A Mathematical Model of Force Generation by Flexible Kinetochore-Microtubule Attachments

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ABSTRACT Important mechanical events during mitosis are facilitated by the generation of force by chromosomal kinetochore sites that attach to dynamic microtubule tips. Several theoretical models have been proposed for how these sites generate force, and molecular diffusion of kinetochore components has been proposed as a key component that facilitates kinetochore function. However, these models do not explicitly take into account the recently observed flexibility of kinetochore components and variations in microtubule shape under load. In this paper, we develop a mathematical model for kinetochore-microtubule connections that directly incorporates these two important components, namely, flexible kinetochore binder elements, and the effects of tension load on the shape of shortening microtubule tips. We compare our results with existing biased diffusion models and explore the role of protein flexibility inforce generation at the kinetochore-microtubule junctions. Our model results suggest that kinetochore component flexibility and microtubule shape variation under load significantly diminish the need for high diffusivity (or weak specific binding) of kinetochore components; optimal kinetochore binder stiffness regimes are predicted by our model. Based on our model results, we suggest that the underlying principles of biased diffusion paradigm need to be reinterpreted.

INTRODUCTION

The process of cell division involves a multitude of complex biochemical and mechanical events that lead to the equal partition of genetic material from the mother cell to the daughter cell. A fascinating and crucial process during division has to do with the generation and control of the movement of replicated chromosomes.

A chromosome must interact with microtubules (Mts), which are part of a dynamic network called the mitotic spindle (1–6). Connections between chromosomes and Mts are mediated by macromolecular structures called kinetochores (kts) (7–10). A variety of proteins that can associate with Mts directly localize at kts, however; Mts also undergo continuous growth and shortening while their ends are attached to the kt sites. A question of considerable interest in this context is how the kt site might function as a force-generating machine capable of moving chromosome several microns. A natural contender for this task would be molecular motor enzymes (11). However, molecular motor enzymes that localize at kts have been shown to be dispensable for kt motion in yeast (12,13). Kt nonmotor components should thus have the ability to generate movement by latching on to Mt tips that constantly lose or gain monomers; how such a task is achieved is not clear. Force generation at kts has consequently attracted considerable interest from both an experimental standpoint and quantitative modeling approaches (14–16).

Many components of kts have become known. Significant technical advances in high-resolution imaging have led to new insights regarding kt component spatial organization and copy numbers inside kts in a variety of organisms (17–19). A few proteins are emerging as important structural components of kts. The Ndc80 complex is an elongated dumbbell-like molecule with a high degree of flexibility because of a hinge site around its halfway point (20–22) that connects on one side to the kt structure and on the other to the Mts (23–25). KMN proteins are conserved kt components that form the primary kt-Mt interface (26); these proteins have affinity for the Mt, and importantly form a scaffold that acts to localize several kt kinases. The Dam1/DASH complex is essential in budding yeast and can form rings or spirals in the presence of Mt (27,28). Although there is no evidence that this complex can form rings in organisms other than yeast, it remains an important component at the kt-Mt interface. Further, it has been shown that Dam1 contain flexible elements for interaction with the kinetochore microtubule (kMt) (29). Finally, the Mis12 complex is another conserved kt component that can directly bind to the chromosomal chromatin (30,31).

Mts are polar hollow filaments composed of $\alpha\beta$ tubulin dimers that are arranged into linear chains called protofilaments. During mitosis, Mts undergo stochastic transitions between states of growth and shortening, known as "dynamic-instability" (32). Mts have a built-in polarity, with the plus-end experiencing faster growth/shortening than the minus-end. Tubulin adds to the Mt lattice in its Guanosine-triphosphate (GTP)-tubulin form; GTP is subsequently hydrolyzed into GDP-tubulin. The hydrolysis state of a tubulin dimer determines its preferred

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conformation: GTP dimers are thought to lie parallel to the Mt lattice, whereas Guanosine-diphosphate (GDP) tubulin prefers to bend away from the lattice (33). When a Mt disassembles, the tubulin at the Mt tips experience both loss of the GTP cap and lateral interactions that causes protofilaments to become relaxed and subsequently flare at the Mt ends (33,34). The plus-ends of Mts are embedded in the kt attachment site, and while attached, growth and shortening prevails. During this process, energy from GTP hydrolysis is released, and presumably this energy can be used by kt sites to generate motion (35–38).

Most of the existing theoretical models of Mt-kt coupling (35,39–42) are based on one of two postulated mechanisms for force generation. In the biased-diffusion model, initially proposed by Hill (35), the plus end of a kMt is assumed to be surrounded by a rigid coaxial "sleeve" the inner surface of which is composed of several binding elements that bind specific kMt sites. The one-dimensional Brownian motion of the sleeve along the axis of the kMt is biased to increase overlap, because a larger number of kMt-sleeve bindings lowers the total energy of the system. The interplay of this biased diffusion and the depolymerization of the kMt gives rise to the pull exerted by the coupler on the kt. The second proposed "power-stroke" coupling mechanism is based on the idea that the curling protofilament tips of a depolymerizing Mt exert a force on a rigid kt-connected sliding ring surrounding the Mt (40,41). These previous models capture several aspects of kt-Mt engagement; however, they ignore important mechanical features of the kt machine such as feedback between kMt protofilament shape and multiple flexible kt binder attachments under load. Specifically, kMt protofilaments at the curling ends of depolymerizing Mts can undergo shape changes when challenged by force. On the other hand, kMt protofilament shape can have significant effects on kMt depolymerization speeds, and on the ability of kt binders to engage with Mt tips. It is reasonable to expect that when modulation of Mt shape and kt binder attachment dynamics under load are combined together, mutual feedback might generate complex attachment responses. Novel modeling approaches are needed to account for these interactions.

Recent experimental results also highlight the need for a novel approach to kt-kMt modeling. There is evidence that kts engage kMts through multivalent attachments that move along Mts consistent with a biased diffusion mechanism (43). However, it also has been recently reported that all kt components are flexible, not rigid (44). Previous theoretical work that studied kt attachments in the biased-diffusion framework (including work from these authors (42)) assumes that kt multivalent attachments are rigid. Importantly, the role of kMt shape in depolymerization dynamics and kt attachments has been largely ignored in previous modeling work. Yet, recent data shows nonmonotonic sensitivity of kt-kMt attachment dynamics on the amount of tension load exerted on kts (45–47). These data collectively indicate that the kt/kMt juncture can respond to force in complex ways and thereby the kt machine may work in regimes where the two standard classes of models currently used do not apply.

The purpose of this paper is to develop a new model of force generation at the kt-Mt interface that incorporates kt-component flexibility, kMt protofilament shape mechanics, and kMt depolymerization kinetics. Using our model, we demonstrate how these features of kt junctures affect the ability of this attachment site to generate force for various parameter regimes. In so doing, we provide an alternate mechanism to rigid sleeve-type biased diffusion for kt force generation.

METHODS AND MATERIALS

Description of the Model

We start by briefly describing the proposed location and geometrical arrangement of the components of the kt site. In Fig. 1 *a* we show a three-dimensional model derived from high-resolution data from a previous study (9), and in Fig. 1 *b* we show a diagram of the attachment site that we use for our model. The key assumption we make in this work is that several flexible kt proteins are bound to Mt protofilaments with variable deformation, generating force depending on the deformation of the flexible protein from its rest position.

Kinetochore binders

We assume that the kt components are uniformly distributed in the radial direction and that kMt protofilaments maintain rotational symmetry, so that the key dynamics of attachment are accurately represented by projecting and tracking the attachment site on a one-dimensional line, as shown in Fig. 1 *b*. We suppose that flexible and stretchable kt components (i.e.,

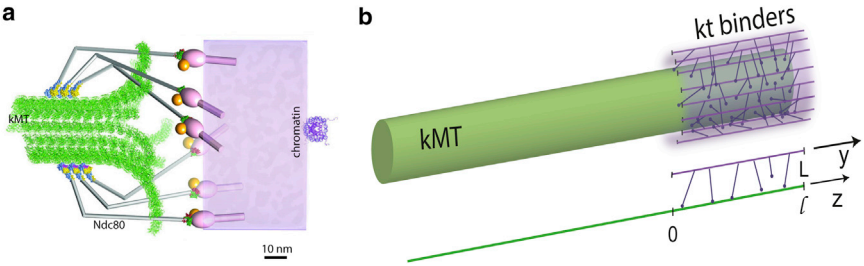

FIGURE 1 Diagram of model components for kt/kMt attachment. (*a*) three-dimensional model derived from high-resolution data of a vertebrate kt attached to a depolymerizing kMt, adapted from (9). Green ribbon representation for the kMt, and inner kt complexes are shown as spheres and rods, as described in (9). A nucleosome is shown as a ribbon model in dark purple, next to a simplified representation of chromatin (*purple*). (*b*) Diagram of our model for kt binders and kMt. The purple structure represents the flexible kMt binder elements, uniformly distributed on the kMt. To see this figure in color, go online.