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Structural Model for Tubulin Recognition and Deformation by Kinesin-13 Microtubule Depolymerases

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SUMMARY

To elucidate the structural basis of the mechanism of microtubule depolymerization by kinesin-13s, we analyzed complexes of tubulin and the *Drosophila melanogaster* kinesin-13 KLP10A by electron microscopy (EM) and fluorescence polarization microscopy. We report a nanometer-resolution (1.1 nm) cryo-EM three-dimensional structure of the KLP10A head domain (KLP10AHD) bound to curved tubulin. We found that binding of KLP10AHD induces a distinct tubulin configuration with displacement (shear) between tubulin subunits in addition to curvature. In this configuration, the kinesin-binding site differs from that in straight tubulin, providing an explanation for the distinct interaction modes of kinesin-13s with the microtubule lattice or its ends. The KLP10AHD-tubulin interface comprises three areas of interaction, suggesting a crossbow-type tubulin-bending mechanism. These areas include the kinesin-13 family conserved KVD residues, and as predicted from the crossbow model, mutating these residues changes the orientation and mobility of KLP10AHDs interacting with the microtubule.

INTRODUCTION

Kinesins are a superfamily of microtubule (MT)-associated ATPases characterized by the presence of a highly conserved catalytic or "motor" head domain (HD, ~340 residues long) containing MT- and ATP-binding sites (Goldstein and Philp, 1999). Although most kinesins work as motile molecular motors generating force and movement along MTs, some kinesins act as MT depolymerases. The latter group includes the kinesin-13 family members, which were the first to be clearly recognized as non-motile kinesins with MT depolymerase activity (Desai et al., 1999).

Kinesin-13s have been reported to play important roles in various cellular processes, including mitosis (Manning et al., 2007; Rogers et al., 2004), cytokinesis (Rankin and Wordeman, 2010), axonal branching (Homma et al., 2003), and ciliogenesis (Kobayashi et al., 2011). Kinesin13s have also attracted considerable attention as potential targets for anticancer therapy due to their effects on MT dynamics and their involvement in mitosis (Sanhaji et al., 2011).

Although much work has been devoted to elucidating the mechanism of action of kinesin motors, it is still not fully clear why some are motile and others are MT depolymerases. The molecular mechanism of kinesin-13 MT depolymerization likely involves the stabilization of a curved tubulin structure, which is incompatible with the formation of lateral interprotofilament contacts in the MT lattice (Desai et al., 1999). However, how kinesin-13s induce tubulin curvature is not well understood. Catalytic activity is coupled to ATP hydrolysis (Hunter et al., 2003) as in other kinesins, but the interaction of kinesin-13s with MTs is very different from that of motile kinesins. Motile kinesins alternate between strong and weak interactions with tubulin as they translocate along the MT lattice. On the other hand, kinesin-13s bind to the MT lattice weakly and undergo unbiased one-dimensional (1D) diffusion until they reach the end of the MT, where they induce depolymerization (Helenius et al., 2006). How kinesin-13 distinguishes tubulin in the MT lattice or at the MT ends is unknown.

As with motile kinesins, many key kinesin-13 functions, such as ATP hydrolysis and tubulin binding, are located in the kinesin-13 HD, but additional areas outside the HD are also functionally important (Hertzer et al., 2006; Maney et al., 2001). A positively charged, ~60-residue-long sequence N-terminal to the HD, known as the neck, promotes MT binding (Cooper et al., 2010) and is critical for normal in vivo MT depolymerization (Ovechkina et al., 2002). The neck may also increase MT depolymerization efficiency by inducing the two HDs of a full-length kinesin-13 dimer to bind adjacent protofilaments (Mulder et al., 2009). However, the kinesin-13 HD alone has the ability to induce tubulin curvature and depolymerize MTs (Moores et al., 2002; Tan et al., 2008). Thus, elucidating how the kinesin-13 HD in particular binds to and bends tubulin is crucial for understanding the mechanism of kinesin-13-mediated MT depolymerization.

Previous lower-resolution electron microscopy (EM) studies have indicated that the kinesin-13 HD binds to curved tubulin in a configuration that is similar to other kinesin HDs bound to straight tubulin in the MT lattice (Tan et al., 2008). This similarity makes it difficult to explain how the kinesin-13 HD binds to and bends tubulin. To address this problem, we analyzed the structure of several complexes of tubulin and the *Drosophila*

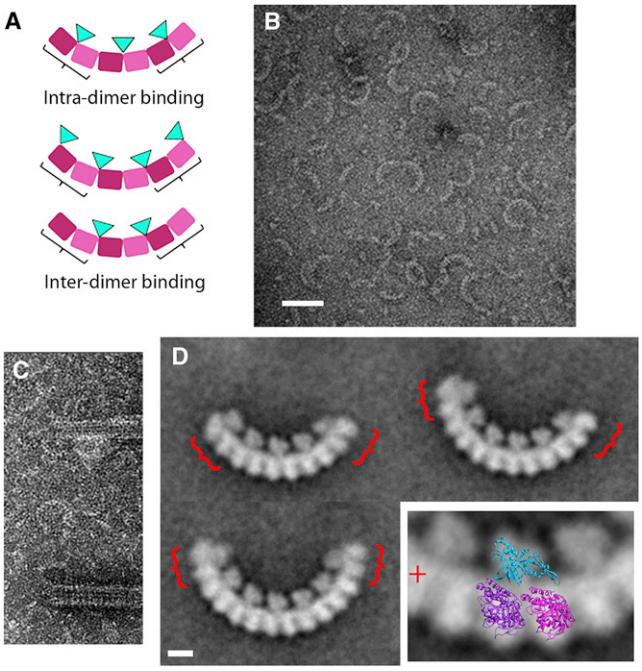

Figure 1. KLP10AHD Binds to the Tubulin Intradimer Interface

(A) Alternative inter- versus intratubulin dimer-binding modes for the KLP10AHD-tubulin complex. Two possibilities for the interdimer case are considered depending on whether the KLP10AHD can bind to a single tubulin subunit at the end of the protofilament. Cyan triangles: KLP10AHD; purple and magenta squares: tubulin.

(B) Electron micrograph of a field with open ring structures formed by incubating free tubulin with KLP10AHD in the presence of AMP-PNP. Scale bars, ~45 nm.

(C) Electron micrographs showing tubulin ring depolymerization intermediates in the background and at the MT ends formed by incubating MTs with KLP10AHD in the presence of ATP.

(D) Three independent image class averages. The tubulin dimer at the protofilament end is indicated by the red brackets. The inset shows atomic models of tubulin (purple and magenta) and the KLP10HD (cyan) superimposed on one of the average images. Scale bars, ~8 nm.

See also Figures S1, S2, and Movie S5.

melanogaster kinesin-13 KLP10AHD using EM and fluorescence polarization microscopy (FPM). Our data identify the kinesin-13 HD binding site on the tubulin intradimer interface, reveal a novel tubulin conformation, and provide an intriguing explanation for the distinct modes of interaction of kinesin-13s with straight or curved tubulin. We propose that the kinesin-13 HD induces a curved-sheared (CS) tubulin configuration by a crossbow-type bending mechanism. In this mechanism, the kinesin-13 HD bends the tubulin dimer by pulling the two tubulin subunits relative to the intradimer interface.

RESULTS

KLP10AHD Binds to the Intradimer Interface

A critical missing piece of information regarding the molecular mechanism of kinesin-13-induced MT depolymerization is the location of the kinesin-13 HD binding site on tubulin. Due to the similarity between α- and β-tubulin, it has not been possible to distinguish whether the kinesin-13 HD binds the tubulin interdimer or intradimer interface. The structures of α- and β-tubulin are only distinguishable at near-atomic resolution (Nogales et al., 1998), which makes it impossible to distinguish between the intra- and interdimer interfaces in medium-resolution EM studies. Additional experiments are needed to make this distinction unambiguously, and, to our knowledge, this has been done only for conventional kinesin (Marx et al., 2006). However, given the sequence similarities between the two tubulin interfaces (Wang and Nogales, 2005), and the completely different functionalities associated with kinesin-1 and kinesin-13s, it cannot simply be assumed that these two kinesin types bind to the same tubulin site.

Table 1. Particle Measurements Summary

	Ring Curvature (Deg/Tub Dimer)					
	Avg	SD	Min	Max	N Part.	KinRing/Tot
AMMPNP	26.3	2.3	12.7	32.1	4,773	0.996
ADP-ALIF$_4^-$	25.6	1.8	20.2	28.33	3,662	0.434
ADP	28.0	2.9	22.5	37.5	499	0.275
NN	–	–	–	–	683	0

Ring curvature was measured in the class-average images. The given statistics are weighted according to the number of particles in each class group. N part. is the total number of particles selected. KinRing/Tot is the ratio of particles that were positively identified as KLP10AHD-tubulin ring complexes to the total number of particles. See also Experimental Procedures and Figures S1 and S2.

To distinguish between α- and β-tubulin in the kinesin-13-tubulin complexes, we sought to visualize the complex at the protofilament ends where the tubulin interdimer interface is exposed (Figure 1A). Figure 1B shows a negative-staining electron micrograph of a field with many tubulin open rings produced by incubating KLP10AHD with tubulin in the presence of the nonhydrolyzable ATP-analog AMP-PNP. Similar rings are observed as depolymerization products when MTs are incubated with KLP10AHD in the presence of ATP (Figure 1C). We observed open and closed rings, as well as longer spirals, but concentrated our analysis on the open ones obtained in the presence of AMP-PNP because they were more numerous and homogeneous. We selected thousands of individual ring images (Table 1) and subjected them to single-particle image analysis to produce class averages with improved signal-to-noise ratio. The classification procedure separated image groups according to the number and curvature of tubulin heterodimers included in the ring, as well as other nonstructure-related image differences (Figures S1 and S2). Figure 1D shows three class averages of particles with distinct numbers of tubulin heterodimers (n = 5, 6, and 7), giving six independent, well-aligned protofilament end averages. In all cases, a density corresponding to KLP10AHD is centered at the intradimer interface, as in the model shown at the top of Figure 1A. Therefore, we conclude that the kinesin-13 HD binds at the tubulin intradimer interface.

Nucleotide Dependence of the KLP10AHD–Curved Tubulin Interaction

To investigate possible changes in the KLP10AHD-tubulin complex related to the kinesin ATPase activity, we analyzed

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