XMAP215 is a long thin molecule that does not increase microtubule stiffness

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INTRODUCTION

Microtubule polymers are dynamic and continually turn over by processes of dynamic instability and/or treadmilling. Dynamic instability is the major pathway of microtubule turnover in cells and describes a two-state behavior of microtubules ends; these ends can exist in either a growth or a shortening state. Abrupt transitions between states are termed catastrophes (the switch from growth to shortening) and rescue (the switch from shortening to growth). Treadmilling, the preferential gain and loss of subunits from opposite microtubule ends, has also been observed in cells (Waterman-Storer and Salmon, 1997; Rodionov and Borisy, 1997).

These dynamic turnover mechanisms are probably regulated by microtubule associated proteins (MAPs), as turnover in vivo can be faster than that observed with purified tubulin in vitro (reviewed by Desai and Mitchison, 1997). One such regulator is XMAP215, a protein initially purified from Xenopus egg extracts, based on its ability to stimulate microtubule plus-end growth (Gard and Kirschner, 1987). Subsequent studies have demonstrated that XMAP215 speeds microtubule plus-end assembly seven- to tenfold, increases shortening rate threefold, and does not change catastrophe frequency (Vasquez et al., 1994). By contrast, XMAP215 had negligible effects on minus-end assembly (Vasquez et al., 1994). Therefore, it is likely that one function of XMAP215 is to speed microtubule plus-end growth, yet allow rapid microtubule turnover in cells. This has been demonstrated in egg extracts where partial depletion of XMAP215 results in approximately twofold slower growth rate in interphase extracts (Tournebize et al., 2000), while 90% depletion nearly eliminates microtubule assembly (Tournebize et al., 2000; Popov et al., 2001). XMAP215 also protects microtubule plus ends from the catastrophe-promoting activity of the Kin I kinesin, XKCM1 (Tournebize et al., 2000). XKCM1 and related kinesins are thought to disrupt microtubule ends by peeling away protofilaments and then releasing tubulin dimers by an ATP-dependent mechanism (Desai et al., 1999). How XMAP215 is able to both speed plus-end assembly and protect microtubule ends from XKCM1 is not understood.

XMAP215 probably functions during spindle assembly, as depletion of this MAP abolishes spindle assembly in Xenopus egg extracts (Tournebize et al., 2000; Wilde and Zheng, 1999). The importance of XMAP215 function during mitosis is also supported by studies of related members of this MAP family: genetic mutations in Drosophila mini-spindles protein (Msp; Cullen et al., 1999), C. elegans Zyg-9 (Matthews et al., 1998), S. cerevisiae Stu2p (Wang and Huffacker, 1997) and S. pombe p93dis1 (Nabeshima et al., 1995; Nabeshima et al., 1998) resulted in defective spindle assembly, short microtubules or poor viability. Finally, depletion of the human homolog, TOGp, from HeLa cell extracts prevented mitotic aster...
assembly (Dionne et al., 2000). XMAP215 and the related MAP family members probably function in mitosis by stimulating microtubule plus-end assembly and counteracting the destabilizing activity of Kin I kinesins (Tournebize et al., 2000; Becker and Gard, 2000), but additionally XMAP215 may serve as a scaffold to localize cyclin B/CDK1 to microtubules during mitosis, as has been observed for TOGp (Charrasse et al., 2000).

Recent sequence analysis suggests that the XMAP215 family members contain a large number of HEAT repeats (Neuwald and Hirano, 2000). While the primary amino acid sequence is not well conserved, each HEAT repeat forms a common structural motif consisting of two α helices that associate together in an antiparallel orientation (reviewed by Kobe et al., 1999). The function of HEAT repeats is not known, but may contribute significantly to the structure of XMAP215.

To develop models of how XMAP215 functions as a regulator of microtubule assembly dynamics or as a scaffold, it is necessary to understand its structure. In the results presented here, we have used electron microscopy to examine the structures of XMAP215 and the partial tubulin rings assembled with this MAP. Additionally, we measured the flexural rigidity of microtubules assembled with XMAP215 to determine whether this MAP stiffens the microtubule lattice. Finally, we use the positions of identified HEAT repeats to align repeated sequences within the N-terminal half of XMAP215.

MATERIALS AND METHODS

Protein purification

XMAP215 was isolated from Xenopus eggs, as described previously (Gard and Kirschner, 1987) with the modifications described in Vasquez et at. (Vasquez et al., 1994). XMAP215 in BRB80 (80 mM Pipes, 1 mM MgCl₂, 1 mM EGTA supplemented with 1 mM DTT, 0.1 mM PMSF, 0.1 μg/ml phenanthroline, 0.1 mM benzamidine HCl and 0.1 μg/ml peptatin) was maintained at 4°C and used within 3-4 days of the final purification step. The two XMAP215 preparations were used here ranged from 50-100 μg/ml concentration, based on comparative staining of XMAP215 and BSA on Coomassie Blue stained SDS gels. Becker and Gard (Becker and Gard, 2000) have identified two isoforms of XMAP215 that differ by alternative splicing. As the XMAP215 used here is isolated from eggs, all considerations of protein sequence use the maternal isoform because this is the isoform present in the egg.

Tubulin was purified from porcine brains as described previously (Vasquez et al., 1994). No MAPs were detected in this preparation after silver staining of over-loaded SDS-PAGE mini-gels (50 μg/lane; not shown).

Glycerol gradient centrifugation

Approximately 25 μg of purified XMAP215 was applied to a 4.5 ml linear gradient of 15 to 40% glycerol in 0.2 M ammonium bicarbonate. Standards consisting of catalase (11.3 S), albumin (4.6 S) and ovalbumin (3.5 S) were loaded on a separate gradient. Samples were spun at 4°C for 16 hours at 130,000 g in an SW 55 rotor, fractionated and examined by Coomassie Blue staining of 10% SDS-PAGE gels. The sedimentation coefficient for XMAP215 was calculated by comparison with the standards by assuming linear separation across the gradient. The maximum sedimentation value was calculated (Ohashi and Erickson, 1997) using 228,000 as the molecular weight of XMAP215 (Tournebize et al., 2000; Becker and Gard, 2000).

Tubulin/XMAP215 partial rings

Tubulin (4 μM) with or without XMAP215 (0.4 μM) was incubated in 0.1xBRB80, 1 mM GTP and 15% glycerol for 1 hour on ice before spraying onto mica and shadowing (described below). Additional tubulin samples (5 μM) lacking XMAP215 were incubated in 0.1xBRB80, 1 mM GTP and 30% glycerol for several hours on ice before spraying onto mica.

C-terminal antibody to TOGp/XMAP215

A C-terminal fragment of TOGp (bp 5421-5961) was cloned into pGEX2T-L (pGEX2T (Amersham) modified to include additional restriction sites; a generous gift from C. Larroque), transformed into Escherichia coli strain BL21 and expressed as a GST-fusion protein after induction by IPTG. The fusion protein was purified on a glutathione-S-transferase column according to the manufacturer’s instructions. This GST-TOGp (amino acids 1807-1987) was used to immunize rabbits (Pocono Rabbit Farms) and generated antiserum that recognized a single band at 215 kDa in HeLa lysates (data not shown). The antiserum was fractionated by ammonium sulfate precipitation (Harlowe and Lane, 1988) and then further purified on a 6xHis-TOGp (amino acids 1807-1987) affinity column. The 6xHis-tagged TOGp (amino acids 1807-1987) was generated by cloning TOGp (bp 5421-5961) into the pQE30 vector (Qiagen) and expression of the fusion protein in E. coli strain M15. 6xHis-TOGp (amino acids 1807-1987) was purified on a nickel column (Qiagen) and coupled to CNBr-activated Sepharose (Pharmacia), according to the protocol provided by the manufacturer. Antibodies were bound to the column in PBS and eluted with 0.1 M glycine, pH 2.5 and each 1 ml fraction was neutralized with 200 μl Tris-HCl, pH 8.5 (Harlowe and Lane, 1988). To demonstrate the specificity of the antibody for XMAP215, purified XMAP215 and a crude Xenopus egg extract were separated on 3-12.5% SDS PAGE gels, transferred to Immobilon membrane (Millipore) and probed with the purified antibodies; antibody binding was detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Arlington Heights, IL.).

Shadowing and electron microscopy

The peak XMAP215 fraction from the glycerol gradient was diluted twofold with 15% glycerol in ammonium bicarbonate and sprayed onto ~1 cm square pieces of freshly cleaved mica. The mica was then dried under vacuum in a Balzers BAE 120 operated at room temperature. Specimens were rotary or uni-directional shadowed by evaporation of platinum onto the mica at ~6° and then coated with carbon at 90°. The platinum/carbon replicas were removed from the mica by flotation on deionized H₂O and mounted on 400 mesh EM grids.

Replicas were examined using a Philips 301 electron microscope operated at 80 kV. Micrographs were taken at a magnification of ~47,000-50,000×. Magnification was calibrated using a negatively stained preparation of bovine tropomyosin paracrystals (Erickson et al., 1981).

Image analysis

EM negatives were scanned at 600 dpi resolution using an Epson Perfection 1200 scanner in negative mode. Measurements were made from the digital images using NIH image software. A distance of 2 nm was subtracted from each length or width measurement to remove the contribution from the shell of metal. The radius of curvature for partial rings was measured using Canvas 6.0 by drawing and overlaying a circle on the partial ring. The radius of each circle was then measured using the radius tool in Canvas. These latter measurements were not corrected for the platinum/carbon coating, as the circle was drawn to bisect the center of the arc of each partial ring. Statistical analysis was performed using analysis of variance in Microsoft Excel.

Measurement of flexural rigidity by thermal fluctuations

For an idealized beam (i.e. a linear rod of homogeneous material and no structural variation), the curvature along its axis gives information about the bending moment associated with the impact of external bending energy, Bending moment and bending energy are coupled by Young’s
elastic modulus (E) and the geometric moment of inertia (I) (Feynman et al., 1964). For an idealized beam, these two parameters can be separated. However, a microtubule, made of subunits of β-tubulin, is not an idealized beam, and its elasticity is often specified by the product EI, which is a measurement of the stiffness or flexural rigidity of the microtubule. Several techniques have been used previously to measure the flexural rigidity of microtubules by correlating bending energy with positional deflections of the microtubule long axis. Bending has been induced by passive excitation through thermal energy (Gittes et al., 1994; Venier et al., 1994; Mickey and Howard, 1995), or by controlled forces applied by optical trapping or hydrodynamic flow (Venier et al., 1994; Kurachi et al., 1995; Felgner et al., 1996; Felgner et al., 1997).

In the present study, microtubule flexural rigidity was derived from measurements of thermal fluctuations at the free ends of clamped microtubules. This type of flexural rigidity analysis relies on the equation (see Appendix for derivation):

$$EI = \frac{k_BT L^3}{3(\sigma^2)},$$

which suggests that the flexural rigidity EI of a microtubule and the mean square deflection <\sigma^2> of an axis point at a distance L from the clamped end are inversely proportional (Venier et al., 1994). This equation assumed that L is much smaller than the persistence length of the microtubule (~6000 μm, Gittes et al., 1994). The remaining parameters are the absolute temperature (T) and the Boltzmann constant (k_B).

Microtubules were assembled from axoneme fragments and imaged using video-enhanced DIC microscopy at video rates (30 frames/second; Vasquez et al., 1994). Tubulin was assembled at 10 μM with or without 0.2 μM XMAP215. The effects of XMAP215 on dynamic instability have been previously published (Vasquez et al., 1994). Tubulin was assembled at 10 μM with or without 0.2 μM XMAP215. The effects of XMAP215 on dynamic instability have been previously published (Vasquez et al., 1994). The video tapes were analyzed here for rigidity by measuring microtubule thermal vibration. Axonemes adhered to the glass surface and therefore acted as a clamped point of the microtubule. Microtubule plus ends of less than 10 μm length and free to vibrate, owing to thermal fluctuation, were selected for analysis. S-VHS tape sequences were digitized and captured into a computer (Power Macintosh) equipped with an image frame grabber board (Scion LG-3 PCI) and running the NIH-Image software. The capture rate was 15 frames/second, which translates to 67 msec intervals between successive frames. For each series of frames describing one microtubule, an arc line perpendicular to the microtubule long axis was drawn near the vibrating microtubule tip. For each advancing image frame, the cursor was overlaid on the image of the microtubule lattice intersecting the arc line, and the x-y coordinates of the position of interest were recorded. The point (x_0, y_0) represents the position of the microtubule at the axoneme attachment site. The 50 collected points ((x_1, y_1), ..., (x_50, y_50)) represent the positions of the vibrating tip of the microtubule for each of the 50 successive frames. The 50 points represent a spread of the different positions of the microtubule tip to the left and right of a mean position (x_{mean}, y_{mean}), where:

$$x_{mean} = \frac{1}{50} \sum_{i=1}^{50} x_i,$$

$$y_{mean} = \frac{1}{50} \sum_{i=1}^{50} y_i.$$  

The mean-squared deflection <\sigma^2> is calculated as:

$$\langle \sigma^2 \rangle = \frac{1}{50} \sum_{i=1}^{50} \sigma_i^2,$$  

where,

$$d^2 = (x_i - x_{mean})^2 + (y_i - y_{mean})^2.$$  

The length L of the microtubule is calculated as:

$$L = \sqrt{(x_0 - x_{mean})^2 + (y_0 - y_{mean})^2}.$$  

RESULTS

XMAP215 is a long, thin protein

Previous sedimentation and chromatography experiments had suggested that XMAP215 is an elongated protein (Gard and Kirschner, 1987). We confirmed this result by sedimentation of XMAP215 on a 15-40% glycerol gradient. In two experiments, XMAP215 sedimented between catalase (11.3 S) and albumin (4.6 S) at approximately 7S (data not shown). The ratio of Sm to S is a useful guide to estimate the shape of proteins, where Sm is the sedimentation coefficient of an unhydrated sphere of radius sufficient to contain the mass of protein, and S is the measured sedimentation coefficient. Sm/S is equal to the frictional ratio, f/l, as defined by Tanford (Tanford, 1961); for globular proteins Sm/S is in the range of 1.2-1.3, while for elongated proteins it is 1.7-2.0 or higher (Tanford, 1961). For the 228 kDa XMAP215, Sm is 14 (see Materials and Methods), giving Sm/S of 2.0. This ratio is the same as that found for a segment of tenascin containing eight fibronectin III domains, which is 25 nm long by 2.5 nm in diameter (Schürmann et al., 2001). Therefore, we conclude that XMAP215 is a highly elongated protein.

The shape of XMAP215 was observed directly by electron microscopy after unidirectional shadowing and appeared as a long, thin molecule of uniform density along its length (Fig. 1A). A corresponding gel of a glycerol gradient fraction used for shadowing is shown in Fig. 1B. Based on electron microscopy, we found no evidence for globular domains within the protein. In general, most XMAP215 molecules had a straight conformation, but several molecules were bent, suggesting that XMAP215 had some flexibility. The average length of an XMAP215 molecule was 60.0±17.7 nm (n=85) (Fig. 1C).

Gard and Kirschner (Gard and Kirschner, 1987) have previously confirmed that XMAP215 is a monomer in solution, based on results from size-exclusion chromatography and sucrose gradient sedimentation. To confirm that XMAP215 is a monomer and ensure that the above length measurement reflects the length of a single protein, we incubated XMAP215 with an affinity purified antibody raised against the C-terminus of TOGP. This antibody also recognizes XMAP215 (Fig. 2A). After unidirectional shadowing, antibody labeling was detected as a knob at one end of the protein (Fig. 2B) and the size of the knob (~13.5 nm) is consistent with the size of single antibody molecules observed by others (Wille et al., 1992). No evidence for XMAP215 dimerization was observed, consistent with previous hydrodynamic experiments (Gard and Kirschner, 1987) and it is therefore highly likely that the long rods shown in Fig. 1A represent the individual XMAP215 molecules.

Based on the molecular mass of XMAP215 (228,000; Tournebize et al., 2000, Becker and Gard, 2000) and the length (60 nm) of a single molecule, XMAP215 has a mass density of ~3800 Da/nm. This mass density is approx. twofold higher than that determined for other MAPs (see Table 1 for comparison).
XMAP215 binds partial tubulin rings

To examine whether XMAP215 can bind tubulin dimers or oligomers, XMAP215 and tubulin were incubated on ice to prevent microtubule assembly. Samples were then rotary shadowed and observed by electron microscopy. Under these conditions, addition of XMAP215 resulted in assembly of partial tubulin rings (Fig. 3). These partial rings were not observed in the absence of XMAP215 (Fig. 3). To compare the XMAP215/tubulin partial rings with those assembled from tubulin alone, we found that a higher glycerol concentration (30%) and a longer incubation time (several hours) resulted in partial ring assembly from pure tubulin (Fig. 3).

The partial rings assembled with XMAP215 appeared uniform in width and were wider than rings assembled from tubulin alone (Fig. 4A); XMAP215/tubulin partial rings had an average width of 8.8±1.8 nm (s.d.; n=59) compared with 5.6±1.1 (s.d.; n=64) for the tubulin partial rings. These mean widths are statistically different (P<0.01) and suggest that XMAP215 adds a width of approximately 3.2 nm to the curved tubulin protofilament. The lengths of the rings (Fig. 4B) also differed significantly (P<0.01); rings assembled from tubulin and XMAP215 measured 59.6±26.2 nm s.d. (n=86) compared with 48.6±24.8 nm (s.d.; n=133) for tubulin alone.

The curvature of the partial rings was also measured by determining the radius of a circle overlapping the arc formed by the partial rings (Fig. 4D). The partial rings had an average radius of curvature of 26.9 nm for tubulin alone and 26.8 nm for samples containing XMAP215 (Fig. 4C). These measured values are close to those reported previously for a protofilament ring (Voter and Erickson, 1979; Mandelkow et al., 1991). Clearly, the presence of XMAP215 had no effect on the curvature of the partial rings.

Microtubule bending stiffness is not altered by XMAP215

To determine whether XMAP215 affected the stiffness of microtubules, we measured microtubule plus end tip deflections and used these displacements to calculate microtubule flexural rigidity (see Materials and Methods). Vibration measurements were made on microtubule plus ends elongating from axoneme fragments in the absence or presence of XMAP215. Plus-end assembly was stimulated eightfold using the maximum possible concentration of XMAP215. Under these conditions, plus-end microtubules assembled without XMAP215 had a flexural rigidity of 17.5±2.2 (10⁻²⁴/Nm²; n=27) compared with 18.5±2.0 (10⁻²⁴/Nm²; n=25) for microtubules assembled from tubulin alone. Thus, XMAP215 did not change microtubule stiffness.

Four N-terminal repeats in XMAP215 are composed of HEAT repeats

Previous alignments of the XMAP215 family protein sequences have recognized that the segment corresponding to XMAP215 264-543 is highly conserved in all family members (Graf et al., 2000; Popov et al., 2001). Cullen et al. (Cullen et
al., 1999) noted further that there were actually four repeats of this subdomain in the N-terminal region of TOGp and
Drosophila Msps. They identified four separate motifs that made a common signature for the subdomain, but the overall sequence identity between the four subdomains is quite limited, making alignment difficult. Recent sequence analysis suggests that the XMAP215 family members contain a large number of HEAT repeats (Neuwald and Hirano, 2000; see Discussion). We found that use of these poorly conserved HEAT repeat sequences provided a guide for alignment within the N terminus of XMAP215 and that this analysis removed most ambiguities. As shown in Fig. 5A, there are five HEAT repeats in each of the four N terminal subdomains (N1-N4), and possibly a sixth one following these not recognized by sequence. The arrangement of HEAT repeats and the subdomains is shown in Fig. 5B.

**DISCUSSION**

The results of our EM observations showed that XMAP215 is a thin, rod-shaped molecule of approximately 60 nm length (Fig. 1A,C). The sedimentation coefficient of 7 S measured in our study is close to the 5 S reported by Gard and Kirschner (Gard and Kirschner, 1987). Both values are consistent with the highly elongated shape seen by EM. The width of the XMAP215 molecule could not be accurately measured, as we used uni-directional shadowing to visualize the protein. However, we could estimate the width of XMAP215 from the difference in width between partial tubulin rings assembled in the presence and absence of XMAP215 (Figs 3, 4); these results suggest that XMAP215 is approximately 3.2 nm wide. The thickness of XMAP215 can also be estimated from its mass density of approximately 3800 Da/nm. If XMAP215

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**Table 1. Mass per unit length for XMAP215 and other MAPs**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mass (Da)</th>
<th>Length (nm)</th>
<th>Mass density (Da/nm)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>XMAP215</td>
<td>228,373</td>
<td>60</td>
<td>3806</td>
<td>Present study</td>
</tr>
<tr>
<td>MAP2</td>
<td>198,859</td>
<td>180</td>
<td>1105</td>
<td>Voter and Erickson, 1982</td>
</tr>
<tr>
<td>MAP1B</td>
<td>270,453</td>
<td>186</td>
<td>1454</td>
<td>Wille et al., 1992</td>
</tr>
<tr>
<td>CLIP-170</td>
<td>156,698</td>
<td>135</td>
<td>1160</td>
<td>Sato-Yoshitake et al., 1989</td>
</tr>
<tr>
<td>Tau</td>
<td>36,737-45,822</td>
<td>35</td>
<td>1469-1309</td>
<td>Schweers et al., 1994</td>
</tr>
</tbody>
</table>

Mass density calculations used the predicted molecular weights determined by sequence analysis; therefore some values listed above will differ from previously published calculations.
were a compact cylinder of protein with a density of 1.37 g/cc, it would have a diameter of 1.3 nm. This would be the smallest possible diameter for a cylinder of this mass and length. If the molecule had a more irregular folding or domain structure, the diameter could easily be 2-3 times greater.

The binding of XMAP215 to partial tubulin rings suggests that XMAP215 binds along a single protofilament, since a tubulin ring consists of a curved protofilament (Voter and Erickson, 1979). This conclusion is consistent with results from experiments with TOGp, demonstrating binding to microtubules, zinc sheets and tubulin rings (Spittle et al., 2000). The 60 nm length of XMAP215 suggests that this MAP could span seven to eight dimers along a protofilament. It is remarkable that the length of the partial tubulin rings assembled with XMAP215 (59.6 nm) is identical to the average length of the MAP (60 nm), suggesting that tubulin dimers may have bound all along the length of a single XMAP215 molecule.

Our results are thus consistent with evidence that XMAP215/TOGp binds to microtubules with a large surface of the MAP bound to the microtubule lattice. Microtubule binding assays of TOGp and fragments demonstrated that one microtubule-binding domain was located within a region of approximately 600 amino acids near the N terminus and a second region in the C-terminal half of the protein was able to bind tubulin dimers or oligomers (Spittle et al., 2000). Expression of truncated segments of XMAP215 in vivo also suggests that the entire protein participates in microtubule binding (Popov et al., 2001). Taken together, these results suggest a widely distributed microtubule binding interface on XMAP215 and are consistent with recent insights into the structure of this protein, as discussed below.

**XMAP215 is composed of multiple HEAT repeats**

Recent iterative sequence analysis identified 20 HEAT repeats in TOGp (Neuwald and Hirano, 2000) and we find that these are also present in XMAP215 (Fig. 5A; data not shown). To compare our EM structure with the structures of well-defined HEAT repeat proteins, we used RASMOL (Sayle and Milner-White, 1995) to measure the spacings of HEAT repeats in PR65/A (PDB # 1B3U), a subunit of PP2A that contains 15 tandem HEAT repeats (Groves et al., 1999). The spacing of 1.15 nm corresponds to a mass density of approximately 3750 Da/nm. This value compares well with the 3800 Da/nm estimated for XMAP215 (Table 1). Additionally, PR65/A has a depth of 2 nm and a width of 3.5 nm (Groves et al., 1999), similar to the 3.2 nm width we estimate for XMAP215, based on its binding to partial tubulin rings. Finally, HEAT repeat proteins are thought to be flexible (Kobe et al., 1999), which is consistent with the variable bending seen in our EM of XMAP215.
Fig. 5. (A) Alignment of the four subdomains within the N-terminal half of XMAP215, labeled N1-N4 and identified specifically on the sequence. (B) A diagram of the TOGp sequence showing the location of HEAT subdomain, but this is not recognizable from the sequence. The analysis shows four segments where three subdomains have HEAT repeats, suggesting that the fourth subdomain also has one, not identified by the sequence analysis. A sixth HEAT repeat may occur in the final part of each subdomain, but this is not recognizable from the sequence. (B) A diagram of the TOGp sequence showing the location of HEAT subdomain, but this is not recognizable from the sequence. The analysis shows four segments where three subdomains have HEAT repeats, suggesting that the fourth subdomain also has one, not identified by the sequence analysis. A sixth HEAT repeat may occur in the final part of each subdomain, but this is not recognizable from the sequence.
may compete for binding sites at microtubule ends and sterically hinder XKCM1 access to the microtubule. Alternatively, Popov et al. (Popov et al., 2001) have suggested that XKCM1 and XMAP215 may interact when not bound to the microtubule. In this case XMAP215 and microtubule ends would compete for binding to XKCM1.

In summary, XMAP215 is a thin protein that has a size and shape consistent with a structure based on multiple HEAT repeats. This structure provides an extended interface for interaction with the microtubule lattice or other binding partners. The proposed domain structure may provide the basis for making smaller expression proteins for biochemical and structural studies; this may lead to an understanding of how XMAP215 functions to speed assembly and protect ends from XKCM1.

APPENDIX

Thermal fluctuation analysis theory

Fig. 6 shows a schematic drawing of a microtubule clamped at one end by attachment to the axoneme, and free at the other end to vibrate in solution due to thermal fluctuation. Let the microtubule profile be represented by the function y(x). The curvature (i.e. inverse of radius-of-curvature) of y(x) is given by the equation:

\[ \frac{1}{R} = \frac{d^2y}{dx^2} \left[ 1 + \left( \frac{dy}{dx} \right)^2 \right]^{3/2}. \]  

(A1)

If the curvature is large (i.e. the deflection d is small) then we can assume \(dy/dx=0\) and Eqn 1 reduces to:

\[ \frac{1}{R} = \frac{d^2y}{dx^2}. \]  

(A2)

The bending moment \(M(x)\) (torque about the neutral axis of any cross section) of the microtubule at a given point from the origin is:

\[ M(x) = F[L-x] = \frac{EI}{R} \frac{d^2y}{dx^2}, \]  

(A3)

where \(F\)=perpendicular force vector at \(x\), \(L=\)total length of beam and \(EI=\)flexural rigidity. The flexural rigidity \(EI\) is the product of \(E\), the Young’s elastic modulus, and \(I\), the moment area of inertia. Rearranging Eqs 2 and 3, we get:

\[ \frac{d^2y}{dx^2} = \frac{F[L-x]}{EI}. \]  

(A4)

We can integrate Eqn. 4 twice to yield,

\[ \int \frac{d^2y}{dx^2} = \frac{F}{EI} \left[ Lx - \frac{x^2}{2} \right] = \frac{dy}{dx}, \]  

(A5)

and,

\[ \int \frac{dy}{dx} = \frac{F}{EI} \left[ \frac{Lx^2}{2} - \frac{x^3}{6} \right] = y(x). \]  

(A6)

At \(x=0\), Eqn 6 reduces to,

\[ y(0) = \frac{FL^3}{3EI}. \]  

(A7)

The quantity \(y(L)\) is the displacement, \(d\), of the tip of the microtubule. The condition at the tip of the clamped microtubule is also analogous to Hooke’s law for a spring. For a spring, the force and potential energy associated with a deflection through a distance \(y\) are:

\[ F(y) = Ky \]  

(A8)

\[ PE(y) = \frac{1}{2} Ky^2, \]  

(A9)

where \(K=\)the spring constant, and \(y=d\). Rearranging Eqns 7 and 8 we arrive at:

\[ \kappa = \frac{3EI}{L^3}. \]  

(A10)

According to the equipartition theorem (Reif, 1965), the mean potential energy is:

\[ \langle PE \rangle = \frac{1}{2} k_BT. \]  

(A11)

where \(k_B=\)Boltzmann constant and \(T=\)absolute temperature. So, from Eqn. 9,

\[ \langle PE \rangle = \frac{1}{2} \kappa \langle y^2 \rangle. \]  

(A12)

Rearranging Eqns 11 and 12, we get:

\[ \frac{1}{2} \kappa \langle y^2 \rangle = \frac{1}{2} k_BT. \]  

(A13)

Substituting Eqn 10 into 13, and rearranging, we get:

\[ \frac{3EI}{L^3} = \frac{k_BT}{\langle y^2 \rangle}, \]  

(A14)

or,

\[ EI = \frac{k_BT L^3}{3\langle y^2 \rangle}. \]  

(A15)

where \(<y^2>=<d^2>\) is the mean square displacement of the microtubule tip.

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REFERENCES


