Friction on MAP Determines Its Traveling Direction on Microtubules

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Microtubule networks generate various forces, and the forces are applied to microtubule-associated proteins (MAPs). Forth et al. (2014) show in a recent issue of *Cell* that asymmetric frictional force between MAPs and microtubules leads to directional movement of MAPs along microtubules, providing insight into the mechanism of microtubule network self-organization.

Microtubules are dynamic protein polymers that play essential roles in various cellular processes such as cell division and motility. Microtubules are often organized into a highly ordered network in which multiple motors and nonmotor MAPs work in concert. To understand how the network is constructed, it is critical to identify the responsible proteins, as well as their biochemical activities toward microtubules. Toward this aim, researchers have typically prepared fluorescent-labeled microtubules and a purified protein of interest (motors/MAPs) labeled with a different color. Observation of the behavior of motors/MAPs on microtubules has provided knowledge as to how proteins bind to and move along microtubules and consequently affect microtubule dynamics and organization. An example is motor proteins; microscopic observation of purified kinesins or dynein has uncovered their directional motility and microtubule depolymerization activity (Vale, 2003). In a similar manner, the nonmotor proteins EB1 and CAMSAP/Nezha were shown to recognize growing plus and/or minus ends of microtubules (Jiang and Akhmanova, 2011).

More sophisticated in vitro assays, in which the cellular environment is mimicked via methods such as increasing component numbers or restricting the reaction volume, have recently been developed (Dogterom and Surrey, 2013). In a recent article in *Cell*, Kapoor and colleagues reconstructed another situation that mimics the cellular microtubule network (Forth et al., 2014). When microtubules are reorganized, polarized, or mobilized by motors, MAPs, or other associated factors such as the membrane, both the microtubules and the associated MAPs are inevitably exposed to external forces. Forth et al. (2014) addressed how nonmotor MAPs behave under such force-applied conditions and uncovered the relationship between force and directional movement of MAPs.

Forth et al. (2014) purified MAPs and microtubules and measured the force dependence of the interaction between a microtubule and a single MAP molecule by a technique combining optical trapping with total internal reflection fluorescence microscopy, utilized previously for motor proteins (Bormuth et al., 2009). Among the three nonmotor MAPs examined, NuMA (Radulescu and Cleveland, 2010) was most extensively analyzed in the study by Forth et al. (2014). When monomeric NuMA was bound to microtubules, it diffused along microtubules in an unbiased manner. However, when force was applied, microtubule-bound NuMA behaved very differently. The frictional force that arose when NuMA was dragged toward the plus end of microtubules was higher than when it was dragged toward the minus end, suggesting that NuMA tends to move in the minus end direction under force (Figure 1A). This behavior differs from the "biased diffusion" previously observed for several motor proteins in which diffusion along the microtubule is biased toward one direction; biased diffusion occurs without external force (Cooper and Wordeman, 2009).

Because NuMA likely functions as a homodimer and crosslinks microtubules in vivo (Radulescu and Cleveland, 2010), Forth et al. (2014) next used a dimeric form of NuMA and observed its behavior on a pair of parallel microtubules that were crosslinked by dimeric NuMA. Interestingly, when force was applied to the crosslinked microtubules, dimerized NuMA migrated to and accumulated at the minus ends of the microtubules. This finding suggests that under force, the combination of asymmetric frictional force along microtubules and dimerization can drive directional movement of a nonmotor MAP in crosslinked microtubules. Forth et al. (2014) constructed mathematical models based on the experimental results using monomeric NuMA and successfully predicted this behavior of dimeric NuMA.

Forth et al. (2014) also analyzed frictional force of two other MAPs that localize at different places along the microtubule. EB1 is a plus-end-tracking protein that recognizes and binds to the growing end of microtubules, whereas PRC1 specifically crosslinks antiparallel microtubules (Figure 1B). Interestingly, Forth et al. (2014) showed that EB1 exhibits lower friction toward the plus end while PRC1 exhibits no directional preference. These data suggest that different MAPs have different properties of frictional force (Figure 1A).

Although the experiments presented by Forth et al. (2014) were performed in an artificial system, the findings may well be relevant in a cellular context. The mitotic spindle or other microtubule-based structures indeed experience various forces produced by motor proteins or other factors. Furthermore, the observed behaviors of the three MAPs are logical with regard to their in vivo localizations and functions (Figure 1B). NuMA is critical for focusing (i.e., bundling) microtubules at the spindle pole, where minus ends of microtubules are predominant (Radulescu and

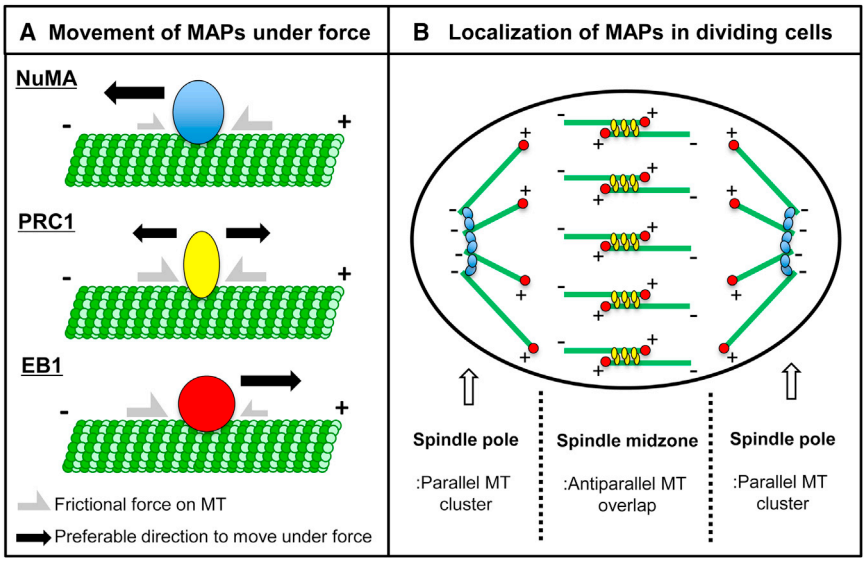

Figure 1. Movement of Nonmotor MAPs under Force and Their Localization in the Mitotic Spindle

(A) Movement of nonmotor MAPs (NuMA, PRC1, and EB1) under force. Under force, each MAP experiences asymmetric or symmetric frictional force along microtubules. The force could affect the distribution of each MAP along microtubules. (B) Localization of NuMA, PRC1, and EB1 during cell division. NuMA accumulates at the spindle pole, at which microtubule minus ends are clustered. EB1 is enriched at plus ends of microtubules. PRC1 localizes in the overlapping region of antiparallel crosslinked microtubules.

Cleveland, 2010). EB1, on the other hand, is enriched on the plus ends and interacts with other structures via their binding partners (i.e., force could be generated at the point of interaction) (Jiang and Akhmanova, 2011). PRC1 localizes in the overlapping region of antiparallel crosslinked and sliding microtubules (Walczak and Shaw, 2010), such that lack of directional preference would be favored. However, it is unlikely that the observed force-dependent movement is the sole mechanism for translocating these proteins in cells. Dynein-dependent transport also supports minus-end-directed motility of NuMA in cells (Radulescu and Cleveland, 2010), while EB1 autonomously tracks plus ends of growing microtubules without external force by directly recognizing plus-end-specific structures (Maurer et al., 2012). To understand the extent to which frictional force between MAPs and microtubules controls localization of MAPs in cells, it is important to design a specific mutant that alters the in vitro behavior and test the functionality of the mutant protein in vivo.

This study by Forth et al. (2014) suggests future experiments. The technique utilized in this study can be applied to other MAPs, in particular those that accumulate at plus or minus ends. The correlation between localization and frictional force asymmetry might be present with other MAPs. It is also of interest to understand the structural basis of the frictional force symmetry or asymmetry; some MAPs may have an unstructured region near the tubulin contact site, as discussed by Forth et al. (2014).

In summary, the study suggests a new mechanism for MAP-dependent organization of cell division machinery, which involves EB1, PRC1, and NuMA. MAP frictional asymmetry may also have a role in organizing other microtubule-based structures with a variety of shapes and sizes, such as neuronal structures (axons and dendrites), cilia, and flagella (Dogterom and Surrey, 2013; Subramanian and Kapoor, 2012). Furthermore, as was suggested by Forth et al. (2014), it would be intriguing to determine whether this type of regulation is conserved in other filamentous networks such as DNA or the actin cytoskeleton.

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