al.*, 2001; our unpublished data). However, deletion studies of MCAK analyzed by the yeast two-hybrid system have indicated that dimerization of Kin Is may also be mediated through domains N-terminal to the catalytic core (Maney *et al.*, 2001). The dimerization of MCAK may be mediated in part by coiled coil interactions. Short (30–40 amino acids), putative coiled coil domains have been identified in the both the N-terminus and C-terminus of XKCM1 and MCAK using the COILS algorithm (www.us.expasy.org). However, only the C-terminal putative coiled coil is identified using PARCOIL (www.us.expasy.org). In contrast, the strong coiled coil region of DmKHC is identified in both programs. We have found that although the C-terminus of XKCM1 is sufficient for dimerization as judged by yeast two-hybrid interactions and hydrodynamic analysis (our unpublished data), the C-terminal putative coiled coil is not sufficient to mediate this interaction. This suggests that regions in addition to the coiled coil must mediate the dimer interaction. Physiologically, it is clear that Kin Is function as homodimers within the cell, but what regions mediate the interaction and the purpose of dimerization remain to be determined.

Cellular assays that measure the destabilization activity of truncated versions of MCAK or XKCM1 show that deleting the C-terminus of the protein does not abolish MT destabilization activity, indicating that dimerization of the molecule is not necessary (Maney *et al.*, 2001; our unpublished data). Indeed, a monomer construct of MCAK or XKCM1 can depolymerize MTs in a manner comparable to the full-length dimerized molecule when assayed *in vitro* (Maney *et al.*, 2001; Ovechkina *et al.*, 2002; our unpublished data). Why are Kin I kinesins dimers, and what can a monomeric Kin I tell us about the mechanism of depolymerization? Mechanistic studies of conventional kinesin have shown that a monomeric kinesin molecule walks along MTs, but it is not processive (Berliner *et al.*, 1995; Inoue *et al.*, 1997; Hancock and Howard, 1998). In order for conventional kinesin to walk processively, it must retain its coiled coil region;

thus it must be a dimer to be processive (Hancock and Howard, 1998). In addition to being necessary for processivity, the C-terminus of kinesin has been shown to inhibit the ATPase activity of the protein (Coy *et al.*, 1999a). It is possible that the C-terminus of the Kin I kinesins may play a similar role. The enzymatic activity of monomeric versus dimeric Kin I kinesins has not been studied, so it is possible that dimerization leads to more efficient depolymerization. In addition, in cellular destabilization assays, it appears that deletion of the C-terminus of MCAK (Maney *et al.*, 2001) or XKCM1 (our unpublished data) results in increased destabilization activity. To date, neither we nor others have been able to reconstitute this inhibition *in vitro*.

WHAT FEATURES OF THE MT ARE RECOGNIZED BY KIN I KINESINS?

Structurally, the interaction between Kin I kinesins and the MT is likely to be different than that of conventional kinesin. It is possible that through the class-specific sequences, Kin I kinesins recognize unique structural features of the MT that allow it to bind to the end of the MT and induce its depolymerization. As an approach towards determining what features of the MT are key in the depolymerization activity of Kin I kinesins, researchers have asked pointed questions to elucidate these structural features.

Do Kin I Kinesins Act on a Single Protofilament?

MTs are hollow tubes consisting of ~13 parallel protofilaments that maintain longitudinal and lateral contacts for stability. Thus, one could imagine two ways in which Kin I kinesins could destabilize MTs: Kin I kinesins could push two adjacent protofilaments apart by disrupting lateral contacts between protofilaments (Figure 5a) or they could peel a single protofilament away from the

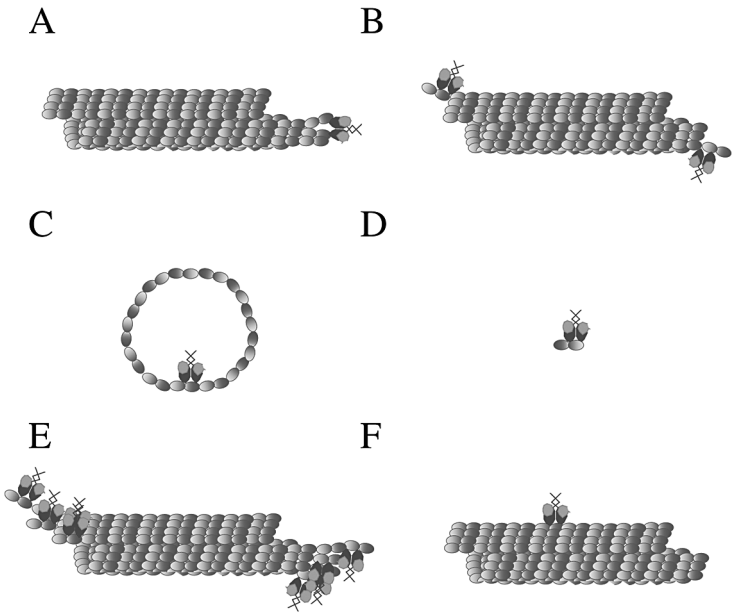

FIGURE 5. Kin I kinesins bind to a variety of MT substrates. Kin I kinesins do not appear to bind to and push apart more than one protofilament (a) in order to depolymerize MTs, as indicated by biochemical assays using macrotubes with antiparallel protofilaments as substrates. However, Kin Is can bind to both plus and minus ends of the MT (b) and can depolymerize from either end, indicating no distinct preference for MT polarity. Kin Is can also bind to and stabilize tubulin rings (c), indicating a possible reaction intermediate. Kin Is can bind to tubulin heterodimer in the presence of AMPPNP, and this binding may reflect a reaction intermediate during the catalytic cycle (d). Kin I kinesins also exhibit cooperativity during the depolymerization of a MT (e). Several Kin Is are present at the ends of a MT at the stoichiometry of approximately one Kin I per protofilament. Kin I kinesins have some affinity for the MT lattice (f), but it is unclear whether this lattice binding is of physiological significance. (Figure appears in color online at www.crbmb.com)

MT by breaking longitudinal tubulin dimer contacts (Figure 5b). In order to avoid the added complication of the inherent dynamic instability of pure tubulin, MT stabilizing agents, such as paclitaxel or the slowly hydolyzable GTP analogue GMPCPP, are often used in assays for MT-destabilizing kinesins. It has been noted that the flexular rigidity of GMPCPP-stabilized MTs is nearly twice that of taxol-stabilized MTs, indicating these two agents are not necessarily interchangeable when elucidating the mechanism of destabilization (Mickey and Howard, 1995). It is well established that Kin I kinesins can readily depolymerize either paclitaxel- or GMPCPP-stabilized MTs (Desai *et al.*, 1999; Maney *et al.*, 2001; Moores *et al.*, 2002; Ovechkina *et al.*, 2002; Homma *et al.*, 2003; Hunter *et al.*, 2003), suggesting that there is no preference for differently stabilized MT structures. Interestingly, we have found that Kin I kinesins can weakly destabilize doubly stabilized (paclitaxel and GMPCPP) MTs as well, albeit less efficiently than singly stabilized MTs. Perhaps a detailed comparison of these activities would provide further mechanistic insight.

It has recently been demonstrated that XKCM1 can depolymerize zinc-induced tubulin macrotubes (Niederstrasser *et al.*, 2002). These polymers are 200–500 nm in diameter, 8–20 times the diameter of a microtubule, and consist of antiparallel protofilaments rather than parallel protofilaments seen in microtubules. These data show that Kin I kinesins act on a single protofilament and are unlikely to push apart adjacent protofilaments as a mechanism for MT destabilization (Figures 5a and 5b). The fact that monomeric Kin I kinesins can depolymerize MTs also supports this finding (Maney *et al.*, 2001).

Do Kin I Kinesins Have Preference for a Particular End of the MT?

XKCM1 has been localized to both the minus end as well as the plus end of MTs (Desai *et al.*, 1999). This suggests that the Kin I kinesins must recognize some unique structural feature that is exposed only at the ends of the MT (Figure 5b). It is unlikely for the binding to be end-on because the two ends of the MT are structurally different: α-tubulin is exposed at the minus end, whereas β-tubulin is exposed at the plus end. In addition, Kin I kinesins can depolymerize MTs from both ends (Desai *et al.*, 1999; Hunter *et al.*, 2003). Recently, however, MCAK has been shown to depolymerize preferentially from one end based on time-lapse analysis of MT depolymerization *in vitro*. Given that the MT polarity in these samples was not known, it is possible that the apparent end preference may simply be a reflection of the difference in depolymerization rates of a faster and slower end (Hunter et al., 2003). It will be interesting to determine whether the faster depolymerizing end does indeed correlate with either the plus or minus end of the MT, as this may provide further insight into the mechanism of depolymerization.

Do Kin I Kinesins Bind to the Inside or the Outside of the MT?

Previous experiments have illustrated that XKCM1 induces protofilament curls, which curve away from the ends of GMPCPP-MTs (Desai *et al.*, 1999), suggesting that the localization of XKCM1 is on the external surface of the MT. In agreement with these results, XKCM1 in the presence of AMPPNP can stabilize GDP-tubulin ring structures and has been visualized bound to these structures (Figure 5c) (Niederstrasser *et al.*, 2002). Unfortunately, due to incompatible buffer conditions, we do not yet know if XKCM1 can depolymerize tubulin rings. Additionally, the pKinI motor has been seen binding to the inner surface of tubulin rings (Moores *et al.*, 2002). Together, these data strongly suggest that Kin I kinesins only bind to the outside of the MT.

Do Kin I Kinesins Require the C-Terminus of Tubulin for Destabilization Activity?

The C-terminus of tubulin is necessary for binding of many MT-associated proteins, as well as for the processivity of conventional kinesin (Okada and Hirokawa, 2000; Thorn *et al.*, 2000; Wang and Sheetz, 2000; Cassimeris and Spittle, 2001). The β-tubulin C-terminus is also important for the depolymerization of MTs by Kin I kinesins, as demonstrated by the inability of Kin I kinesins to destabilize subtilisin-treated MTs (Moores *et al.*, 2002; Niederstrasser *et al.*, 2002). Though Kin I kinesins appear to need the C-terminus of tubulin to depolymerize MTs, this region is not necessary for binding to the MT, as it has been shown that Kin I kinesins still pellet with subtilisin-MTs (Niederstrasser *et al.*, 2002). However, the affinity of this interaction has not been determined. Data showing that the positively charged neck as well as the negatively charged tubulin C-terminus are important for depolymerization suggest an interaction such as the KIF1A K-loop interaction with the C-terminus of tubulin

(Okada and Hirokawa, 2000). The C-terminus of tubulin has also been implicated in the stability of the MT polymer. It has been proposed that loss of the C-terminus creates a more stable MT and facilitates polymer formation (White *et al.*, 1987; Mejillano and Himes, 1991; Sackett, 1995); thus it will be important to distinguish whether or not the C-terminus of β-tubulin interacts with Kin I kinesins or if its loss creates a polymer too stable for adequate conformational change necessary to destabilize the polymer.

Do Kin I Kinesins Remain Bound to the MT During Depolymerization?

In addition to binding to MTs, Kin I kinesins are proposed to interact with tubulin as it is released from the MT after the induction of a catastrophe event (Figure 5d). Evidence that Kin I kinesins bind to tubulin comes from gel filtration experiments with KIF2, which show that the enzyme binds to tubulin in the presence of AMPPNP but not in ATP (Desai *et al.*, 1999). In addition, MCAK and XKCM1 exhibit tubulin-stimulated ATPase activity, suggesting a role for tubulin interaction during the catalytic cycle of the enzyme (Hunter *et al.*, 2003; our unpublished data). Additional studies are required to fully understand this aspect of the biochemical mechanism.

What Is the Extent of the Preference of Kin I Kinesins for MT Ends?

Kin I kinesins exhibit specific targeting to both ends of the MT at levels substoichiometic to tubulin when assayed *in vitro* by immunofluorescence microscopy. This end targeting is clearly of functional importance. Hunter *et al.* (2003) have shown that the rate of depolymerization saturates with a very low ratio of Kin I:tubulin, suggesting that depolymerization activity occurs only at the ends of MTs. They have estimated the number of MCAK molecules bound to the end of the MT and determined that one MCAK is bound per protofilament. This suggests that multiple MCAK molecules bind to the end of a MT in order to initiate a depolymerization event, although this has not yet been determined experimentally (Figure 5e). Though Kin I kinesins bind with increased affinity to MT ends, they do retain the ability to bind to the MT lattice (Figure 5f). This property is demonstrated by the decoration of MTs by the catalytic core of pKinI as well as the binding of XKCM1 to GDP-tubulin rings (Moores *et al.*, 2002; Niederstrasser *et al.*, 2002). Lattice binding has also been seen *in vivo* when GFP-MCAK is overexpressed in CHO cells and then assayed for rigor binding by fluorescence microscopy of permeabilized cells (Maney *et al.*, 1998; Wordeman, 1999). Given the high sequence conservation between the Kin I kinesins and other members of the kinesin superfamily, it is perhaps not surprising that the Kin I kinesins retain the ability to bind to the lattice. Together, these data indicate that the binding site for Kin I kinesins on the MT lattice or the MT end are different, and that certain structural features that vary in these regions may be responsible for the change in affinity from weaker sidewall binding to strong end binding. For example, certain lateral sites on the tubulin heterodimer that are not usually exposed in the MT lattice may be exposed at the end of the MT. Alternatively, the nucleotide state of Kin I kinesins may regulate binding to these substrates as well. Moreover, unique sequences either within the motor domain or the neck region (as discussed above) may play a role in determining the binding affinity during the catalytic cycle. These questions may be addressed by site-directed mutagenesis or deletion studies in the future.

How Do Kin I Kinesins Target to the Ends of MTs?

Kin I kinesins have not shown MT-based motility, so how do these enzymes reach the ends of the MT? Because XKCM1 can reach the end of the MT in the presence of ATP or AMPPNP (Desai *et al.*, 1999), we know that ATP hydrolysis is not necessary for the enzyme to reach the MT ends. Alternatively, Hunter *et al.* (2003) have proposed that MCAK uses 1D diffusion to target effectively to the ends of the MT (Figure 6a). An on rate of 54 $\mu M^{-1}s^{-1}$ has been measured for the targeting of MCAK to the ends of MTs (Hunter *et al.*, 2003), which is 20 times faster than the targeting of conventional kinesin to the lattice (Hackney, 1995). This 1D diffusion could explain the ability of Kin I kinesins to target to both ends of the MT. It would be expected that binding to the lattice would represent a weaker binding state in which Kin I kinesins would bind first to the MT lattice and then target to the ends. Interestingly, the 1D diffusion of MCAK was possible only in the presence of nucleotide (ATP or ADP) (Hunter *et al.*, 2003). In contrast, the ATP state is proposed to be the tight MT-binding state of kinesins (Crevel *et al.*, 1996). A truncated Ncd protein (a minus-end directed Kin C kinesin-related protein) does not show the directed motility of

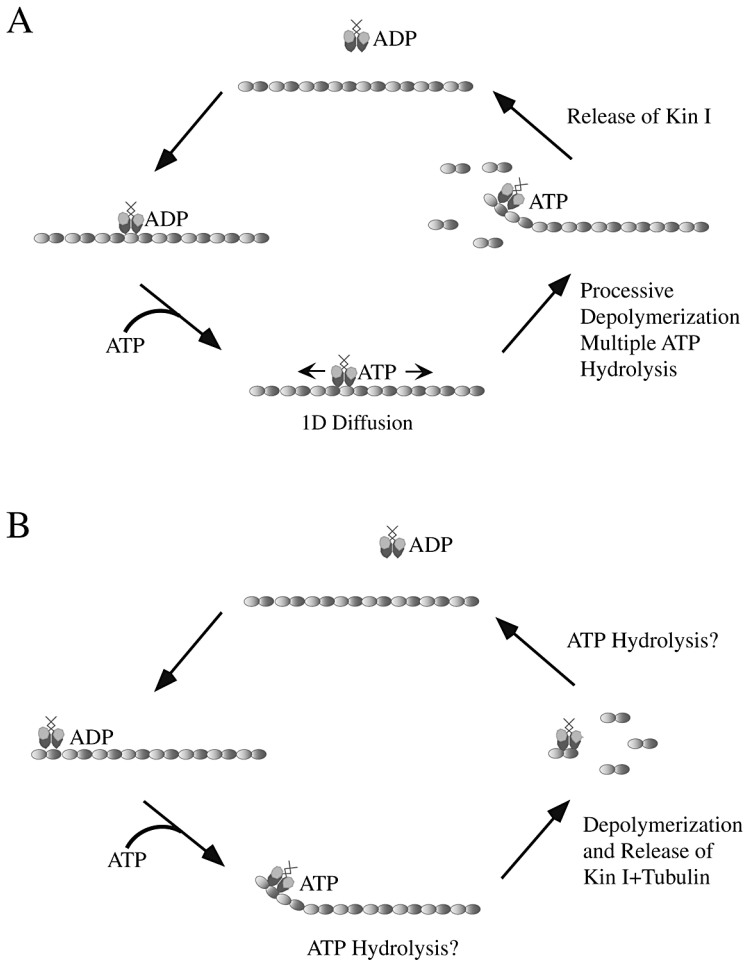

FIGURE 6. The catalytic cycle of Kin I kinesins. Hunter *et al.* (2003) propose that Kin Is are processive depolymerases that remain on the MT during multiple rounds of ATP hydrolysis (a). The Kin I binds to the MT lattice (shown here as a single protofilament for clarity), where, in the ATP bound state, it uses 1D diffusion to reach the MT end. Once it has reached its target, its high affinity binding at the end induces depolymerization. The Kin I remains bound to the MT, releasing ~20 tubulin heterodimers, using 1–5 ATP per heterodimer released. Eventually the Kin I is released and recycled. In contrast, the model proposed by Desai *et al.* (1999) (b) argues that the Kin I first binds to the end of the MT. ADP is exchanged for ATP, and the exchange of nucleotide may induce a structural change which in turn causes the conformational strain in the MT that leads to its subsequent depolymerization. The point at which ATP is hydrolyzed is unknown, but this hydrolysis is required for catalytic activity. The Kin I is released from the MT still bound to a tubulin heterodimer and is later released to start another round of depolymerization. Adapted from Walczak (2003). (Figure appears in color online at www.crbmb.com)

full-length Ncd but does exhibit 1D diffusion (Chandra *et al.*, 1993a). Similar to MCAK, this diffusion is seen in the presence of ATP. Weaker diffusional activity is seen without added nucleotide for this Ncd construct, and this movement is inhibited by AMPPNP, mimicking the nucleotide-dependent binding affinity of kinesins for MTs (Chandra *et al.*, 1993a). However, using *in vitro* assays, XKCM1, at concentrations substoichiometric to tubulin, still targets to the ends of MTs in the presence of AMPPNP or without added nucleotide (Desai *et al.*, 1999; our unpublished data). In contrast to our findings, Hunter *et al*. (2003) report that MCAK can bind to the MT lattice in the absence of nucleotide (cited as unpublished data). Clearly, this is an issue that needs to be resolved in the future because it will provide essential data to understand how the Kin Is get to the ends of MTs. Perhaps there is another mechanism for end binding other than diffusion from the lattice, or perhaps the affinity of different nucleotide

states for the MT end/lattice are distinct for Kin I kinesins.

ATPASE CYCLE OF KIN I KINESINS

Kin I kinesins hydrolyze ATP; however, hydrolysis is not necessary for the destabilization of MTs (Desai *et al.*, 1999; Moores *et al.*, 2002). Hydrolysis is proposed to be required for the enzyme to complete multiple rounds of depolymerization (Desai *et al.*, 1999). Like other kinesin-related proteins, Kin I kinesins have very low basal ATPase activity that is stimulated in the presence of MTs (Hunter *et al.*, 2003; our unpublished data). This MT-stimulated activity is lower than conventional kinesin, most likely because it results from end stimulation. Indeed, increasing the number of MT ends by shearing while keeping the tubulin concentration constant increases the ATPase activity (Hunter *et al.*, 2003). Another difference between Kin I kinesins and other kinesins is that Kin I kinesin ATPase activity is stimulated in the presence of tubulin heterodimer (Hunter *et al.*, 2003; our unpublished data). This suggests that either there are unique features on the tubulin dimer that are recognized by the Kin I kinesins or that there is a role for tubulin in the catalytic cycle of Kin I kinesins that is unlike other kinesin-related proteins.

Conventional kinesin tightly couples the hydrolysis of ATP to movement along the MT, hydrolyzing 1 ATP per 8 nm step (Coy *et al.*, 1999b). According to recent kinetic experiments with MCAK, Kin I kinesins hydrolyze 5 ATP per tubulin dimer released from a stabilized GMPCPP-MT substrate (Hunter *et al.*, 2003). The authors propose that the MCAK molecule slips along the MT lattice to release additional dimers before dissociation from the MT end. However, only 2 ATP are needed per tubulin heterodimer removed from paclitaxel-stabilized MTs (Hunter *et al.*, 2003). Given the stability of the MT substrate used in these experiments, it is likely that the number of ATP hydrolyzed in the presence of dynamic MTs would be less. Previous models of Kin I catalytic activity have proposed that the function of the hydrolysis of ATP is to release the Kin I from depolymerization products. Hunter *et al.* (2003) argue that MCAK is not released from the MT after each round of ATP hydrolysis, but that it is a processive enzyme that releases 20 tubulin dimers before disassociating from the MT (Figure 6a). To better understand how Kin I kinesins use ATP to function as a catalytic depolymerase we must determine at what point in the depolymerization reaction ATP is hydrolyzed and whether or not the tubulin heterodimer plays a prominent role.

Some progress has been made towards understanding the catalytic cycle by examining the affinity of XKCM1 for MTs in the presence of various nucleotides. These assays have hinted that the Kin I kinesins are similar to other kinesin-related proteins in this regard specifically. That is, ATP-bound Kin I kinesins have a higher affinity for MTs than ADP-bound Kin I kinesins (our unpublished results). In addition, experiments with pKinI have indicated that the affinity for MTs is less in the ADP + Pi state than in the ADP state (Moores *et al.*, 2002). This finding is similar to that of Ncd and other kinesins (Crevel *et al.*, 1996; Foster *et al.*, 1998). More work needs to be done in this area of research to truly understand how ATP hydrolysis is coupled to the destabilization of MTs.

INSIGHTS INTO THE MECHANISM OF DEPOLYMERIZATION

Understanding the mechanism of Kin I kinesins involves knowledge of the depolymerization products and reaction intermediates. Once a catastrophe occurs, tubulin heterodimers are released, but what happens to the Kin I kinesin? Is the enzyme released from the MT attached to a tubulin heterodimer or does it remain on the MT to start another round of depolymerization? Microscopy and turbidity assays have recently established a rate of depolymerization for MCAK, similar to that determined for XKCM1, of $\sim$0.9 μm/min. In addition, there is evidence for cooperativity in depolymerization; multiple Kin I kinesin molecules at the end of the MT are required to reach a maximum depolymerization rate (Hunter *et al.*, 2003). It will be interesting in the future to determine the exact number of Kin I kinesins needed to induce destabilization and to see if the processive depolymerization can be visualized.

It is likely that soluble tubulin dimer is the end product of the depolymerization reaction, since initial gel filtration studies of depolymerization products of GMPCPP-stabilized MTs have indicated that the ultimate product is tubulin dimer (Desai *et al.*, 1999). However, it is not clear if there are intermediate oligomeric states during the depolymerization cycle. Protofilament peels can be seen at the end of GMPCPP-MTs incubated with Kin I kinesins in the presence of ATP, suggesting that multiple tubulin dimers remain associated during the initial

depolymerization cycle of stabilized MTs (Desai *et al.*, 1999). Moreover, Moores *et al.* (2002) noticed the appearance of protofilament rings as depolymerization products when pKinI was incubated with MTs in the presence AMPPNP. Rings were formed from paclitaxel-stabilized MTs as well as from GMPCPP-stabilized MTs. This is different from XKCM1, in which protofilament peels could not be seen during the depolymerization of paclitaxel-stabilized MTs (Desai *et al.*, 1999). It is possible that the presence of stoichiometic levels of the pKinI catalytic domain may stabilize the ring structure. Perhaps these rings represent reaction intermediates of stabilized MTs during the depolymerization cycle. However, these reaction intermediates cannot be compared directly to the reaction products one would find *in vivo*, in which the substrate would be a dynamic MT. The observation that pKinI can bind to protofilament rings has provided structural insight into the placement of the Kin I catalytic domain onto curved protofilaments.

In support of this idea, the pKinI catalytic domain was visualized binding along the inside of the tubulin ring at a ratio of 1 catalytic domain to 1 tubulin heterodimer. The binding of pKinI in these ratios is most likely due to the stoichiometry of the Kin I in the reaction, which results in binding to the MT lattice as well as MT ends. Image averaging has permitted comparison of Kin I tubulin interactions relative to the positioning of Kin N kinesins on tubulin. Kin I kinesins sit more squarely and more centered at the intradimer interface than Kin N kinesins (Rice *et al.*, 1999; Moores *et al.*, 2002). Such a binding position may allow the Kin I kinesin to deform the contacts between tubulin monomers, creating a curved protofilament structure and destabilization of the MT.

MODEL OF ENZYMATIC ACTIVITY

There are currently two models of Kin I activity. The original model by Desai *et al.* (1999) (Figure 6b) proposes that the Kin I kinesin binds to the end of the MT, with either ADP or ATP bound. However, it is most likely the ATP bound state that induces a conformational change that makes it possible to cause the catastrophe at the end of the MT, because Kin Is induce protofilament peels in the presence of AMPPNP (Desai *et al.*, 1999). This theory is also supported by the finding that pKinI can cause depolymerization in the presence of AMPPNP (Moores *et al.*, 2002). It has been proposed that the Kin I is then released from the end of the MT bound to a tubulin heterodimer and recycled for another round of depolymerization. ATP is hydrolyzed at some point after the catastrophe event, possibly to release the Kin I from the tubulin subunit.

Hunter *et al.* (2003) have recently presented an alternative model in which Kin Is are processive enzymes that stay bound to the MT while releasing tubulin subunits (Figure 6a). They also suggest that Kin Is initially bind to the lattice and, with either ATP or ADP + Pi bound, then diffuse along the MT. Once the enzyme reaches the high affinity binding site at the end of the MT, it induces the destabilization of the MT. As it depolymerizes the MT, it uses between 1–5 ATP per tubulin subunit removed and remains attached to the MT lattice until on the order of 20 tubulin dimers are released.

There are several clear differences between these two models, which will provide incentive for creative experiments in the future. Hunter *et al.* (2003) propose that Kin Is are processive, and remain bound to the MT while shifting away from the depolymerizing end during the catalytic cycle, whereas Desai *et al.* (1999) believe that after each round of the catalytic cycle the Kin I is removed from the end. Experiments focused on establishing whether or not there is a role for tubulin in the catalytic cycle of Kin Is will help to determine whether or not Kin Is are truly processive. In particular, assays including dynamic MTs, though difficult, will provide more information on the reaction products generated. In addition, Hunter *et al.* (2003) suggest that, unlike other kinesin superfamily members, the ATP bound state is not a high affinity binding state for MTs. Thus in this model Kin Is diffuse in the presence of ATP or ADP + Pi along the MT until reaching the end of the MT. Why, then, does XKCM1 target to the ends of MTs in the presence of AMPPNP when this nucleotide analogue inhibits 1D diffusion of other molecules (Chandra *et al.*, 1993a)? Can Kin Is diffuse to the ends of MTs in the presence of AMPPNP? It will be important to resolve this question, as well as define which nucleotide states encourage high or low affinity MT binding, in order to understand how Kin Is reach the ends of the MT.

There are many questions remaining concerning the mechanism of Kin I kinesins. One ambiguous point is at which moment in the catalytic cycle ATP is hydrolyzed. Because MT-stimulated ATPase activity is much higher than that of tubulin heterodimer, it is possible that hydrolysis occurs on the MT, though it is not needed to actually induce a catastrophe. It is still unclear how the Kin I would remain on the MT after causing this

conformational change and why it would need to remain during the removal of 20 tubulin heterodimers, especially considering the nature of dynamic MTs. It is possible that the dimeric Kin I could mediate processivity by allowing one head of the Kin I to remain bound to one tubulin heterodimer while the other completes its catalytic cycle, as with conventional kinesin. If dimerization allows for processivity, is a monomeric Kin I no longer processive? Further kinetic analysis is needed to determine if Kin Is are indeed processive on physiological substrates and how this processivity is mediated. Perhaps single-molecule tracking will allow visualization of processive depolymerization. In addition, although both models are in agreement that Kin Is cause a structural change at the MT end, the mechanics of this structural change are elusive. Structural information about the unique interaction between Kin I kinesins and the MT would help elucidate this change.

In addition to these mysteries in the Kin I mechanism, if tubulin is indeed involved in the catalytic cycle, how would its release from the Kin I be regulated? Furthermore, the tubulin heterodimer has many sites exposed which are similar to the end of a MT. How is this affinity weaker than the affinity of the MT end? What additional sequences of Kin Is or MTs are necessary for targeting to MT ends? How is this targeting mediated? How do Kin Is recognize the ends specifically and with higher affinity than the sidewall of the MT? These questions are a few of the many left unanswered by current research. Much work on the mechanism of Kin I kinesins has been performed on stabilized MT substrates. Perhaps in the future, some of our questions may be addressed in assays using dynamic MTs. In addition, hopefully crystal structures catching Kin Is in the act will elucidate the exact conformational changes leading to depolymerization of the MT.

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