Cell

Insights into Antiparallel Microtubule Crosslinking by PRC1, a Conserved Nonmotor Microtubule Binding Protein

Radhika Subramanian,[1] Elizabeth M. Wilson-Kubalek,[2] Christopher P. Arthur,[2] Matthew J. Bick,[3] Elizabeth A. Campbell,[3] Seth A. Darst,[3] Ronald A. Milligan,[2] and Tarun M. Kapoor[1,*]
[1]Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York, NY 10065, USA
[2]Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037, USA
[3]Laboratory of Molecular Biophysics, The Rockefeller University, New York, NY 10065, USA
*Correspondence: kapoor@mail.rockefeller.edu
DOI 10.1016/j.cell.2010.07.012

SUMMARY

Formation of microtubule architectures, required for cell shape maintenance in yeast, directional cell expansion in plants and cytokinesis in eukaryotes, depends on antiparallel microtubule crosslinking by the conserved MAP65 protein family. Here, we combine structural and single molecule fluorescence methods to examine how PRC1, the human MAP65, crosslinks antiparallel microtubules. We find that PRC1's microtubule binding is mediated by a structured domain with a spectrin-fold and an unstructured Lys/Arg-rich domain. These two domains, at each end of a homodimer, are connected by a linkage that is flexible on single microtubules, but forms well-defined crossbridges between antiparallel filaments. Further, we show that PRC1 crosslinks are compliant and do not substantially resist filament sliding by motor proteins in vitro. Together, our data show how MAP65s, by combining structural flexibility and rigidity, tune microtubule associations to establish crosslinks that selectively "mark" antiparallel overlap in dynamic cytoskeletal networks.

INTRODUCTION

The dynamic reorganization of microtubule networks plays a critical role in diverse biological processes, including cell migration, neuronal transport and cell division. It is now clear that different cytoskeletal architectures arise from the interplay between motor proteins, which can crosslink and move microtubules relative to one another, and nonmotor microtubule associated proteins (MAPs), which can crosslink microtubules to stabilize specific orientations (Glotzer, 2009; Manning and Compton, 2008). While we have good biophysical and structural models for motor proteins that crosslink microtubules, much less is known about nonmotor microtubule crosslinking proteins.

Several nonmotor MAPs that crosslink microtubules (e.g., MAP65, NuMA, NuSAP and Mia1p) are now known to play important roles in dividing and nondividing cells (Ribbeck et al., 2006; Sasabe and Machida, 2006; Schuyler et al., 2003; Thadani et al., 2009; Zeng, 2000). Current models for the functions of these proteins are based on cellular localizations and loss-of-function studies. However, we lack any structural data to explain how microtubule crosslinking is achieved by these MAPs. Recently, there have been several advances in our understanding of the structure of nonmotor MAPs. Among the best characterized class of MAPs are the +TIP proteins (e.g., XMAP215, EB1 and CLIP170), which can dynamically track the growing end of a microtubule. Microtubule binding in these proteins is mediated by calponin-homology, CAP/Gly or TOG domains (Slep and Vale, 2007). Similarly, structural work on Ndc80, a conserved mitotic MAP, has revealed how a calpolin-homology domain may be used to establish kinetochore-microtubule associations during cell division (Ciferri et al., 2008; Wei et al., 2007; Wilson-Kubalek et al., 2008). However, due to lack of similarity in primary sequence, it is not very likely that these structural models will shed light on nonmotor MAPs that can crosslink two microtubules.

As a step toward developing structural models for how nonmotor MAPs may crosslink microtubules, we focused on the conserved MAP65 family, which plays key roles in microtubule organization in eukaryotes. Since their initial discovery in budding yeast, microtubule crosslinking functions of the MAP65 proteins have been shown to be required for cell shape maintenance in yeast cells, directional cell expansion in plants and formation of the central spindle in eukaryotes (Chan et al., 1999; Jiang et al., 1998; Loiodice et al., 2005; Yamashita et al., 2005). Currently, at least three activities have been ascribed to these proteins. First, MAP65s can selectively crosslink microtubules in an antiparallel orientation (Gaillard et al., 2008; Loiodice et al., 2005). Second, these nonmotor crosslinkers can oppose filament movements driven by motor proteins. For example, Ase1, the fungal MAP65, is proposed to antagonize kinesin-14 driven filament sliding required for organizing microtubules during interphase (Janson et al., 2007). Third, these crosslinking proteins can recruit signaling proteins or kinesins to the microtubule structures they stabilize. For example, the recruitment of Polo-like kinase to the central spindle during cytokinesis is mediated via interactions with PRC1, the human MAP65 (Neef

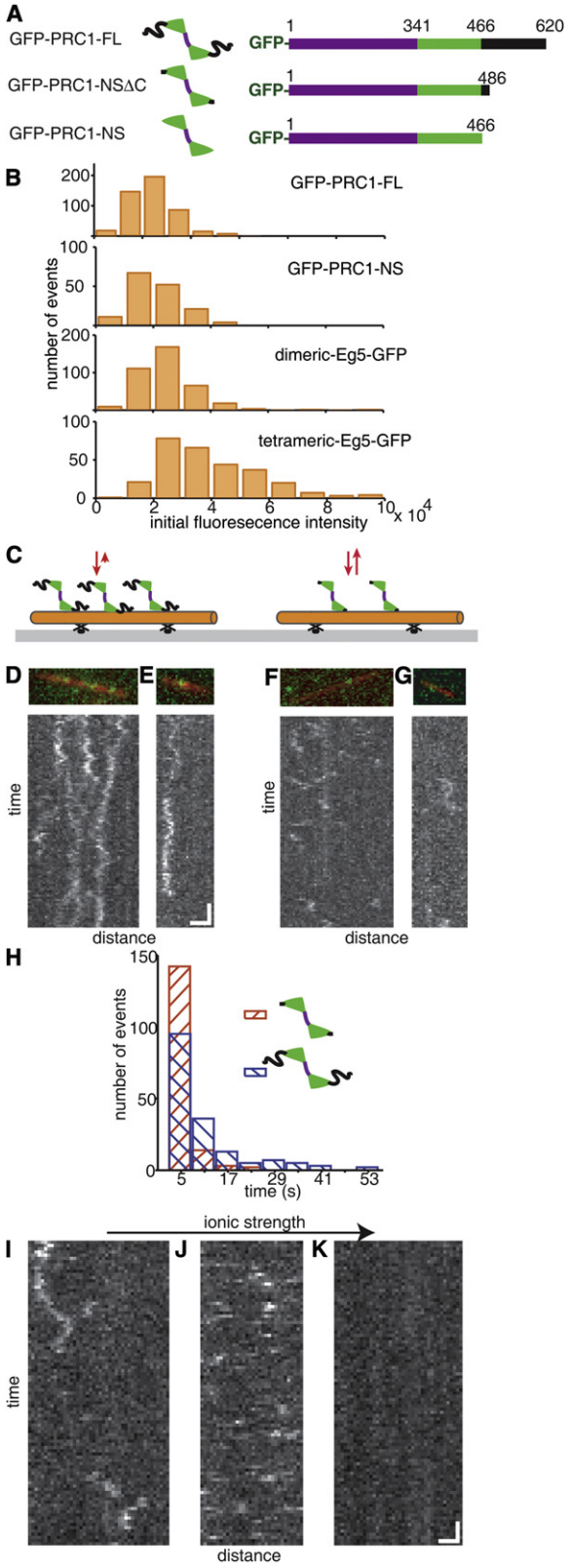

Figure 1. Single-Molecule Analysis of Microtubule Binding by PRC1

(A) Schematic of PRC1's domain organization and a guide for constructs used in the fluorescence microscopy assays (purple: coiled-coil domain; green: microtubule binding domain; black: C-terminal domain).

(B) Fluorescence intensity analysis of two PRC1 constructs, GFP-PRC1-FL (aa: 1–620; intensity = $2.5 \times 10^4 \pm 0.9 \times 10^4$, N = 469) and GFP-PRC1-NS (aa: 1–466; intensity = $2.0 \times 10^4 \pm 0.8 \times 10^4$, N = 156). Dimeric-Eg5-GFP (Intensity = $2.5 \times 10^4 \pm 1.0 \times 10^4$, N = 377) and tetrameric-Eg5-GFP (Intensity = $4.2 \times 10^4 \pm 2.2 \times 10^4$, N = 290) were used as references. Intensities are reported as mean ± SD.

(C–H) Single molecule TIRF assay was used to examine the association of PRC1 constructs (green) with microtubules (orange) immobilized on a glass surface. (C) Schematic for assay showing the two constructs, GFP-PRC1-FL and GFP-PRC1-NSΔC (aa 1–486). Single frames showing two-color overlays (top) and associated kymographs (below) of GFP-PRC1-FL (D and E) or GFP-PRC1-NSΔC (F and G). (H) Distribution of microtubule association lifetimes for GFP-PRC1-FL (blue) and GFP-PRC1-NSΔC (red).

(I–K) Microtubule association of GFP-PRC1-NSΔC under different ionic strength conditions. Representative kymographs from assays at 0.75x motility buffer (I), motility buffer (J), motility buffer+20 mM KCl. (K) The scale bar represents 1.5 μm, 10 s. See also Figure S1 and Figure S3.

et al., 2007) and kinesin-5 driven microtubule sliding during anaphase depends on Ase1 (Khmelinskii et al., 2009). Currently, we do not have a structural framework to explain how these MAPs specifically crosslink antiparallel microtubules. Moreover, the activities of MAP65s have not been reconstituted in the presence of motor proteins to test if MAP65s resist filament sliding by motor proteins or if their main function is to act as "marks" that recruit other proteins to regions of antiparallel microtubule overlap in dynamic networks.

Here we show, using single molecule fluorescence microscopy assays, X-ray crystallography and electron microscopy that PRC1 uses structured and unstructured domains to bind microtubules. These domains at each end of a PRC1 homodimer are connected by a linker that adopts a rigid conformation only when crosslinking microtubules. We also show, in assays combining TIRF and fluorescent speckle microscopy (FSM), that PRC1 does not substantially resist filament sliding by kinesin-5. Based on these results, we propose a model for how a crosslinking MAP can achieve specific and compliant crosslinking of microtubules by balancing structural rigidity and flexibility.

RESULTS

Structured and Unstructured Domains Mediate Microtubule Binding in PRC1

PRC1, like other Map65 family proteins, has a modular architecture with an N-terminal coiled-coil domain, a central region that can mediate microtubule binding, and a C-terminal regulatory domain (Figure 1A). While the central domain is thought to be required for microtubule binding, the contributions of the C-terminal domain remain poorly characterized. To address this, we analyzed the microtubule binding activity of PRC1 using two approaches, a TIRF microscopy assay to examine the properties of single molecules and a microtubule cosedimentation assay to analyze equilibrium binding.

For visualizing PRC1 molecules by fluorescence microscopy we expressed and purified recombinant GFP-tagged full-length PRC1 in bacteria. We found that C-terminal tags on PRC1