We showed previously that stable, detyrosinated (Glu) microtubules function to localize vimentin intermediate filaments in fibroblasts (Gurland, G., and Gundersen, G. G. (1995) J. Cell Biol. 131, 1275–1290). To identify candidate proteins that mediate the Glu microtubule-vimentin interaction, we incubated microtubules with microtubule-interacting proteins and saturating levels of antibodies to Glu or tyrosinated (Tyr) tubulin. Antibodies to Glu tubulin prevented the microtubule binding of kinesin obtained from fibroblast or brain extracts more effectively than antibodies to Tyr tubulin. Scatchard plot analysis showed that kinesin heads bound to Glu microtubules with an ~2.8-fold higher affinity than to Tyr microtubules. Purified brain kinesin cosedimented with vimentin, but not with neurofilaments, indicating that kinesin specifically associates with vimentin without accessory molecules. Kinesin binding to vimentin was not sensitive to ATP, and kinesin heads failed to bind to vimentin. By SDS-polyacrylamide gel electrophoresis, a kinesin heavy chain of ~120 kDa and a light chain of ~64 kDa were detected in vimentin/kinesin pellets. The light chain reacted with a general kinesin light chain antibody, but not with two other antibodies that recognize the two known isoforms of kinesin light chain in brain, suggesting that the kinesin involved in binding to vimentin may be a specific one. These results demonstrate a kinesin-based mechanism for the preferential interaction of vimentin with detyrosinated microtubules.

Even though tubulin detyrosination accompanies the stabilization of MTs (4–9), several studies have demonstrated that the elevated Glu tubulin level is not responsible for the increased stability of Glu MTs (5, 6, 10). Nonetheless, tubulin detyrosination can serve as a marker for stable MTs in vivo.

The role of enhanced tubulin modification in MT function had remained elusive until recently, when studies from this laboratory showed that stable Glu MTs function to localize vimentin intermediate filaments (IFs) in polarized fibroblasts (1). In this study, IFs were found to coalign with Glu MTs, and this coalignment could be disrupted by microinjection of affinity-purified antibodies to Glu tubulin, but not to Tyr tubulin. In a more recent study, microinjection of nonpolymerizable Glu tubulin, but not Tyr tubulin, into the cytoplasm disrupted the distribution of IFs without affecting the level of stable MTs. These results conclusively demonstrate that tubulin detyrosination, rather than increased MT stability, is responsible for the Glu MT-IF interaction. We hypothesize that Glu tubulin may function as a signal for the recruitment of IFs, and perhaps other cellular components, onto stable MTs in polarized cells.

One critical element to understand the preferential interaction of IFs with Glu MTs is to determine the factors involved in mediating the interaction. Previous studies have provided evidence for cross-bridging structures or molecules that might mediate the interaction between IFs and MTs (11, 12); however, none of these candidates has been shown to interact selectively with Glu versus Tyr tubulin. In this study, we have pursued the identity of factors that might be mediating the preferential interaction between IFs and Glu MTs by using antibodies to Glu tubulin as competitors for the binding of the putative cross-bridging molecule to Glu MTs in vitro. We found that antibodies to Glu tubulin reduced the binding of a kinesin to MTs more effectively than antibodies to Tyr tubulin. By directly measuring the binding affinity of kinesin for pure Glu and Tyr MTs, we confirm that kinesin binds to Glu MTs with a 2.8-fold higher affinity than to Tyr MTs. In further experiments, we found that there is a kinesin that specifically cosediments with vimentin IFs, but not with neurofilaments or actin filaments. This kinesin is composed of kinesin heavy chain and a light chain that might be novel. Taken together, our results show that kinesin can mediate the preferential interaction of vimentin IFs with Glu MTs.

Kinesin Is a Candidate for Cross-bridging Microtubules and Intermediate Filaments

SELECTIVE BINDING OF KINESIN TO DETYROSINATED TUBULIN AND VIMENTIN

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Microtubules (MTs)§ are highly dynamic structures that are present in nearly every eukaryotic cell. During cellular polarization and differentiation, MTs become stabilized, and the tubulin subunits composing these MTs become post-translationally modified. One of these modifications is detyrosination, which involves the removal of the C-terminal tyrosine residue from α-tubulin (2). This generates α-tubulin with a glutamate residue at the C terminus. The two forms of α-tubulin are

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The abbreviations used are: MTs, microtubules; Glu MTs, detyrosinated microtubules; Tyr MTs, tyrosinated microtubules; IF, intermediate filament; mAb, monoclonal antibody; Pipes, 1,4-piperazinediethanesulfonic acid; AMP-PNP, adenosine 5′-β,γ-imino-triphosphate; PAGE, polyacrylamide gel electrophoresis; MAP, microtubule-associated protein.

EXPERIMENTAL PROCEDURES

Materials—Taxol was a gift of Dr. Ven L. Narayanan (NCI, Bethesda, MD). Monoclonal antibody (mAb) to kinesin heavy chain, H2 (13), was generously provided by Dr. G. S. Bloom (University of Texas, Southwestern, Dallas, TX). Polyclonal antibody to kinesin heavy chain, HD (14), was provided by Dr. V. I. Gelfand (University of Illinois, Urbana, IL). Monoclonal antibodies to kinesin light chain, L1 and L2

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9797
Kinesin-mediated Interaction of Vimentin with Microtubules

Affinity-purified Antibodies to Glu Tubulin Reduce the Binding of Kinesin to MTs—In a search for the cross-briding molecule that mediates the Glu MT-IF interaction, we assembled Taxol-stabilized MTs from purified calf brain tubulin (an ~50:50 mixture of Glu and Tyr tubulin) and incubated them with saturating levels of affinity-purified antibodies to either Glu (SG) or Tyr (W2) tubulin and subsaturating levels of preparations of MT-interacting proteins. If there is a Glu tubulin-specific cross-bridging molecule, antibodies to Glu tubulin, but not to Tyr tubulin, should sterically interfere with the binding of the cross-bridging molecule to MTs. When we incubated MTs with SG or W2 and crude preparations of brain MT-associated proteins (MAPs), we did not see significant inhibition of individual MAP binding with either antibody (data not shown). These results are consistent with previous observations (10, 21, 26).

With preparations of MT motor proteins from a 3T3 cell extract, SG (but not W2) blocked the sedimentation of an ~110-kDa polypeptide with MTs (Fig. 1a). At a 1:1 molar ratio of antibody to tubulin, SG significantly reduced the binding of the 110-kDa protein to MTs, whereas the level of the 110-kDa protein in the W2-treated sample was comparable to that in the control sample, where no antibody was added. At a 4:1 ratio of antibody to tubulin, the binding of both SG and W2 to MTs was saturated (data not shown), and the 110-kDa protein was not detectable in the SG-treated sample, but was still present in the W2-treated sample (Fig. 1a). Other polypeptide bands were not significantly affected by either antibody (e.g. bands at ~70 and ~50 kDa). The 110-kDa protein ran as a doublet in some of the samples (see control (C) lane in Fig. 1a). A mAb to Glu tubulin, 48S1 (1) (from J. C. Bulinski, Columbia University), also inhibited the binding of the 110-kDa protein to MTs more effectively than a mAb to Tyr tubulin, YL1/2 (27) (data not shown).

We identified the 110-kDa polypeptide as a member of the kinesin family based on a number of criteria. The 110-kDa polypeptide bound to MTs in the absence of ATP and was eluted from MTs with 10 mM ATP (data not shown), consistent with characteristics of kinesin motor proteins (28). Moreover, a mAb to kinesin heavy chain, H2, recognized the 110-kDa protein in the W2-treated sample (Fig. 1a). Another mAb to kinesin (SU4K) and a polyclonal antibody to kinesin (HD) also reacted with the 110-kDa protein (data not shown). Experiments with preparations of MT motor proteins from brain extracts showed similar results, except that the polypeptide that was inhibited from binding to MTs by SG migrated at ~120 kDa (see below, Fig. 1c). These results demonstrate that Glu antibody is capable of inhibiting the binding of two different kinesins to MTs.

We next analyzed the level of kinesin binding to MTs in the presence of SG or W2 using Western blotting with H2 antibody. As shown in Fig. 1c, SG prevented more kinesin from binding to MTs than W2. We also analyzed the MT pellets to determine the level of tubulin antibodies bound to MTs. No significant difference was observed in the levels of IgG that cosedimented with MTs (Fig. 1d). These results rule out the possibility that the differential capability of SG and W2 to inhibit the binding of kinesin to MTs was due to differential binding of SG and W2 to MTs.
MT motor proteins prepared from brain extract were added to Taxol-stabilized MTs (8 μg of DEAE-purified brain tubulin was used in each sample) in the presence of saturating levels of affinity-purified SG or W2. The amount of kinesin bound to MT pellets was quantified by quantitative Western blotting and is reported as percent of control (level of kinesin bound to MTs in the absence of antibodies). The amount of SG or W2 IgG bound to MT pellets was also quantified by quantitative Western blotting using rabbit IgG as a standard. At equivalent levels of MT-bound antibodies, SG inhibited the binding of kinesin to MTs 1.7-fold more than W2, (% inhibition of kinesin binding/μg of W2/% inhibition of kinesin binding/μg of SG) = 1.7.

| Exp. | SG W2 |
|------|-------|
| 1    | 44 75 |
| 2    | 33 55 |
| 3    | 30 62 |
| Average | 36±6 74±8 |

Levels of kinesin and SG or W2 antibody bound to MTs were measured by quantitative Western blotting with H2 antibody and a commercial antibody to rabbit IgG, respectively. Table I shows the individual determinations from three identical experiments (only two determinations of the amount of bound antibody were made). On average, SG inhibited the binding of kinesin to MTs by ~64%, whereas W2 inhibited binding only by 36%. When these levels are normalized against the amount of antibody bound to MTs, we found that at equivalent levels of antibody bound to MTs, SG inhibited the binding of kinesin to MTs 1.7-fold more than W2 did (Table I). Since we used low, subsaturating levels of kinesin in these experiments, these data suggest that kinesin was preferentially bound to Glu rather than Tyr tubulin, so that when Glu tubulin sites were blocked, more kinesin was displaced.

Kinesin heads bind to Glu MTs with a higher affinity than to Tyr MTs—To directly compare the binding affinity of kinesin for Glu and Tyr MTs, we prepared tubulin from HeLa cells to generate MTs composed of pure Glu and Tyr tubulin. Tubulin purified from HeLa cells is reported to be >90% Tyr tubulin (21) and can be converted to Glu tubulin by carboxypeptidase A treatment (9). Moreover, tubulin prepared from HeLa cells is not as complex as brain tubulin since it does not contain several post-translational modifications abundant in brain tubulin (e.g. polyglutamylation (29) and nontyrosinatable tubulin (30)). We have confirmed that our preparations of HeLa cell tubulin and carboxypeptidase A-treated HeLa cell tubulin were predominantly Tyr (90%) and Glu (100%) tubulin, respectively. To measure the binding affinity of kinesin for Glu and Tyr MTs, we used a recombinant head domain of squid kinesin heavy chain, K394 (16). We incubated increasing amounts of K394 with Taxol-stabilized Glu or Tyr MTs and separated the bound kinesin from unbound by sedimenting the MTs. The amount of kinesin bound was analyzed by quantitative Western blotting.

As shown in Fig. 2a, more K394 bound to Glu MTs than to Tyr MTs at all concentrations. Scatchard plot analysis of binding assays showed that K394 bound to Glu and Tyr MTs with Kd values of 29 and 81 nM, respectively (Fig. 2b). These results directly demonstrate that kinesin preferentially binds to Glu MTs compared with Tyr MTs.
The amount of sedimented Glu or Tyr tubulin was the same in each incubation (data not shown). The kinesin in the preparation did not sediment alone (Fig. 3a, lanes 2 and 4), 0.24 μM (lanes 3 and 7), or 0.48 μM (lanes 4 and 8) and either 0.8 μM Glu tubulin (lanes 1–4) or 0.8 μM Tyr tubulin (lanes 5–8). b, Scatchard plot analysis of the binding of K394 to Glu and Tyr MTs. Data are pooled from three separate binding experiments.

**Kinesin Cosediments with Vimentin IFs**—We showed above that antibodies to Glu tubulin reduced the binding of a 110-kDa (3T3 cell) and a 120-kDa (brain) kinesin to MTs more effectively than antibodies to Tyr tubulin and that recombinant kinesin heads bound to Glu MTs with a higher affinity than to Tyr MTs. These results strongly suggest that a kinesin may mediate the preferential interaction of vimentin IFs with Glu MTs. To test this possibility directly, we assembled vimentin IFs from purified recombinant vimentin and tested whether a kinesin could interact with vimentin IFs. We found a 120-kDa kinesin in the MT motor protein preparation from bovine brain cosedimented with purified vimentin IFs, as assessed by both protein staining and Western blotting (Fig. 3a, lanes 1 and 4). Experiments with preparations of MT motor proteins from 3T3 cell extracts showed a 110-kDa kinesin sedimenting with vimentin (data not shown). The kinesin in the preparation did not sediment alone (Fig. 3a, lanes 3 and 6). The sedimentation of this kinesin with vimentin IFs was not due to trapping by filamentous proteins since significantly less kinesin was found in the pellet from an equivalent amount of actin filaments (Fig. 3b, compare lanes 2 and 4 with lanes 1 and 3).

To distinguish whether the 120-kDa kinesin from preparations of brain MT motor proteins associated with vimentin IFs directly or through some accessory molecules, we purified kinesin from brain (see “Experimental Procedures”). Analysis of the preparation through the sequential steps of the purification (MT motor proteins (lane M), gel filtration (lane G), ion exchange (lane I), and sucrose density gradient ultracentrifugation (lane S)) showed the enrichment of the kinesin heavy (120 kDa) and light (64 kDa) chains (Fig. 4a). For unknown reasons, our purified kinesin preparation contained relatively more light chain than that detected in kinesin preparations obtained by other laboratories (20). After the final sucrose gradient centrifugation step, the preparation contained only the 120-kDa heavy and 64-kDa light chains (Fig. 4a, lane S). As shown in Fig. 4b, with the 120-kDa kinesin obtained from different stages of purification, comparable levels of kinesin (even with the purest fraction) sedimented with vimentin IFs, indicating that the 120-kDa kinesin associated with vimentin without the need for an accessory protein (however, see below for an analysis of light chain binding). More important, the 120-kDa kinesin from the purest fraction did not cosediment significantly with a comparable amount of neurofilament proteins (Fig. 4c), showing that the interaction of kinesin is specific for vimentin IFs.

**Differences in Kinesin Binding to MTs and Vimentin IFs**—Conventional kinesin holenzyme is a tetramer composed of two ~120-kDa heavy chains and two 60–70-kDa light chains (31). The head domain of kinesin heavy chain is conserved among different isoforms of kinesin and kinesin-like proteins and contains both MT- and ATP-binding sites (for review, see Ref. 32). We determined if kinesin head was involved in the association of kinesin with vimentin by testing whether recom-
binant squid kinesin head, K394, cosedimented with vimentin. As shown in Fig. 5a, no K394 was found in the pellet after centrifugation of a vimentin/K394 incubation mixture, showing that the vimentin-binding site of kinesin is not located in the head domain. While the binding of kinesin to MTs is normally inhibited by millimolar levels of ATP, cosedimentation of the 120-kDa kinesin with vimentin IFs was not affected by the presence of 2 mM ATP (Fig. 5b). These results show that the binding of kinesin to vimentin occurs by a mechanism distinct from that involved in kinesin-MT binding.

A Specific Kinesin Light Chain Is Involved in Binding to Vimentin—Kinesin can be divided into three regions: a globular head domain formed by the two heavy chains, a stalk domain formed primarily by the heavy chains, and a fan-like tail domain formed by the light chains and the heavy chain carboxyl-terminal domain (for reviews, see Refs. 32 and 33). The kinesin tail domain is thought to participate in cargo binding, and recent evidence supports this notion (34, 35). We have shown above that kinesin head is not responsible for the association of kinesin with vimentin. By SDS-PAGE analysis, a polypeptide of 64 kDa cosedimented with the 120-kDa kinesin heavy chain and was present in approximately equal stoichiometry (Fig. 6a).

To further characterize the kinesin light chain involved in vimentin binding, we probed the kinesin that cosedimented with vimentin with several well characterized monoclonal antibodies to kinesin light chains, L1 and L2 (13) and 63–90 (15). We first used L1, which was reported to detect two known isoforms of kinesin light chain (13), on a blot of vimentin/kinesin pellets already probed with antibody to kinesin heavy chain (L1, L2, and 63–90). P and S, pellet and supernatant of vimentin + kinesin, respectively.

![Fig. 4. Purified kinesin binds to vimentin IFs.](image)

![Fig. 5. Characterization of the binding of kinesin to vimentin.](image)

![Fig. 6. Cosedimentation of kinesin light chains with vimentin IFs.](image)
chain, H2. As shown in Fig. 6b, L1 failed to detect the light chain in the kinesin that sedimented with vimentin (lane 1), but reacted with the kinesin light chains in an equivalent amount of purified bovine brain kinesin (lane 2). Similar results were observed in cosedimentation experiments directly probed with L1 or L2, i.e. both L1 and L2 failed to detect a kinesin light chain in the kinesin that pelleted with vimentin, but both reacted with kinesin light chains remaining in the supernatant (Fig. 6c). However, 63–90, a monoclonal antibody against a conserved domain near the amino terminus of kinesin light chains, reacted with kinesin light chains in both the pellet and the supernatant (Fig. 6c). These results show that not all kinesin holoenzymes are capable of binding to vimentin and raise the possibility that there is a specific kinesin light chain that, in combination with a kinesin heavy chain, directs kinesin to vimentin. We are currently pursuing the identity of this kinesin light chain.

**DISCUSSION**

**Preferential Binding of Kinesin to Glu MTs Compared with Tyr MTs**—In this study, we have provided two lines of evidence that kinesin preferentially binds to Glu MTs compared with Tyr MTs. First, using saturating levels of anti-Glu and anti-Tyr tubulin antibodies as competitors for the binding of subsaturating levels of MT motor proteins to MTs, kinesin was prohibited from binding to MTs more effectively by anti-Glu tubulin antibody than by anti-Tyr tubulin antibody. Second, when we used pure Glu and Tyr MTs as the binding substrates of kinesin, we found that recombinant kinesin heads bound to Glu MTs with an ∼2.8-fold higher affinity than to Tyr MTs. These results revealed for the first time a differential interaction of Glu and Tyr tubulin with another protein, namely kinesin. The ability of kinesin to differentially interact with Glu and Tyr tubulin is so far unique among MT-interacting proteins. Previous studies showed no obvious difference in the binding of MAP4 and brain MAPs to Tyr and Glu MTs (21), and the mAb to Tyr tubulin, YL1/2, had no effect on the incorporation of MAP2 and tau into MTs in *vitro* (26). These results show that tubulin desymmetry is not involved in the regulation of the binding of MAPs to MTs, and this is also confirmed by our current observation that antibodies to Glu and Tyr tubulin had little effect on the binding of brain MAPs to MTs.

We found in this study that the difference between the binding affinity of kinesin for Glu and Tyr MTs is ∼2.8-fold. If kinesin is the motor for moving vimentin on MTs, is this difference in binding affinity sufficient to account for the *in vivo* observation that vimentin IFs are preferentially localized on Glu MTs? We think that a straight 2.8-fold difference in binding affinity may not be sufficient to account for the strong preference of vimentin IFs for Glu MTs in 3T3 cells; however, we can envision several factors that might magnify this difference and contribute to the Glu MT-IF association. One possibility is that the affinity difference is magnified each step kinesin moves along a MT. According to this scenario, an IF that associates with a Glu MT would be more likely to stay on a MT than one that associates with a Tyr MT. Another possibility is that a vimentin IF requires the association of multiple kinesins with a MT before it will move. Since this would be more likely on Glu MTs than on Tyr MTs, IFs may be restricted to Glu MTs. A final possibility is that the kinesin that is specific for cross-bridging vimentin and MTs might have a much larger preference for Glu MTs than the K394 kinesin head we used in our binding experiments. We think that the difference in kinesin binding affinity that we measured, in combination with one or more of these factors, is sufficient to explain the *in vivo* finding that vimentin IFs are preferentially localized on Glu MTs. It will be interesting to check whether other MT-dependant, peripherally localized organelles, such as the endoplasmic reticulum, mitochondria, and lysosomes, are also preferentially distributed on Glu MTs.

**Regulation of Kinesin Activity by Post-translational Modification of the Substrate**—Our results demonstrated for the first time that the binding of a MT motor protein, kinesin, to MTs can be regulated at the substrate level by post-translational modification. Regulation of kinesin binding by another form of tubulin modification, glutamylation, was inferred in a study by Larcher et al. (36), although in their study, only antibody inhibition data from blot overlay assays of tubulin (not MTs) were obtained, and no direct binding studies with glutamylated and non-glutamylated tubulin were performed. Nevertheless, this supports the involvement of the C terminus of tubulin in regulation of the kinesin-MT interaction (see below). At this point, we do not have information on whether and how the differential binding of kinesin to Glu and Tyr MTs changes the mechanistic parameters of kinesin activities on these two substrates. It will be interesting to determine if kinesin moves with a different processivity and/or velocity, if kinesin partitions differently between a stepping pathway and a static pathway, or if the load capacity of kinesin is different on Glu and Tyr MTs.

**Kinesin as a Candidate for Cross-bridging MT-IF**—The fact that antibodies to Glu tubulin (1) and nonpolymerizable Glu tubulin2 disrupted the distribution of IFs *in vivo*, coupled with our current observation that Glu antibodies interfered with the binding of kinesin to MTs, suggests that kinesin might be the motor responsible for the extension of IFs onto MTs. This notion is supported by a previous observation that microinjection of antibodies to kinesin heavy chain collapsed IFs to the perinuclear area (14). In preliminary studies, we have confirmed this observation by showing that microinjection of a number of antibodies to tubulin heavy chain or light chain disrupted the distribution of IFs. In order for kinesin to move vimentin IFs on MTs, kinesin has to be able to associate with vimentin either directly or indirectly through some accessory molecules. Indeed, we provided evidence here that kinesin can associate with vimentin IFs directly. The interaction of kinesin with vimentin is specific since we did not observe significant cosedimentation of kinesin with actin or with neurofilaments. Moreover, the light chain involved in the kinesin-vimentin interaction was recognized by one monoclonal antibody to kinesin light chain, but not by two others, suggesting that not all kinesins are capable of interacting with vimentin and that the kinesin responsible for cross-bridging IF-MT is a specific one. At this point, we do not have information on whether the kinesin heavy chain involved is also specific for vimentin or is a universal one that can associate with multiple kinds of kinesin light chain to form a kinesin holoenzyme. We are currently preparing enough “vimentin kinesin” so that we can address these issues.

Our results demonstrating the involvement of a specific kinesin light chain in vimentin binding, along with the fact that the vimentin-binding domain was not localized in the head domain of kinesin, provide a molecular explanation for the preferential localization of vimentin IFs on Glu MTs that we observed earlier (1): namely, that a kinesin for vimentin IFs binds to the cargo (i.e. vimentin IFs) using the specific light chain and selectively binds to and moves along MTs enriched in Glu tubulin with its motor domain. A more direct demonstration of kinesin as the motor that drives the movement of IFs along MTs will require the reconstitution of IF movement *in vitro*.

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3. G. Liao and G. G. Gundersen, unpublished observations.
Kinesin-mediated Interaction of Vimentin with Microtubules

The Kinesin-binding Site on MTs—Our results that antibodies to Glu tubulin inhibited the binding of kinesin to MTs strongly suggest that the α-subunit of the αβ-tubulin dimer either is part of the site that kinesin binds to or regulates kinesin binding and that this site is most likely located in the C-terminal region of tubulin. Initially, results from cross-linking studies suggested that kinesin interacts only with β-tubulin (16). However, more recent studies have consistently observed cross-linking between kinesin and both α- and β-tubulin (37, 38). It is likely that in the earlier studies (16), either the cross-linker failed to cross-link the kinesin-α-tubulin complex, or the recognition site for the α-tubulin antibody used was blocked in the cross-linked kinesin-α-tubulin complex. Supportive evidence for the interaction between kinesin and α-tubulin is also provided by structural studies that showed that kinesin motor domains made extensive contacts with both the α- and β-tubulin monomers (39). In agreement with our conclusion that kinesin interacts with tubulin in the C-terminal region, mapping studies of cross-linked kinesin-tubulin suggest that cross-linking to kinesin occurs in the C-terminal region of α- and β-tubulin subunits (38). The fact that antibody to glutamylated tubulin blocked the binding of kinesin to tubulin (36) also supports the notion that the kinesin-binding site is in the C-terminal region of tubulin, where the glutamylation occurs. In preliminary studies, we found that a fragment of the C-terminal region, mapping studies of cross-linked kinesin-tubulin suggest that cross-linking to kinesin occurs in the C-terminal region of α- and β-tubulin subunits (38). The fact that antibody to glutamylated tubulin blocked the binding of kinesin to tubulin (36) also supports the notion that the kinesin-binding site is in the C-terminal region of tubulin, where the glutamylation occurs. In preliminary studies, we found that a fragment of the C-terminal region of α-tubulin effectively inhibited the binding of kinesin to MTs, further supporting the notion that kinesin interacts with α-tubulin in the C-terminal region.

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