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Motor function in interpolar microtubules during metaphase

J.M. Deutsch, Ian P. Lewis

Department of Physics, University of California, Santa Cruz, CA 95064, United States

HIGHLIGHTS

- We examine the mechanism that results in constant spindle spacing during prometaphase.
- We analyze *in vitro* gliding assays of two antagonistic motors related to this process.
- Microtubule motion ceases with the right balance of these motor species.
- We show that these results can be explained by considering concentration fluctuations.
- These fluctuations pin the microtubule to points where it can only jitter randomly.

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ABSTRACT

We analyze experimental motility assays of microtubules undergoing small fluctuations about a "balance point" when mixed in solution of two different kinesin motor proteins, KLP61F and Ncd. It has been proposed that the microtubule movement is due to stochastic variations in the densities of the two species of motor proteins. We test this hypothesis here by showing how it maps onto a one-dimensional random walk in a random environment. Our estimate of the amplitude of the fluctuations agrees with experimental observations. We point out that there is an initial transient in the position of the microtubule where it will typically move of order its own length. We compare the physics of this gliding assay to a recent theory of the role of antagonistic motors on restricting interpolar microtubule sliding of a cell's mitotic spindle during prometaphase. It is concluded that randomly positioned antagonistic motors can restrict relative movement of microtubules, however they do so imperfectly. A variation in motor concentrations is also analyzed and shown to lead to greater control of spindle length.

1. Introduction

During mitosis, pole spacing is regulated by a system of interpolar microtubules. It has been proposed that the interpolar microtubules can be moved in two directions by opposing motors (Heck et al., 1993; Cole et al., 1994; Cottingham et al., 1999; Enos and Morris, 1990; Saunders and Hoyt, 1992; Sawin et al., 1992; Straight et al., 1998), but the details of such a proposed system are not yet well understood (Sharp et al., 1999; Brust-Mascher and Scholey, 2002). The interpolar microtubules are likely bundled and moved by two families of kinesin motor proteins; kinesin-5 and kinesin-14. Experiments with *Drosophila melanogaster* suggest that a kinesin-5 motor protein, KLP61F, plays a large role in creating the spindle during prometaphase (Heck et al., 1993). It has also been shown that kinesin-5 forms cross-bridges between interpolar microtubules in the centralspindlin (Sharp et al., 1999). Further experiments suggest that the same motor drives the separation of the poles during metaphase and anaphase (Brust-Mascher and Scholey, 2002; Tao et al., 2006a). In vitro experiments show that KLP61F slides antiparallel microtubules apart on motility assays, where motor proteins are bound to glass slides and move microtubules that are added to the solution (Tao et al., 2006a).

All of the above results show that kinesin-5 plays an important role in controlling the spindle spacing. Being a tetramer with both dimers at the N-terminus, the motor can walk toward the plus ends of two antiparallel microtubules, thus forcing the poles apart.

The kinesin-5 are antagonized by the kinesin-14, which walk toward the minus end of the microtubules. In vitro experiments show that a kinesin-14, Ncd, is capable of bundling microtubules and driving an inward sliding of the interpolar microtubules (Sharp et al., 1999). With one motor able to separate the poles, and one able to bring them closer, it seems possible that the two motors are responsible for maintaining spindle spacing and moving the poles apart. The net force exerted by the two motor species could govern the direction and rate of pole movement.

Recently, seminal work has been done in trying to understand how outward microtubule sliding generated by the kinesin-5 and inward sliding generated by the kinesin-14 could result in the stable, steady-state spindle spacing during prometaphase. A balance of forces could

E-mail addresses: josh@ucsc.edu (J.M. Deutsch), iplewis@ucsc.edu (I.P. Lewis).

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result in a stationary spindle, but it is unclear how the "collective antagonism" could occur (Tao et al., 2006a). In the following section, we will discuss one group's proposed solution to the problem.

1.1. Experimental work

Experiments with in vitro motility assays were performed to see if KLP61F and Ncd could interact to control the speed and polarity of microtubules' motility and whether the antagonism between the motors could stall microtubule sliding enough to produce the stable steady-state spindle spacing observed during prometaphase (Tao et al., 2006a). Before combining both motors in an assay, each motor was observed moving microtubules in motility assays as expected. KLP61F moved microtubules at 0.04 μm/s with the minus ends leading and Ncd moved microtubules at 0.1 μm/s with the plus ends leading (Tao et al., 2006a). Further experiments also showed that KLP61F alone, Ncd alone, and mixtures of the two motors bundled microtubules under conditions with physiological ATP concentrations (Tao et al., 2006a).

To see how the two species of motors would interact, different molar ratios of KLP61F and Ncd were mixed and microtubule motility was measured. A balance point at a mole fraction of 0.7 Ncd was found where microtubules displayed a mean velocity of approximately zero (Tao et al., 2006a). For greater mole fractions of Ncd, the mean velocity was plus end directed. Conversely, for smaller mole fractions of Ncd, the mean velocity was minus end directed, as shown in Fig. 5(a) of Tao et al. (2006a). The slope of the lines fit to the two sides of the balance point in this figure suggests that KLP61F is a strong, slow motor that is not slowed down easily by the weak, fast Ncd motor, which in turn is slowed down easily by KLP61F (Tao et al., 2006a). At the balance point, the microtubules were observed to display oscillatory motion between KLP61F and Ncd directed movement with intermediate rates of roughly 0.02 μm/s, as shown in Fig. 5(b) of Tao et al. (2006a). This is reproduced in Fig. 1(a), and the initial transient behavior is shown in Fig. 1(b).

Tao et al. (2006a) suggest that KLP61F and Ncd motors could act synchronously to antagonize one another. However, being an inherently stochastic process, it is hard to see how motor power stroking could become synchronized. In later work (Civelekoglu-Scholey et al., 2010), a fully stochastic force dependent detachment rate (FDDR) model was devised and tested numerically, and the synchronization was made more physically viable by a detailed model of the two motors' response to force and displacement. The model incorporates stochastic binding of a number of antagonistic motors to microtubules. One motor will "win" over the other kind, leading to motion in that direction. The losing motor will continue to try to bind to the microtubule, however the model posits a detachment rate that depends strongly on the force applied. Above some threshold force, these motors only bind for short times before detaching. This implies that once a direction has been chosen, it is hard for the losing motor to oppose this motion. Simulations show that only occasional changes in the sign of velocity can occur, on the scale of hundreds of seconds, happening only when an unlikely circumstance allows the losing motors to gain control. This kind of bistability in directionality has been considered before in the case of a single motor (Jülicher and Prost, 1995, 1997; Duke, 2002; Guérin et al., 2010), and also with antagonistic interactions (Gilboa et al., 2009). Therefore there are systems where this kind of behavior is well established. In order for this to be a viable explanation, first, the size of stochastic fluctuations has to be large enough to allow switching between the two opposing states (Badoual et al., 2002). Second, because the position of the microtubule remains constant during prometaphase, while additional forces act on it, the position of the microtubule should remain almost constant under the application of a small but finite force. We will discuss to what extent this bistability can explain this requirement in the following section.

In addition to bistability, Tao et al. (2006a) made the important observation that microtubules could be gliding on a spatially varying landscape, with varying densities of KLP61F and Ncd motors (Tao et al., 2006a). Periods of directional movement would be due to the patches in the environment where one motor is dominant. It is possible that the microtubule finds a "valley" in the landscape where it oscillates between patches of motors that move it back towards the balance point. It is this idea that we will attempt to model in the following section.

We show that the phenomenon is quite general and independent of the details in the parameters. If the system is rescaled to be dimensionless in length and time, we find that the behavior is only controlled by one parameter; the effective "temperature" of the system. A detailed understanding of the motors will only change this effective temperature and nothing else, since scaling laws for spatio-temporal fluctuations are universal. A study from this perspective also elucidates other aspects of this system, such as the nature of initial transients in motion of the microtubules in these assays before they reach a quasi-steady state. These transients have interesting implications, as we show that they also should occur for interpolar microtubules during metaphase.

2. Physical analysis of antagonistic motor assay

2.1. Average force-velocity dependence of antagonistic motors

We first consider the problem of a single molecular motor, such as kinesin, with the tail tethered to a substrate such as a glass plate while the heads can freely interact with a long microtubule, as

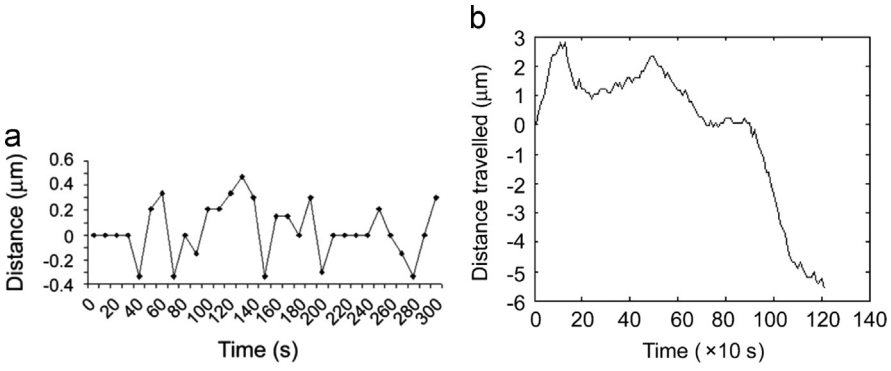

Fig. 1. (a) Plot of displacements versus time for a typical microtubule at the balance point. Reproduction of Fig. 5B from Tao et al. (2006a). (b) Initial transient behavior of this system. Reproduction of Fig. S3A from Tao et al. (2006a).