Biophysics of Microtubule End Coupling at the Kinetochore

Ekaterina L. Grishchuk

Abstract The main physiological function of mitotic kinetochores is to provide durable attachment to spindle microtubules, which segregate chromosomes in order to partition them equally between the two daughter cells. Numerous kinetochore components that can bind directly to microtubules have been identified, including ATP-dependent motors and various microtubule-associated proteins with no motor activity. A major challenge facing the field is to explain chromosome motions based on the biochemical and structural properties of these individual kinetochore components and their assemblies. This chapter reviews the molecular mechanisms responsible for the motions associated with dynamic microtubule tips at the single-molecule level, as well as the activities of multimolecular ensembles called couplers. These couplers enable persistent kinetochore motion even under load, but their exact composition and structure remain unknown. Because no natural or artificial macro-machines function in an analogous manner to these molecular nano-devices, understanding their underlying biophysical mechanisms will require conceptual advances.

Key terminology

Microtubule end-tracking	ability of the kinetochore (or isolated protein) to move with a dynamic microtubule end;
Microtubule end conversion	transition from microtubule wall binding to microtubule-end attachment and subsequent microtubule end-tracking;

E.L. Grishchuk (✉)
Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
e-mail: gekate@mail.med.upenn.edu

B.E. Black (ed.), *Centromeres and Kinetochores*, Progress in Molecular and Subcellular Biology 56, DOI 10.1007/978-3-319-58592-5_17

Microtubule end coupler	a mobile protein-mediated link between a dynamic microtubule end and a cargo (chromosome or microbead);
Load-bearing	ability of microtubule end coupling to persist under tension.

1 The Kinetochore as a Versatile Molecular Machine

Kinetochores are multicomponent molecular assemblies that are capable of various modes of microtubule-dependent motility (reviewed in Mitchison 1988; Rieder and Salmon 1998; McIntosh et al. 2002). Elucidating how the underlying connections are established, maintained, and regulated is crucial for our understanding of normal cell division.

1.1 Microtubule Wall-to-End Transition

Early in mitosis, kinetochores often bind to the walls of spindle microtubules (Hayden et al. 1990; Tanaka et al. 2005; Magidson et al. 2011). The initial binding is facilitated by an expansion of the outer kinetochore layer, called the corona, which is rich in dynein and the kinetochore-localized kinesin CENP-E (Pfarr et al. 1990; Steuer et al. 1990; Cooke et al. 1997; Yao et al. 1997; Putkey et al. 2002; Wan et al. 2009). These mechanochemical enzymes use ATP to drive kinetochore motion along the microtubule. This mode of kinetochore motility is similar to the transport of other intracellular organelles containing motors of opposite polarities, e.g., during axonal or intraflagellar transport. In many cell types, mitotic kinetochores simultaneously contain dynein, a minus-end–directed transporter that moves the chromosomes to the spindle poles, and CENP-E kinesin, a plus-end-directed motor that helps to gather chromosomes at the spindle equator (Fig. 1a).

Many questions persist regarding the coordination of these opposing activities, and the role of a "polar wind," a microtubule-dependent force acting on the chromosome arms and pushing chromosomes away from the spindle poles (Ke et al. 2009; Cheerambathur et al. 2013; Barisic et al. 2014). Collectively, these forces ultimately transport kinetochores to the spindle midzone, where the microtubule plus ends are located. Not all chromosomes in a typical mammalian cell undergo long-distance transport along microtubule walls before coming into contact with the plus ends, and many of the translocations that do occur are probably too short to be detected (Magidson et al. 2011). Furthermore, some microtubule ends

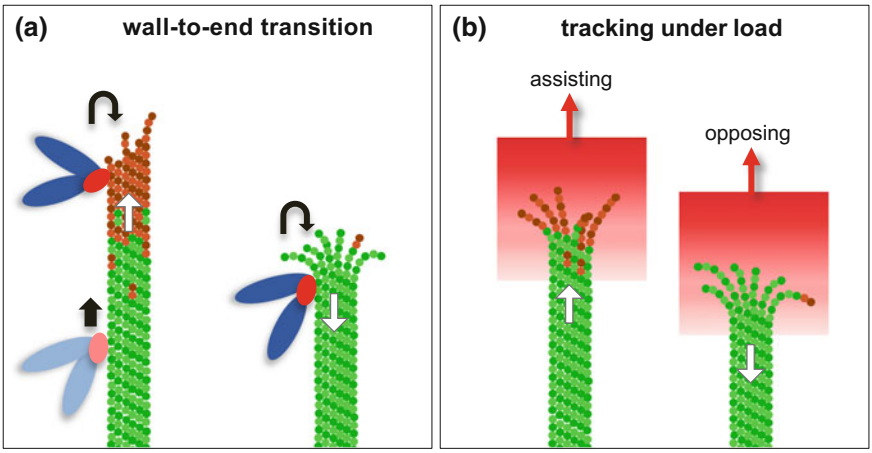


Fig. 1 Modes of kinetochore motility. Each cartoon shows a microtubule plus end interacting with a kinetochore (in *red*). In the microtubules, GTP-bound tubulin dimers are shown in brown and GDP-tubulin dimers in *green*. *Black arrows* indicate kinetochore motion, and *white arrows* indicate direction of microtubule dynamics. **a** Pathways that bring a laterally attached kinetochore in contact with the plus end of a dynamic microtubule. *Left* Plus-end-directed kinetochore-localized kinesin CENP-E transports the kinetochore, dragging it along the microtubule wall. *Right* When the microtubule depolymerizes, its plus end reaches the wall-bound kinetochore. The kinetochore rotates to assume microtubule end-on attachment due to the forces exerted by other spindle microtubules (not shown), which orient the sister kinetochore pair coaxially with the spindle (e.g., see Zaytsev and Grishchuk 2015). For simplicity, the kinetochore is drawn as a small oval, whereas in reality the size of the vertebrate kinetochore is 10–20-fold larger than the diameter of the microtubule; chromosome arms (*blue*) are also depicted on a much smaller scale. **b** In the end-on attachment mode, the kinetochore tracks the dynamic microtubule ends bidirectionally and processively. In metaphase, force acting from the sister kinetochore (*upward red arrow*) assists the motion of the kinetochore when it moves at the polymerizing end, but exerts a load when the kinetochore reverses its motion and tracks the end of depolymerizing microtubule. The fine structure of the kinetochore-bound microtubule ends is not known, but the ends of polymerizing and depolymerizing kinetochore microtubules appear quite similar, with protofilament flare smaller than that in freely depolymerizing microtubules, but larger than that in freely polymerizing microtubules in vitro (McIntosh et al. 2008)

become captured by kinetochores via either a direct encounter or following depolymerization of the laterally bound microtubule (Fig. 1a). Through these various mechanisms, the predominantly lateral microtubule attachments are gradually replaced by attachment to microtubule ends, often referred to as “end-on” binding, a prerequisite for normal segregation (reviewed in Tanaka 2010; Cheerambathur and Desai 2014). No other essential cellular cargo undergoes such a dramatic wall-to-end transition, and the biophysical mechanisms underlying this dynamic process remain poorly understood.

1.2 Bidirectional Processive Tracking of the Dynamic Microtubule Ends

The mechanism by which kinetochores maintain their persistent association with microtubule ends also remains to be elucidated (Inoué and Salmon 1995; Maiato et al. 2004). Textbook images of the kinetochores, e.g., in PtK1 cells, show kinetochore "plates," but these dense structures are seen only on a subset of kinetochores after conventional chemical fixation (Rieder 1982; O'Connell et al. 2012). Methods that preserve cellular structures more accurately, such as rapid freezing and freeze substitution, reveal subtler plates resembling a fibrous meshwork (McEwen et al. 1998; Dong et al. 2007; McIntosh et al. 2008, 2013). Microtubule plus ends become embedded within this meshwork while exhibiting a conspicuous flaring of linear tubulin arrays called protofilaments (Fig. 1).

Remarkably, the attachments between the embedded ends and kinetochore are not static, but instead exhibit dynamic instability (Mitchison et al. 1986; Skibbens et al. 1993), although with different rates and transition frequencies than unbound microtubule ends. As tubulin subunits are added or lost from these ends, the kinetochores move concomitantly (reviewed in Rieder and Salmon 1998; Maiato et al. 2004; Cheeseman and Desai 2008; Santaguida and Musacchio 2009), a behavior referred to as microtubule tip- or end-tracking. Thus, during microtubule end conversion, the kinetochores first transit from wall-to-end binding, then continuously track the dynamic microtubule ends. Kinetochore tracking is bidirectional during chromosome oscillations in metaphase, whereas during anaphase the tip-tracking motion is almost exclusively toward the spindle poles. Strikingly, the fine structure of kinetochore–microtubule ends differs only slightly during these stages (McIntosh et al. 2008). This observation is contrary to expectations based *on* in vitro studies with purified tubulin, in which the structures of polymerizing and depolymerizing ends are dramatically different. At the kinetochore, both polymerizing and depolymerizing microtubule ends contain flaring protofilaments (Fig. 1b). As described later in this chapter, several molecular mechanisms could in principle explain bidirectional kinetochore tracking of dynamic microtubule ends, but many questions remain regarding the identity of the molecules involved and their respective contributions.

1.3 Load-Bearing by Tracking Kinetochores

In cells, kinetochore–microtubule connections are maintained even in the presence of opposing forces (Fig. 1b), as evidenced by chromatin stretching between bi-oriented sister kinetochores in metaphase (reviewed in Rieder and Salmon 1998; Rago and Cheeseman 2013). The magnitudes of the forces at these stretched

kinetochores, and those that move in anaphase, remain unknown. Because chromosome motions at the ends of kinetochore microtubules in metaphase and anaphase are relatively slow (1–3 μm/min), the force required to move a typically sized mammalian chromosome in the end-on configuration is very small, on the order of 1 pN (Nicklas 1965; Taylor 1965; Alexander and Rieder 1991). Nonetheless, some estimates suggest that mitotic kinetochores experience forces up to 10 pN (reviewed in Asbury et al. 2011), whereas other studies indicate that the physiological load at the kinetochore may reach hundreds of pN (Alexander and Rieder 1991; Ye et al. 2016). On the scale of intracellular transport, this is a very large force; by comparison, the CENP-E kinesin stall force is 5 pN (Yardimci et al. 2008). Micromanipulation studies in grasshopper spermatocytes suggest that end-on attached kinetochores can move even under loads of 400–600 pN (Nicklas 1983). Analogous experiments in vertebrate cells are lacking because of the difficulty of applying calibrated forces in these more fragile cells (Skibbens and Salmon 1997). Although laser trapping of segregating chromosomes in different cell types shows that they can be stalled by forces on the order of 2–10 pN (Ferraro-Gideon et al. 2013), the direct manipulation of chromosomes with a powerful laser beam is likely to be harmful. Kinetochore particles isolated from budding yeast can stay attached to dynamic microtubule ends under the forces of up to 10 pN (Akiyoshi et al. 2010). Thus, although direct measurements of average physiological loads and maximal (stall) forces in live dividing cells are still lacking, it is plausible that larger kinetochores, such as those of human cells, which connect to 15–20 microtubules, are built to withstand significant forces.

The ability of kinetochores to continue their motions despite significant opposing loads may constitute a fail-safe mechanism for preventing chromosome loss, which could result from resistance due to cellular obstacles or from counteraction by improperly attached (merotelic) microtubules in anaphase (Cimini et al. 2004). The specific molecular mechanisms that govern kinetochore motility under load remain largely unknown (reviewed by Inoué and Salmon 1995; Joglekar et al. 2010). Accordingly, the overarching goal of this field is not only to identify the key components, but also to understand the underlying biophysical mechanisms, which are far from trivial. Indeed, the coexistence of the two major properties of the kinetochore (the processive tracking and persistence under force) is counterintuitive, because tracking implies mobility (i.e., constant dissolution of existing kinetochore–microtubule bonds and formation of new bonds), whereas stability of attachment is most feasibly ensured by static bonds (reviewed in Mitchison 1988; Inoué and Salmon 1995; Grishchuk et al. 2012). Understanding of such intricate biophysical relationships has been facilitated by a combination of in vitro reconstitutions and quantitative experimental and theoretical approaches, as summarized briefly in the following sections.

2 Molecular Mechanisms of Microtubule Tip-Tracking

Classical experiments identified the kinetochore as the major site of force generation for chromosome motion (Nicklas 1989; Khodjakov and Rieder 1996). However, subsequent analyses revealed that kinetochore-localized microtubule-dependent motors are largely dispensable for chromosome motions (Grishchuk and McIntosh 2006; Tanaka et al. 2007), implying that the dynamics of end-on attached microtubules are the primary driver of chromosome motility (reviewed in Inoué and Salmon 1995; McIntosh et al. 2010). In this sense, the kinetochore is not a motor per se, but rather a complex macromolecular device that couples chromosomes to the ends of microtubules that do the actual work of translocation. One possible explanation of the ability of kinetochores to track microtubule ends is that it results directly from the activities of various kinetochore-localized microtubule-binding proteins that are capable of tracking at a single-molecule level. Such molecules could create individual mobile molecular bonds between kinetochores and microtubules, enabling processive kinetochore motions. Alternatively, kinetochore tip-tracking could emerge from the collective behavior of multiple molecules that are individually incapable of tracking (or track poorly) at the single-molecule level. Therefore, it is important to identify the tip-tracking abilities of all kinetochore-localized microtubule binders and their multi-molecular assemblies, elucidate the underlying biophysical mechanisms, and determine the respective contribution of these mechanisms to kinetochore tracking.

2.1 Affinity-Based Microtubule Tip-Tracking

2.1.1 Polymerizing Microtubule Ends

The EB proteins are a well-characterized example of affinity-based tracking of polymerizing microtubule tips (reviewed in Akhmanova and Steinmetz 2010). These proteins discriminate between different types of microtubule lattices, as demonstrated by their strong preference for tubulin polymerized with non-hydrolysable GTP analogs in vitro (Zanic et al. 2009; Maurer et al. 2011). Because microtubule tips grow by addition of tubulins bound to GTP that is later hydrolyzed to GDP, the nascent microtubule wall is rich in GTP-tubulin (Desai and Mitchison 1997). When microtubules polymerize in the presence of soluble fluorescently labeled EB proteins, their growing ends are highlighted and appear as moving "comets," whereas the shortening ends are not labeled. Individual EB molecules bind to the tip region very transiently (0.05–0.8 s) (Bieling et al. 2008; Chen et al. 2014). Although a single EB molecule is capable of diffusive motions on the microtubule wall, the transient nature of this interaction prevents the individual molecule from moving processively (see below). Hence, tip-tracking by EB is merely apparent, rather than reflecting actual motion, and consequently represents

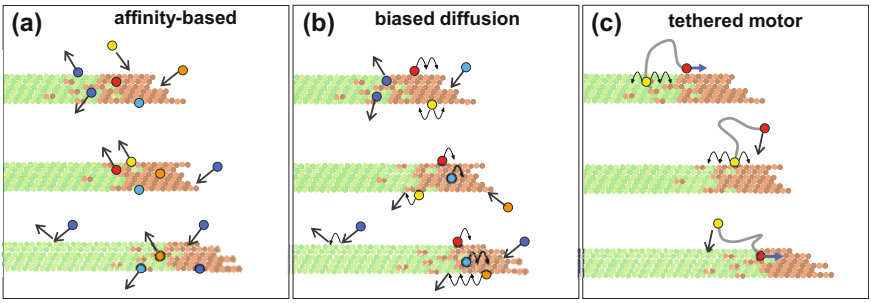


Fig. 2 Mechanisms of single-molecule tracking of polymerizing microtubule tip. In each panel, elongating microtubule end is depicted with three consecutive configurations. *Straight arrows* indicate molecular binding/unbinding events; *curved arrows* correspond to diffusional hopping. **a** MAP molecules are shown in different colors, so that they can be traced easily on the microtubule lattice. In the affinity mechanism, individual MAPs do not move relative to the lattice, but binding of multiple molecules leads to continuous decoration of the growing microtubule end. **b** In contrast to tracking that is based on affinity alone, in this mechanism the individual molecules change their position on the microtubule lattice due to diffusion. The outcome of this diffusion is biased by affinity: MAP molecules that happen to diffuse in the direction of microtubule elongation will retain their binding to the microtubule for a longer time due to their preference for the biochemical or structural features of tubulins at the tip. **c** A motor domain (*red circle*) walks processively to the plus end (*blue arrow*), where it dissociates, while the tail (*yellow circle*) behaves as a diffusing MAP with a limited residency time. When these two domains are tethered together, as in full-length CENP-E, the molecule can track microtubule ends processively and bidirectionally. For simplicity, only a single chain of this homodimeric motor is shown

treadmilling (Fig. 2a). The conspicuous microtubule comets seen in the presence of soluble EB result from multiple individual EB molecules quickly binding and unbinding to the moving microtubule tip.

Transient microtubule interactions can lead to lasting attachments when multiple molecules are involved, even if the binding of individual molecules is stochastic and uncoordinated (Zaytsev et al. 2013). Thus, although binding by a single EB molecule is too transient to lead to processive tracking, multiple EB molecules connected to a surface or scaffold could in principle provide a loose "glue" on the microtubule tip capable of sustaining the motion of small objects. For example, in vitro, short actin filaments can move with growing microtubule ends in the presence of EB and a linking protein that binds both EB and actin (Preciado López et al. 2014). In mitotic cells, EB proteins do not constitutively associate with the kinetochore, but they are seen at the ends of growing kinetochore-bound microtubules (Tirnauer et al. 2002; Armond et al. 2015). It is conceivable that in this capacity, multiple EB molecules binding to a more permanent kinetochore protein, e.g., Ska1 (Thomas et al. 2016), could contribute to kinetochore tracking at polymerizing microtubule ends.

A similar affinity-based mechanism may facilitate tracking with polymerizing microtubule tips by the bona fide kinetochore microtubule-associated proteins (MAPs). For example, the Dam1 heterodecameric protein complex, which is

persistently associated with metaphase kinetochores in yeast, prefers to bind GMPCPP-containing microtubule walls in vitro (Westermann et al. 2005). Not surprisingly, small Dam1 oligomers can move with the growing microtubule ends (Lampert et al. 2010), although it remains unclear whether these motions are processive and how they depend on the number of Dam1 subunits. Microbeads coated with Dam1 heterodecamers travel continuously with growing microtubule ends (Asbury et al. 2006), indicating that multiple scaffold-bound Dam1 complexes can readily sustain processive tip-tracking. Likewise, the major microtubule-binding kinetochore component, Ndc80 complex (reviewed in Cheeseman and Desai 2008), can support polymerization-driven motion of microbeads (Powers et al. 2009), but tracking of growing microtubule tips by single Ndc80 molecules is very poor (Lampert et al. 2010). Although Ndc80 has not been reported to have preference for the GTP-containing microtubule lattice, its ability to support the polymerization-driven motion of the microbeads suggests that it recognizes some feature of the growing microtubule tip. Even a small difference in binding affinity will be amplified when multiple bead-bound molecules interact with the microtubule tip, explaining the processive tracking.

In addition, the TOG domain protein XMAP215 has also been reported to move with the growing microtubule tips at a single-molecule level (Brouhard et al. 2008; Widlund et al. 2011) and can sustain the motion of beads (Trushko et al. 2013). Tip-tracking by single XMAP215 molecules is not highly processive, and is thought to involve XMAP215 catalytic activity specifically at the microtubule tip (Kerssemakers et al. 2006; Ayaz et al. 2014). Interestingly, the yeast homolog of XMAP215, Stu2, does not autonomously follow growing microtubule tips in mitotic cells; instead, it is delivered to this location by kinesin-dependent transport (Gandhi et al. 2011). Stu2 localization at the kinetochore is very transient, with a half-life less than 50 s (Aravamudhan et al. 2014). Despite this dynamicity, kinetochore particles purified from Stu2-depleted yeast cells have been reported to interact with MT ends in vitro differently than the wild type particles (Miller et al. 2016), so a minor population of Stu2 must be binding strongly to the kinetochores. This stably bound Stu2 has been proposed to mediate complex tension-sensitive and microtubule dynamics-sensitive responding of yeast kinetochore (Miller et al. 2016).

Thus, many candidate molecules could potentially contribute to affinity-based recognition of growing microtubule ends at the kinetochore. It remains unclear, however, whether the main role of these MAPs is to provide end-tracking motion that pushes the kinetochore away from the pole (so-called AP motion). In vertebrate cells, a kinetochore moving with polymerizing microtubule ends is thought to be dragged by the pulling force acting through its poleward-moving sister (Skibbens et al. 1993; Waters et al. 1996). In this view, the role of kinetochore MAPs with high affinity for the polymerizing microtubule tip would be to provide molecular "friction" that prevents the AP kinetochore from slipping from the growing end (Maddox et al. 2003; Dumont et al. 2012), rather than to actively transport this kinetochore via tip-tracking mechanisms.

2.1.2 Depolymerizing Microtubule Ends

On the other hand, affinity-based tracking of depolymerizing microtubule ends is much less well understood. Two MAPs that might move via this motility mechanism are the human kinetochore proteins Ska1 and CENP-F, which can track depolymerizing microtubule ends in vitro (Schmidt et al. 2012; Volkov et al. 2015). CENP-F exhibits a weak preference for protofilament curls, which decorate the ends of shortening microtubules, whereas human Ska1 has similar affinities to tubulins in microtubule walls and curls. It remains to be seen whether tip-tracking by these proteins is truly affinity-driven, or instead occurs by a biased-diffusion mechanism, as described in the following sections. Individual protofilament curls are highly transient structures, with a lifetime of less than 0.1 s (assuming protofilament length of five dimers and a microtubule depolymerization rate of 25 μm/min). Thus, only proteins with a strong preference for GDP-tubulin curls should be capable of apparent tracking of shortening ends in vitro. The lifetime of the protofilament curls is much shorter than that of the GTP-tubulins in the growing tip. Thus, it is challenging to study depolymerization-driven motions at a single-molecule level, and consequently this type of non-processive tracking remains to be examined.

2.2 Biased-Diffusion Tracking of Polymerizing Microtubule Ends

Single molecules of most examined MAPs can diffuse on the microtubule wall (reviewed in Cooper and Wordeman 2009; Grishchuk et al. 2012; Reithmann et al. 2015). Diffusion along a polymer is not unique to microtubules, and has also been observed for actin-binding and DNA-binding proteins. The underlying biophysical mechanisms of microtubule-dependent diffusion have not been elucidated, but this phenomenon is likely to rely on the presence of multiple microtubule-binding sites within the diffusing protein molecule. Importantly, thermal motions are not directional and cannot by themselves lead to tip-tracking. However, they can be biased in the direction of microtubule dynamics via various mechanisms. At the growing microtubule tip, such bias is provided by an increased affinity for this structure. All kinetochore MAPs described in the previous section diffuse on the microtubule wall to some extent, so their tip-tracking may involve these diffusive motions. As pointed out above, a nondiffusing protein can lead only to apparent tip-tracking, whereas true processivity requires the molecule to move along the microtubule. Diffusing molecules can exhibit more persistent tracking because they can “hop” from one binding site to the next at the microtubule tip without fully dissociating from it (Fig. 2b). Such tracking cannot be highly processive because eventually a diffusing molecule will hop by chance in the wrong direction, away from the high-affinity sites at the tip, quickly losing its microtubule attachment.

No general theory has yet been developed to predict how the rate of MAP diffusion and its affinity for different features at the microtubule tip vs. wall will affect the processivity of tracking by single and multiple molecules. Vertebrate kinetochore represents a spatially distributed ensemble of various MAPs capable of microtubule binding and diffusion, probably 20 or more such molecules per microtubule end (Lawrimore et al. 2011; Suzuki et al. 2015). The weakly processive motion of a single molecule should be greatly enhanced when many such molecules are bunched together, enabling persistent association of the kinetochore with the growing microtubule ends. However, the rate of collective diffusion is expected to be much slower than mobility of a single molecule (Volkov et al. 2013). Given the large number of kinetochore MAPs, such as Ndc80, CENP-F, Ska1, Knl1, TOG domain proteins, microtubule-binding domains of CENP-E kinesin and dynein, and other factors (Cheeseman and Desai 2008; Nagpal and Fukagawa 2016), it is surprising that microtubule ends can polymerize while bound to the kinetochores of isolated mammalian chromosomes (Mitchison and Kirschner 1985; Hunt and McIntosh 1998). A rigorous understanding of this phenomenon will require thorough quantification of the diffusion rates and microtubule residency times of all kinetochore MAPs, as well as application of advanced mathematical models capable of incorporating these kinetic features and the mechanical properties of the MAPs and their kinetochore receptors.

2.3 Biased-Diffusion Tracking of Depolymerizing Microtubule Ends

2.3.1 Single Molecules

Tracking with the depolymerizing microtubule end can also take place by biased diffusion, driven by thermal energy in association with the unidirectionality of tubulin disassembly. In this case, the bias arises as a direct consequence of microtubule shortening. For successful operation of this mechanism, it is imperative that when a MAP molecule diffusing along a microtubule encounters the end, its probability of detachment is low. Mathematical models that assume this property—e.g., the "burnt bridge" model, in which the molecule's detachment from the end is simply prohibited (Mai et al. 2001)—can recapitulate end-tracking. Because this postulate does not rely directly on a specific mechanochemical pathway of microtubule disassembly, this type of tip-tracking is possible for any shortening polymer, not just microtubules. The mechanisms that could prevent a diffusing molecule from falling off the microtubule end are not well understood. Such property, however, has been reported for the microtubule-binding domain in the tail of CENP-E kinesin (Gudimchuk et al. 2013). When random motions bring the purified CENP-E Tail to the microtubule end, the Tail falls off only infrequently, and most of the time bounces off the end and continues to diffuse, as if the

microtubule end constituted a reflective barrier. This remarkable behavior has also been noticed for Ndc80 protein (Powers et al. 2009). While it is likely that other MAPs may also be "reflected" by the microtubule tip, it remains unclear whether this will turn out to be a general feature of all microtubule diffusers.

Even if a MAP is capable of both wall diffusion and bouncing off the tip, such a MAP will not necessarily exhibit processive tracking at a single-molecule level. This is because successful tip-tracking requires an intricate balance between the rate of MAP diffusion and the rate of tubulin dissociation from the shortening microtubule tip. For example, the CENP-E Tail does not track the depolymerizing tip because its diffusion is too fast (1 $\mu m^2/s$) relative to microtubule shortening. After the Tail molecule bounces off the tip, it moves ahead of it much more rapidly than tubulin disassembly (Fig. 3). Thus, while the Tail's random motion is biased by microtubule depolymerization, this molecule spends most of the time diffusing on the wall and only a small fraction of time at the microtubule end (Gudimchuk et al. 2013). A molecule diffusing on microtubule wall ten times slower, e.g., Dam1 heterodecamer or Ndc80 protein ($\sim$0.1 $\mu m^2/s$) (Gestaut et al. 2008; Grishchuk et al. 2008b; Powers et al. 2009; Volkov et al. 2013; Zaytsev et al. 2015), will not move far on the microtubule wall before the shortening polymer end catches up. Thereafter, such MAP molecule will exhibit directed motion, staying close to the shortening tip, because its slow diffusion becomes rate-limiting for microtubule disassembly (Grishchuk et al. 2012). MAP molecules that diffuse even more slowly will simply block microtubule depolymerization until either the molecule dissociates or the terminal tubulin falls off, taking the MAP molecule with it (Fig. 3). Thus, successful tracking also depends on how long the diffusing molecule can remain associated with the microtubule. For all MAPs studied to date, the duration of diffusive motion is brief: Dam1 heterodecamer remains bound to microtubules for only 2 s (Gestaut et al. 2008), whereas Ndc80 kinetochore protein detaches in less than 0.5–1 s (Powers et al. 2009; Zaytsev et al. 2015). Because kinetochore–microtubules disassemble at 1–2 μm/min, a kinetochore would move with one such molecule for only $\sim$40 nm, the length of five tubulin dimers, before disconnecting from the microtubule. In vitro, microtubules depolymerize much faster, so small oligomers of Dam1 can move for longer distances. Despite having a similar rate of diffusion, single Ndc80 molecules are poor trackers of shortening microtubule ends (Powers et al. 2009; Umbreit et al. 2012; Schmidt et al. 2012).

2.3.2 Multiple Molecules Attached to a Microbead

As discussed in regard to polymerization-dependent tracking, motions at the shortening microtubule end are also expected to be much more processive when multiple MAP molecules are involved. Coupling by multiple molecules is usually tested in vitro using microbeads randomly coated with a MAP. This approach has revealed that numerous MAPs, not just those localized to kinetochores, can support transport of beads with microtubule disassembly to varying extents (reviewed in McIntosh et al. 2010). One of the best proteins for bead coupling is Dam1, which

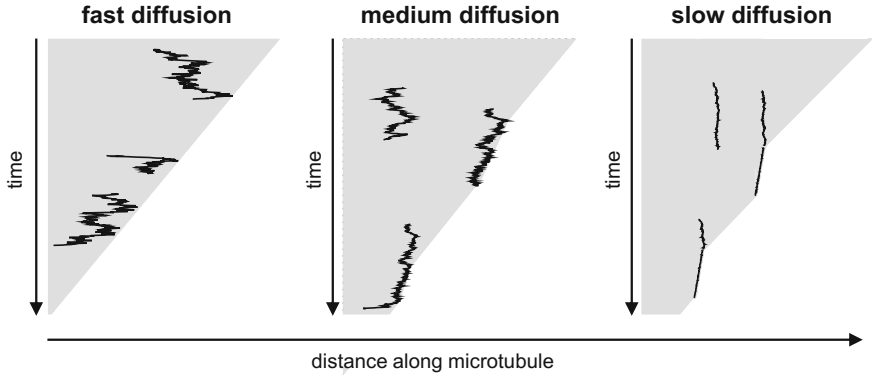


Fig. 3 Quantitative features of the single-molecule tracking of depolymerizing microtubule end. Drawings represent kymograph-like plots for three microtubules (in *grey*, *horizontal arrow* points to the plus-end direction). With time, each microtubule shortens, so the position of the disassembling plus end corresponds to the rightmost side of the *grey triangle*. The slopes of these lines correspond to depolymerization velocities. Each panel also shows changes in position versus time for three diffusing molecules (*black lines*). All molecules have roughly similar residency times, but the rates of diffusion are different in each panel. Rapidly diffusing molecules (diffusion coefficient ~ 1 $\mu m^2/s$, leftmost kymograph) spend too little time at the microtubule tip, so they do not really track it. A moderate rate of diffusion (~ 0.1 $\mu m^2/s$) is optimal for tip-tracking in vitro, because such molecules can move significant distances, staying in close vicinity with the tip. With slower diffusion, however, microtubule depolymerization becomes severely inhibited because the molecular hops are too infrequent. Drawings are based on calculations in Grishchuk et al. (2012)

depending on soluble Dam1 concentration forms small oligomeric patches, microtubule-encircling rings, or stabilizing spirals (Westermann et al. 2005). When soluble Dam1 is present, the Dam1-coated beads "slide" along microtubules, just as expected if the beads were transported by the bead-bound Dam1 ring encircling the microtubule (Grishchuk et al. 2008b). However, when soluble Dam1 is not included in the assay buffer, Dam1-coated beads can still follow the shortening microtubule ends, but under these conditions they roll on the microtubule surface. Because this complex motion is likely to reflect the biased rotational diffusion of the beads (Peskin and Oster 1995), it represents a poor model for coupling at mitotic chromosomes. Furthermore, small non-protein particles with polyvalent positive bonds can also diffuse on the microtubule surface (Minoura et al. 2010) and follow shortening microtubule tips (unpublished observation). Apparently, the negative charges associated with tubulin globular domains and tails, which collectively form a relatively large interaction surface on the multiprotofilament microtubule, can support multivalent interactions with the positively charged molecular clusters. Processive motion of these clusters during microtubule depolymerization could arise through translational or rotational diffusive motions, or represent their combination. Such transport may not be physiological, so caution should be exercised when interpreting depolymerization coupled motility of protein clusters/aggregates, especially under experimental conditions that slow the rate of microtubule

disassembly or use buffers with lower than physiological ionic strength. In this regard, a recently developed procedure for clustering kinetochore proteins in a highly controlled manner with the help of origami scaffolds is very promising (Verma et al. 2015). Such precise and quantitative approaches should help overcome the significant technical limitations of randomly coated microbeads, advancing experimental studies of microtubule end-tracking by molecular ensembles with defined compositions.

2.4 *Tethered Motor Mechanism of Bidirectional Tip-Tracking*

This unusual, ATP-dependent mode of tracking has been proposed for kinetochore kinesin CENP-E, which has two microtubule-binding domains: one with motor activity at the N-terminus of the molecule, and one with weak microtubule-binding affinity at the C-terminal tail (Liao et al. 1994; Wood et al. 1997; Kim et al. 2008; Espeut et al. 2008). In vitro studies have shown that this dimeric motor can walk along microtubule walls much faster than the rate at which microtubules polymerize (Yardimci et al. 2008), allowing it to catch up with the growing tip. Afterward, CENP-E does not dissociate, but instead continues to move processively at the rate of microtubule elongation (Gudimchuk et al. 2013). When the microtubule switches into depolymerization, CENP-E also reverses direction and tracks with the shortening end. Although these motions are not highly processive, lasting only for tens of seconds, they can be sustained by a single molecule. This is remarkable because it demonstrates that an individual kinetochore component can possess intrinsic abilities analogous to the physiological behavior of an intact kinetochore: motion along the microtubule wall, transition into tip-binding mode, and bidirectional tip-tracking.

This bidirectional tip-tracking does not rely on the increased CENP-E affinity for microtubule tips, but is instead an emergent behavior of the distinct activities of its two microtubule-binding domains (Fig. 2c). None of these domains exhibit a preference for polymerizing or depolymerizing tips, and neither can track these dynamic ends on their own. When the CENP-E molecule reaches a microtubule plus end, the CENP-E motor domains fall off the tip. Because they are tethered to the microtubule wall by the tail domains of this molecule, the motor domains rebind quickly. The tethering via CENP-E tails is very short (0.5 s), but it is sufficient for rebinding that is estimated to occur within a millisecond. Thus, CENP-E molecule tracks microtubule tips, both assembling and disassembling, by repeating the cycles of plus-end-directed walking, motor domains dissociation, and rebinding. As a tip-tracking protein, CENP-E kinesin can potentially provide bidirectional mobile links between the kinetochore and microtubules (Gudimchuk et al. 2013; Shrestha and Draviam 2013; Vitre et al. 2014), thereby enhancing the ability of chromosomes to follow microtubule ends (Lombillo et al. 1995).

Other plus-end-directed kinesins, e.g., Kif18A and Kip3, can associate processively with the growing microtubule tips in vitro, assisted by the microtubule-binding activity of the C-terminal tails (Mayr et al. 2011; Su et al. 2011; Weaver et al. 2011). However, these kinesins fail to track with microtubule disassembly. This is likely to be due to the slow diffusion of their tail domains. The tail of Kif18A, which diffuses 100-times slower than the tail of CENP-E, is expected to dissociate rapidly from shortening microtubule end, because the end catches up repeatedly with a molecule diffusing slowly in front of the wave of tubulin depolymerization. Each of these encounters may cause stochastic loss of the terminal tubulin dimer together with the bound molecule, so a slowly diffusing tail is not an effective tether for the motor domains on the shortening microtubule. It remains to be seen if the tethered motor mechanism is involved in tracking by these and other kinesin motors of the polymerizing microtubule ends.

3 Load-Bearing Coupling to Dynamic Microtubule Ends

In cells, kinetochore–microtubule connections persist despite the stochasticity of tubulin dynamics even in the presence of variable forces, both assisting and opposing. Load-bearing (processive motion under the force that acts oppositely to the vector of motion, Fig. 1b) is not a single-molecule phenomenon, and instead requires operation of multi-molecular ensembles, called kinetochore couplers. Load-bearing by couplers moving with a polymerizing microtubule is inherently limited because the microtubule buckles when its growing tip experiences a resisting force (Dogterom and Yurke 1997). Nonetheless, couplers based on multiple independent EB binders are strong enough to bear forces that can sustain microtubule bending for $\sim$100 times longer than the binding time of a single EB molecule (Chen et al. 2014; Doodhi et al. 2014). The assisting force, which acts in the same direction as the vector of microtubule dynamics (Fig. 1b), can be applied at the growing microtubule end coupled to a microbead coated with purified proteins or yeast kinetochore particles (Akiyoshi et al. 2010). The latter can maintain persistent attachment to the assembling tips for tens of minutes under an assisting force of 2–6 pN. Such coupling is thought to rely on the affinity-biased and force-biased diffusion of the bead-bound molecular components, but the underlying theory has not yet been developed. Also, possible contributions from more complex phenomena, such as force-induced modification of the MAPs' hopping rates or attachment times, as well as the bead's rotational diffusion, have not yet been examined.

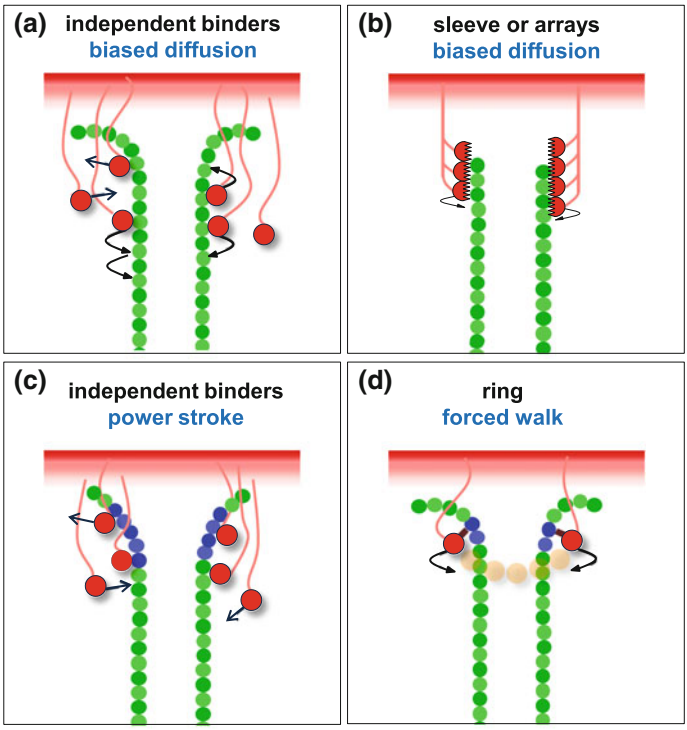
By contrast, load-bearing by couplers moving at depolymerizing microtubule ends has been investigated both theoretically and experimentally, and these results are covered in more detail in the following sections. Although the ultimate role of such couplers is to capitalize on the unidirectionality of microtubule disassembly, the energy for transporting a load can come from two sources: thermal motions (biased-diffusion mechanism) or changes in tubulin conformation (power stroke

mechanism). The exact composition and structure of the kinetochore couplers is not yet known, but theoretical analyses have deepened our understanding of the physically possible coupling mechanisms (reviewed in Grishchuk et al. 2012). Sections below summarize these findings to emphasize that the requirements for processive tracking with no load can be readily met by couplers with different designs, moving via either the biased diffusion or power stroke mechanism. However, large load-bearing can be achieved only by couplers that can capture energy from tubulin power strokes. Moreover, the biomechanical properties of such couplers must be finely tuned to enable them to move at the force-generating microtubule end without detaching. Advanced experimental approaches are needed to test these models and reconstitute the couplers acting at kinetochores in different cell types.

3.1 Load-Bearing by Couplers Moving via the Biased-Diffusion Mechanism

3.1.1 Single Molecules

Biased-diffusion tracking of depolymerizing microtubule tip is fundamentally similar to the work of other "thermal ratchets" (Peskin et al. 1993; Reimann 2002; Mogilner and Oster 2003). Force for MAP motion is generated by thermal fluctuation, and the role of microtubule disassembly is to rectify the resulting motion. In this mechanism, the ultimate role of GTP hydrolysis is to control the rate and location of depolymerization, but it does not directly provide the energy for this motion. A single MAP molecule tracking a dynamic microtubule tip by the biased-diffusion mechanism is a poor vehicle for cargo transport. This is because a molecule can be mobile only if its binding energy is low enough for thermal fluctuation to cause the molecule to hop from one binding site to another. Thus, forces that exceed thermal (0.5–1 pN, assuming 4–8 nm step size), should easily detach such a molecule from the microtubule. To prevent this undesirable outcome, it is usually assumed that the energy required to detach a molecule completely from the microtubule is greater than the energy needed for the molecule to hop. Indeed, some MAPs diffuse on microtubules for much longer than would be expected based on the frequency of hops (Powers et al. 2009), indicating that the assumption of two different energies is reasonable. In this case, however, another problem arises, because instead of detaching, the load will pull the mobile molecule along the microtubule until it reaches the end. As discussed above for diffusing tip-tracking molecules, in order to avoid the loss of the MAP molecule bound to the terminal tubulin subunit, the rate of microtubule depolymerization must decrease and become limited by the rate of the MAP's diffusion (Fig. 3). Thus, load-bearing by a diffusing MAP is limited by the requirement for a fluctuation in thermal energy that can overcome the opposing force and advance the molecule (and its load) away



from the tip. Because thermal energy on average generates a small force, even a relatively small load of <1 pN can block tip-tracking of a single MAP, so the biased-diffusion mechanism of tip-tracking has an inherent limit on the load it can carry at physiological velocities (Grishchuk et al. 2012). This conclusion is consistent with the general tendency of thermal ratchets to perform poorly relative to power stroke–dependent motors (Wagoner and Dill 2016).

3.1.2 Multiple Independent Binders

Load capacity should obviously improve if multiple MAP molecules are involved. The simplest such coupler could consist of multiple "independent binders" (Fig. 4a) (Zaytsev et al. 2013), similar to the EB-dependent coupler for polymerizing microtubule tips, considered earlier in this review. In this model, each MAP hops randomly along the microtubule wall in steps of 4 or 8 nm (step size is dictated by the spacing of the binding sites on the microtubule wall), biased by the moving reflective boundary (i.e., the shortening end). It is challenging to predict the load that can be borne by this multimolecular ensemble without a detailed mathematical

◂**Fig. 4** Designs of the most popular models for multimolecular couplers for the depolymerizing microtubule end. **a** In the "independent binders" coupler moving by biased-diffusion mechanism, each MAP binds and diffuses on the microtubule stochastically and independently, analogous to the behavior of molecules in Fig. 2b. The reflection of diffusing molecules at the tip is thought to occur due to some specific feature of bending protofilaments. For example, Ndc80 molecules have weaker affinity for bent tubulin protofilaments than to the tubulins in the cylindrical wall (Alushin et al. 2010; Schmidt et al. 2012). The major concern with this coupling design is that a large pulling load will collect all MAPs at the affinity boundary, where they will either detach or block tubulin disassembly. A corresponding theoretical analysis of these effects, as well as their possible remedies, has not yet been performed. **b** Schematics corresponding to the cross section of a cylindrical sleeve, which completely encircles the microtubule wall, but also illustrates the oligomeric arrays, i.e., groups of three to five MAP subunits with strong lateral bonds that are all bound to the same protofilament. The "teeth" in each MAP symbolize the 0.6-nm step size for coordinated diffusion, the characteristic feature of Hill's design. Also, in this design, the biased motion of the coupler under load arises only in the presence of the "overhangs," i.e., the parts of the cylinder or the arrays that are not bound to the microtubule. Consequently, Hill's design does not function when tubulin ram's horns are present, and this coupler cannot follow polymerizing microtubule ends. **c** The design of the "independent binders" coupler moving via the power stroke mechanism is highly similar to that shown in panel A. It differs only in that the affinity of the MAPs for the microtubule wall is stronger, so they have no or little diffusion. This distinction is similar to that between the affinity-based and biased-diffusion-based mechanisms of tracking of growing microtubule tips (Fig. 2). Thus, in the coupler in *panel A*, the individual MAPs can move processively by thermal hopping, whereas in the power stroke dependent coupler shown here the individual MAPs are not processive. Instead, they bind to straight tubulin in the microtubule wall, mediate pulling on the fibril when the attached tubulin is curling (in *blue*), and dissociate after the tubulin becomes completely bent. **d** Ring couplers can move via different mechanisms. This drawing shows the predicted position of the ring, moving as described in the forced-walk model (Efremov et al. 2007). In this model, the ring binds strongly to the microtubule wall and does not diffuse. The ring moves (*curved arrows*) only when the tubulins at the base of the curls (in *blue*) push on the flexible linkers (*dark bars*). The linkers connect ring subunits with the microtubule wall, both in the models and in the real Dam1 ring (Wang et al. 2007)

model that considers tubulin subunit dynamics and the quantitative characteristics of the MAP's diffusion, its residency time at the microtubule wall and tip, and other details. One estimate for 13 independent MAPs that diffuse similarly to Ndc80 but without detaching from the microtubule wall, suggests that tracking would stall at 6–7 pN (Grishchuk et al. 2012). However, the time of Ndc80 diffusion on the microtubule wall is brief (<1 s) (Powers et al. 2009; Zaytsev et al. 2015). Although with no load these transient individual interactions can sustain lasting attachment of the coupler to the microtubule (Zaytsev et al. 2013), when load is applied the "independent binders" coupler is likely to detach before the stalling force is reached, so its ability to bear a load is probably even smaller. In vitro, Ndc80 molecules conjugated randomly to beads can sustain only 1–2 pN at the ends of depolymerizing microtubules (Powers et al. 2009), consistent with the idea that under these conditions Ndc80 molecules operate independently, coupling the bead to dynamic microtubule via biased diffusion.

3.1.3 Sleeve and Oligomeric Arrays

Another possibility is that at the kinetochore the Ndc80 molecules do not operate as independent binders, but instead move as in Hill's model for a cylindrically shaped "sleeve" coupler (Hill 1985; Powers et al. 2009). Hill and Kirschner pioneered the theoretical investigation of end-on coupling mechanisms (Hill and Kirschner 1982), and Hill was the first to introduce the biased-diffusion mechanism to explain kinetochore load-bearing (Hill 1985; reviewed and analyzed in Mogilner and Oster 1996; Joglekar and Hunt 2002; Shtylla and Keener 2011; Grishchuk et al. 2012). There is a common misconception in the mitosis field that all biased-diffusion couplers move in the same fashion as the Hill's sleeve (Fig. 4b), but this is not so. Rather, there are multiple designs, and accordingly multiple underlying molecular and physical mechanisms that could explain how the biased diffusion of multiple MAPs couples the kinetochore to the disassembling microtubule tip. Indeed, in the coupler with independent binders, considered in previous paragraph (Fig. 4a), the individual MAPs bind and unbind the microtubule wall stochastically and diffuse in an uncoordinated manner. By contrast, in the sleeve coupler the individual MAPs do not operate independently, but hop synchronously in the same direction. As a result, the sleeve's tracking is biased by its motion toward the thermodynamic energy minimum, which is characterized by the maximal number of microtubule-bound MAPs. On the other hand, for the independent binders coupler the entropic component plays a significant role, and at steady-state the number of microtubule-bound MAPs is less than maximal, as dictated by their molecular kinetic constants (Zaytsev et al. 2013). Thus, although both types of couplers exhibit biased diffusion and rectify thermal motions, their underlying mechanisms are very different.

The sleeve design, as proposed many years ago by Hill, deviates in many ways from our current knowledge regarding the molecular and structural biology of the kinetochore (discussed in Efremov et al. 2007), and most researchers agree that such a coupling design is unlikely. It is certainly not a good model for Ndc80, which binds the microtubule wall with 4 nm spacing (Alushin et al. 2010; Zaytsev et al. 2015). In the sleeve model of Ndc80 coupling, the molecular clusters are assumed to diffuse with 0.6 nm step (Powers et al. 2009), while using the physiologically accurate step size (4 nm) should significantly decrease the predicted load-bearing by such a coupler. Another proposed structural arrangement for Ndc80 involves the oligomeric arrays of three to five Ndc80 molecules (Alushin et al. 2010). Such oriented "high-affinity" cluster is thought to maintain microtubule attachment while diffusing along a microtubule protofilament (reviewed in Alushin and Nogales 2011; Tooley and Stukenberg 2011). However, fluorescence analysis of Ndc80 molecules in vitro revealed that this protein has little tendency to form such diffusing arrays (Zaytsev et al. 2015), and the degree of cooperativity implied by electron microscopy studies, which gave rise to this model, appears to be unrealistically high (Zaytsev et al. 2013). Moreover, Ndc80 is not a very fast diffuser, and if three to five Ndc80 molecules were clustered together, such an array would diffuse so slow that it would not be capable of keeping up with the rate of microtubule dynamics at the kinetochore (Zaytsev et al. 2015). Thus, although

Ndc80 remains the main candidate responsible for load-bearing end-on kinetochore attachment in vertebrate cells, the exact design of the Ndc80-containing coupler and whether it moves by the biased-diffusion mechanism is controversial.

3.2 *Microtubule Depolymerization as a Powerful Motor*

It remains unknown how strong the end-on coupler needs to be in order to safely segregate mitotic chromosomes in different cell types, largely because, as pointed out above, the magnitude of forces acting at the kinetochore is not known with certainty. If the load were relatively small, not exceeding 10–15 pN, a well-designed multi-molecular coupler moving via the biased-diffusion mechanism would probably be sufficient to safely transport mitotic chromosomes. However, a different alternative is needed in organisms in which moving chromosomes may encounter large opposing forces or if large pulling forces are needed to assist proper bi-orientation of sister chromatids. Perhaps cells rely on microtubules to transport chromosomes because microtubules are unusual polymers in that they permit not only the biased-diffusion mechanism for processive motility but also a mechanism that can generate force that exceeds the force from thermal fluctuations (reviewed in Inoué and Salmon 1995). Depolymerizing microtubules generate force, and can work as depolymerization motor, thanks to the specific pathway by which tubulin adds to and leaves the microtubule end (reviewed in Desai and Mitchison 1997; Nogales 2001). Each microtubule is like a loaded spring because, during polymerization, only GTP-bound tubulin assembles at the tip and the GTP becomes hydrolyzed. Some of the chemical energy liberated from GTP hydrolysis is stored in conformational strain in the microtubule wall (Caplow et al. 1994; Alushin et al. 2014). During depolymerization of the GDP-containing microtubule wall, linear strands of tubulin, called protofilaments, curl to form "ram's horns" (Mandelkow et al. 1991). This curling protofilaments have been proposed to be capable of delivering a power stroke (Koshland et al. 1988), thereby moving the chromosomes (reviewed in McIntosh et al. 2010). If all the energy from hydrolyzed GTP were channeled into mechanical stroke, the thermodynamically maximal force that depolymerizing microtubule could generate is $\sim$80 pN (Molodtsov et al. 2005). This force is large enough to explain why chromosome motion is not blocked by the experimental application of hundreds of pN (Nicklas 1983), and to rationalize the large force estimate obtained by using fluorescent sensors at the kinetochores in mammalian cells (Ye et al. 2016). Such a force-producing mechanism could also explain how shortening microtubules move chromosomes in purified systems in vitro (Coue et al. 1991). In contrast to the biased-diffusion mechanism, the tip-tracking coupler does not have to wait for thermal fluctuations to overcome the force field of an opposing load, making transport of large loads at physiological velocities possible. However, in the power stroke mechanism, the kinetochore-associated couplers must not only ensure the processivity of tracking, but should also be capable of capturing the energy from microtubule

depolymerization. The final sections of this review describe two different ways in which this could be achieved: via couplers with non-processive elements, or via a ring-shaped processive coupler.

3.3 *Load-Bearing by a Coupler with Non-processive Binding Elements*

Force from bending protofilaments has been shown to act on a micron-size streptavidin-coated bead stably attached to a wall of biotinylated microtubule (Grishchuk et al. 2005). This finding is interesting because biotin–streptavidin attachment is so strong that on the intact microtubule wall the bead is completely immobile, and the force from laser tweezers cannot rupture the underlying bonds. Very strong bonds do not necessarily ensure processivity, and streptavidin bead fails to move with the shortening microtubule end, even in the absence of load. However, as the microtubule depolymerizes at the bead attachment site, the bead exhibits a small jerk in the direction of microtubule shortening, just as expected from the shape of curving protofilaments. Because this motion can be observed in the absence of soluble proteins and nucleotides, the pull must have been generated by conformational changes that took place at the shortening microtubule end as it passed by the bead. This "single-shot" force is only a fraction of a pN, which is much smaller than the rupture force for the bead from the microtubule. This is because these forces are different in nature, and rupture does not represent the force with which the microtubule pulls on the associated coupler. "Single-shot" force exerted by bending protofilaments on the laterally attached micron-size bead is so small, that such a bead would be a terrible coupler from the standpoint of chromosome motion.

Interestingly, both experiments and calculations indicate that if the bead were much smaller, similar in size to a protein molecule, the force it could transduce would have been much greater (Grishchuk et al. 2008a). Moreover, many such "mini-beads" bunched together in a multiprotein coupler could potentially transduce even larger force, since they would have experienced the jerks from all bending protofilaments, not just the one or two protofilaments that interact with the laterally attached microbead. Moreover, with a different design, strong static bonds may become advantageous. This idea was tested with a mathematical model in which multiple MAPs were placed at the ends of elongated fibrils (Fig. 4c) (McIntosh et al. 2008). This design is analogous to the "independent binders" coupler, which was discussed earlier in this chapter as a candidate for biased-diffusion-based coupling. It could be converted into a coupler for the power stroke mechanism by assuming that MAP binding to tubulins in the cylindrical microtubule wall is fairly strong, so under a load they stay attached to the protofilaments long enough to experience the initial stages of their bending. However, to recycle these MAPs, allowing them to rebind microtubule wall and

undergo new single-shot pulls, the MAPs must detach fairly quickly from the fully bent tubulins in the ram's horns. This aspect of the power stroke mechanism is frequently misunderstood, because it seems intuitive that bending protofilament generates the power stroke all along its length. However, the main stroke is exerted by the tubulins that undergo conformational changes, i.e., first two or three tubulins at the base of the curl (Molodtsov et al. 2005). The rest of the curl contains tubulin in the fully bent state, so it represents the "exhaust" of the depolymerization motor. For this reason, the non-processive coupler must first attach to tubulin in the wall of the microtubule, and this mechanism will not work if the MAP's only attachment is to the fully bent (i.e., low-energy) form of tubulin. Modeling shows that, indeed, under the power strokes from curling tubulins (shown in blue in Fig. 4), each wall-bound MAP in the coupler can pull transiently on the fibril, whose other end is attached to a cargo. Even though individual MAPs do not diffuse on the microtubule surface, their collective jerks can move the cargo processively against a significant load. In silico, such a coupler becomes stalled at only $\sim$70 pN, so this transporting mechanism with depolymerizing microtubule end is both feasible and potentially very powerful (McIntosh et al. 2008). This coupler could be adapted to move with the polymerizing microtubule tip by the affinity-based mechanism, as in Fig. 2a, but such model has not yet been developed.

Indirect evidence that force at the kinetochores is generated by protofilament power strokes is provided by electron tomography of mammalian kinetochores, which has revealed slender fibrils connected with the curved protofilaments at the end-on attached microtubules (McIntosh et al. 2008, 2013). The fibril-bound protofilaments observed in these studies were slightly straighter than in the typical ram's horns (Fig. 4c), suggesting that they were pulling against a significant load. Currently, however, there is no direct evidence that kinetochore couplers work through high-affinity non-processive binders. The molecular identity of the kinetochore fibril remains unknown, but many fibrillar MAPs could be involved (reviewed in Cheeseman and Desai 2008; Nagpal and Fukagawa 2016). The main candidates are Ndc80 and CENP-F proteins, each comprising a microtubule-binding domain and a highly elongated fibrillar domain. However, when coupled to the microbeads, they can sustain only a small force of several pN (Powers et al. 2009; Volkov et al. 2015), presumably because these MAPs have moderate microtubule-binding affinity. Thus, other kinetochore MAPs would need to be involved in order to realize successfully this coupling mechanism.

Obviously, other coupling designs could be developed based on the ensemble of nondiffusing MAPs, and the movement of such couplers should not necessarily rely on the energy from protofilament power strokes. In one study (Civelekoglu-Scholey et al. 2013), the coupling is hypothesized to involve a viscoelastic protein that does not diffuse and has long residency time and complex force-dependent detachment kinetics. In the "flexible" coupler model (Keener and Shtylla 2014), the MAPs are arranged as in Hill's sleeve, but the sleeve is not rigid, and the nondiffusive MAPs bind to the microtubule wall independently. In the future, this field will undoubtedly see more theoretical studies that analyze the performance of various coupling designs in the context of biophysical properties of specific kinetochore MAPs.

3.4 Ring-Shaped Couplers

3.4.1 Theoretical Motility Mechanisms

Mitchison and colleagues were the first to suggest that a microtubule-encircling ring coupler would be advantageous because it could move processively along the microtubule wall without detaching (Koshland et al. 1988; Mitchison 1988). Ring coupling is particularly interesting because in theory it permits motion by both the thermally driven biased-diffusion mechanism and the diffusion-free mechanism, in which the motion is due to protofilament power strokes (Mitchison 1988; Efremov et al. 2007). This is because the mechanism of motion is not defined by the coupler's geometry so much as by microtubule-binding affinity, as discussed for the "independent binders" designs. If the ring subunits bind the microtubule wall weakly enough to permit the ring's diffusion, its "random walk" (Pearson 1905; Howard 2002) will be biased by the flared protofilaments at the end of the shortening microtubule, assuming that these structures are persistent. As expected for all biased-diffusion couplers, calculations have shown that the opposing load pulls the low-affinity ring to the microtubule tip (Efremov et al. 2007), where the ring will either resist further depolymerization or detach quickly if the protofilament curls are lost, e.g., through stochastic fluctuations or straightening under a load.

To force the loaded ring to move away from the terminal tip subunits and permit microtubule disassembly, tubulin power strokes are necessary; accordingly, this mode of tip-tracking has been termed the "forced walk" (Efremov et al. 2007). The primary distinction between the biased-walk and forced-walk mechanisms is related not to the presence or absence of the "conformational wave" of tubulin bending (Koshland et al. 1988), but rather to the role it plays in the ring's motion (Mitchison 1988). In the biased-diffusion mechanism, the "conformational wave" forms a reflective barrier, whereas in the forced walk this conformational change represents a force-producing element. As the name "forced walk" implies, in this mechanism the MAP elements of the ring coupler move processively under the influence of the depolymerization force. This contrasts with the operation of the independent binders coupler in Fig. 4c, which is also moved by power strokes. In that design, however, the motions of the individual MAPs are not processive, so this variation of the "independent binders" coupler does not operate via the forced-walk mechanism. Hopefully, this consideration demonstrates the diversity and multitude of possible coupling designs, as well as the need to rigorously analyze them via quantitative modeling. The important lesson from theoretical studies thus far is that the competing constraints on the molecular parameters of each design arise from the nontrivial requirement of processivity of tracking under a large load at the force-generating end of the polymer.

Theoretical analyses of ring coupling have allowed evaluating the relative benefits of various mechanisms of operation (Efremov et al. 2007). One important finding relates to the ability of the power stroke mechanism to work in conjunction with rings that bind to the microtubule wall much more strongly than is permissive

for ring's diffusion, so such rings cannot be moved by the biased-diffusion mechanism. Stronger ring binding is highly beneficial for chromosome segregation because ring's adhesion represents a back-up mechanism to prevent ring's slipping from the microtubule end that is not protected by the protofilament flare, which can be temporarily lost due to a stochastic fluctuation or disassembly pausing (Zakharov et al. 2015). Such protection would come at the expense of maximum load-bearing by the ring coupler, because the energy from power strokes will now have to be spent moving the wall-binding ring, not just the load. Obviously, if the binding is excessive, the internal friction of this coupler may become so strong that the depolymerization motor will not be able to move such a ring, and microtubule disassembly will be blocked until the ring falls off. Thus, the back-up mechanism to prevent ring's slipping and load-bearing impose competing constraints on the strength of ring's binding to microtubule wall. When it is optimal, the power stroke mechanism can transport larger load and provide better stability of end-attachment than the biased-diffusion mechanism. On the other hand, load-bearing by such a ring is smaller than that by a coupler with non-processive independent binders (as in Fig. 4c) because the latter do not "walk," and therefore such a coupler has no internal friction (McIntosh et al. 2008). Other models for a ring coupler have also been proposed (Liu and Onuchic 2006; Armond and Turner 2010; Vichare et al. 2013), so theoretical investigations evaluating different ring coupling designs are ongoing.

3.4.2 The Yeast Dam1 Ring

The ring coupler is also unusual and exciting because it can be studied experimentally using Dam1 protein complex from the yeast kinetochore, which oligomerizes spontaneously to form microtubule-encircling rings in vitro (Miranda et al. 2005; Westermann et al. 2005). The electron-dense core of the Dam1 ring is separated from the outer microtubule wall by a 3–6 nm gap, spanned by flexible linkages (Miranda et al. 2007; Wang et al. 2007). Such large ring diameter is optimally suited for the power stroke mechanism because it maximizes force transduction by the ring (Molodtsov et al. 2005). Although Dam1 heterodecamers, the individual subunits of the ring, diffuse well on microtubule walls (Westermann et al. 2006; Gestaut et al. 2008; Grishchuk et al. 2008b), their collective microtubule affinity is so strong that the 16-subunit ring diffuses extremely slowly (Volkov et al. 2013); for alternative view on ring's diffusion see (Ramey et al. 2011). If such slow ring had to move via biased diffusion, the rate of microtubule disassembly would have decreased below 0.1 μm/min. On a side note, this behavior is significantly different from that of the sleeve coupler, because the latter was designed to always track at the same velocity by adjusting the size of the overhangs; ring, however, has all subunits bound to the microtubule wall. Real Dam1 ring appears to be able to track the shortening microtubule ends in vitro much faster than would be expected

for the biased-diffusion mechanism, at 7–10 μm/min (Grishchuk et al. 2008a). Collectively, this evidence points to the forced-walk mechanism of yeast Dam1 ring tracking. Such nondiffusive coupler with strong microtubule adhesion would be particularly useful in budding yeast, in which each kinetochore is attached to only one microtubule; consequently, loss of this attachment cannot be permitted.

Additional evidence for the forced-walk mechanism of yeast Dam1 ring tracking came from measurements of the load-bearing by this coupler. The relatively strong microtubule wall binding by Dam1 ring, as deduced from its negligible diffusion, implies that it should be able to carry ~40 pN load (Efremov et al. 2007). The initial experiments, however, found that microtubule depolymerization force transduced by Dam1 did not exceed 5 pN (Asbury et al. 2006; Grishchuk et al. 2008a). This is much larger than the force measured with streptavidin beads, but much smaller than predicted by the forced-walk model. This discrepancy could be explained by differences in the geometry of load application between theory and experiment. The experimental measurements were carried out with Dam1 rings attached laterally to the microbeads, whereas the theoretical prediction was for suspended rings (Fig. 4d), such as those that are thought to operate at yeast kinetochores (Gonen et al. 2012). Indeed, when Dam1 was linked to beads with the help of elongated fibrillar tethers, force transduction increased dramatically (Volkov et al. 2013). This force still has not reached the theoretical maximum, presumably because experimental rings can rupture, whereas theoretical rings are rupture-free. Weak ring integrity may limit load-bearing capacity at the kinetochore, or may simply reflect the inadequacy of in vitro systems that use purified components. One important outcome from experiments using the suspended Dam1 ring is that its load-bearing appears to be similar to that of isolated kinetochore particles tracking depolymerizing microtubule ends (Akiyoshi et al. 2010; Volkov et al. 2013). Therefore, it is possible that the suspended Dam1 ring moving via the forced-walk mechanism represents the main force-transducing unit of yeast kinetochore. Elongated tethers are highly important for ring's load-bearing because they allow the load to become aligned along the microtubule axis, thereby minimizing the lever-arm effect that limits force transduction by the wall-bound beads (Grishchuk et al. 2005). Long tethers also permit distributing the load evenly among the microtubule protofilaments. Although ring-dependent coupling may be unique to the yeast kinetochore, fibrillar elements will undoubtedly be found to play essential roles in load-bearing by kinetochore couplers in other cell types.

Acknowledgements The author thanks F. Ataullakhanov for critical reading of the manuscript, and M. Godzi for technical assistance with preparing the manuscript and figures. Funding for this work was provided by the National Institutes of Health grant GM-R01098389. Numerical calculations in Fig. 3 were carried out with support from Russian Science Foundation (grant # 16-14-00-224). E.L.G. is supported in part by the American Cancer Society grant RSG-14-018-01-CCG.

References

Akhmanova A, Steinmetz MO (2010) Microtubule + TIPs at a glance. J Cell Sci 123:3415–3419. doi:10.1242/jcs.062414

Akiyoshi B, Sarangapani KK, Powers AF, Nelson CR, Reichow SL, Arellano-santoyo H, Gonen T, Ranish J a, Asbury CL, Biggins S (2010) Tension directly stabilizes reconstituted kinetochore- microtubule attachments. Nature 468:576–579. doi:10.1038/nature09594

Alexander SP, Rieder CL (1991) Chromosome motion during attachment to the vertebrate spindle: initial saltatory-like behavior of chromosomes and quantitative analysis of force production by nascent kinetochore fibers. J Cell Biol 113:805–815

Alushin G, Nogales E (2011) Visualizing kinetochore architecture. Curr Opin Struct Biol 21:661–669. doi:10.1016/j.sbi.2011.07.009

Alushin GM, Lander GC, Kellogg EH, Zhang R, Baker D, Nogales E (2014) High-resolution microtubule structures reveal the structural transitions in αβ-tubulin upon GTP hydrolysis. Cell 157:1117–1129. doi:10.1016/j.cell.2014.03.053

Alushin GM, Ramey VH, Pasqualato S, Ball DA, Grigorieff N, Musacchio A, Nogales E (2010) The Ndc80 kinetochore complex forms oligomeric arrays along microtubules. Nature 467:805–810. doi:10.1038/nature09423

Aravamudhan P, Felzer-Kim I, Gurunathan K, Joglekar AP (2014) Assembling the protein architecture of the budding yeast kinetochore-microtubule attachment using FRET. Curr Biol 24:1437–1446. doi:10.1016/j.cub.2014.05.014

Armond JW, Turner MS (2010) Force transduction by the microtubule-bound Dam1 ring. Biophys J 98:1598–1607. doi:10.1016/j.bpj.2010.01.004

Armond JW, Vladimirou E, Erent M, McAinsh AD, Burroughs NJ (2015) Probing microtubule polymerisation state at single kinetochores during metaphase chromosome motion. J Cell Sci 128:1991–2001. doi:10.1242/jcs.168682

Asbury CL, Gestaut DR, Powers AF, Franck AD, Davis TN (2006) The Dam1 kinetochore complex harnesses microtubule dynamics to produce force and movement. Proc Natl Acad Sci U S A 103:9873–9878. doi:10.1073/pnas.0602249103

Asbury CL, Tien JF, Davis TN (2011) Kinetochores' gripping feat: Conformational wave or biased diffusion? Trends Cell Biol 21:38–46. doi:10.1016/j.tcb.2010.09.003

Ayaz P, Munyoki S, Geyer EA, Piedra FA, Vu ES, Bromberg R, Otwinowski Z, Grishin NV, Brautigam CA, Rice LM (2014) A tethered delivery mechanism explains the catalytic action of a microtubule polymerase. Elife 3:e03069. doi:10.7554/eLife.03069

Barisic M, Aguiar P, Geley S, Maiato H (2014) Kinetochore motors drive congression of peripheral polar chromosomes by overcoming random arm-ejection forces. Nat Cell Biol 16:1249–1256. doi:10.1038/ncb3060

Bieling P, Kandels-Lewis S, Telley IA, van Dijk J, Janke C, Surrey T (2008) CLIP-170 tracks growing microtubule ends by dynamically recognizing composite EB1/tubulin-binding sites. J Cell Biol 183:1223–1233. doi:10.1083/jcb.200809190

Brouhard GJ, Stear JH, Noetzel TL, Al-Bassam J, Kinoshita K, Harrison SC, Howard J, Hyman AA (2008) XMAP215 Is a processive microtubule polymerase. Cell 132:79–88. doi:10.1016/j.cell.2007.11.043

Caplow M, Ruhlen RL, Shanks J (1994) The free energy for hydrolysis of a microtubule-bound nucleotide triphosphate is near zero: All of the free energy for hydrolysis is stored in the microtubule lattice. J Cell Biol 127:779–788. doi:10.1083/jcb.127.3.779

Cheerambathur DK, Desai A (2014) Linked in: Formation and regulation of microtubule attachments during chromosome segregation. Curr Opin Cell Biol 26:113–122. doi:10.1016/j.ceb.2013.12.005

Cheerambathur DK, Gassmann R, Cook B, Oegema K, Desai A (2013) Crosstalk between microtubule attachment complexes ensures accurate chromosome segregation. Science 342:1239–1242. doi:10.1126/science.1246232

Cheeseman IM, Desai A (2008) Molecular architecture of the kinetochore–microtubule interface. Nat Rev Mol Cell Biol 9:33–46. doi:10.1038/nrm2310

Chen Y, Rolls MM, Hancock WO (2014) An EB1-kinesin complex is sufficient to steer microtubule growth in vitro. Curr Biol 24:316–321. doi:10.1016/j.cub.2013.11.024

Cimini D, Cameron LA, Salmon ED, Hill C, Carolina N (2004) Anaphase spindle mechanics prevent mis-segregation of merotelically oriented chromosomes. Curr Biol 14:2149–2155. doi:10.1016/j.cub.2004.11.029

Civelekoglu-Scholey G, He B, Shen M, Wan X, Roscioli E, Bowden B, Cimini D (2013) Dynamic bonds and polar ejection force distribution explain kinetochore oscillations in PtK1 cells. J Cell Biol 201:577–593. doi:10.1083/jcb.201301022

Cooke CA, Schaar B, Yen TJ, Earnshaw WC (1997) Localization of CENP-E in the fibrous corona and outer plate of mammalian kinetochores from prometaphase through anaphase. Chromosoma 106:446–455. doi:10.1007/s004120050266

Cooper JR, Wordeman L (2009) The diffusive interaction of microtubule binding proteins. Curr Opin Cell Biol 21:68–73. doi:10.1016/j.ceb.2009.01.005

Coue M, Lombillo VA, McIntosh JR (1991) Microtubule depolymerization promotes particle and chromosome movement in vitro. J Cell Biol 112:1165–1175. doi:10.1083/jcb.112.6.1165

Desai A, Mitchison TJ (1997) Microtubule polymerization dynamics. Annu Rev Cell Dev Biol 13:83–117. doi:10.1146/annurev.cellbio.13.1.83

Dogterom M, Yurke B (1997) Measurement of the force-velocity relation for growing microtubules. Science 80(278):856–860. doi:10.1126/science.278.5339.856

Dong Y, Vanden Beldt KJ, Meng X, Khodjakov A, McEwen BF (2007) The outer plate in vertebrate kinetochores is a flexible network with multiple microtubule interactions. Nat Cell Biol 9:516–522. doi:10.1038/ncb1576

Doodhi H, Katrukha EAEA, Kapitein LCLC, Akhmanova A (2014) Mechanical and geometrical constraints control kinesin-based microtubule guidance. Curr Biol 24:322–328. doi:10.1016/j.cub.2014.01.005

Dumont S, Salmon ED, Mitchison TJ (2012) Deformations within moving kinetochores reveal different sites of active and passive force generation. Science 337:355–358. doi:10.1126/science.1221886

Efremov A, Grishchuk EL, McIntosh JR, Ataullakhanov FI (2007) In search of an optimal ring to couple microtubule depolymerization to processive chromosome motions. Proc Natl Acad Sci U S A 104:19017–19022. doi:10.1073/pnas.0709524104

Espeut J, Gaussen A, Bieling P, Morin V, Prieto S, Fesquet D, Surrey T, Abrieu A (2008) Phosphorylation relieves autoinhibition of the kinetochore motor Cenp-E. Mol Cell 29:637–643. doi:10.1016/j.molcel.2008.01.004

Ferraro-Gideon J, Sheykhani R, Zhu Q, Duquette ML, Berns MW, Forer A (2013) Measurements of forces produced by the mitotic spindle using optical tweezers. Mol Biol Cell 24:1375–1386. doi:10.1091/mbc.E12-12-0901

Gandhi SR, Gierliński M, Mino A, Tanaka K, Kitamura E, Clayton L, Tanaka TU (2011) Kinetochore-dependent microtubule rescue ensures their efficient and sustained interactions in early mitosis. Dev Cell 21:920–933. doi:10.1016/j.devcel.2011.09.006

Gestaut DR, Graczyk B, Cooper J, Widlund PO, Zelter A, Wordeman L, Asbury CL, Davis TN (2008) Phosphoregulation and depolymerization-driven movement of the Dam1 complex do not require ring formation. Nat Cell Biol 10:407–414. doi:10.1038/ncb1702

Gonen S, Akiyoshi B, Iadanza MG, Shi D, Duggan N, Biggins S, Gonen T (2012) The structure of purified kinetochores reveals multiple microtubule-attachment sites. Nat Struct Mol Biol 19:925–929. doi:10.1038/nsmb.2358

Grishchuk EL, Efremov AK, Volkov VA, Spiridonov IS, Gudimchuk N, Westermann S, Drubin D, Barnes G, McIntosh JR, Ataullakhanov FI (2008a) The Dam1 ring binds microtubules strongly enough to be a processive as well as energy-efficient coupler for chromosome motion. Proc Natl Acad Sci U S A 105:15423–15428. doi:10.1073/pnas.0807859105

Grishchuk EL, McIntosh JR (2006) Microtubule depolymerization can drive poleward chromosome motion in fission yeast. EMBO J 25:4888–4896. doi:10.1038/sj.emboj.7601353

Grishchuk EL, McIntosh JR, Molodtsov MI, Ataullakhanov FI (2012) Force generation by dynamic microtubule polymers. In: Goldman YE, Ostap EM (eds) Comprehensive biophysics. Elsevier, Netherlands, pp 93–117

Grishchuk EL, Molodtsov MI, Ataullakhanov FI, McIntosh JR (2005) Force production by disassembling microtubules. Nature 438:384–388. doi:10.1038/nature04132

Grishchuk EL, Spiridonov IS, Volkov V, Efremov A, Westermann S, Drubin D, Barnes G, Ataullakhanov FI, McIntosh JR (2008b) Different assemblies of the DAM1 complex follow shortening microtubules by distinct mechanisms. Proc Natl Acad Sci U S A 105:6918–6923. doi:10.1073/pnas.0801811105

Gudimchuk N, Vitre B, Kim Y, Kiyatkin A, Cleveland DW, Ataullakhanov FI, Grishchuk EL (2013) Kinetochore kinesin CENP-E is a processive bi-directional tracker of dynamic microtubule tips. Nat Cell Biol 15:1079–1088. doi:10.1038/ncb2831

Hayden JH, Bowser SS, Rieder CL (1990) Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt lung cells. J Cell Biol 111:1039–1045. doi:10.1083/jcb.111.3.1039

Hill TL (1985) Theoretical problems related to the attachment of microtubules to kinetochores. Cell Biol 82:4404–4408. doi:10.1073/pnas.82.13.4404

Hill TL, Kirschner MW (1982) Bioenergetics and kinetics of microtubule and actin filament assembly-disassembly. Int Rev Cytol 78:1–125. doi:10.1016/S0074-7696(08)60105-9

Howard J (2002) Mechanics of motor proteins. Physics of bio-molecules and cells. Physique des biomolécules et des cellules. Springer, Berlin, pp 69–94

Hunt AJ, McIntosh JR (1998) The dynamic behavior of individual microtubules associated with chromosomes in vitro. Mol Biol Cell 9:2857–2871. doi:10.1091/mbc.9.10.2857

Inoué S, Salmon ED (1995) Force generation by microtubule assembly/disassembly in mitosis and related movements. Mol Biol Cell 6:1619–1640. doi:10.1091/mbc.E12-02-0146

Joglekar AP, Bloom KS, Salmon ED (2010) Mechanisms of force generation by end-on kinetochore-microtubule attachments. Curr Opin Cell Biol 22:57–67. doi:10.1016/j.ceb.2009.12.010

Joglekar AP, Hunt AJ (2002) A simple, mechanistic model for directional instability during mitotic chromosome movements. Biophys J 83:42–58. doi:10.1016/S0006-3495(02)75148-5

Ke K, Cheng J, Hunt AJ (2009) The distribution of polar ejection forces determines the amplitude of chromosome directional instability. Curr Biol 19:807–815. doi:10.1016/j.cub.2009.04.036

Keener JP, Shtylla B (2014) A mathematical model of force generation by flexible kinetochore-microtubule attachments. Biophys J 106:998–1007. doi:10.1016/j.bpj.2014.01.013

Kerssemakers JWJ, Munteanu EL, Laan L, Noetzel TL, Janson ME, Dogterom M (2006) Assembly dynamics of microtubules at molecular resolution. Nature 442:709–712. doi:10.1038/nature04928

Khodjakov A, Rieder CL (1996) Kinetochores moving away from their associated pole do not exert a significant pushing force on the chromosome. J Cell Biol 135:315–327. doi:10.1083/jcb.135.2.315

Kim Y, Heuser JE, Waterman CM, Cleveland DW (2008) CENP-E combines a slow, processive motor and a flexible coiled coil to produce an essential motile kinetochore tether. J Cell Biol 181:411–419. doi:10.1083/jcb.200802189

Koshland DE, Mitchison TJ, Kirschner MW (1988) Polewards chromosome movement driven by microtubule depolymerization in vitro. Nature 331:499–504. doi:10.1038/331499a0

Lampert F, Hornung P, Westermann S (2010) The Dam1 complex confers microtubule plus end-tracking activity to the Ndc80 kinetochore complex. J Cell Biol 189:641–649. doi:10.1083/jcb.200912021

Lawrimore J, Bloom KS, Salmon ED (2011) Point centromeres contain more than a single centromere-specific Cse4 (CENP-A) nucleosome. J Cell Biol 195:573–582. doi:10.1083/jcb.201106036

Liao H, Li G, Yen TJ (1994) Mitotic regulation of microtubule cross-linking activity of CENP-E kinetochore protein. Science 80(265):394–398. doi:10.1126/science.8023161

Liu J, Onuchic JN (2006) A driving and coupling "Pac-Man" mechanism for chromosome poleward translocation in anaphase A. Proc Natl Acad Sci 103:18432–18437. doi:10.1073/pnas.0608962103

Lombillo VA, Nislow C, Yen TJ, Gelfand VI, McIntosh JR (1995) Antibodies to the kinesin motor domain and CENP-E inhibit microtubule depolymerization-dependent motion of chromosomes in vitro. J Cell Biol 128:107–115. doi:10.1083/jcb.128.1.107

Maddox P, Straight A, Coughlin P, Mitchison TJ, Salmon ED (2003) Direct observation of microtubule dynamics at kinetochores in Xenopus extract spindles: implications for spindle mechanics. J Cell Biol 162:377–382. doi:10.1083/jcb.200301088

Magidson V, O'Connell CB, Lončarek J, Paul R, Mogilner A, Khodjakov A (2011) The spatial arrangement of chromosomes during prometaphase facilitates spindle assembly. Cell 146:555–567. doi:10.1016/j.cell.2011.07.012

Sokolov Mai, Blumen A (2001) Directed particle diffusion under "burnt bridges" conditions. Phys Rev E—Stat Nonlinear, Soft Matter Phys 64:011102/1–011102/4. doi:10.1103/PhysRevE.64.011102

Maiato H, DeLuca J, Salmon ED, Earnshaw WC (2004) The dynamic kinetochore-microtubule interface. J Cell Sci 117:5461–5477. doi:10.1242/jcs.01536

Mandelkow E, Mandelkow E, Milligan RA (1991) Microtubule dynamics and microtubule caps: a time-resolved cryo- electron microscopy study. J Cell Biol 114:977–991. doi:10.1083/jcb.114.5.977

Maurer SP, Bieling P, Cope J, Hoenger A, Surrey T (2011) GTPgammaS microtubules mimic the growing microtubule end structure recognized by end-binding proteins (EBs). Proc Natl Acad Sci U S A 108:3988–3993. doi:10.1073/pnas.1014758108

Mayr MI, Storch M, Howard J, Mayer TU (2011) A non-motor microtubule binding site is essential for the high processivity and mitotic function of kinesin-8 Kif18A. PLoS ONE 6:e27471. doi:10.1371/journal.pone.0027471

McEwen BF, Hsieh C-EE, Mattheyses AL, Rieder CL (1998) A new look at kineochore structure in vertebrate somatic cells using high-pressure freezing an freeze substitution. Chromosoma 107:366–375. doi:10.1007/s004120050320

McIntosh JR, Grishchuk EL, Morphew MK, Efremov AK, Zhudenkov K, Volkov VA, Cheeseman IM, Desai A, Mastronarde DN, Ataullakhanov FI (2008) Fibrils connect microtubule tips with kinetochores: a mechanism to couple tubulin dynamics to chromosome Motion. Cell 135:322–333. doi:10.1016/j.cell.2008.08.038

McIntosh JR, Grishchuk EL, West RR (2002) Chromosome-microtubule interactions during mitosis. Annu Rev Cell Dev Biol 18:193–219. doi:10.1146/annurev.cellbio.18.032002.132412

McIntosh JR, O'Toole E, Zhudenkov K, Morphew M, Schwartz C, Ataullakhanov FI, Grishchuk EL (2013) Conserved and divergent features of kinetochores and spindle microtubule ends from five species. J Cell Biol 200:459–474. doi:10.1083/jcb.201209154

McIntosh JR, Volkov V, Ataullakhanov FI, Grishchuk EL (2010) Tubulin depolymerization may be an ancient biological motor. J Cell Sci 123:3425–3434. doi:10.1242/jcs.067611

Miller MP, Asbury CL, Biggins S (2016) A TOG protein confers tension sensitivity to kinetochore-microtubule attachments. Cell 165:1428–1439. doi:10.1016/j.cell.2016.04.030

Minoura I, Katayama E, Sekimoto K, Muto E (2010) One-dimensional brownian motion of charged nanoparticles along microtubules: a model system for weak binding interactions. Biophys J 98:1589–1597. doi:10.1016/j.bpj.2009.12.4323

Miranda JJL, De Wulf P, Sorger PK, Harrison SC (2005) The yeast DASH complex forms closed rings on microtubules. Nat Struct Mol Biol 12:138–143. doi:10.1038/nsmb896

Miranda JJL, King DS, Harrison SC (2007) Protein arms in the kinetochore-microtubule interface of the yeast DASH complex. Mol Biol Cell 18:2503–2510. doi:10.1091/mbc.E07-02-0135

Mitchison T, Evans L, Schulze E, Kirschner M (1986) Sites of microtubule assembly and disassembly in the mitotic spindle. Cell 45:515–527. doi:10.1016/0092-8674(86)90283-7

Mitchison TJ (1988) Microtubule dynamics and kinetochore function in mitosis. Annu Rev Cell Biol 4:527–549. doi:10.1146/annurev.cb.04.110188.002523
Mitchison TJ, Kirschner MW (1985) Properties of the kinetochore in vitro. II. Microtubule capture and ATP-dependent translocation. J Cell Biol 101:766–777. doi:10.1083/jcb.101.3.766
Mogilner A, Oster G (1996) Cell motility driven by actin polymerization. Biophys J 71:3030–3045. doi:10.1016/S0006-3495(96)79496-1
Mogilner A, Oster G (2003) Polymer motors: pushing out the front and pulling up the back. Curr Biol 13:R721–R733. doi:10.1016/j.cub.2003.08.050
Molodtsov MI, Grishchuk EL, Efremov AK, McIntosh JR, Ataullakhanov FI (2005) Force production by depolymerizing microtubules: a theoretical study. Proc Natl Acad Sci U S A 102:4353–4358. doi:10.1073/pnas.0501142102
Nagpal H, Fukagawa T (2016) Kinetochore assembly and function through the cell cycle. Chromosoma 125:645–659. doi:10.1007/s00412-016-0608-3
Nicklas RB (1965) Chromosome velocity during mitosis as a function of chromosome size and position. J Cell Biol 25:119–135. doi:10.1083/jcb.25.1.119
Nicklas RB (1983) Measurements of the force produced by the mitotic spindle in anaphase. J Cell Biol 97:542–548. doi:10.1083/jcb.97.2.542
Nicklas RB (1989) The motor for poleward chromosome movement in anaphase is in or near the kinetochore. J Cell Biol 109:2245–2255. doi:10.1083/jcb.109.5.2245
Nogales E (2001) Structural insights into microtubule function. Annu Rev Biochem 69:277–302. doi:10.1146/annurev.biochem.74.061903.155440
O'Connell CB, Khodjakov A, McEwen BF (2012) Kinetochore flexibility: creating a dynamic chromosome-spindle interface. Curr Opin Cell Biol 24:40–47. doi:10.1016/j.ceb.2011.12.008
Pearson K (1905) The problem of the random walk. Nature 72:294
Peskin CS, Odell GM, Oster GF (1993) Cellular motions and thermal fluctuations: the Brownian ratchet. Biophys J 65:316–324. doi:10.1016/S0006-3495(93)81035-X
Peskin CS, Oster GF (1995) Force production by depolymerizing microtubules: load-velocity curves and run-pause statistics. Biophys J 69:2268–2276. doi:10.1016/S0006-3495(95)80097-4
Pfarr CM, Coue M, Grissom PM, Hays TS, Porter ME, McIntosh JR (1990) Cytoplasmic dynein is localized to kinetochores during mitosis. Nature 345:263–265. doi:10.1038/345263a0
Powers AF, Franck AD, Gestaut DR, Cooper J, Gracyzk B, Wei RR, Wordeman L, Davis TN, Asbury CL (2009) The Ndc80 Kinetochore complex forms load-bearing attachments to dynamic microtubule tips via biased diffusion. Cell 136:865–875. doi:10.1016/j.cell.2008.12.045
Preciado López M, Huber F, Grigoriev I, Steinmetz MO, Akhmanova A, Koenderink GH, Dogterom M (2014) Actin-microtubule coordination at growing microtubule ends. Nat Commun 5:4778. doi:10.1038/ncomms5778
Putkey FR, Cramer T, Morphew MK, Silk AD, Johnson RS, McIntosh JR, Cleveland DW (2002) Unstable kinetochore-microtubule capture and chromosomal instability following deletion of CENP-E. Dev Cell 3:351–365. doi:10.1016/S1534-5807(02)00255-1
Rago F, Cheeseman IM (2013) The functions and consequences of force at kinetochores. J Cell Biol 200:557–565. doi:10.1083/jcb.201211113
Ramey VH, Wang H-W, Nakajima Y, Wong A, Liu J, Drubin D, Barnes G, Nogales E (2011) The Dam1 ring binds to the E-hook of tubulin and diffuses along the microtubule. Mol Biol Cell 22:457–466. doi:10.1091/mbc.E10-10-0841
Reimann P (2002) Brownian motors: noisy transport far from equilibrium. Phys Rep 361:57–265. doi:10.1016/S0370-1573(01)00081-3
Reithmann E, Reese L, Frey E (2015) Quantifying protein diffusion and capture on filaments. Biophys J 108:787–790. doi:10.1016/j.bpj.2014.12.053
Rieder CL (1982) The formation, structure, and composition of the mammalian kinetochore and kinetochore Fiber. Int Rev Cytol 79:1–58. doi:10.1016/S0074-7696(08)61672-1
Rieder CL, Salmon ED (1998) The vertebrate cell kinetochore and its roles during mitosis. Trends Cell Biol 8:310–318. doi:10.1016/S0962-8924(98)01299-9

Santaguida S, Musacchio A (2009) The life and miracles of kinetochores. EMBO J 28:2511–2531. doi:10.1038/emboj.2009.173

Schmidt JC, Arthanari H, Boeszoermenyi A, Dashkevich NM, Wilson-Kubalek EM, Monnier N, Markus M, Oberer M, Milligan RA, Bathe M, Wagner G, Grishchuk EL, Cheeseman IM (2012) The kinetochore-bound Ska1 complex tracks depolymerizing microtubules and binds to curved protofilaments. Dev Cell 23:968–980. doi:10.1016/j.devcel.2012.09.012

Shrestha RL, Draviam VM (2013) Lateral to end-on conversion of chromosome-microtubule attachment requires kinesins cenp-e and MCAK. Curr Biol 23:1514–1526. doi:10.1016/j.cub.2013.06.040

Shtylla B, Keener JP (2011) A mathematical model for force generation at the kinetochore-microtubule Interface. SIAM J Appl Math 71:1821–1848. doi:10.1137/100802645

Skibbens RV, Skeen VP, Salmon ED (1993) Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: A push-pull mechanism. J Cell Biol 122:859–875. doi:10.1083/jcb.122.4.859

Skibbens RV, Salmon ED (1997) Micromanipulation of chromosomes in mitotic vertebrate tissue cells: tension controls the state of kinetochore movement. Exp Cell Res 235:314–324. doi:10.1006/excr.1997.3691

Steuer ER, Wordeman L, Schroer TA, Sheetz MP (1990) Localization of cytoplasmic dynein to mitotic spindles and kinetochores. Nature 345:266–268. doi:10.1038/345266a0

Su X, Qiu W, Gupta ML, Pereira-Leal JB, Reck-Peterson SL, Pellman D (2011) Mechanisms underlying the dual-mode regulation of microtubule dynamics by Kip3/Kinesin-8. Mol Cell 43:751–763. doi:10.1016/j.molcel.2011.06.027

Suzuki A, Badger BL, Salmon ED (2015) A quantitative description of Ndc80 complex linkage to human kinetochores. Nat Commun 6:8161. doi:10.1038/ncomms9161

Tanaka K, Kitamura E, Kitamura Y, Tanaka TU (2007) Molecular mechanisms of microtubule-dependent kinetochore transport toward spindle poles. J Cell Biol 178:269–281. doi:10.1083/jcb.200702141

Tanaka K, Mukae N, Dewar H, van Breugel M, James EK, Prescott AR, Antony C, Tanaka TU (2005) Molecular mechanisms of kinetochore capture by spindle microtubules. Nature 434:987–994. doi:10.1038/nature03483

Tanaka TU (2010) Kinetochore-microtubule interactions: steps towards bi-orientation. EMBO J 29:1–13. doi:10.1038/emboj.2010.294

Taylor EW (1965) Brownian and saltatory movements of cytoplasmic granules and the movement of anaphase chromosomes. In: Lee EH (ed) Proceedings of the fourth international congress on rheology. Interscience, New York, pp 175–191

Thomas GE, Bandopadhyay K, Sutradhar S, Renjith MR, Singh P, Gireesh KK, Simon S, Badarudeen B, Gupta H, Banerjee M, Paul R, Mitra J, Manna TK (2016) EB1 regulates attachment of Ska1 with microtubules by forming extended structures on the microtubule lattice. Nat Commun 7:11665. doi:10.1038/ncomms11665

Tirnauer JS, Canman JC, Salmon ED, Mitchison TJ (2002) EB1 targets to kinetochores with attached, polymerizing microtubules. Mol Biol Cell 13:4308–4316. doi:10.1091/mbc.E02

Tooley J, Stukenberg PT (2011) The Ndc80 complex: integrating the kinetochore's many movements. Chromosome Res 19:377–391. doi:10.1007/s10577-010-9180-5

Trushko A, Schäffer E, Howard J (2013) The growth speed of microtubules with XMAP215-coated beads coupled to their ends is increased by tensile force. Proc Natl Acad Sci U S A 110:14670–14675. doi:10.1073/pnas.1218053110

Umbreit NT, Gestaut DR, Tien JF, Vollmar BS, Gonen T, Asbury CL, Davis TN (2012) The Ndc80 kinetochore complex directly modulates microtubule dynamics. Proc Natl Acad Sci U S A 109:16113–16118. doi:10.1073/pnas.1209615109

Verma V, Mallik L, Hariadi RF, Sivaramakrishnan S, Skiniotis G, Joglekar AP (2015) Using protein dimers to maximize the protein hybridization efficiency with multisite DNA origami scaffolds. PLoS ONE 10(9):e0137125

Vichare S, Jain I, Inamdar MM, Padinhateeri R (2013) Forces due to curving protofilaments in microtubules. Phys Rev E 88:62708. doi:10.1103/PhysRevE.88.062708

Vitre B, Gudimchuk N, Borda R, Kim Y, Heuser JE, Cleveland DW, Grishchuk EL (2014) Kinetochore-microtubule attachment throughout mitosis potentiated by the elongated stalk of the kinetochore kinesin CENP-E. Mol Biol Cell 25:1–26. doi:10.1091/mbc.E14-01-0698

Volkov VA, Grissom PM, Arzhanik VK, Zaytsev AV, Renganathan K, McClure-Begley T, Old WM, Ahn N, Richard McIntosh J (2015) Centromere protein F includes two sites that couple efficiently to depolymerizing microtubules. J Cell Biol 209:813–828. doi:10.1083/jcb.201408083

Volkov VA, Zaytsev A V., Gudimchuk N, Grissom PM, Gintsburg AL, Ataullakhanov FI, McIntosh JR, Grishchuk EL (2013) Long tethers provide high-force coupling of the Dam1 ring to shortening microtubules. Proc Natl Acad Sci U S A 110:7708–7713. doi:10.1073/pnas.1305821110/-/DCSupplemental. www.pnas.org/cgi/doi/10.1073/pnas.1305821110

Wagoner JA, Dill KA (2016) Molecular motors: power strokes outperform brownian ratchets. J Phys Chem B 120:6327–6336. doi:10.1021/acs.jpcb.6b02776

Wan X, O'Quinn RP, Pierce HL, Joglekar AP, Gall WE, DeLuca JG, Carroll CW, Liu S-T, Yen TJ, McEwen BF, Stukenberg PT, Desai A, Salmon ED (2009) Protein architecture of the human kinetochore microtubule attachment site. Cell 137:672–684. doi:10.1016/j.cell.2009.03.035

Wang H-W, Ramey VH, Westermann S, Leschziner AE, Welburn JPI, Nakajima Y, Drubin DG, Barnes G, Nogales E (2007) Architecture of the Dam1 kinetochore ring complex and implications for microtubule-driven assembly and force-coupling mechanisms. Nat Struct Mol Biol 14:721–726. doi:10.1038/nsmb1274

Waters JC, Skibbens RV, Salmon ED (1996) Oscillating mitotic newt lung cell kinetochores are, on average, under tension and rarely push. J Cell Sci 109:2823–2831

Weaver LN, Ems-McClung SC, Stout JR, Leblanc C, Shaw SL, Gardner MK, Walczak CE (2011) Kif18A uses a microtubule binding site in the tail for plus-end localization and spindle length regulation. Curr Biol 21:1500–1506. doi:10.1016/j.cub.2011.08.005

Westermann S, Avila-Sakar A, Wang HW, Niederstrasser H, Wong J, Drubin DG, Nogales E, Barnes G (2005) Formation of a dynamic kinetochore-microtubule interface through assembly of the Dam1 ring complex. Mol Cell 17:277–290. doi:10.1016/j.molcel.2004.12.019

Westermann S, Wang H-W, Avila-Sakar A, Drubin DG, Nogales E, Barnes G (2006) The Dam1 kinetochore ring complex moves processively on depolymerizing microtubule ends. Nature 440:565–569. doi:10.1038/nature04409

Widlund PO, Stear JH, Pozniakovsky A, Zanic M, Reber S, Brouhard GJ, Hyman AA, Howard J (2011) XMAP215 polymerase activity is built by combining multiple tubulin-binding TOG domains and a basic lattice-binding region. Proc Natl Acad Sci U S A 108:2741–2746. doi:10.1073/pnas.1016498108

Wood KW, Sakowicz R, Goldstein LSB, Cleveland DW (1997) CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. Cell 91:357–366. doi:10.1016/S0092-8674(00)80419-5

Yao X, Anderson KL, Cleveland DW (1997) The microtubule-dependent motor centromere-associated protein E (CENP-E) is an integral component of kinetochore corona fibers that link centromeres to spindle microtubules. J Cell Biol 139:435–447. doi:10.1083/jcb.139.2.435

Yardimci H, van Duffelen M, Mao Y, Rosenfeld SS, Selvin PR (2008) The mitotic kinesin CENP-E is a processive transport motor. Proc Natl Acad Sci U S A 105:6016–6021. doi:10.1073/pnas.0711314105

Ye AA, Cane S, Maresca TJ (2016) Chromosome biorientation produces hundreds of piconewtons at a metazoan kinetochore. Nat Commun 7:13221. doi:10.1038/ncomms13221

Zanic M, Stear JH, Hyman AA, Howard J (2009) EB1 recognizes the nucleotide state of tubulin in the microtubule lattice. PLoS ONE 4:e7585. doi:10.1371/journal.pone.0007585

Zakharov P, Gudimchuk N, Voevodin V, Tikhonravov A, Ataullakhanov FI, Grishchuk EL (2015) Molecular and mechanical causes of microtubule catastrophe and aging. Biophys J 109 (12):2574–2591. doi:10.1016/j.bpj.2015.10.048

Zaytsev AV, Ataullakhanov FI, Grishchuk EL (2013) Highly transient molecular interactions underlie the stability of kinetochore-microtubule attachment during cell division. Cell Mol Bioeng 6:393–405. doi:10.1007/s12195-013-0309-4

Zaytsev AV, Grishchuk EL (2015) Basic mechanism for biorientation of mitotic chromosomes is provided by the kinetochore geometry and indiscriminate turnover of kinetochore microtubules. Mol Biol Cell 26:3985–3998. doi:10.1091/mbc.E15-06-0384

Zaytsev AV, Mick JE, Maslennikov E, Nikashin B, DeLuca JG, Grishchuk EL (2015) Multisite phosphorylation of the NDC80 complex gradually tunes its microtubule-binding affinity. Mol Biol Cell 26:1829–1844. doi:10.1091/mbc.E14-11-1539