

*Order from Chaos: How kinetochores organize
microtubules in the mitotic spindle*

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Abstract

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Accurate chromosome segregation during mitosis relies on the precise coordination of kinetochore-attached microtubules. While individual microtubules exhibit highly variable, stochastic growth and shortening dynamics, they must cooperate within the spindle to prevent chromosome mis-segregation and aneuploidy. This dissertation investigates the biophysical mechanisms that organize this stochastic chaos into robust cellular behaviors. First, I evaluate how mechanical forces promote chromosome biorientation with a computational model of the budding yeast spindle. I show that traditional error-correction mechanisms—including catch-bond behavior, tension-sensitive kinase activity, and geometric bias—are insufficient on their own to achieve timely biorientation. However, incorporating a direction-sensitive grip that specifically destabilizes side-attached kinetochores sliding toward the pole successfully recapitulates in vivo biorientation times. Second, using a novel dual laser trap assay and data-driven stochastic modeling, I demonstrate that pairs of growing microtubules are synchronized via mechanical coupling under a shared tensile load. Finally, I outline a framework to extend our models of microtubule growth to spindles with bundled kinetochore-microtubules in growth and shortening. Together, this work reveals that direction-sensitive grip and mechanical load-sharing act as critical, self-correcting organizing influences that tame microtubule heterogeneity to ensure mitotic fidelity.

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Chapter 1: Life is beautifully orchestrated and chaotic.

Incredible complexity from chaotic building blocks

The complexity of life is astounding. Organisms reproduce, metabolize nutrients, excrete waste, and adapt to their environment, to name just a few of many basic functions. Each of these functions is governed by a myriad of subprocesses—for example, for an organism to successfully adapt to its environment, it must sense environmental signals and react to them in a way that is beneficial to its survival. Environmental signals can take many forms, from light, to sound, to heat, to pain and so on. Each of these unique stimuli results in a specific response, and each of those responses often consists of many subprocesses.

Similarly, to reproduce, cells must duplicate all of their chromosomes and essential organelles, then divide those copies equally between daughter cells. Inheriting chromosomes is an intricate process in which the cell must duplicate, condense, align, and separate chromosomes exactly equally between the two daughter cells. Once again, each of these steps requires many substeps—to align chromosomes, for instance, the cell must assemble a bipolar mitotic spindle of long protein filaments called microtubules ([Figure 1.1](#)) as well as many microtubule-associated proteins. Large, many-protein complexes called kinetochores assemble on chromosomes to connect them to microtubules, allowing microtubules to pull chromosomes into alignment at the spindle equator. Microtubules then pull sister chromatids to opposite spindle poles upon chromosomal cleavage in anaphase. Failure of individual proteins to perform their expected function can lead to cell arrest and death ([Biggins et al., 1999](#); [Miller et al., 2019](#)). Organelles have their own methods of inheritance—for instance, Golgi bodies are inherited by their association with spindle poles and their associated microtubules ([Shorter and Warren, 2002](#)), while vacuole inheritance is driven by actin ([Weisman, 2006](#)), a different long, filamentous protein.

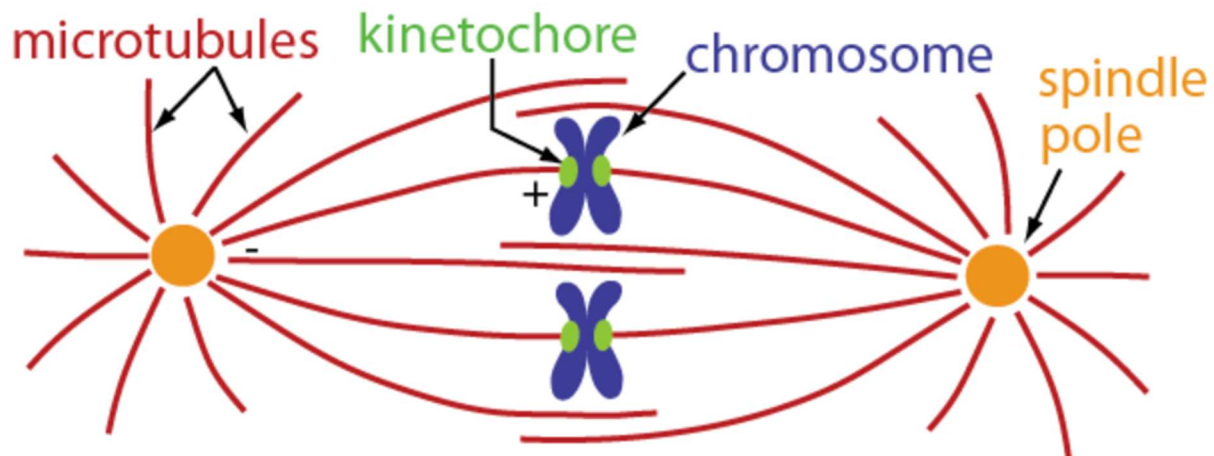


Figure 1.1: Microtubules form a bipolar mitotic spindle. Microtubule plus ends attach to chromosomes via kinetochores while microtubule minus ends attach to spindle poles. During metaphase, microtubules align chromosomes between the two spindle poles.

These networks of interconnected biological processes are especially fascinating because each component is governed by stochastic elements at multiple levels. Stochastic processes are those that occur with a certain probability and for which the outcome is uncertain. For example, at the molecular level, chemical reactions, transient binding and unbinding, and thermal motion are all stochastic ([Lakic and Mason, 2025](#)). As emergent behaviors of these molecular processes, neuron firing ([Harrison et al., 2005](#)), protein expression ([Raj and Oudenaarden, 2011](#)), and proliferation ([Ortega-Sabater et al., 2022](#)) are just a few examples of stochasticity at the cellular level. Based on the probability of the stochastic event, a mean behavior emerges, often with a wide distribution. For instance, protein expression levels in cells can fluctuate greatly ([Raj and Oudenaarden, 2011](#)).

Cells can overcome variability from stochastic chaos in a number of ways. Necessary interactions can be made all but guaranteed if the reactants are plentiful or they are very energetically favorable. For instance, ions are abundant in the cytoplasm, so even though each individual ion behaves probabilistically, there are enough ions in the right place at the right time to bind as cofactors for their protein partners ([Andreini et al., 2008](#)). Another example is kinetochore components, which must assemble on the centromere to bind microtubules and perform other cell-cycle signaling functions ([Navarro and Cheeseman, 2022](#)). Although kinetochore components are sparse ([Beck et al., 2011](#)), they bind each other tightly ([Miller et al., 2008](#)), and so have a high probability of associating when they happen to move close enough together, as well as a low probability of dissociating before they are purposefully degraded or removed from the kinetochore after mitosis is complete ([Chen et al., 2025](#); [Liao et al., 1995](#)). Cells can also mitigate variability with negative feedback—for example, tubulin, the protein subunit comprising microtubules, triggers the degradation of the mRNA coding for tubulin, thereby decreasing its own expression levels when tubulin levels get too high ([Cleveland et al., 1981](#); [Hopfler et al., 2023](#); [Lin et al., 2019](#)).

Alternatively, cells sometimes use feedback systems to embrace the chaos and improve their adaptability. If positive feedback is incorporated, a system can gain additional stable states ([Ingolia and Murray, 2007](#)). For example, microtubules use positive feedback to switch stochastically between quasi-stable states of growth and shortening, termed ‘dynamic instability.’ Microtubules are made of many tubulin protofilaments arranged in a hollow cylinder and grow and shorten from stochastic addition and subtraction of tubulin from their tips ([Brouhard and Rice, 2018](#)). Dynamic instability emerges from positive feedback that promotes shortening in shortening microtubules and growth in growing microtubules. When microtubules shorten, their protofilaments curve away from each other and create a conformational wave that often propagates until the microtubule has fully disassembled ([Murray et al., 2022](#)). When a microtubule grows, it incorporates GTP-tubulin from solution in a cap that stabilizes lateral tubulin interactions and makes self-propagating protofilament curls less likely to emerge ([Estevez-Gallego et al., 2026](#); [Manka and Moores, 2019](#); [VanBuren et al., 2002](#)).

These different modes of microtubule behaviors can be extremely beneficial to the cell, which can modulate the relative amount of microtubule growth and shortening. For instance, cells can adjust the activity of microtubule-associated proteins that alter microtubule growth, shortening, and switch rates between growth and shortening throughout the cell cycle ([Rusan et al., 2001](#); [Tournebise et al., 1999](#); [Verde et al., 1992](#)). This adjustability means that during interphase, microtubules can act as structural components and stable, elongatable highways for

intracellular transport ([Bloom and Goldstein, 1998](#)), whereas in mitosis, their ability to grow and more frequently shorten allows them to capture, align, and segregate chromosomes ([Biggins and Walczak, 2003](#); [Paul et al., 2009](#)). Microtubules' ability to shorten is also important after mitosis, when the cell has to disassemble the spindle ([Woodruff et al., 2010](#)).

How do cells efficiently biorient all of their chromosomes at once?

In addition to microtubule dynamic instability, cells must also curb the chaos created by stochastic chromosome capture ([Nicklas, 1997](#)). Chromosomes can attach to spindle poles either correctly, with one sister kinetochore attached to each pole ("bioriented"), or incorrectly, for example, with both sister kinetochores attached to the same pole. For successful mitosis, in a human cell, all 46 chromosomes must be attached correctly at the same time. Failure to attach to opposite spindle poles results in aneuploidy, which can lead to daughter cell death ([Rodrigues-Ferreira and Nahmias, 2020](#)) and is associated with cancer ([Sdeor et al., 2024](#)). Despite the difficulty of achieving simultaneous correct attachments from trial-and-error, mitosis is remarkably accurate, with a less than 0.3% loss rate for individual chromosome per division in mammalian cells ([Burns et al., 1999](#); [Klaasen and Kops, 2022](#); [Zaytsev and Grishchuk, 2015](#)). It is also fast, with the time from initial chromosome capture by spindle poles to chromosome separation being less than 60 min in many cell types ([Araujo et al., 2016](#); [Leitao and Kellogg, 2017](#); [Sim et al., 2024](#)).

One critical mechanism that cells employ against the inherent randomness of chromosome capture is to make incorrect attachment less likely with sister kinetochores that face in opposite directions ([Indjeian and Murray, 2007](#); [Nicklas and Ward, 1994](#); [Zaytsev and Grishchuk, 2015](#)). Another important mechanism is error correction—if an incorrect attachment is made, the cell can sense and destabilize it so that the stochastic attachment process can try again. Both error avoidance and error correction can suffer from drawbacks. Decreasing the likelihood of sister chromatids binding to the same pole might make it more difficult for microtubules to bind overall ([Nicklas, 1997](#)). Meiotic and mitotic kinetochores represent two different compromises to this issue—meiotic kinetochores protrude more than mitotic kinetochores and suffer more from syntelic attachments, while mitotic kinetochores suffer more from unattached kinetochores ([Nicklas, 1997](#)). Likewise, if error correction is too aggressive at destabilizing incorrect kinetochore-microtubule attachments, it might also destabilize correct ones.

In [Chapter 2](#), I developed a series of computational models to explore how proposed mechanisms of error avoidance and correction can lead to timely, simultaneous biorientation of all sixteen chromosomes in the budding yeast mitotic spindle. I found that even with a large geometric bias toward correct attachment, tension-based methods of error correction could not explain timely biorientation. However, these mechanisms were sufficient upon the addition of direction-sensitive grip, which specifically destabilizes kinetochores sliding toward the pole and so uniquely destabilizes incorrect attachments. My work illustrates how the cell can almost always achieve correct attachment of all of its chromosomes even with the unpredictability of attachment through trial-and-error, with a combination of bias toward correct attachment and careful weeding out of incorrect attachments.

How do kinetochore-microtubules grow and shorten as a single unit to move chromosomes?

Controlling microtubule dynamic instability parameters changes their mean length, but the combination of dynamic instability and variability in microtubule growth and shortening from stochastic addition and subtraction of tubulin still creates wide length distributions (Kiewisz et al., 2022; Verde et al., 1992). Yet precise control over not only the mean, but also the variability of microtubule lengths underlies cell division. In mammals, a kinetochore on a single sister chromatid will often bind a bundle of ten or more microtubules. The individual microtubules in these ‘k-fibers’ grow and shorten with remarkable synchrony in order to align and keep chromosomes at the spindle equator (O'Toole et al., 2020). Although microtubule tips within a bundle are kept in close proximity, different k-fibers in the same spindle often have variable lengths. This suggests the intriguing possibility that somehow, via the kinetochore, microtubules within a bundle can influence each other such that when one microtubule grows or shortens at a particular rate, the others are more likely to grow or shorten at that rate.

In [Chapters 3-5](#), I describe my progress toward explaining how tension-dependent microtubule dynamics and load-sharing via the kinetochore can synchronize microtubules within a k-fiber. I developed a new type of laser trap experiment and computational models to understand how intrinsic variability in growth rates can be coordinated for a pair of growing microtubules, as explained in [Chapter 3](#). I found that kinetochore-microtubule growth can be coordinated by mechanical coupling. I also found that microtubule pausing, another stochastic behavior of microtubules where they neither grow nor shorten during periods of overall growth, is suppressed by tension. Models including tension-dependent pausing explained both the observed variability in microtubule growth as well as the degree of coordination achieved by mechanical coupling. In [Chapters 4 and 5](#), I discuss my work to extend these computational models to many microtubules in a heterogeneous state of growth and shortening. This would allow us to assess how much of the incredible synchrony between microtubule tips in a k-fiber can be explained by mechanical coupling via the kinetochore.

My work illustrates another way that cells can control stochastic chaos—by coupling inherently stochastic elements together, those elements start to behave as a single unit. Fast microtubules are slowed by their generally slower compatriots, producing a more averaged and predictable overall behavior that can nonetheless be controlled by tension. When sister chromatids are linked and correctly attached in prometaphase, tension is high, and k-fibers are long so chromosomes can be aligned at the spindle equator. In anaphase, when sisters are no longer physically linked, tension is low, and k-fibers shorten to the spindle pole. The chaotic nature of microtubules makes them adaptable tools. Mechanical coupling is one way that cells can take advantage of microtubules’ adaptability while still being able to control their behavior.

As in biology, so in completing a thesis—life is all about using the surrounding chaos to best advantage. Cells need to solve huge numbers of problems while dealing with an inherently chaotic universe. It is up to them, and us, to harness that chaos.

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Chapter 2: Direction-sensitive grip with other error-avoidance methods explains timely chromosome biorientation.

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My role in this work

In this work, I conceptualized a series of computational models of budding yeast biorientation incorporating key elements of error correction with my advisor, Chip Asbury. I built and analyzed these models based on my extensive research of mitosis in general and experimental studies of mitotic error correction in budding yeast in specific. I wrote the first-draft manuscript and edited that manuscript with Chip. We plan to submit this work for publication to a peer-reviewed, primary scientific journal shortly.

Abstract

Mitotic cells separate their chromosomes with astounding speed and accuracy through a trial-and-error process where duplicated sister chromatid pairs initially attach randomly to microtubules emanating from either side of the cell. Attachments that happen to form correctly, with sisters attached to microtubules from opposite sides, are then selectively retained while incorrect attachments are released, giving the sisters another chance to form correct attachments. At least four mechanisms have been proposed to underlie this error correction process, including: (i) catch bond-like, tension-stabilized interactions between kinetochores and microtubule tips, (ii) selective kinase-triggered release of tip-attached kinetochores that lack tension, (iii) back-to-back sister kinetochore geometry that biases sisters toward capturing microtubules from opposite sides, and (iv) the newly-discovered direction-sensitive grip of kinetochores on microtubule sides. To test the sufficiency of these candidate mechanisms, we describe here a simple computational approach focused on budding yeast mitosis and constrained directly by cytological and biophysical measurements. Our simulations suggest that the intrinsic catch-bond behavior of kinetochores stabilizes both correct and incorrect attachments. Kinase-based release of low-tension attachments increases the selectivity for correct attachments, while geometric bias toward capturing microtubules from opposite sides speeds biorientation. However, these three mechanisms combined are not sufficient to explain the high efficiency of biorientation in vivo. Incorporating direction-sensitive grip specifically destabilizes incorrect attachments and allows simulated cells to biorient all their chromosomes as quickly as real cells.

Introduction

For accurate mitosis, every pair of replicated sister chromatids must become bioriented, with one chromatid attached to microtubules from the left side of the mitotic spindle and its

sister attached to microtubules from the right (Figure 2.1). Many cell types, from mammals to budding yeast, achieve complete biorientation of all their chromosomes in sixty minutes or fewer (Araujo et al., 2016; Leitao and Kellogg, 2017; Sim et al., 2024), and prolonged mitosis can result in cell arrest and death (Fulcher et al., 2025; Meitinger et al., 2024; Uetake and Sluder, 2018). Despite the limited time, cell division is nearly always accurate. The loss rate for an individual chromosome is less than 0.3% per division in mammalian cells (Burns et al., 1999; Klaasen and Kops, 2022; Zaytsev and Grishchuk, 2015) and only 0.0001% in budding yeast (Lacefield et al., 2009). High accuracy ensures daughter cells are viable and prevents aneuploidies, which are associated with cancer (Sdeor et al., 2024).

The high accuracy and speed of mitosis is astounding because biorientation is achieved by trial-and-error (Nicklas, 1997). Each replicated sister chromatid attaches to microtubules via a large protein complex called the kinetochore. Sister kinetochore pairs are initially 'monotelic' (Figure 2.1a), with only one microtubule-attached sister (McEwen et al., 1997; Nicklas and Ward, 1994). When the second sister attaches, it can either form a correct 'amphitelic' attachment (Figure 2.1b), or an incorrect 'syntelic' attachment (Figure 2.1c). The incorrect, syntelic attachments must then be selectively released, while correct, amphitelic attachments must be selectively retained. The opposing microtubules exert pulling forces, so correctly attached amphitelic sisters experience high tension, while incorrectly attached syntelic sisters lack tension. The high tension on correct attachments stabilizes them, while lack of tension on incorrect attachments causes them to detach and try again (Nicklas and Koch, 1969; Nicklas and Ward, 1994).

Two mechanisms have been proposed to explain this tension-dependent stabilization, which is widely believed to be a primary basis for mitotic accuracy. First, kinetochores exhibit an intrinsic catch-bond behavior which stabilizes tension-bearing tip-attachments (Akiyoshi et al., 2010). Second, kinases such as Aurora B (Ipl1 in budding yeast) and possibly Mps1 are thought to selectively phosphorylate low-tension kinetochores, weakening their attachments and causing them to release while leaving high-tension tip-attached kinetochores untouched (Biggins et al., 1999; de Regt et al., 2022; Maure et al., 2007; Mukherjee et al., 2019; Sarangapani et al., 2021; Tanaka et al., 2002). While cells without Aurora B or Mps1 fail to biorient many of their chromosomes (Biggins et al., 1999; Maure et al., 2007; Tanaka et al., 2002), it is unclear whether this failure is solely due to tension-sensitive kinetochore-microtubule release or if these kinases have additional functions that help cells avoid or correct erroneous attachments. Tension-dependent stabilization by both catch-bond behavior and kinases probably helps promote biorientation, but by themselves these mechanisms might not be sufficient. In addition, it remains mysterious how tension-sensitive kinases and catch-bond behavior can destabilize low-tension syntelic attachments without overly destabilizing monotelics, which also lack tension but must be formed and maintained on the pathway to amphitelic attachments.

A third error-avoidance mechanism is suggested by the geometric arrangement of sister kinetochores. In many cell types, sister chromatids are built back-to-back such that sister kinetochores face opposite directions. Their back-to-back arrangement is evident in electron micrographs of mitotic chromosomes from animal cells (Journey and Whaley, 1970; Ris and Witt, 1981) and may predispose monotelic pairs to catch microtubules emanating from the correct side of the spindle (Nicklas and Ward, 1994). This so-called 'geometric bias' may favor

creation of new amphitelic pairs over syntelic pairs and push the cell closer to biorientation of all its chromosomes. In budding yeast, neither kinetochores nor chromosomes are distinguishable by electron microscopy. However, chromosome segregation accuracy is improved in budding yeast when attachments form after spindle bipolarization, suggesting that a geometric bias helps monotelic pairs form correct attachments ([Indjeian and Murray, 2007](#)). Still, the strength of this geometric bias is unknown, and it is unclear how important it might be for timely biorientation.

A recent discovery suggests a fourth mechanism for error correction that specifically affects side-attached kinetochores. Usually, a kinetochore will first attach to the side of a microtubule and subsequently find the plus end ([Doodhi et al., 2021](#); [Rieder et al., 1990](#); [Tanaka et al., 2007](#)), either when external force slides it to the end or when the microtubule plus end shortens to the side-attached kinetochore. Recent work shows that side-attached kinetochores grip much more tightly when they are pulled toward the plus end than the minus end ([Demidov et al., 2025](#); [Larson et al., 2025](#)). Because of the polarity of microtubules in the spindle, with minus ends embedded at the poles and plus ends pointing toward the chromosomes ([McIntosh and Euteneuer, 1984](#)), the side-attached kinetochore in an amphitelic pair will be dragged by its tip-attached sister toward the plus end ([Figure 2.1b](#)). In this direction, the side-attached kinetochore has a strong grip and is likely to keep its hold until reaching the plus end. Conversely, the side-attached kinetochore in a syntelic pair will be dragged toward the minus end ([Figure 2.1c](#)). In this direction, the side-attached sister has a weak grip and is likely to detach quickly. Notably, unlike tension-dependent error-correction mechanisms ([Akiyoshi et al., 2010](#); [Doodhi et al., 2021](#); [Kalantzaki et al., 2015](#)), this direction-sensitive grip should not cause monotelic attachments to release since they do not slide toward minus ends.

All four mechanisms — catch-bond behavior, tension-sensitive kinase activity, geometric bias, and direction-sensitive grip — potentially help to efficiently biorient chromosomes. However, their distinct roles are unclear, and it remains uncertain whether together they are sufficient to explain timely biorientation. These are difficult questions to answer with traditional cytological or genetic approaches because the proteins involved in these mechanisms are either unknown or involved in many other aspects of mitosis, so clean separation-of-function mutants are unlikely to exist. We therefore developed a computational approach. To avoid the complexities of bundled kinetochore-attached microtubules, we focused on the budding yeast spindle, where kinetochores attach only one microtubule each ([Winey et al., 1995](#)). Moreover, biophysical measurements of isolated kinetochore behaviors are uniquely available for budding yeast kinetochores, so we could derive almost every model parameter directly from experimental data ([Tables 2.1 and 2.2](#)).

While previous models have explored three of the four error correction mechanisms either individually or in combination ([Edelmaier et al., 2020](#); [Paul et al., 2009](#); [Tubman et al., 2017](#); [Zaytsev and Grishchuk, 2015](#)), few have considered kinetochore attachments to the sides of microtubules, and none have included direction-sensitive grip, which was discovered only recently. In addition, recent measurements of kinetochore-microtubule release by Aurora B ([de Regt et al., 2022](#)) allowed us to incorporate tension-sensitive kinase activity directly from measurements and thereby to assess whether this activity, catch-bond behavior, or both are sufficient to explain timely biorientation. We found that even combined, catch-bond behavior and tension-sensitive kinase activity were not sufficient— instead, both geometric bias and

direction-sensitive grip were necessary for cells to efficiently biorient all of their chromosomes. Our work illustrates how direction-sensitive grip complements the other three mechanisms and suggests that the four mechanisms together are sufficient to explain the ability of dividing yeast to biorient all their chromosomes accurately and quickly.

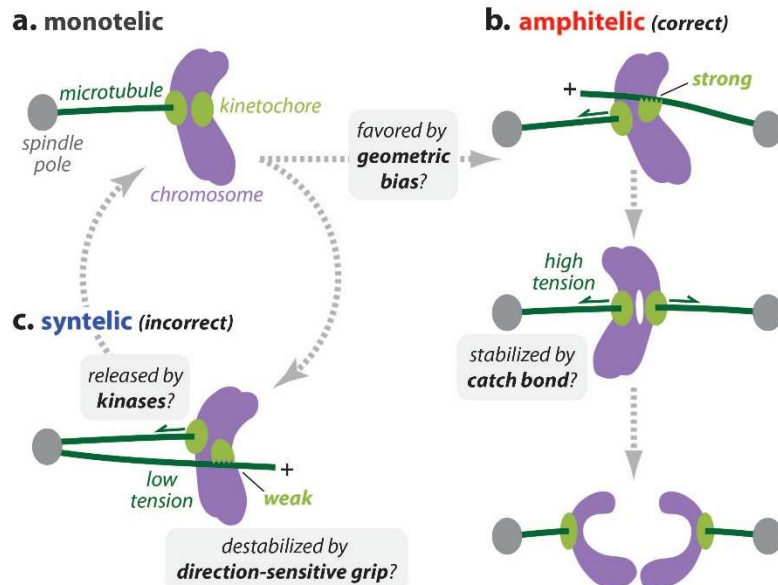


Figure 2.1. Multiple mechanisms proposed to promote chromosome biorientation. **a.** Biorientation of sister kinetochores occurs through trial-and-error. Initially, when only one sister is attached to a microtubule, the pair is called 'monotelic' (where the prefix mono- from ancient Greek means 'alone'). The second sister can attach either correctly or incorrectly. The back-to-back configuration of sister kinetochores on chromosomes might cause a geometric bias, favoring formation of correct over incorrect attachments. **b.** If the second sister attaches correctly, the pair is called 'amphitelic' (where amphi- means 'on both sides'). Opposing microtubules exert pulling forces so amphitelic attachments come under high tension, which stabilizes them until the sisters separate during anaphase. This tension-dependent stabilization might depend on the intrinsic catch-bond behavior of kinetochore-microtubule attachments. **c.** If the second sister attaches incorrectly, the pair is called 'syntelic' (where syn- means 'together') and lacks tension due to the absence of opposing microtubules. Kinases like Aurora B might selectively phosphorylate low-tension tip-attached kinetochores, causing their release. Conversely, the high tension on correctly attached kinetochores might protect them from release by kinases. An intrinsically direction-sensitive grip might also cause the release of side-attached kinetochores specifically when they are in a syntelic configuration.

Results

Two-stage strategy for simulating biorientation of sister kinetochore pairs

To examine the roles and sufficiency of the four candidate error correction mechanisms, we took a two-stage approach. In stage 1, we simulated individual sister kinetochore pairs and examined how effectively correct, amphitelic attachments were stabilized relative to incorrect, syntelic attachments. We successively incorporated catch-bond behavior, tension-sensitive kinase release, and direction-sensitive grip. To compare different scenarios, we computed a "selectivity ratio" for each model, defined as its mean amphitelic lifetime divided by its mean syntelic lifetime. We successively incorporated catch-bond behavior, tension-sensitive kinase release, and direction-sensitive grip to compare the selectivity ratios of different combinations of error correction mechanisms. Higher selectivity ratios indicate higher relative stabilization of

amphitelic over syntelic. In stage 2, we used the distributions of attachment lifetimes predicted in stage 1 to simulate how long it would take for individual yeast cells to simultaneously biorient all sixteen of their sister kinetochore pairs, with and without geometric bias. We then compared simulated biorientation times to mitotic durations measured in budding yeast. With successful combinations of error correction mechanisms, simulated cells bioriented all sixteen chromosomes in ≤ 40 min, the time required for budding yeast to go from pole separation to anaphase onset (Leitao and Kellogg, 2017; Sim et al., 2024). The specific mechanisms included in each model are indicated above each figure (“Model features”) together with the relevant simulation stage, 1 or 2. For simplicity, we restricted our models to one-dimension and assumed a fixed spindle length of $1.5\ \mu\text{m}$, the length of budding yeast spindles at metaphase (Marco et al., 2013; Sim et al., 2024).

Simulations produce realistic kinetochore positions and forces

Each stage 1 simulation began with a sister kinetochore pair side-attached to a pair of dynamic microtubules in either an amphitelic, syntelic, or monotelic configuration (Figures 2.2, S2.1, and Video 2.1-2.3; see Methods 1.1 and 1.2). Sister kinetochores were linked by a simple elastic spring (see Methods 1.3). Microtubules were programmed to grow, shorten, and stochastically switch between growth and shortening with rates chosen to match microtubules in vivo (see Table 2.1 and Methods 1.4). Kinetochore behavior essentially as measured in vitro, including the tension-dependent effects responsible for catch-bond behavior (see Methods 1.4 and Figures S2.2a-d) and the sliding of side-attached kinetochores along the microtubule lattice (see Methods 1.5). In addition to passively sliding along the lattice under external force, side-attached kinetochores also actively moved away from the pole at a slow speed ($0.5\ \mu\text{m}\cdot\text{s}^{-1}$ as reported in (Torvi et al., 2022); see also Methods 1.6). Incorporating this anti-poleward movement prevented kinetochores from becoming permanently stuck at the poles.

For each simulation, we recorded the lifetime of the attachment as well as the kinetochore positions and forces over time (Figures 2.2a, 2.2d, S2.1a, and Video 2.1-2.3). By running many simulations, we obtained distributions of positions and forces (Figures 2.2b and 2.2e) and calculated means. After a brief startup period, amphitelic kinetochores (one sister attached to each pole) tracked persistently with microtubule tips, which repeatedly pulled sisters apart (Figures 2.2a and 2.2b). Just as in cells, simulated amphitelic kinetochores formed a bilobed distribution with sisters positioned $0.4 \pm 0.3\ \mu\text{m}$ from their respective poles (mean $\pm$ SD at 20 min, $N = 5,071$ simulated pairs (Chacon et al., 2014; He et al., 2001; Marco et al., 2013)). At steady state, they sustained $10 \pm 3\ \text{pN}$ of force (mean $\pm$ SD at 20 min for tip-attached kinetochores only, $N = 10,142$ kinetochores), similar to force estimates for bioriented kinetochores in metaphase yeast cells (Chacon et al., 2014; Mukherjee et al., 2019). Attachment lifetimes varied widely with a mean of 31 min ($N = 10,000$ pairs; Figure 2.2c). Thus, once formed, an individual amphitelic pair could often persist from pole separation until anaphase onset.

Model features: Catch bond ☒ Tension-sensitive kinase ☐ Geometric bias ☐ Direction-sensitive grip ☐
Stage 1: Simulation of individual sister kinetochore pairs

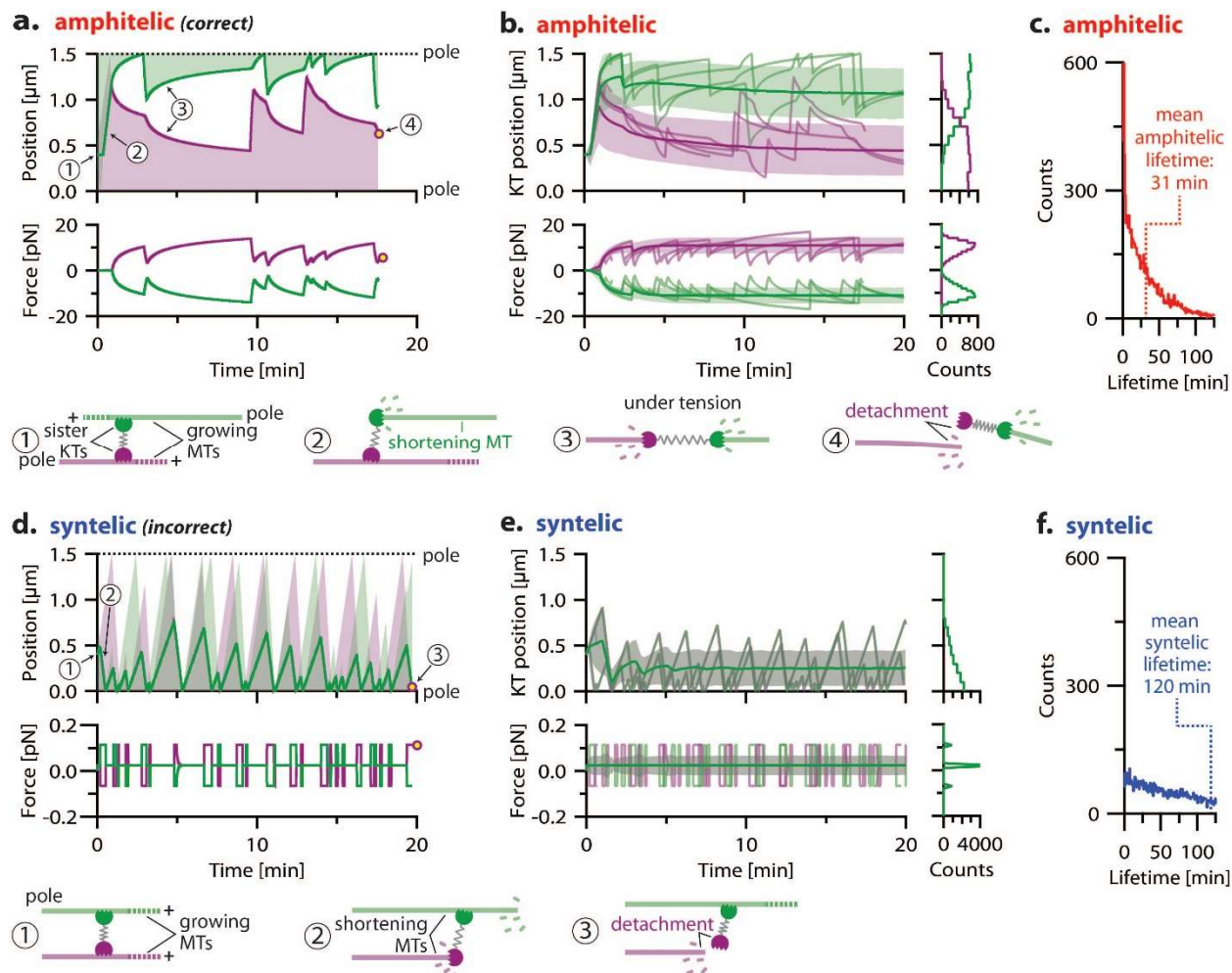


Figure 2.2. Simulations produce realistic kinetochore positions and forces. **a.** Example stage 1 simulation (same as [Video 2.1](#)) of an amphitelic sister kinetochore pair showing kinetochore position (*top*) and force (*bottom*) over time. Microtubule lattice is indicated by shaded regions with colors corresponding to the attached kinetochores. Poles are located at 0 and 1.5 μm . Cartoons below depict kinetochore-microtubule configurations at indicated timepoints: (1) Kinetochore are initially side-attached; (2) One microtubule begins to shorten and its kinetochore becomes tip-attached. The tip-attached kinetochore drags its side-bound sister toward a microtubule plus end at sub-pN forces; (3) Kinetochore are pulled apart by shortening microtubule tips and experience higher forces of 5 to 15 pN; (4) One kinetochore detaches stochastically from its shortening microtubule tip according to force, the most common way for amphitelic simulations to end when only catch-bond behavior is included. Detachment is marked by yellow circles with outlines corresponding to the color of the detached kinetochore. **b.** (*Left*) Average amphitelic kinetochore (KT) position (*top*) and force (*bottom*) plotted in bold with shaded area indicating standard deviation ($N = 10,000$ simulated kinetochore pairs). Five example simulations, including the one shown in panel a, are also plotted as lighter curves. (*Right*) Histograms show kinetochore positions (*top*) and forces (*bottom*) at 20 min ($N = 5,748$ pairs). **c.** Histogram of amphitelic attachment lifetimes with mean of 31.0 ± 0.3 min ($\pm$ SEM, $N = 10,000$ pairs). **d.** Example stage 1 simulation (same as [Video 2.2](#)) of a syntelic sister kinetochore pair arranged as in panel a. (1) Kinetochore are initially side-attached; (2) One microtubule begins to shorten and its kinetochore becomes tip-attached, then drags its side-bound sister toward the microtubule minus end at sub-pN forces; (3) One kinetochore detaches

stochastically from its shortening microtubule tip according to force, the sole way for syntelic simulations to end when only catch-bond behavior is included. **e.** (*Left*) Average syntelic kinetochore (KT) position (*top*) and force (*bottom*) plotted in bold with shaded area indicating standard deviation ($N = 10,000$ pairs). Three example simulations, including the one shown in panel d, are also plotted as lighter curves. (*Right*) Histograms show kinetochore positions (*top*) and forces (*bottom*) at 20 min ($N = 8,493$ pairs). **f.** Histogram of syntelic attachment lifetimes with mean of 120 ± 1 min ($\pm$ SEM, $N = 10,000$ pairs).

Syntelic kinetochores (both sisters attached to the same pole) spent much more time on microtubule sides instead of tracking with tips. Without the resisting force of inter-kinetochore tension, microtubules tended to pull syntelic kinetochores closer to the pole, to a distance of $0.3 \pm 0.2 \mu\text{m}$ (mean $\pm$ SD at 20 min, $N = 8,493$ pairs; [Figures 2.2d](#) and [2.2e](#)), similar to observations in cells ([Marco et al., 2013](#)). As expected, they experienced feeble, sub-piconewton forces of 0.09 ± 0.01 pN (mean $\pm$ SD at 20 min for tip-attached kinetochores only, $N = 2,524$ kinetochores). Syntelic attachment lifetimes varied widely with a mean lifetime of 120 min ($N = 10,000$ pairs; [Figure 2.2f](#)). Thus, on average, with catch-bond behavior alone, incorrect syntelic attachments lasted about four times as long as correct amphitelic attachments.

Monotelic kinetochores (one sister kinetochore attached to a microtubule and the other sister unattached) behaved similarly to syntelic attachments, staying relatively close to the pole at a distance of $0.3 \pm 0.3 \mu\text{m}$ (mean $\pm$ SD, $N = 9,654$ pairs; [Figures S2.1a](#) and [S2.1b](#)), similar to observations in cells ([Marco et al., 2013](#)). Simulated monotelic attachments only ever experienced the tiny force that drives anti-poleward movement ([Torvi et al., 2022](#)). Monotelic lifetimes were broadly distributed and considerably longer than amphitelic and syntelic lifetimes with a mean of 481 min ($N = 10,000$ pairs; [Figure S2.1c](#)). Overall, the positions and forces of simulated sister kinetochore pairs in all three configurations matched behaviors measured in cells, with amphitelic dynamically stretching apart and relaxing together in the middle of the spindle, while syntelics and monotelics clustered closer to a pole. However, in these simulations, syntelic lifetimes are much longer than amphitelic lifetimes, so catch-bond behavior by itself is unlikely to be sufficient to explain timely biorientation.

Triphasic catch bond preserves both amphitelic and syntelic attachments

To compare the relative stability of correct versus incorrect attachments, we then calculated the selectivity ratio, defined as the mean amphitelic lifetime divided by the mean syntelic lifetime ([Figure 2.3a](#)). Having a selectivity ratio greater than one indicates amphitelic are stabilized over syntelics and is probably necessary for efficient biorientation. With catch-bond behavior alone, the selectivity ratio was only 0.25. Poor selectivity occurred in this case because simulated kinetochores were programmed to remain stably tip-attached when the force was near zero, consistent with observations *in vivo* and *in vitro* that kinetochores without external force rarely detach, even when tracking with disassembling tips ([Doodhi et al., 2021](#); [Kalantzaki et al., 2015](#); [Tanaka et al., 2007](#); [Torvi et al., 2022](#)). Together with the other tension-dependent kinetochore behaviors measured *in vitro* ([Akiyoshi et al., 2010](#)), this produces what we call a triphasic catch bond: The mean lifetime of a tip-attached kinetochore is long at forces near zero, quickly drops as piconewton forces are applied, then gradually peaks again as force increases to ~ 9 pN ([Figures 2.3b](#) and [2.3c](#); see also [Methods 1.4](#), [Methods 3.1](#), and [Figures S2.2a-d](#)). Amphitelic sisters generally experienced high tension, 10 pN on average, so they spent

the majority of their time in the high-lifetime peak (Figure 2.3b). However, because syntelic pairs experienced very low tension, they spent most of their time in the stable area near zero force (Figure 2.3c). Syntelic kinetochores also spent less time at microtubule tips and more time attached to microtubule sides, where they were protected from detachment (Figures 2.2d, 2.2e, S2.1a, and S2.1b). Thus, while the triphasic catch bond produces long amphitelic lifetimes comparable to the duration of mitosis, it does not effectively destabilize syntelic attachments.

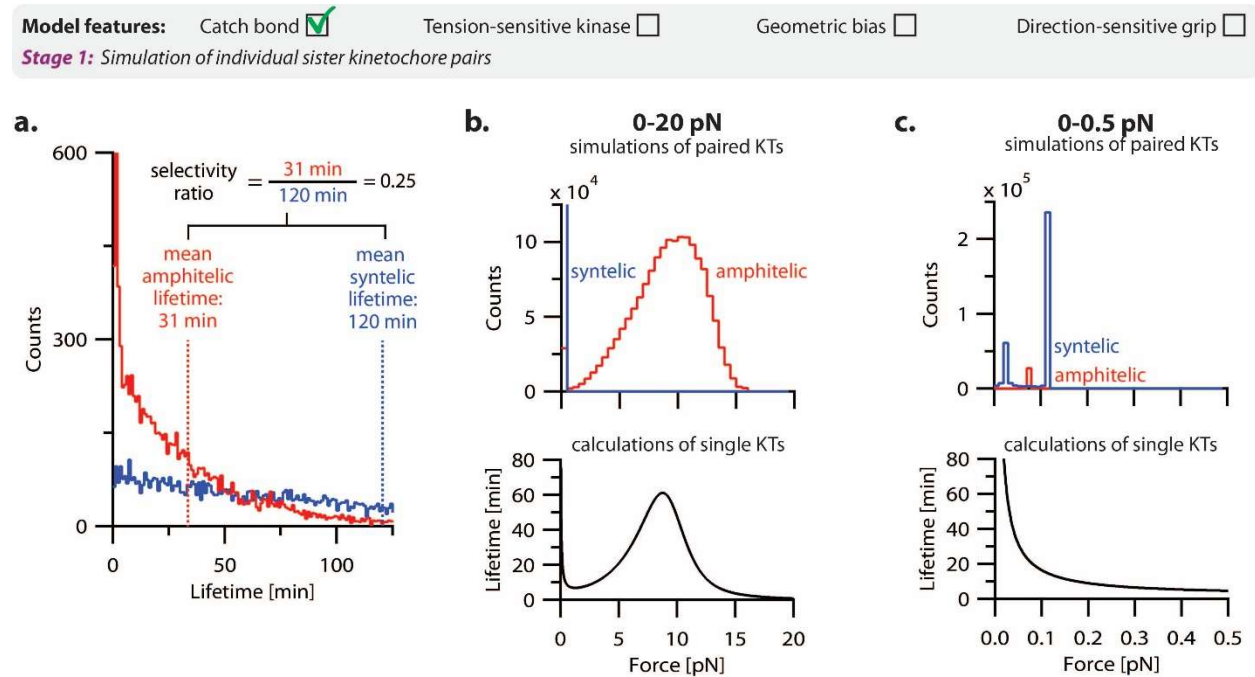


Figure 2.3. Triphasic catch bond preserves both amphitelic and syntelic attachments. **a.** Lifetimes of amphitelic (red) and syntelic (blue) attachments replotted from Figures 2.2c and 2.2f for comparison ($N = 10,000$ simulated kinetochore pairs each). **b.** (Top) Distributions of forces on tip-attached kinetochores during simulations of amphitelic (red) and syntelic (blue) attachments ($N = 100$ pairs each). (Bottom) Calculated attachment lifetime versus force for an individual hypothetical kinetochore on a microtubule tip. Calculations assume that the kinetochore is held at constant force and behaves according to the dynamic rates used in our simulations (Figure S2.2 and Table 2.1; see also Methods 3.1). **c.** Same as panel b, but showing only the low-force region between 0 and 0.5 pN.

The poor selectivity ratio produced by the triphasic catch bond was nevertheless better than a slip-bond alternative. Native kinetochore particles purified from yeast strains depleted of the TOG-family protein, Stu2 (ch-TOG in humans), lose their catch bond-like behavior, instead displaying a more conventional slip-bond behavior where tip-attachment lifetime decreases monotonically with increasing force (Miller et al., 2016). This loss of catch-bond behavior arises from differences in the force-dependent detachment rates for Stu2-depleted kinetochores relative to wild type (Figure S2.2a). Incorporating the Stu2-depleted rates into our simulations (see Methods 1.7) reproduced slip-bond behavior (Figure S2.2d) and severely reduced amphitelic attachment lifetimes (1.1 min mean lifetime, $N = 10,000$ simulated pairs) while increasing syntelic attachment lifetimes (mean > 167 min, $N = 10,000$ simulated pairs; Figure

S2.2e). Consequently, the selectivity ratio became miniscule (< 0.007), demonstrating that the triphasic catch bond strongly favors amphitelic attachments relative to a slip-bond alternative.

Tension-sensitive kinase activity lowers syntelic lifetimes

Kinases, especially Aurora B, are a primary mechanism for releasing incorrect attachments (Biggins et al., 1999; Maure et al., 2007; Tanaka et al., 2002). Previous work showed that Aurora B triggers kinetochore detachment at low forces ~ 1.5 pN while leaving high-force attachments ~ 5 pN untouched (de Regt et al., 2022). We fit these measurements with a double exponential such that kinetochores under zero force experience no added detachment (Figure 2.4a), consistent with the stability of kinetochore-microtubule attachments in the absence of external force observed in vivo and in vitro (Doodhi et al., 2021; Kalantzaki et al., 2015; Tanaka et al., 2007; Torvi et al., 2022). According to this fit, kinetochores experience a maximum of 0.8 kinase-triggered detachments per min at 0.3 pN and are almost completely protected above 5 pN. We incorporated this mechanism into our simulations by adding the extra kinase-triggered detachment to the spontaneous detachment rate from catch-bond behavior (Methods 1.8).

Tension-sensitive kinase activity dramatically destabilized syntelic attachments, reducing their mean lifetime more than 10-fold from 120 to 7.4 min ($N = 10,000$ pairs; Figures 2.4b and S2.3a). Monotelic lifetimes were also dramatically reduced, from 481 min to 25 min, showing that even the small anti-poleward force they experienced was enough to allow significant kinase-triggered release (Figure S2.3b). Amphitelic lifetimes were also reduced, from a mean of 31 to 23 min ($N = 10,000$ pairs; Figures 2.4b and S2.3c), because they inevitably transited a vulnerable low-force startup phase before they achieved the persistent, high-force tip-attachments that would protect them from kinase-triggered release (Figures S2.3d and S2.3e). Nevertheless, the selectivity ratio with both tension-sensitive kinase activity and catch-bond behavior was 3.1, implying that amphitelic attachments were about three times more stable than syntelics, an approximately 10-fold improvement over simulations with catch-bond behavior alone. However, it remained unclear whether this level of selectivity would be sufficient to biorient all sixteen sister kinetochore pairs as quickly as must occur in vivo.

Model features: Catch bond ☒ Tension-sensitive kinase ☒ Geometric bias ☐
Stage 1: Simulation of individual sister kinetochore pairs Direction-sensitive grip ☐

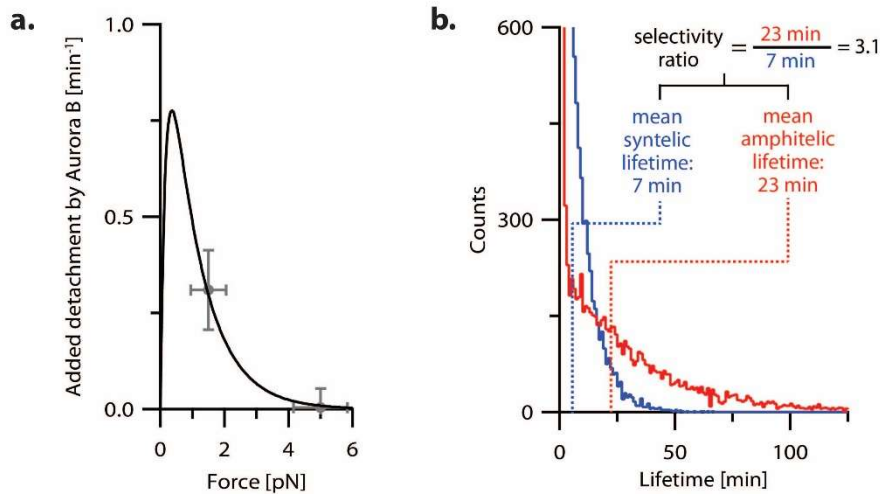


Figure 2.4. Tension-sensitive kinase activity lowers syntelic lifetimes. **a.** Model for kinase-triggered detachment as a function of force (*black curve*) based on in vitro measurements of Aurora B (*gray symbols* show measurements and uncertainties from (de Regt et al., 2022)). **b.** Histograms of simulated amphitelic (*red*) and syntelic (*blue*) attachment lifetimes when kinase-triggered, force-dependent detachment was added to simulations with catch-bond behavior ($N = 10,000$ simulated kinetochore pairs each).

Tension-dependent mechanisms alone are insufficient to explain in vivo biorientation speed

To test whether our model including tension-sensitive kinase activity and catch-bond behavior could explain the high efficiency of biorientation in vivo, we developed stage 2 of our approach, where lifetimes predicted in stage 1 were used to simulate biorientation of all sixteen kinetochore pairs in a cell (Figure 2.5a; see also Methods 2.1). Stage 2 simulations began with eight amphitelic, eight syntelic, and zero monotelic pairs, all with the same initial side-attached configuration as in Stage 1 (see Methods 2.2). Each amphitelic and syntelic pair had a lifetime drawn at random from the distributions generated in stage 1 (e.g., Figure 2.4b). At the end of each lifetime, one sister detached and the pair became monotelic. Monotelic pairs reattached stochastically, becoming either amphitelic or syntelic after an average delay of 1.5 min, which we chose to match reattachment events observed in yeast cells ((Mukherjee et al., 2019); see Methods 2.3). Initially, we did not include any geometric bias, so monotelics were equally likely to reattach either correctly or incorrectly (i.e., the probability of amphitelic reattachment was 50%).

After an initial startup phase, the number of amphitelic, syntelic and monotelic pairs reached a quasi-steady-state (Figures 2.5a and 2.5b). The number of amphitelics fluctuated around the steady state value as attachments formed and broke, until by chance, all sixteen pairs became bioriented. The cell could then proceed into anaphase without error, and we defined this as the time required for full biorientation.

Model features: Catch bond ☒ Tension-sensitive kinase ☒ Geometric bias ☒ Direction-sensitive grip ☐
Stage 2: Simulation of all sixteen sister pairs in a cell

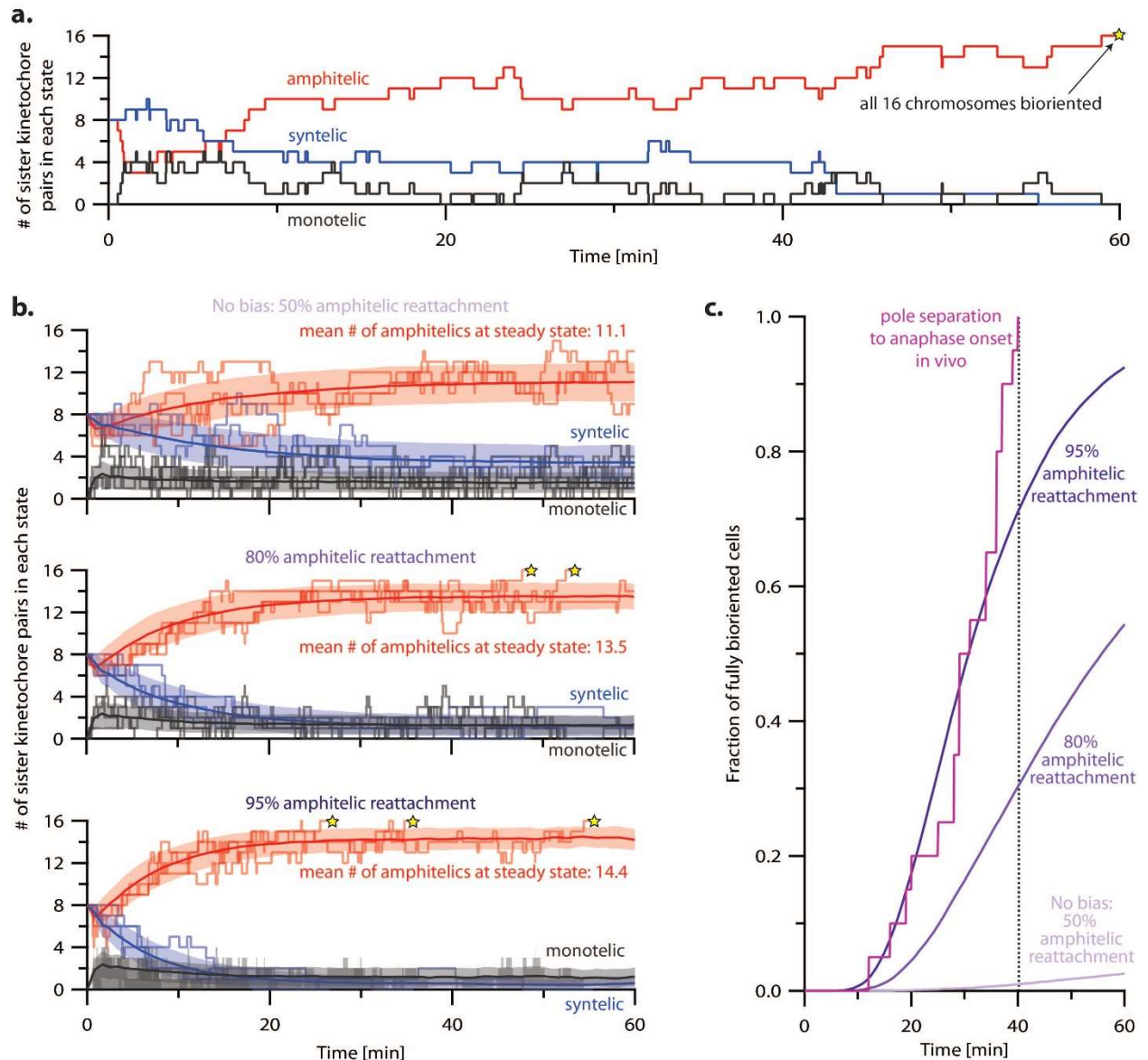


Figure 2.5. Geometric bias toward amphitelic reattachment speeds biorientation. **a.** Example stage 2 simulation of the biorientation of all sixteen sister kinetochore pairs in a budding yeast cell. The numbers of pairs attached amphitelic (red), syntelic (blue), and monotelic (gray) are plotted versus time. A cell was considered fully bioriented after all sixteen pairs were correctly attached for at least 1 min (at ~58 min here and marked by star, see [Methods 2.3](#) for details). In this example, no geometric bias was included (50% amphitelic reattachment). **b.** Mean numbers of sister pairs attached amphitelic (red), syntelic (blue), and monotelic (gray) plotted versus time with no geometric bias (top, No bias: 50% amphitelic reattachment) or with geometric bias such that monotelic sister pairs had a higher chance of amphitelic reattachment. The amount of bias included was either modest (middle, 80% amphitelic reattachment), or high (bottom, 95% amphitelic reattachment). Means are displayed as bold trajectories with shading to indicate standard deviations ($N = 2,000$ simulated cells each). Three individual examples are also plotted for each case as lighter trajectories. Stars mark detachment events. **c.** Fraction of fully bioriented cells over

time from simulations with no geometric bias (*light purple, No bias: 50% amphitelic reattachment*), modest bias (*medium purple, 80% amphitelic reattachment*), and high bias (*dark purple, 95% amphitelic reattachment, $N = 2,000$ simulated cells each*) compared to times from pole separation to anaphase onset in vivo (*magenta, (Sim et al., 2024), $N = 20$ real cells*). Vertical dashed line shows 40 min maximum time that real cells require for this process.

In the example shown in [Figure 2.5a](#), all sixteen pairs became bioriented at 58 min. However, full biorientation of all sixteen pairs in simulations combining only catch-bond behavior and tension-sensitive kinase activity was very rare. At steady state, the mean number of amphitelic pairs was only 11.1 ($N = 2,000$ simulated cells), so on average, five more amphitelic pairs were needed for full biorientation ([Figure 2.5b](#), top graph). During the time required for five new amphitelic pairs to form, others usually broke, making full biorientation unlikely. Plotting the fraction of fully bioriented cells over time showed that only 1% achieved full biorientation in 40 min ([Figure 2.5c](#), 50% amp reattach). This predicted behavior is much worse than in vivo, where yeast cells become fully bioriented in ≤ 40 min ([\(Leitao and Kellogg, 2017\)](#); [Figure 2.5c](#), in vivo measurement histogram from [\(Sim et al., 2024\)](#)).

Because in vitro Aurora B activity may differ somewhat in magnitude from the activity of multiple tension-sensitive kinases in vivo, we tested whether changing the detachment amplitude would allow cells to efficiently biorient their chromosomes ([Methods 1.8](#)). When we altered our model of kinase-based release to be 2-fold stronger than measured in vitro, only 2% of cells were able to fully biorient in 40 min ([Table 2.3](#)). Therefore, catch-bond behavior and tension-sensitive kinase activity together were not sufficient to explain timely biorientation, underscoring the need for additional mechanisms to either correct or avoid attachment errors.

Geometric bias toward amphitelic reattachment speeds biorientation time

Back-to-back sister kinetochore geometry is thought to promote biorientation by biasing monotelic pairs toward capturing microtubules from the correct side, thereby favoring the formation of amphitelic over syntelic pairs ([Figure 2.1c](#), [\(Indjeian and Murray, 2007; Ris and Witt, 1981\)](#)). To quantify the efficacy of this mechanism, we compared simulated cells with different levels of geometric bias, all including catch-bond behavior and tension-sensitive kinase activity ([Figures 2.5b and 2.5c](#), $N = 2,000$ simulated cells each). When monotelics had an 80% bias to reattach correctly, similar to what has been observed before for grasshopper spermatocytes ([Nicklas and Ward, 1994](#)), the mean number of amphitelic pairs at steady-state was 13.5 ([Figure 2.5b](#), middle graph), putting cells significantly closer to full biorientation than without geometric bias. The time required to reach this steady-state was also reduced from ~ 40 min to ~ 20 min. Accordingly, the fraction of cells that achieved full biorientation in 40 min rose to 29% ([Figure 2.5c](#), 80% amp reattach).

When amphitelic reattachment bias was increased further to 95%, the mean number of amphitelic pairs at steady-state was elevated to 14.4 ([Figure 2.5b](#), bottom graph) and 70% of cells were fully bioriented in 40 min ([Figure 2.5c](#), 95% amp reattach), confirming that geometric bias effectively speeds biorientation. However, simulated cells still bioriented more slowly than real cells, which nearly always achieve full biorientation in 40 min or fewer ([Leitao and Kellogg, 2017; Sim et al., 2024](#)). Increasing the amplitude of tension-sensitive kinase release either 1.5- or 2-fold resulted in nearly identical biorientation efficiency, with 75% and 72% of cells achieving

full biorientation in less than 40 min, respectively (Table 2.3). Thus, even combined with a strong geometric bias toward forming amphitelic attachments, catch-bond behavior and tension-sensitive kinase activity were not quite sufficient to explain how real cells achieve timely biorientation.

Model with direction-sensitive grip achieves in vivo biorientation efficiency

Recent work revealed that side-attached kinetochores have a strikingly direction-sensitive grip on the microtubule lattice, gripping more tightly when pulled toward the plus end than the minus end (Larson et al., 2025). To investigate how this might influence biorientation, we added a constant detachment rate for side-attached kinetochores specifically during sliding toward the minus end into our simulations (which had previously allowed kinetochore detachment only from the microtubule tip; see Methods 1.9). Because amphitelic and monotelic kinetochores never slid toward the minus end, amphitelic and monotelic lifetimes were unaffected and only syntelic lifetimes were reduced. Incorporating this direction-sensitive grip into stage 1 simulations with catch-bond behavior and tension-sensitive kinase activity decreased the mean syntelic lifetime from 7.4 to 3.2 min, resulting in a selectivity ratio of 7.2 (Figure 2.6a). Therefore, with catch-bond behavior, tension-sensitive kinase activity, and direction-sensitive grip combined, amphitelic attachments were about seven times more stable than syntelic attachments.

Notably, a specific reduction of syntelic lifetimes without changing amphitelic lifetimes was unique to the direction-sensitive grip mechanism. Catch-bond behavior and tension-sensitive kinase activity caused detachment in response to low tension, making them prone to off-target effects because amphitelic attachments invariably experienced a low-tension startup period (Figure S2.3). By targeting a state uniquely visited by syntelics, direction-sensitive grip had a strong selectivity advantage over the other two mechanisms. When we simulated the biorientation of all sixteen kinetochore pairs using lifetimes predicted for the combination of direction-sensitive grip, catch-bond behavior, and tension-sensitive kinase activity, assuming a 95% amphitelic reattachment bias, a vast majority of cells (93%) achieved full biorientation in 40 min ($N = 2,000$ cells). Thus, the combination of all four mechanisms was sufficient to explain timely biorientation in budding yeast.

Model features: Catch bond ✓ Tension-sensitive kinase ✓ Geometric bias ✓
Direction-sensitive grip ✓

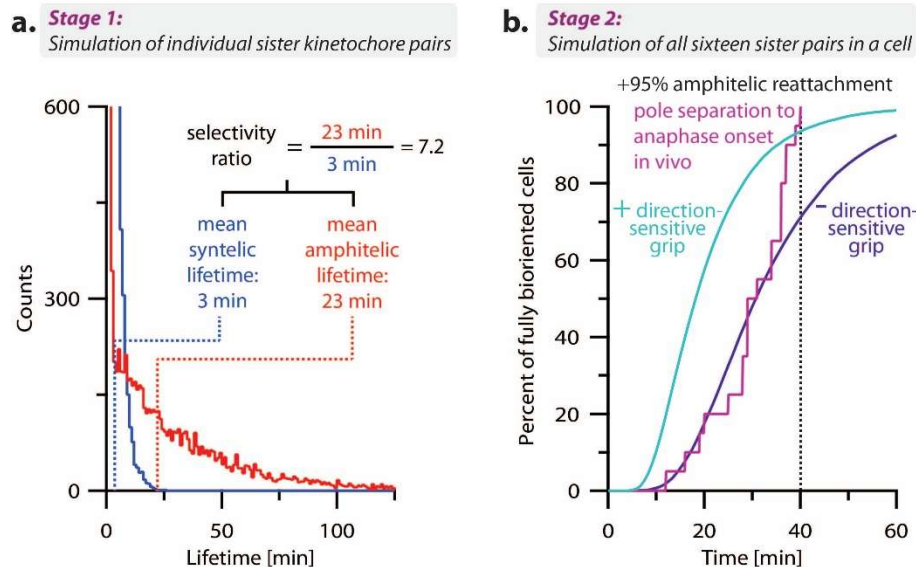


Figure 2.6. Direction-sensitive grip combined with catch-bond behavior, tension-sensitive kinase activity, and geometric bias can explain in vivo biorientation times. **a.** Histograms of amphitelic (red) and syntelic (blue) attachment lifetimes from stage 1 simulations ($N = 10,000$ simulated kinetochore pairs each) including direction-sensitive grip, so kinetochores detached from the microtubule lattice (at 1 min^{-1}) specifically when pulled toward the microtubule minus end. **b.** Fraction of fully bioriented cells over time from stage 2 simulations with (cyan) and without (purple) direction-sensitive grip ($N = 2,000$ simulated cells each) compared to in vivo times from pole separation to anaphase onset (magenta, (Sim et al., 2024)). The stage 2 simulations here included high geometric bias (95% amphitelic reattachment). Those with direction-sensitive grip were based on the stage 1 lifetimes in panel a, while those without direction-sensitive grip were replotted from Figure 2.5c and based on lifetimes in Figure 2.4b.

Discussion

To achieve timely biorientation, cells must choose correct attachments over incorrect ones, so amphitelic lifetimes must be much longer than syntelic lifetimes. However, this is not the only important time requirement. Another important requirement is that monotelic attachments must last long enough for the unattached sister to bind a microtubule. This seems unlikely to pose a problem because, in our simulations, even with tension-sensitive kinase activity, monotelic attachments lasted over 25 min on average and therefore were highly likely to outlast the 1.5 min estimated reattachment time. Another, more stringent requirement is that amphitelic attachments must frequently last as long or longer than the time from initial bipolarization to anaphase onset. In our simulations, including all mechanisms as in Figure 2.6, amphitelic attachments lasted 23 min on average compared to the simulated median full biorientation time of 19 min. Thus, some amphitelic attachments formed early in prometaphase could endure until all chromosomes were bioriented and the error avoidance process didn't need to be repeated. Biorientation was greatly affected by monotelic reattachment and post-biorientation delay time, both of which are currently constrained by limited data (see Methods 2.3).

In our model, it is impossible for tension-dependent error correction to destabilize low-tension syntelic kinetochores without also destabilizing low-tension monotelics and immature amphitelic attachments. The triphasic catch bond produced long-lived amphitelic and monotelic attachments, but did little to destabilize syntelic attachments (Figures 2.2 and 2.3). Tension-sensitive kinase activity greatly improved the selectivity ratio by reducing mean syntelic lifetime more than ten-fold (Figures 2.4b and S2.3a). This also destabilized monotelics, which were always at 0.024 pN (Figure S2.3b), close to the 0.3 pN where tension-sensitive kinases are most active in our model (Figures 2.4b and S2.3d). Most importantly, tension-sensitive kinase activity released immature amphitelic kinetochores, both during sliding and initial tip-attached sister separation (see Methods 1.5).

Destabilization of immature amphitelic attachments could be mitigated by multiple mechanisms. This idea has been previously discussed as the “Initial Problem of Biorientation” (Edelmaier et al., 2020; Kalantzaki et al., 2015; Tubman et al., 2017). In budding yeast, kinetochore pairs with one sister attached to each pole may quickly gain tension as the poles spring from side-by-side to 1 or more μm apart (Leary et al., 2019), allowing initial amphitelic attachments to escape the danger zone (Figures S2.3d and S2.3e). However, this mechanism does not help kinetochore pairs that are initially syntelic or monotelic at spindle pole separation. Immature amphitelic attachments may be protected if kinases trigger low-tension detachment only after a threshold number of sites have been phosphorylated (Tubman et al., 2017). Even if such mitigation methods exist, neither catch-bond behavior nor tension-sensitive kinase activity can destabilize low-tension attachments too much without overly weakening immature amphitelic attachments. This makes additional error correction methods that can more specifically target syntelic attachments especially valuable.

Direction-sensitive grip is an effective mechanism to specifically destabilize syntelic kinetochores while leaving amphitelic and monotelic kinetochores unaffected. However, it is possible that as with tension-sensitive kinase activity and catch-bond behavior, this mechanism can't be too potent without also weakening immature amphitelic and monotelic attachments, as thermal fluctuations may cause chromosomes to move a little toward the minus end even when their net motion is toward the plus end. Kinetochores may detach spontaneously while sliding toward the minus end because kinetochores have an intrinsically weaker grip in that direction (Demidov et al., 2025; Larson et al., 2025), although kinetochore detachment from microtubule sides under force has yet to be measured. In addition, when sliding toward the minus end, kinetochores adopt a compact configuration unlike when they are plus-end-attached or sliding toward the plus end (Larson et al., 2025). Kinases like Aurora B or Mps1 may target this irregular configuration as part of their function of destabilizing incorrect attachments (Biggins et al., 1999; de Regt et al., 2022; Maure et al., 2007; Mukherjee et al., 2019; Sarangapani et al., 2021; Tanaka et al., 2002).

The importance of geometric bias for rapid biorientation of all sixteen chromosomes is striking (Figure 2.5) and consistent with previous models of many different organisms (Edelmaier et al., 2020; Indjeian and Murray, 2007; Paul et al., 2009; Zaytsev and Grishchuk, 2015). The most direct tests of geometric bias come from grasshopper spermatocytes, which can be manipulated to face towards or away from the pole and show ~83% bias toward attaching to the facing pole (Nicklas and Ward, 1994). Experiments like these are much more difficult with mitotic chromosomes, where sister kinetochores are close together and difficult to

manipulate independently. Perhaps the reattachment time of individual, unreplicated mitotic chromatids could be measured while facing towards or away from a spindle pole, similar to experiments in grasshopper spermatocytes, to quantify the degree to which back-to-back geometry can bias mitotic sister kinetochores toward catching a microtubule from the correct pole.

The proportion of fully bioriented cells in our simulations including all four error correction mechanisms are shifted to lower times than in vivo times from pole separation to anaphase onset (Figure 2.6). However, this is not in fact a problem. While it is critical for full biorientation to occur before anaphase to prevent aneuploidy, it does not necessarily occur at the same time as anaphase onset. Other factors may delay anaphase independent of biorientation. For instance, the cell may wait until the spindle is sufficiently long (Galli and Morgan, 2016) or cell-cycle-determining cyclins have accumulated to some threshold level (Visintin et al., 1997; Yeong et al., 2000). The spindle assembly checkpoint may also delay anaphase onset for minutes after full biorientation, as the checkpoint signal takes time to leave the kinetochore (Kuhn and Dumont, 2017). Indeed, PtK1 cells experience a 23 min delay on average between full alignment at the spindle equator and anaphase onset (Rieder et al., 1994), although some of this delay may be for the cell to resolve any remaining merotelic attachments (Zaytsev and Grishchuk, 2015). This post-biorientation delay is independent of alignment time (Rieder et al., 1994), suggesting that once biorientation has been completed, cells require a fixed amount of time to finish other tasks required for division (although this time may vary between cell types).

In principle, each of the error correction mechanisms described here could also promote biorientation in organisms with kinetochore-microtubule bundles. However, error correction with bundles poses unique challenges as well as potential advantages. Individual microtubules can bind and unbind from amphitelic kinetochores without the entire kinetochore detaching. Therefore, individual kinetochore-microtubule detachment rates can be higher than in budding yeast, even under tension. Indeed, the microtubule half-life in kinetochore-microtubule bundles is only 2-4 minutes in mammalian cells during prometaphase (reviewed in (Zaytsev and Grishchuk, 2015)). On the other hand, correcting syntelics is more complicated because many individual kinetochore-microtubule attachments must be released.

Bundled kinetochore-microtubules have the additional problem of 'merotelic' attachment errors, when a single sister in a kinetochore pair attaches microtubules from both poles (Cimini et al., 2003). Interestingly, it is possible that direction-sensitive grip may help to correct merotelic attachments. Merotelic attachments may form when one sister of an amphitelic pair catches a microtubule from the opposite side as its primary bundle (Zaytsev and Grishchuk, 2015). The kinetochore is likely to initially side-attach to the new microtubule (Doodhi et al., 2021; Rieder et al., 1990; Tanaka et al., 2007). If the primary microtubule bundle is lengthening, the kinetochore moves toward the plus-end of the primary bundle and the minus-end of the new microtubule. Direction-sensitive grip may then destabilize the newly-formed attachment during sliding, preventing it from reaching the plus end where it may experience stabilizing high tension as it pulls against the microtubules of the primary bundle (Cimini et al., 2004). Chromosomes in many organisms undergo synchronized oscillations during prometaphase, where one kinetochore-microtubule bundle lengthens while its sister shortens. These oscillations have been proposed to help chromosomes 'shake off' erroneous attachments

([Wakiya et al., 2021](#); [Wordeman et al., 2007](#)). Direction-sensitive grip may be at least one mechanism behind this type of error-correction, as the primary bundle must be lengthening to force lateral merotelic attachments to slide toward the minus end and detach. It will be fascinating to see if direction-sensitive grip along with catch-bond behavior, tension-sensitive kinase activity, and geometric bias can successfully explain timely biorientation in simulations of cells with bundled kinetochore-microtubules, as they were in our simulations of budding yeast.

Supplemental figures

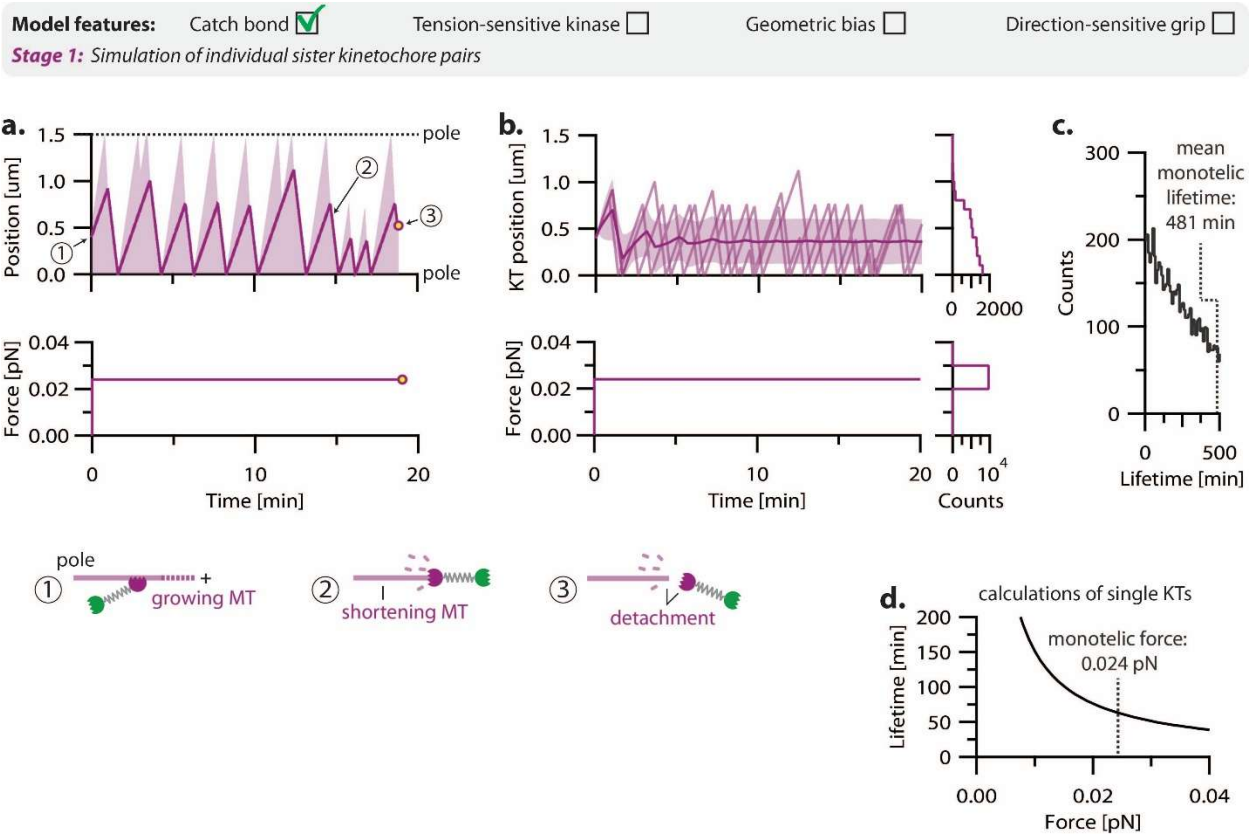


Figure S2.1. Simulated monotelic kinetochores have realistic positions and forces. **a.** Example stage 1 simulation (same as Video 2.3) of a monotelic sister kinetochore pair showing kinetochore position (top) and force (bottom) over time. Microtubule lattice is indicated by shaded regions with color corresponding to the attached kinetochore. Cartoons below depict kinetochore-microtubule configurations at indicated timepoints: (1) One kinetochore is initially side-attached; (2) The microtubule begins to shorten and its kinetochore becomes tip-attached. The attached kinetochore is pulled toward the pole, dragging its unattached sister with it; (3) The kinetochore detaches stochastically from its shortening microtubule tip according to force, the sole way for monotelic simulations to end when only catch-bond behavior is included. **b.** (Left) Average monotelic kinetochore (KT) position (top) and force (bottom) plotted in bold with shaded area indicating standard deviation ($N = 10,000$ simulated kinetochore pairs). Three example simulations, including the one shown in panel a, are also plotted as lighter curves. Because the simulated monotelic kinetochores only ever experience 0.24 pN force, all example force traces overlap exactly with the mean and the standard deviation is zero. (Right) Histograms show kinetochore positions (top) and forces (bottom) at 20 min ($N = 9,654$ simulated kinetochore pairs). **c.** Histogram of monotelic attachment lifetimes with mean of 481 ± 4 min ($\pm$ SEM, $N = 10,000$ pairs). **d.** Calculated attachment lifetime versus force for an individual hypothetical kinetochore on a microtubule tip assuming the kinetochore is held at constant force and behaves according to the dynamic rates used in our simulations (Figure S2.2 and Table 2.1; see also Methods 3.1).

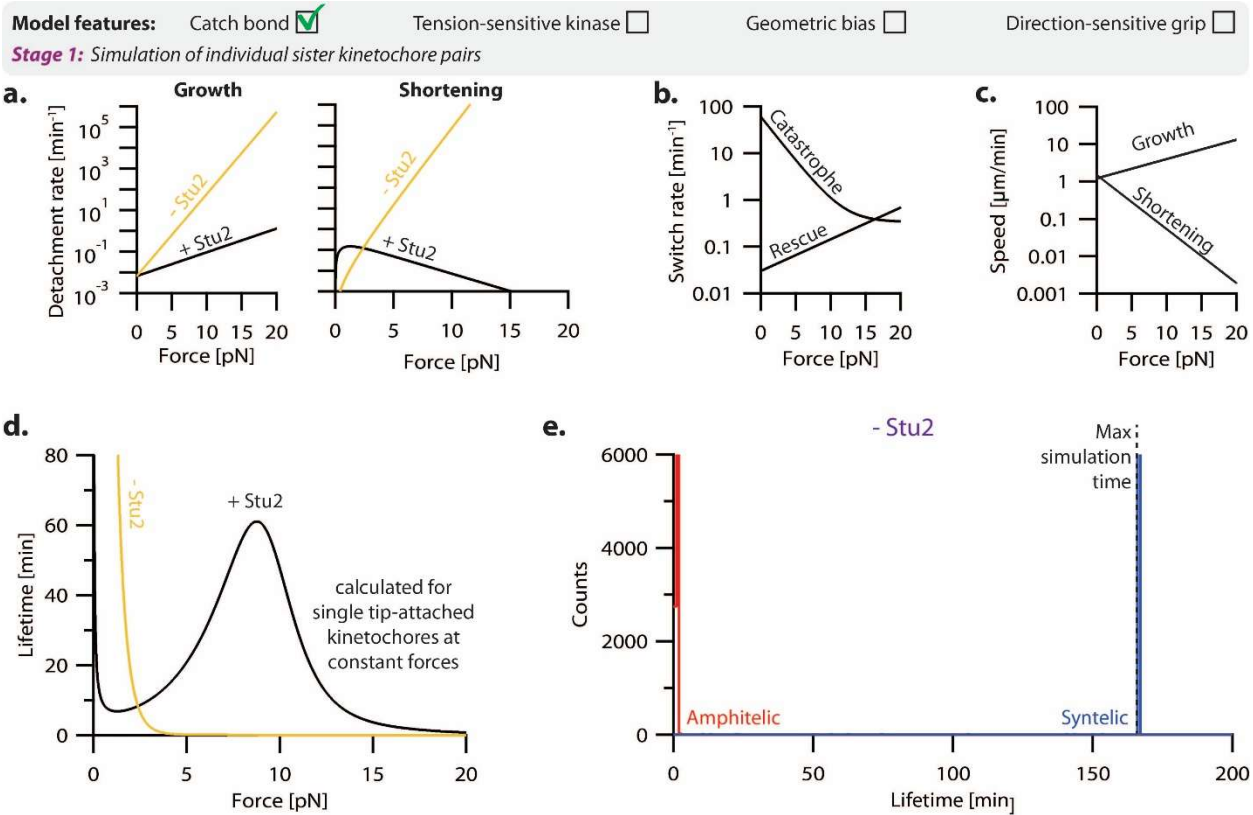


Figure S2.2. Simulated kinetochores without Stu2 have reduced amphitelic lifetimes and increased syntelic lifetimes. **a.** Detachment rate during growth (*left*) and shortening (*right*) for individual kinetochores used in simulations with Stu2 (*black*) and without Stu2 (*yellow*) as a function of force. **b.** Catastrophe and rescue rates for individual kinetochores used in simulations as a function of force. Simulated catastrophe and rescue rates were identical with and without Stu2. **c.** Growth and shortening speeds for individual kinetochores used in simulations as a function of force. Speeds were identical with and without Stu2. **d.** Calculated attachment lifetime versus force for an individual hypothetical kinetochore on a microtubule tip with Stu2 (*black*) and without Stu2 (*yellow*). Calculations assume that the kinetochore is held at constant force and behaves according to the dynamic rates used in our simulations and displayed in panels a-c (Table 2.1). **e.** Histograms of amphitelic lifetimes (*red*) and syntelic lifetimes (*blue*) without Stu2 ($N = 10,000$ simulations each). Dashed line indicates the maximum simulation time of ~167 min (10,000 s). In almost every syntelic simulation, the maximum simulation time was reached before a kinetochore detached.

Model features: Catch bond ☒ Tension-sensitive kinase ☒ Geometric bias ☐ Direction-sensitive grip ☐
 Stage 1: Simulation of individual sister kinetochore pairs

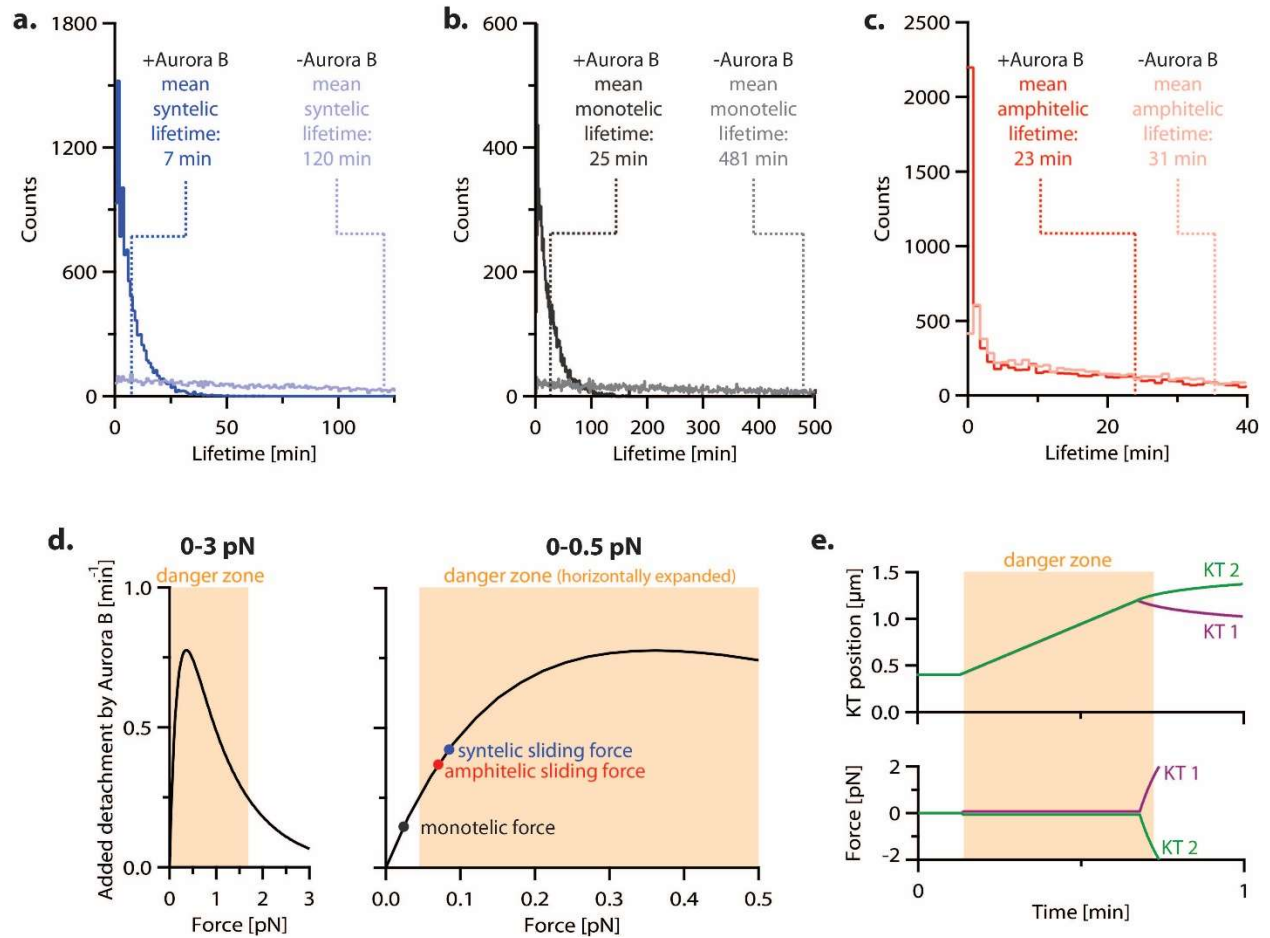


Figure S2.3. Tension-sensitive kinase activity lowers amphitelic, syntelic, and monotelic lifetimes to different extents. **a.** Comparison of syntelic lifetime distributions with (dark blue) and without (light blue) tension-sensitive kinase release. **b.** Comparison of monotelic lifetime distributions with (dark gray) and without (light gray) tension-sensitive kinase release. **c.** Comparison of amphitelic lifetime distributions with (dark red) and without (light red) tension-sensitive kinase release. Note that all the axes in syntelic (a), monotelic (b), and amphitelic (c) lifetime distributions are scaled differently to emphasize the changes resulting from added tension-sensitive kinase activity. **d.** Kinase-triggered detachment rate used in stage 1 simulations as a function of force from 0-3 pN (left) and 0-0.5 pN (right). These curves are replotted from Figure 2.4a to emphasize the “danger zone” tip-attached kinetochores experience when the rate of kinase-triggered detachment is greater than 0.25 per min (between 0.044 and 1.67 pN). Kinase-triggered detachment rates of tip-attached monotelic kinetochores (gray circle), syntelic kinetochores pulling their side-attached sisters (blue circle), and amphitelic kinetochores pulling their side-attached sisters (red circle) are also displayed. **e.** Example simulation with kinetochore (KT) position (top) and force (bottom) showing that amphitelic attachments primarily experience danger zone forces during sliding and as kinetochores become tip-attached and separate.

Description	Symbol	Value	Source
Growth speed, no tip-bound KT	v_{growth_bare}	1.21 $\mu\text{m}/\text{min}$	(Gupta et al., 2006)
Zero-force growth speed with tip-bound KT	$v_{growth0}$	1.21 $\mu\text{m}/\text{min}$	(Gupta et al., 2006)
Shortening speed, no tip-bound KT	v_{short_bare}	3 $\mu\text{m}/\text{min}$	(Bergman et al., 2018; Tanaka et al., 2007; Torvi et al., 2022)
Zero-force shortening speed with tip-bound KT	v_{short0}	1.5 $\mu\text{m}/\text{min}$	(Tanaka et al., 2007)
Catastrophe rate, no tip-bound KT	k_{cat_bare}	0.58 min^{-1}	(Gupta et al., 2006)
Zero-force catastrophe rate with tip-bound KT	k_{cat0}	60 min^{-1}	Chosen, see Methods 1.4
Rescue rate, no tip-bound KT	k_{res_bare}	0.882 min^{-1}	(Gupta et al., 2006)
Zero-force rescue rate with tip-bound KT	k_{res0}	0.03 min^{-1}	Chosen, see Methods 1.4
Zero-force detachment rate during growth	$k_{det_growth0}$	0.0067 min^{-1}	(Akiyoshi et al., 2010)
Detachment rate amplitude during shortening	k_{det_short0}	0.33 min^{-1}	(Akiyoshi et al., 2010), see Methods 1.4
Growth speed force sensitivity	F_{growth}	8.4 pN	(Akiyoshi et al., 2010)
Shortening speed force sensitivity	F_{short}	-3 pN	(Akiyoshi et al., 2010)
Catastrophe rate force sensitivity	F_{cat}	-2.3 pN	(Akiyoshi et al., 2010)
Rescue rate force sensitivity	F_{res}	6.4 pN	(Akiyoshi et al., 2010)
Detachment rate force sensitivity during growth	F_{det_growth}	3.8 pN	(Akiyoshi et al., 2010)
Detachment rate force sensitivities during shortening	F_{det_short1} F_{det_short2}	-1 pN -2.6 pN	(Akiyoshi et al., 2010)
Zero-force detachment rate during growth, no Stu2	$k_{det_growth_noStu2_0}$	0.007 min^{-1}	(Miller et al., 2016), see Methods 1.7
Detachment rate amplitude during shortening, no Stu2	$k_{det_short_noStu2_0}$	0.006 min^{-1}	(Miller et al., 2016)
Detachment rate force sensitivity during growth, no Stu2	$F_{det_growth_noStu2}$	1.1 pN	(Miller et al., 2016)
Detachment rate force sensitivities during shortening, no Stu2	$F_{det_short_noStu2_1}$ $F_{det_short_noStu2_2}$	1 pN -4.7 pN	(Miller et al., 2016)

Table 2.1. Microtubule dynamics used in simulations.

Description	Symbol	Value	Source
Kinase-triggered detachment rate force-sensitivities	F_{Aur1} F_{Aur2}	0.2 pN 1 pN	(de Regt et al., 2022)
Lattice detachment rate toward the minus-end	None	1 min ⁻¹	Chosen, see Discussion
Plus-end directed force	None	0.024 pN	(Bormuth et al., 2009; Torvi et al., 2022)
Monotelic kinetochore average amphitelic reattachment time	None	1.5 min	(Mukherjee et al., 2019)
Step size along MT	δ	4.8 nm	(Larson et al., 2025)
Step rate along MT	k_{step}	1.05 min ⁻¹	(Larson et al., 2025)
Directional asymmetry of sliding	Δ	-1.8 nm	(Larson et al., 2025)
Hookean spring constant	κ	15.5 pN/μm	(Chacon et al., 2014)
Inter-KT separation with no force in <i>S. cerevisiae</i>	x_{rest}	0 μm	(Chacon et al., 2014)
Metaphase spindle length	None	1.5 μm	(Marco et al., 2013; Sim et al., 2024)
Delay time before simulated cells with 16 bioriented KT pairs are considered “fully bioriented”	None	1 min	Chosen, see Methods 2.1 , Methods 2.3 , and Discussion
Number of additional MTs per pole	None	2 MTs	(Winey et al., 1995)

Table 2.2. Other simulation parameters.

Stage 1 parameters			Stage 1 results				Stage 2 parameter	Stage 2 results		
Stu2-AID?	Kinase amplitude (1/min)	Direction-sensitive grip amplitude (1/min)	Amp lifetime (min)	Syn lifetime (min)	Mono lifetime (min)	Selectivity ratio	Geometric bias	% cells fully bioriented at 40 min	5% biorientation time (min)	95% biorientation time (min)
n	0	0	31.0	120.5	480.7	0.26	0.5	0	> 1666	> 1666
n	0	0	31.0	120.5	480.7	0.26	0.8	0	> 1666	> 1666
n	0	0	31.0	120.5	480.7	0.26	0.95	0	224	> 1666
n	1.33	0	23.2	7.4	25.4	3.12	0.5	0.9	85	> 1666
n	1.33	0	23.2	7.4	25.4	3.12	0.8	29	21	161
n	1.33	0	23.2	7.4	25.4	3.12	0.95	73	15	66
n	1.33	1	22.8	3.2	25.4	7.24	0.5	12	27	455
n	1.33	1	22.8	3.2	25.4	7.24	0.8	70	12	78
n	1.33	1	22.8	3.2	25.4	7.24	0.95	93	9	43
y	0	0	1.1	165.7	1643.2	0.01	0.95	0	> 1666	> 1666
y	1.33	0	1.0	8.0	26.4	0.12	0.95	0	> 1666	> 1666
y	1.33	1	1.0	3.2	26.4	0.30	0.95	0	> 1666	> 1666
n	0	1	31.0	4.8	480.7	6.41	0.5	14	27	370
n	0	1	31.0	4.8	480.7	6.41	0.8	76	12	65
n	0	1	31.0	4.8	480.7	6.41	0.95	97	10	36
n	1	0	24.4	9.8	33.3	2.48	0.5	0.3	147	> 1666
n	1	0	24.4	9.8	33.3	2.48	0.95	55	18	80
n	1	1	24.7	3.4	33.3	7.20	0.5	14	26	402
n	1	1	24.7	3.4	33.3	7.20	0.8	74	12	70
n	1	1	24.7	3.4	33.3	7.20	0.95	95	9	40
n	2	0	19.7	5.1	17.2	3.83	0.5	2	66	> 1666
n	2	0	19.7	5.1	17.2	3.83	0.95	75	13	66
n	2	1	19.6	2.7	17.2	7.38	0.5	9	29	599
n	2	1	19.6	2.7	17.2	7.38	0.8	61	12	97
n	2	1	19.6	2.7	17.2	7.38	0.95	86	9	55
n	0.66666667	0	26.5	14.1	47.9	1.88	0.95	31	23	105
n	0.66666667	1	26.1	3.8	47.9	6.96	0.5	15	25	383
n	0.66666667	1	26.1	3.8	47.9	6.96	0.8	76	11	68
n	0.66666667	1	26.1	3.8	47.9	6.96	0.95	96	9	37
n	1.66	0	17.40	3.87	12.8	4.50	0.5	2	59	> 1666
n	1.66	0	17.40	3.87	12.8	4.50	0.95	72	12	74

Table 2.3. Stage 1 and 2 results for combinations of error correction mechanisms described in the text. Stage 1 and 2 results were shaded so that the darker the cell's color, the higher the value. To emphasize the most important comparisons, monotelic lifetimes were colored such that those 50 min and longer were darkest, times until 5% of cells were fully bioriented were colored such that times 30 min and longer were darkest, and times until 95% of cells were fully bioriented were colored such that times 100 min and longer were darkest.

Methods

1.1 Stage 1 simulations: General procedure

Each stage 1 simulation consists of a sister kinetochore pair interacting with dynamic microtubules in a one-dimensional, 1.5 μm long spindle (Figures 2.1, S2.1a-c, and Video 2.1-2.3), approximately the length of a budding yeast spindle in mid-metaphase (Marco et al., 2013; Sim et al., 2024). Microtubules grow, shorten, and switch between growth and shortening. During shortening, microtubules drag tip-attached kinetochores toward their attached pole and kinetochores exert reciprocal forces on the attached microtubule, influencing microtubule dynamics. These forces arise from chromatin and kinetochore stretching as the two sisters in the kinetochore pair separate. Kinetochores detach from microtubule tips stochastically according to force and we measure these attachment lifetimes.

Stage 1 simulations were performed with a modified Gillespie algorithm (Gardner and Odde, 2010; Gillespie, 1977). All simulations used a maximum timestep of 0.1 s. The timestep was chosen to be small enough that force changed negligibly between consecutive timesteps, which is important because the Gillespie algorithm assumes constant growth, shortening,

switch, and detachment rates, and each of these rates is force-dependent. Stage 1 simulations used a maximum simulation time of 10,000 s, except for simulations of syntelic and monotelic attachments with catch-bond or slip-bond behavior alone, which used a maximum simulation time of 100,000 s. Maximum simulation time was chosen to be long enough that simulations nearly always included a detachment event, allowing for accurate lifetime measurements to calculate means, selectivity ratios, and for use in stage 2 simulations.

1.2 Stage 1 simulations: Initial conditions

Both kinetochores started side-attached, 0.1 μm from the tip of their attached microtubule and 0.4 μm from the left pole (Figures 2.2a, 2.2d, and S2.1a). This is near the center of a kinetochore “lobe” (Chacon et al., 2014; He et al., 2001; Marco et al., 2013) and close to the average distance of simulated monotelic, syntelic, and amphitelic kinetochores from the pole (Figures 2.2b, 2.2e, and S2.1b). Thus, amphitelic simulations represent a situation where the unattached kinetochore in a monotelic kinetochore pair has just reattached to the opposite pole from its sister. Syntelic simulations represent a situation where the unattached kinetochore in a monotelic kinetochore pair has just reattached to the same pole as its sister. Monotelic simulations represent a situation where one of the kinetochores of an amphitelic or syntelic kinetochore pair detaches. Simulations began with growing microtubules, since simulated bare microtubule tips are most frequently in growth (Figures 2.1d and S2.1a).

1.3 Stage 1 simulations: Simple inter-kinetochore spring model

We modeled the connection between sister kinetochores as a simple spring using the equation:

$$F = \kappa(x - x_{rest}) \quad (2.1)$$

In this equation, F is the spring force, κ is the strain-stiffening constant, x is the separation between sisters, and x_{rest} is the separation between sisters without force (Table 2.2).

We estimated x_{rest} based on previous work from (Chacon et al., 2014), where average inter-kinetochore stiffness was quantified based on thermal fluctuations in position of fluorescent kinetochores in metaphase budding yeast. Chacon et al. observed that nocodazole-treated cells had sister centromeres that appeared as a single spot, consistent with inter-kinetochore rest length from 0 to 0.17 μm , the resolution limit of their assay. A nonzero rest length is difficult to include realistically in a one-dimensional simulation, so we assumed that the rest length was zero. We used a κ of 15.5 pN/ μm as measured by (Chacon et al., 2014).

1.4 Stage 1 simulations: Microtubule dynamics

Bare microtubule tips grew and switched stochastically between growth and shortening according to the measured dynamics of pre-anaphase, cytoplasmic, non-kinetochore-microtubules ((Gupta et al., 2006), v_{grow_bare} , k_{cat_bare} , and k_{res_bare} in Table 2.1). Bare microtubules shortened at twice the speed of kinetochore-bound microtubules without force, consistent with previous observations ((Bergman et al., 2018; Tanaka et al., 2007; Torvi et al., 2022), v_{short_bare} in Table 2.1). Microtubules with tip-attached kinetochores under no force

shortened at speeds measured in vivo ((Tanaka et al., 2007), v_{short0} in Table 2.1) and grew at the same speed as bare tips ($v_{growth0}$ in Table 2.1), since measurements of kinetochore dynamics on growing microtubule tips without force do not exist. Microtubules could only experience tension—forces toward their minus ends cause kinetochores to become side-bound and slide along the lattice.

Kinetochore-bound microtubule tips were assigned different switch rates from bare tips because the average kinetochore-microtubule length is 0.4-0.5 μm in budding yeast spindles (Chacon et al., 2014; Marco et al., 2013; O'Toole et al., 1999; Sim et al., 2024; Yang et al., 2008), which can be achieved either by increasing catastrophe and/or decreasing rescue from those recorded for bare tips. Both increased catastrophe and decreased rescue are consistent with observations that kinetochores immediately shorten upon reaching a microtubule tip and continue to shorten until they reach the seed (in vitro, (Torvi et al., 2022)) or pole (in vivo, (Tanaka et al., 2007)). Therefore, we increased catastrophe rate without force to 1 per min and decreased rescue rate without force to 0.0005 per min when microtubules have tip-attached kinetochores (k_{cat0} and k_{res0} in Table 2.1). To constrain our model to a 1.5 μm spindle, microtubules were also forced to rescue when they shortened to their own pole and forced to catastrophe when they grew to the opposing pole. Under these conditions, at 20 min, simulated amphitelic kinetochore-microtubules were $0.467 \pm 0.004 \mu\text{m}$ in length (mean $\pm$ SEM, Figure 2.2a), similar to in vivo measurements.

Each tip-attached kinetochore-microtubule had growth, shortening, detachment, and switch rate force-sensitivities based on measurements of microtubules under constant tension (Akiyoshi et al., 2010). With the exception of detachment during shortening, these dynamic rates were fit to measurements from (Akiyoshi et al., 2010) with a single exponential as a function of force using the equation:

$$r = r_0 e^{F/F_{sens}} \quad (2.2)$$

In this equation, r is the dynamic rate, r_0 is the dynamic rate without force, F is the force on the kinetochore, and F_{sens} is the e-fold force sensitivity of that rate.

We derived the e-fold force sensitivity of each of rate from these fits, then inserted those force sensitivities in the following equations to calculate and simulate dynamic rates:

$$v_{growth} = v_{growth0} e^{F/F_{gr}} \quad (2.3)$$

$$v_{short} = v_{short0} e^{F/F_{short}} \quad (2.4)$$

$$k_{cat} = k_{cat0} e^{F/F_{cat}} \quad (2.5)$$

$$k_{res} = k_{res0} e^{F/F_{res}} \quad (2.6)$$

$$k_{det_gro} = k_{det_growth0} e^{F/F_{det_gr}} \quad (2.7)$$

In this equation, v_{growth} and v_{short} are the growth and shortening speeds, $v_{growth0}$ and v_{short0} are the growth and shortening speeds without force, F is the force on the kinetochore, and F_{growth} and F_{short} are the force sensitivities of growth and shortening speed. k_{cat} and k_{res} are the rates of catastrophe (switch from growth to shortening) and rescue (switch from shortening to growth), k_{cat0} and k_{res0} are the catastrophe and rescue rates without force, and F_{cat} and F_{res} are the force sensitivities of catastrophe and rescue rates. k_{det_growth} is the detachment rate of a tip-attached kinetochore during growth, $k_{det_growth0}$ is the detachment rate of a tip-attached kinetochore during growth without force, and F_{det_growth} is the force sensitivity of

detachment rate of a tip-attached kinetochore during growth. Like the force sensitivities, the detachment rate during growth without force (k_{det_growth}) was derived from the single exponential fit of detachment rate during growth versus force to the measurements from (Akiyoshi et al., 2010). See Table 2.1 for all microtubule dynamic parameters used in these simulations.

Measurements of detachment rate during shortening from (Akiyoshi et al., 2010) were fit with a double exponential to explain both the observed decrease in detachment during shortening with increasing force and the ability of monotelic attachments to persist on shortening tips (Doodhi et al., 2021; Kalantzaki et al., 2015; Tanaka et al., 2007; Torvi et al., 2022):

$$k_{det_short} = k_{det_short0} e^{\frac{F}{F_{det_short}}} \left(1 - e^{\frac{F}{F_{det_short}}} \right) \quad (2.8)$$

In this equation, k_{det_short} is the detachment rate of a tip-attached kinetochore during shortening, k_{det_short0} is the detachment rate of a tip-attached kinetochore during shortening without force, F is the force on the kinetochore, F_{det_short} is the first force sensitivity of detachment rate of a tip-attached kinetochore during shortening, F_{det_short} is the second force sensitivity of detachment rate of a tip-attached kinetochore during shortening (Table 2.1). We used this fit to calculate and simulate detachment rate during shortening with one major alteration. Because microtubule shortening is about 10-fold slower in vivo (and in our model) than in vitro, kinetochores may be better at persisting on slower shortening microtubule tips in vivo than suggested from in vitro measurements by (Akiyoshi et al., 2010). For this reason, we used a 10-fold lower detachment rate during shortening without force (k_{det_short0}) than predicted by the double exponential fit from (Akiyoshi et al., 2010). Without this decreased detachment rate during shortening, even with the catch-bond, tension-sensitive kinase activity, and direction-sensitive grip (Figure 2.6), the selectivity ratio is only 0.8 compared to 7.2 with decreased detachment rate during shortening. This is because amphitelic attachments only last 2 min without compared to 23 min with decreased detachment rate during shortening. Therefore, stabilizing tip-attached kinetochores during shortening is critical for the triphasic catch bond to preserve amphitelic attachments, and together with tension-sensitive kinase activity and direction-sensitive grip, to selectively stabilize amphitelic over syntelic attachments.

1.5 Stage 1 simulations: Side-attached kinetochore dynamics

The sliding speed of side-attached kinetochore was calculated as a function of force using the equation for asymmetric sliding of a diffusor along a lattice proposed by (Bormuth et al., 2009):

$$v = \delta k_{step} \left(e^{\frac{(\frac{1}{2}\delta + \Delta)F}{k_B T}} - e^{\frac{-(\frac{1}{2}\delta - \Delta)F}{k_B T}} \right) \quad (2.9)$$

In this equation, v is the kinetochore velocity, δ is the step size of the diffusor along the lattice, k_{step} is the rate at which the diffusor takes steps, Δ is the asymmetry parameter corresponding to the distance between the midpoint of the binding sites and the energy barrier, F is force on the kinetochore, k_B is Boltzmann's constant, and T is temperature in Kelvin. We fit this equation

to binned measurements of the velocity of side-attached kinetochores as a function of force on microtubules in vitro from ((Larson et al., 2025), δ , Δ , and k_{step} in Table 2.2).

Because in vivo budding yeast microtubules shorten at only 1.5 μm per min, microtubules dragged toward the plus and minus end experience almost identical forces, 0.070 pN compared to 0.066 pN, respectively (the mean of 0.09 pN occurs because the plus-end directed force is additive for syntelic kinetochores). Therefore, tip-attached kinetochores dragging their side-attached sister have almost the same detachment rate when attached amphitelicly or syntelicly. This means that neither catch-bond behavior nor tension-sensitive kinase activity can selectively destabilize syntelic kinetochores pulling their side-bound sisters without also destabilizing amphitelic ones. In fact, the small additional force experienced by amphitelic kinetochores from asymmetric friction makes them more vulnerable (Figures 2.3c and S2.3d). Because sliding kinetochores experience less than 0.2 pN of force, increasing force brings kinetochores closer to ~ 1.5 pN, where they are most unstable from the triphasic catch bond, and ~ 0.3 pN, where tension-sensitive kinase activity is highest in our model. Therefore, it is unlikely that drag force from the side-attached amphitelic kinetochore is sufficient to protect its tip-attached sister from low-tension detachment.

1.6 Stage 1 simulations: Plus-end-directed force

(Torvi et al., 2022) measured that kinetochores in extract move toward the microtubule plus end at 0.5 per min. We found that a force of 0.024 pN is required to push a kinetochore toward the microtubule plus end at this speed using equation (2.9). Plus-end movement is dependent on Kip3 (Torvi et al., 2022), which can diffuse along the microtubule lattice and accumulate at the microtubule tips (Varga et al., 2009). Movement toward the spindle equator has also been reported in *S. pombe* due to the kinesin Cut7 and in humans due to CENP-E (Aker et al., 2015), and was used in the error-correction model of *S. pombe* of (Edelmaier et al., 2020). Therefore, we applied this small plus-end-directed force to each side-attached and tip-attached kinetochore, preventing kinetochores from becoming stuck at the spindle poles.

1.7 Stage 1 simulations: Simulated kinetochore-microtubules without Stu2

Simulated kinetochores without Stu2 had detachment rates during growth and shortening based on measurements of tip-attached kinetochores without Stu2 as a function of force from ((Miller et al., 2016), Figure S2.2a). We fit the measurements of detachment rate during growth with a single exponential as we did for kinetochores with Stu2:

$$k_{det_grow_noStu} = k_{det_growth_noStu2_0} e^{F/F_{det_grow_noStu2}} \quad (2.10)$$

In this equation, $k_{det_grow_noStu}$ is the detachment rate of a tip-attached kinetochore without Stu2 during growth, $k_{det_growth_noStu2_0}$ is the detachment rate of a tip-attached kinetochore without Stu2 during growth without force, F is the force on the kinetochore, and $F_{det_grow_noStu2}$ is the force sensitivity of detachment rate of a tip-attached kinetochore without Stu2 during shortening (Table 2.1).

We fit the measurements of detachment rate during shortening with a double exponential as we did for kinetochores with Stu2:

$$k_{det_short_no} = k_{det_short_noStu2_0} e^{\frac{F}{F_{det_short_noS_1}}} \left(1 - e^{\frac{F}{F_{det_short_noStu2_2}}} \right) \quad (2.11)$$

In this equation, $k_{det_short_no}$ is the detachment rate of a tip-attached kinetochore without Stu2 during shortening, $k_{det_short_noStu2_0}$ is the detachment rate of a tip-attached kinetochore without Stu2 during shortening without force, F is the force on the kinetochore, $F_{det_short_no_Stu2_1}$ is the first force sensitivity of detachment rate of a tip-attached kinetochore without Stu2 during shortening, and $F_{det_short_no_Stu2_2}$ is the second force sensitivity of detachment rate of a tip-attached kinetochore without Stu2 during shortening (Table 2.1). As we did for kinetochores with Stu2, this fit (specifically, $k_{det_short_noStu2_0}$) was divided by 10 (see Methods 1.4). Simulated kinetochores without Stu2 had identical growth, shortening, catastrophe, and rescue rates as kinetochores with Stu2 (Figures S2.2b and S2.2c).

1.8 Stage 1 simulations: Model for tension-sensitive kinase release

As with detachment during shortening, we fit the measurements from (de Regt et al., 2022) with a double exponential so kinetochores without force experience no added detachment due to tension-sensitive kinase release, consistent with observations in vivo and in vitro that kinetochores rarely detach from microtubules without force (Doodhi et al., 2021; Indjeian and Murray, 2007; Kalantzaki et al., 2015; Tanaka et al., 2007; Torvi et al., 2022):

$$k_{Aur} = k_{Aur0} \left(1 - e^{-\frac{F}{F_{Aur1}}} \right) e^{-\frac{F}{F_{Aur2}}} \quad (2.12)$$

In this equation, k_{Aur} is the rate of tension-sensitive kinase release, k_{Au} is the amplitude, F_{Aur1} is the force sensitivity in the left section of the tension-sensitive kinase release curve where detachment rate is increasing, and F_{Au} is the force sensitivity in the right section of the tension-sensitive kinase release curve where detachment rate is decreasing (Figures 2.4a, S2.3d, Table 2.2). This fit was performed with F_{Au} fixed at 1 pN because the fit equation has 3 parameters and the measurements from (de Regt et al., 2022) only include Aurora B-triggered detachment rates at two forces (1.5 ± 0.3 pN and 5.0 ± 0.5 pN, mean $\pm$ SEM). We chose $F_{Aur2} = 1$ pN because it resulted in a qualitatively reasonable fit of these measurements without creating a high peak in tension-sensitive kinase release at low forces that is unsupported by direct measurements. We increased or decreased the amplitude of tension-sensitive kinase release by increasing or decreasing k_{Aur0} as indicated in Table 2.3.

1.9 Stage 1 simulations: Direction-sensitive grip

Direction-sensitive grip was applied as a constant, velocity-independent detachment rate affecting side-attached kinetochores only while being pulled toward the minus end (1 min^{-1} in Figure 2.6). This effect is based on studies showing that side-attached kinetochores experience higher friction when sliding toward the plus end than the minus end (Demidov et al., 2025; Larson et al., 2025). In addition, (Larson et al., 2025) found that kinetochores dragging to minus ends adopt a more compact architecture compared to both kinetochores dragging to plus ends and correct plus-end-attached kinetochores. These findings suggest that kinetochores being dragged to minus ends may be prone to spontaneous detachment from their intrinsic instability and/or specific detachment by kinases targeting this uniquely incorrect state.

Because tip-attached kinetochores slow microtubule shortening, when a syntelic, tip-attached kinetochore is pulling its sister toward the pole, the shortening microtubule of the side-attached sister will sometimes catch up to its kinetochore. In this case, we considered this kinetochore to still be side-attached in that it could detach from the lattice from direction-sensitive grip but could not detach from the tip, either spontaneously or from tension-sensitive kinase release. However, the side-attached microtubule was still considered to have the altered dynamics (slower shortening, faster catastrophe, and slower rescue) of kinetochore-attached microtubule tips. This avoided ringing behavior where syntelic sisters take turns dragging each other poleward, as the microtubule with the side-attached kinetochore continually outpaces the other.

2.1 Stage 2 simulations: General procedure

Amphitelic and syntelic attachments have lifetimes drawn at random from the distributions of predicted amphitelic and syntelic lifetimes generated in stage 1 simulations. At the end of each lifetime, one of the kinetochores in the sister pair detaches and the pair becomes monotelic. Monotelic sister kinetochores reattach stochastically, becoming either amphitelic or syntelic after an average of 1.5 min, consistent with *in vivo* observations (([Mukherjee et al., 2019](#)); see also [Methods 2.3](#)).

After a transient startup phase, the number of amphitelic, syntelic, and monotelic attachments approaches a quasi-steady-state ([Figures 2.5a](#) and [2.5b](#)). The steady-state numbers of amphitelic attachments reported above are recorded at 3950 s, the timepoint before the maximum simulation time of 4000 s, after nearly all simulations have reached equilibrium numbers of amphitelic, syntelic, and monotelic attachments. The number of amphitelic attachments fluctuates around the steady state as attachments form and break until all 16 kinetochore pairs are bioriented for at least 1 min. We define this moment as the biorientation time. The 1 min delay time was chosen because amphitelic kinetochores take about 1 min to become tip-attached in stage 1 simulations ([Figure 2.2b](#)) and there is evidence that tip-attachment is required to silence the spindle assembly checkpoint ([Kuhn and Dumont, 2017](#)). It is possible that real cells require even more time once chromosomes are fully bioriented to begin anaphase (see [Discussion](#) and [Methods 2.3](#)). Once all 16 kinetochore pairs are amphitelic and tip-attached, the spindle assembly checkpoint is satisfied and the cell can proceed in anaphase.

2.2 Stage 2 simulations: Initial conditions

Stage 2 simulations began with eight amphitelic, eight syntelic (four associated with each pole), and zero monotelic kinetochore pairs. These initial conditions represent a situation where after spindle pole duplication and before spindle pole separation, all kinetochore pairs behave as syntelic attachments and become monotelic again frequently. Detached sisters have an equal likelihood of reforming an attachment to either pole, so eventually, on average, 50% of pairs will have one sister attached to each pole, 25% of pairs will have both sisters attached to the mother pole, and 25% of pairs will have both sisters attached to the daughter pole. Two non-kinetochore-microtubules were also associated with each pole, which is important for reattachment kinetics (see [Methods 2.3](#)).

2.3 Stage 2 simulations: Reattachment kinetics and geometric bias

Reattachment of monotelic kinetochore pairs either syntelically or amphitelically was assumed to be a stochastic, first-order process such that the reattachment rate scaled linearly with the number of non-kinetochore-microtubules available to bind. We assumed that each spindle pole nucleated 18 microtubules, one for each of the 16 kinetochore pairs and two extra. This configuration is consistent with observations that budding yeast spindle poles only nucleate a limited number of microtubules, on average 22, just two more than the 20 that is sufficient to form 16 amphitelic attachments and 4 interpolar microtubule attachments (Winey et al., 1995). Including at least one extra microtubule per pole means that a lone monotelic kinetochore pair (with all 15 other kinetochore pairs amphitelic or syntelic) does not necessarily reform the same attachment (amphitelic or syntelic) as the one that just broke.

We used the following equation to calculate the reattachment rate used in the Gillespie algorithm to generate each stochastic timestep:

$$k_{re} = N_{amp}k_{single_syn} \frac{(B_{reattach-amp})}{(100 - B_{reattach-amp})} + N_{syn}k_{single_syn} \quad (2.13)$$

In this equation, $k_{reattach}$ is the reattachment rate of a simulated monotelic kinetochore pair, N_{amp} is the number of non-kinetochore-microtubules that could form amphitelic attachments to the monotelic kinetochore pair, k_{single_syn} is the attachment rate of a monotelic kinetochore to a lone microtubule coming from the same pole as the already attached sister, N_{syn} is the number of non-kinetochore-microtubules that could form syntelic attachments to the monotelic kinetochore pair, and $B_{reattach}$ is the percent bias toward amphitelic reattachment reported in the main text.

We empirically determined k_{single_syn} for each combination of lifetimes simulated in stage 1 and amphitelic reattachment bias used in stage 2 to keep the average time for amphitelic reattachment within 1 s of the 1.5 min⁻¹ estimated in (Mukherjee et al., 2019). We calculated the average time for amphitelic reattachment, T_{amp_reat} , with the inverse of the first term of equation (2.13):

$$T_{amp_reattach} = \frac{1}{k_{amp_reattach}} = \frac{100 - B_{reattach-amp}}{N_{amp}k_{single_syn}B_{reattach-amp}} \quad (2.14)$$

Adjusting k_{single_syn} for different combinations was necessary because reattachment rate depends both on the percent bias toward amphitelic reattachment and the average number of non-kinetochore-microtubules attached to either pole, which changes based on amphitelic and syntelic attachment lifetimes. If lifetimes are long relative to reattachment time, there will be fewer non-kinetochore-microtubules because most of the 18 microtubules from each pole will be attached to a kinetochore.

In budding yeast, it is especially important for amphitelic attachments to endure for the majority of mitosis to avoid having to repeat the error-correction or avoidance process (see Discussion). The longer the reattachment time or post-biorientation delay time, the longer amphitelic attachments have to last. Unfortunately, there is very little data at the moment describing reattachment times of individual kinetochore-microtubules in wild-type mitotic spindles. To our knowledge, the only such data are from (Mukherjee et al., 2019), where only two amphitelic reattachments were observed. In budding yeast, interpretation of these measurements, as well

as measurements of time from biorientation to anaphase (Marco et al., 2013), are made complicated because biorientation is determined by the separation of fluorescent operons inserted adjacent to sister centromeres. After separation, these spots periodically rejoin (“breathing”), which is thought to (at least in part) reflect the dynamics of the cohesin bonds linking the pericentromere rather than necessarily being an indication that the pair became monotelic (Richeldi et al., 2023). In addition, both monotelic and syntelic attachments appear as a single spot, so it is impossible to measure the time required for monotelics to become syntelic. Still, further sister centromere tracking or reinterpretation of previous experiments would be invaluable to measure amphitelic lifetimes, amphitelic reattachment times, and post-biorientation delay in budding yeast, each of which are critical parameters in our error-correction model.

3.1 Calculated attachment lifetime at constant forces

To calculate the attachment lifetime of a single kinetochore-microtubule at constant force, we used the equation:

$$\langle t \rangle = \frac{k_{cat} + k_{res} + k_{dshort}}{k_{cat}k_{dshort} + k_{res}k_{dgrow} + k_{dgrow}k_{dshort}} \quad (2.15)$$

In this equation, $\langle t \rangle$ is the mean attachment lifetime, k_{cat} is the catastrophe rate, k_{res} is the rescue rate, k_{dshort} is the detachment rate during shortening, and k_{dgrow} is the detachment rate during growth. All rates are force-dependent (see Methods 1.4), so these calculations assume a single (constant) force throughout the kinetochore-microtubule attachment lifetime. All rates and their force-dependencies are identical to those used in stage 1 simulations. Importantly, this equation only describes the dissociation of kinetochores from the microtubule tip.

Calculating the mean attachment lifetime of a single tip-attached kinetochore under constant force is useful to understand what forces most (de)stabilize tip-attached kinetochores (Figures 2.3, S2.1, and S2.2). However, these calculations do not directly predict the attachment lifetimes of stage 1 simulations for a number of reasons. First, the number of tip-attached kinetochores varies from zero to two between simulations and over time. Amphitelic kinetochore pairs spend the majority of their time tip-attached where both sisters are prone to stochastic detachment (Figure 2.2a and Video 2.1). Syntelic kinetochore pairs spend much of their time with both sisters side-attached, where they are protected from detachment, and some of their time with one sister tip-attached and the other side-attached (Figure 2.2d and Video 2.2). Monotelic kinetochore pairs spend the majority of their time with one sister side-attached and the other sister detached (thereby “protected” from further stochastic detachment), and the rest of their time with one kinetochore tip-attached and the other detached (Figure S2.1a and Video 2.3). Second, amphitelic kinetochores spend the majority of their time on shortening tips, where they are protected from detachment at high force (Figures 2.2a, S2.2a, and Video 2.1). When an amphitelic sister switches to growth, high tension makes it grow rapidly towards its sister (Figure S2.2b), relieving the force on both kinetochores and making them less prone to detachment during growth. Low force biases kinetochore-microtubules to switch to shortening (Figure S2.2c), where they are somewhat prone to detachment. However, shortening is fast under low force, so kinetochores quickly regain the

higher forces that are protected during shortening. For these reasons, using constant force calculations to estimate the attachment lifetimes simulated in stage 1 should be undertaken with caution.

3.2 Software

Calculations, curve fits, and stochastic simulations were performed using custom software in Igor Pro 9 (Wavemetrics). Curve-fitting was performed with Igor Pro's default Levenberg-Marquardt (damped least-squares) fit algorithm ([Press et al., 1992](#)). Source code will be available on Zenodo.

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Chapter 3: Mechanical coupling coordinates microtubule growth.

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My role in this work

In this work, I performed and optimized a new type of dual laser-trap experiment. I hired and trained an undergraduate researcher, Katelyn Kostello, to help me collect data using this new experimental approach, since two pairs of hands were required to manage the two required laser traps. I built the data analysis software to analyze both the dual-trap data and previously-collected force-clamp data. I analyzed these data to build models of microtubule variability and force-dependence from force-clamp data that I tested on dual-trap data. I hired and trained another undergraduate researcher, Yuna Liu, to assist in extending these models to more microtubules (see [Chapter 5](#)) and quantitatively evaluating which model fit dual-trap data best. I wrote the first-draft manuscript, including figures, and edited that manuscript with my advisor, Chip Asbury. In addition, I secured the Hurd Fellowship, which provided my salary for two years of this approximately four-year endeavor.

Abstract

During mitosis, kinetochore-attached microtubules form bundles (k-fibers) in which many filaments grow and shorten in near-perfect unison to align and segregate each chromosome. However, individual microtubules grow at intrinsically variable rates, which must be tightly regulated for a k-fiber to behave as a single unit. This exquisite coordination might be achieved biochemically, via selective binding of polymerases and depolymerases, or mechanically, because k-fiber microtubules are coupled through a shared load that influences their growth. Here, we use a novel dual laser trap assay to show that microtubule pairs growing in vitro are coordinated by mechanical coupling. Kinetic analyses show that microtubule growth is interrupted by stochastic, force-dependent pauses and indicate persistent heterogeneity in growth speed during non-pauses. A simple model incorporating both force-dependent pausing and persistent growth speed heterogeneity explains the measured coordination of microtubule pairs without any free fit parameters. Our findings illustrate how microtubule growth may be synchronized during mitosis and provide a basis for modeling k-fiber bundles with three or more microtubules, as found in many eukaryotes.

Introduction

In the mitotic spindles of many eukaryotes, each chromosome is attached to a bundle of microtubules ([Kiewisz et al., 2022](#); [McDonald et al., 1992](#); [O'Toole et al., 2020](#); [VandenBeldt et](#)

al., 2006) that grow and shorten in unison, acting as a single unit to produce the forces necessary to move the chromosome (Cimini et al., 2004). Microtubules are microns-long protein polymers composed of $\alpha\beta$ -tubulin dimers with their 'minus' ends oriented toward the spindle poles and their more dynamic 'plus' ends oriented toward the chromosomes. Microtubule plus-ends, or tips, attach to chromosomes via protein complexes called kinetochores to form 'k-fiber' bundles. Kinetochores attached tips within a single k-fiber remain very close to one another (within $\sim 0.2 \mu\text{m}$ along the bundle axis) (O'Toole et al., 2020) even as the bundle grows and shortens (Cimini et al., 2004) by the addition and loss of tubulin subunits at each tip (Maddox et al., 2003). The number of microtubules per k-fiber varies across species; human RPE1 cells have ten to fifteen microtubules per k-fiber, while marsupial PTK2 cells have more than twenty, and cervid Indian Muntjac cells have up to sixty (O'Toole et al., 2020; Drpic et al., 2018). Budding yeast lack k-fiber bundles, having just a single microtubule per kinetochore (Winey et al., 1995), although microtubules attached to sister kinetochores on opposite sides of the yeast spindle must still coordinate with each other. Uncovering how microtubule tips maintain a high degree of synchrony within k-fibers of up to sixty microtubules is critical to understanding how chromosomes are accurately segregated during normal mitosis and why even modest perturbations of kinetochore-attached microtubule dynamics can lead to mis-segregation (Bakhoun & Compton, 2012; Bakhoun et al., 2009; Ertych et al., 2014; Stumpff et al., 2014).

The tightly coordinated growth of microtubules within a k-fiber bundle is remarkable because individually, microtubule tips exhibit highly variable growth speeds and switch stochastically between distinct states of growth and shortening (Brouhard, 2015). Microtubule growth speed varies considerably in purified tubulin systems (Figure 3.1a) (Cleary et al., 2022; Gildersleeve et al., 1992; Mahserejian et al., 2022), in cell extracts (Geisterfer et al., 2020), and in live cells (Cassimeris et al., 1988; Gaudenz et al., 2010; Yamashita et al., 2015). Part of this heterogeneity can be attributed to pausing, when microtubules stochastically stop and start growing during periods of net growth (Amoah-Darko & White, 2022; Mahserejian et al., 2022). How the intrinsic heterogeneity of microtubule growth is coordinated to maintain sub-micrometer proximity between k-fiber tips remains a mystery.

One possible mechanism that might coordinate k-fiber microtubules is selective binding of microtubule-regulatory proteins (Maiato, Rieder, et al., 2003; McAinsh & Meraldi, 2011; Tirnauer et al., 2002). Many microtubule-associated proteins that are known to regulate growth in vitro are implicated in modulating k-fiber dynamics and kinetochore movements in cells, including TOG-family proteins such as XMAP215 (Brouhard et al., 2008) and CLASP (Al-Bassam et al., 2010; Maiato, Fairley, et al., 2003; Sousa et al., 2007), mitotic kinesins such as MCAK and Kif18A (Sanhaji et al., 2011; Stumpff et al., 2012), and other plus-end-trackers such as EB1 (reviewed in (Amin et al., 2019)). The localization of some of these regulators is enriched near kinetochores specifically when a kinetochore is moving anti-poleward and its k-fiber is growing longer (Amaro et al., 2010; Castrogiovanni et al., 2021; Tirnauer et al., 2002). However, whether

such selective enrichment is sufficient to explain the very tight coordination across many individual microtubule tips within a k-fiber is unclear.

Another, simpler mechanism is suggested by the fact that microtubule dynamics are also strongly affected by mechanical force. Tension applied to individual microtubules in vitro biases them toward growth (Figure 3.1a) (Akiyoshi et al., 2010), while compression biases them toward shortening (Dogterom & Yurke, 1997; Janson et al., 2003). Theoretically, these mechanical effects should promote synchronization of any group of microtubules whose tips, like the tips of kinetochore-attached microtubules within a k-fiber, are all coupled to the same load (Banigan et al., 2015; Schwieter & Kierfeld, 2020; Skibbens et al., 1993). To illustrate how, consider a hypothetical k-fiber composed of just two microtubules sharing a total load, F_{TOT} . The load is transmitted to the microtubule tips through the kinetochore, which has elasticity (Cojoc et al., 2016; Renda et al., 2020) that we represent as spring-like connections from the kinetochore to each tip (Figure 3.1b). Initially, the two microtubules might have equal lengths and share the load equally – each one carrying half of F_{TOT} (Figure 3.1b, step 1). If one tip happens to grow more slowly than the other, then its spring will be stretched more, and it will start carrying more tension than the other tip (Figure 3.1b, step 2). Conversely, the faster-growing tip will start carrying less tension. Because tension accelerates microtubule growth, the lagging tip will tend to accelerate, while the leading tip will tend to slow down, such that, on average, they return to having equal lengths and tensions (Figure 3.1b, step 3).

Mechanical coupling was proposed decades ago as an explanation for coordination between sister k-fibers (Gardner et al., 2005; Khodjakov et al., 1996; Skibbens et al., 1995; Skibbens et al., 1993; Wan et al., 2012), but how effectively it can coordinate microtubules within a k-fiber has not been determined. Compressive forces can cause a bundle of microtubules growing in vitro to switch cooperatively into a shortening state (Laan et al., 2008), but k-fiber bundles are usually under tension, not compression (Khodjakov & Rieder, 1996; Waters et al., 1996). Theoretical studies have explored the effects of shared tensile and compressive loads on cooperative switching and bi-stability in microtubule bundles (Banigan et al., 2015; Das et al., 2014; Ghanti & Chowdhury, 2015; Schwieter & Kierfeld, 2020; Zelinski & Kierfeld, 2013). However, these studies have generally neglected any differences in growth speed between microtubules and therefore cannot address how much variability exists, nor how such variability is suppressed. Furthermore, to our knowledge, the possibility that part of this variability (and suppression thereof) can be attributed to tension-dependent pausing during microtubule growth has not been explored.

Here, we use a novel dual laser trap assay to test directly how well a constant shared load can coordinate a pair of microtubules growing in vitro. We show that when microtubule pairs are mechanically coupled to a single load through a material of sufficient stiffness, their growth is tightly coordinated. By re-analyzing recordings of individual microtubules growing under constant force (from (Akiyoshi et al., 2010)) and examining their stochastic pausing kinetics, we

show that pausing is suppressed by tension. We then develop a theoretical model, based on the measured behaviors of individual growing microtubules, to describe both the natural variability and force dependence of growth using no free fit parameters. Simulations based on this model fit the dual-trap data well. Altogether, this work illustrates how mechanical coupling may coordinate microtubule growth and provides a basis for modeling k-fibers of three or more microtubules under a shared load.

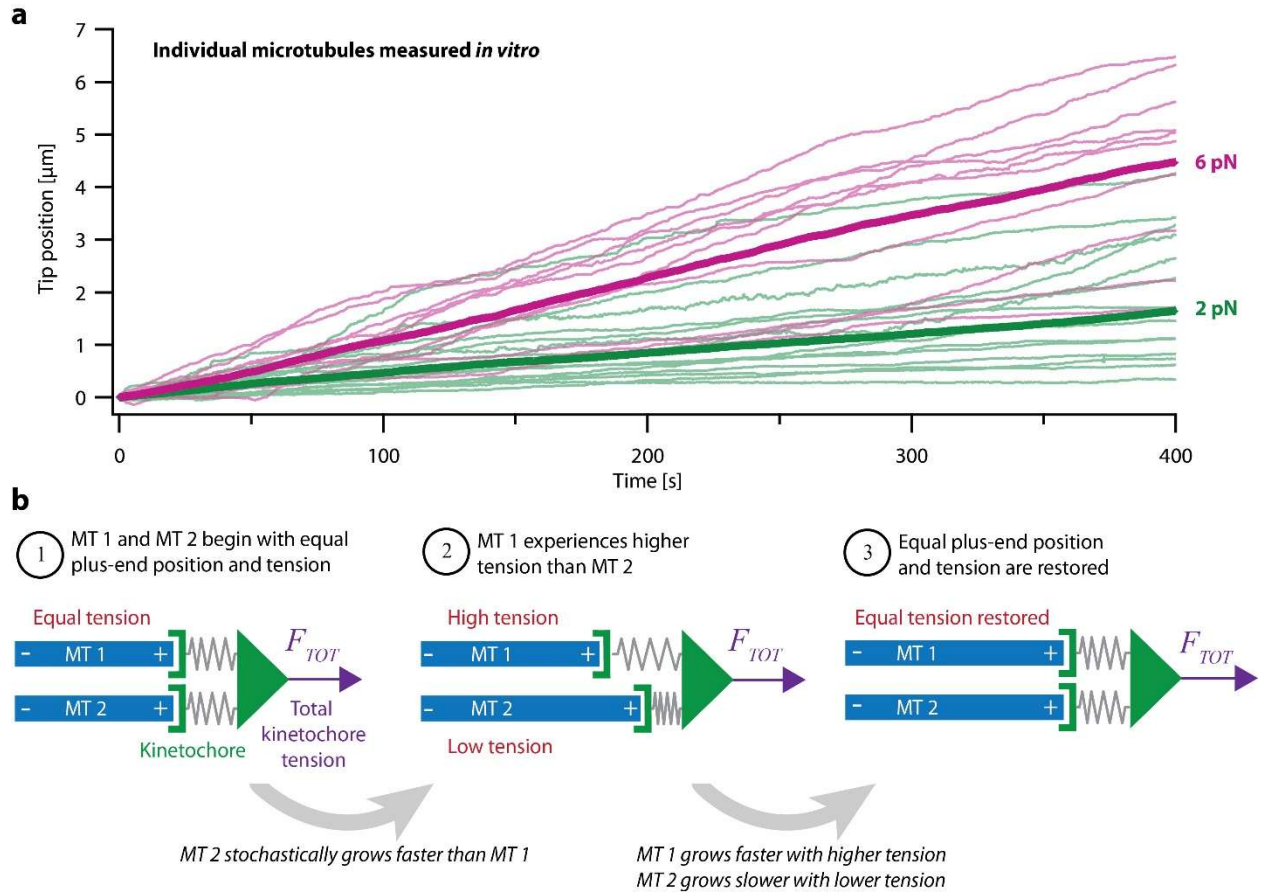


Figure 3.1. Intrinsic variability in microtubule growth could be coordinated by mechanical coupling. (a)

Microtubules (MTs) grow at intrinsically variable rates that increase with force, on average. Example recordings show plus-end position versus time for individual growing microtubules subject to a constant tensile force of either 2 or 6 pN (thin green or magenta traces, respectively). At each force, 10 to 14 example recordings are shown, together with the mean position versus time (thick traces, computed from $N = 25$ or $N = 10$ individual recordings at 2 or 6 pN, respectively). Individual traces are from (Akiyoshi et al., 2010). **(b)** Mechanism by which mechanical coupling could coordinate growing microtubules. In this example, two microtubules share a total force, F_{TOT} , through two spring-like connections to their plus-ends. If one microtubule stochastically lags behind, it will experience more tension than the leading microtubule due to differential stretching of their spring-connections. Because tension tends to accelerate plus-end growth (as illustrated in panel a), the higher tension will tend to accelerate the growth of the lagging microtubule, causing it to catch up.

Results

Dual-trap assay to measure coordination between paired microtubules in vitro

To study how mechanical coupling affects microtubule growth, we developed a novel technique which we call the dual-trap assay ([Figure 3.2](#)). The dual-trap assay is based on our previously developed single-trap (force-clamp) assay ([Akiyoshi et al., 2010](#); [Miller et al., 2016](#); [Sarangapani et al., 2013](#)), in which dynamic microtubules are grown from stabilized seeds bound to a biotinylated coverslip. Using the single laser trap, we attach an individual bead decorated with isolated yeast kinetochores to the growing plus-end of a single microtubule. A computer then continuously measures the bead position and adjusts the trap to exert a precise, constant level of tension on the microtubule via the kinetochore-decorated bead. Under this persistent feedback-controlled tension, kinetochore-beads typically track with the microtubule tips even as the tips stochastically grow and shorten.

Our new dual-trap assay uses two separate laser trapping microscopes, located adjacent to one another in the same room and connected to a single computer. On each of the two instruments, we attach a kinetochore-decorated bead to a dynamic microtubule plus-end. The computer then simultaneously monitors and controls the forces on both microtubules. Rather than keeping the force constant on each microtubule, the computer adjusts the forces dynamically to simulate an elastic coupling of both plus-ends to a single shared load ([Figure 3.2a](#)). Thus far, we have simulated only purely elastic couplers, where both coupling springs are linear (Hookean) with stiffness, κ ([Figure 3.2b](#)). In the future, more complex coupling materials with viscoelasticity ([Cojoc et al., 2016](#)) or strain-stiffening ([Schwietert et al., 2022](#); [Volkov et al., 2018](#)) could also be simulated.

To begin a dual-trap experiment, we first choose the spring stiffness, κ , and the total shared load, F_{TOT} , which are kept constant ([Figure 3.2a](#), step 1). After a kinetochore-decorated bead is attached to a growing plus-end on each of the two instruments, feedback-control is initiated and the two plus-ends are arbitrarily considered to be parallel, with tips side-by-side (i.e., both at $x_1 = x_2 = 0$) and sharing the load equally ($F_1 = F_2 = \frac{1}{2} \cdot F_{TOT}$) ([Figure 3.2a](#), step 2). Because microtubule growth is intrinsically variable, the two microtubules subsequently grow at different speeds ([Figure 3.2a](#), step 3). The computer then dynamically monitors the bead positions ([Figure 3.2a](#), step 4) and adjusts the forces, F_1 and F_2 ([Figure 3.2a](#), step 5), according to the elastic coupling model ([Figure 3.2b](#)). For purely elastic couplers, the force difference across the two microtubules equals the tip separation, $(x_2 - x_1)$, multiplied by the coupling stiffness (κ). When one microtubule grows more quickly than the other, tension on the leading (faster-growing) microtubule decreases and tension on the lagging (slower-growing) microtubule increases to maintain a constant total force on the pair, F_{TOT} . Forces in our dual-trap feedback system are updated at a rate of 50 Hz, which is more than sufficient for accurately simulating the elastic coupling between two microtubules ([Figure S3.3-1](#)). For all the experiments described below, $F_{TOT} = 8$ pN. Thus, each microtubule generally experiences

between 1 and 7 pN of tension, close to the estimated average of 4 to 6 pN experienced by kinetochore-microtubules in budding yeast (Chacon et al., 2014; Parmar et al., 2023).

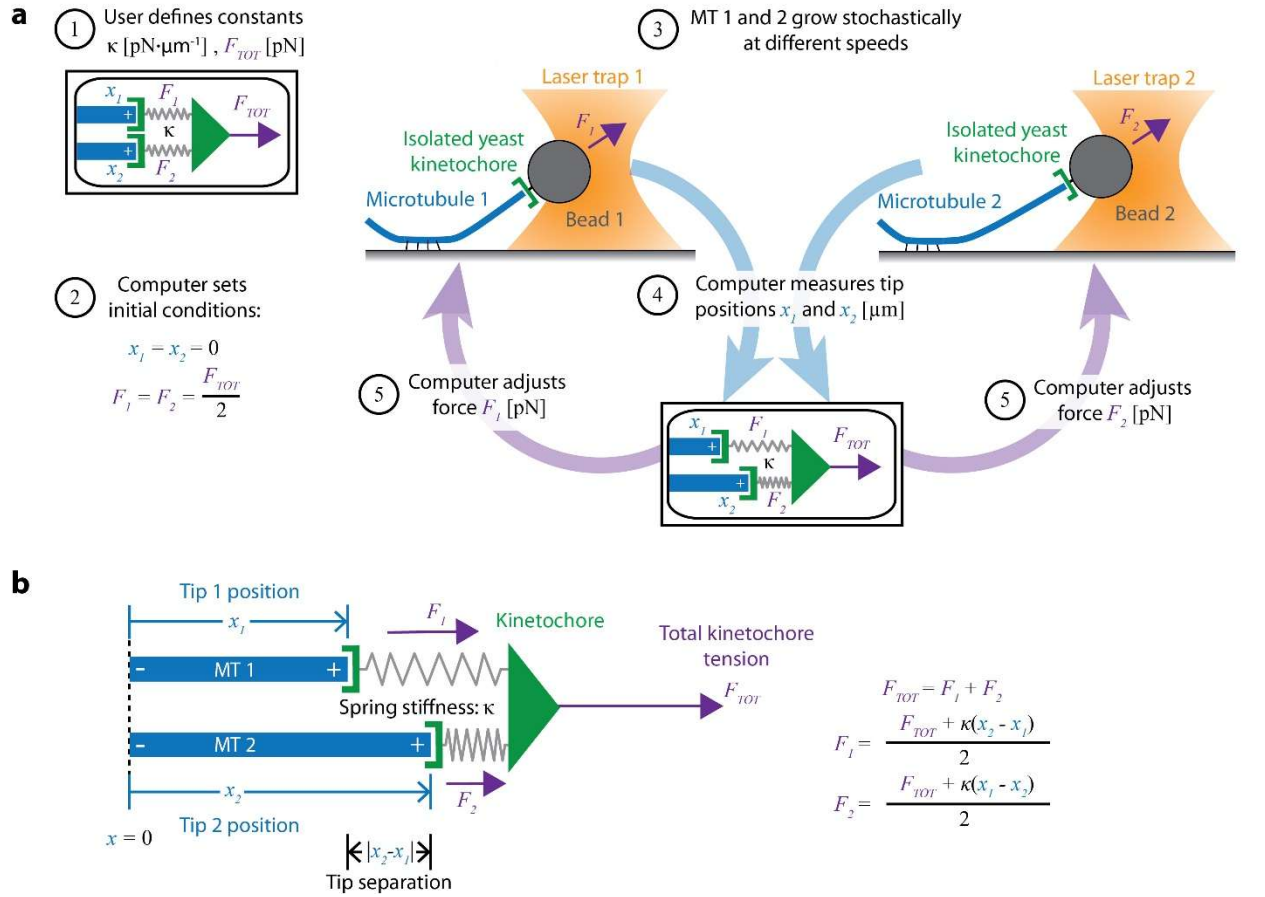


Figure 3.2. Dual-trap assay to measure the coordination of two real microtubules in vitro coupled together by simulated spring-like connections to a shared tensile load. (a) Schematic of the dual-trap assay, which measures the effects of the spring-coupler model (detailed in b) on real microtubules (MTs) growing in vitro. Two dynamic microtubules are grown from seeds anchored to two separate coverslips on two separate stages, each with their own microscope and laser trap. Each laser trap applies tension to its respective microtubule plus-end via a bead decorated with isolated yeast kinetochores, and each tension is adjusted dynamically under feedback control by a shared computer. Initially (steps 1 and 2), microtubule plus-ends are considered to have the same position ($x_1 = x_2 = 0$) so that each bears half of the total user-input tension (F_{TOT}). Over time, as microtubules grow at different rates (step 3), the computer dynamically adjusts the tension on each microtubule according to the spring-coupler model (steps 4 and 5), so that the higher the tip separation ($|x_1 - x_2|$) and stiffness (κ), the larger the difference in tension between the two microtubules. (b) Spring-coupler model used to represent the physical connection between two microtubule plus-ends via a kinetochore. Both springs have identical stiffnesses (κ). The kinetochore is assumed to be in force equilibrium such that the sum of the spring forces is equal to the total external force, F_{TOT} , which is kept constant in the current study.

Mechanical coupling coordinates microtubule pairs in vitro

To measure the effects of mechanical coupling, we recorded the growth of many pairs of microtubules coupled to a constant shared load ($F_{TOT} = 8$ pN) either through relatively soft springs, with $\kappa = 1$ pN· μm^{-1} , or through springs with five-fold greater stiffness, $\kappa = 5$ pN· μm^{-1} . These two stiffnesses span a range near the minimum elasticities estimated for mitotic chromosomes (Nicklas, 1983; Poirier et al., 2002), for pericentromeres (Chacon et al., 2014), and for elements of the kinetochore (Schwietert et al., 2022; Volkov et al., 2018). When coupled through soft springs ($\kappa = 1$ pN· μm^{-1}), microtubule pairs tended to grow at different rates and their tips often became separated by more than a micrometer (Figure 3.3a, left, and Figure S3.3-1, left). After 300 s, the average tip separation for soft-coupled pairs grew to 0.8 ± 0.2 μm (mean $\pm$ SEM from $N = 10$ pairs) (Figure 3.3b, left). When coupled through stiff springs ($\kappa = 5$ pN· μm^{-1}), growth speeds were better matched, and the microtubule tips remained closer together (Figure 3.3a, right, and Figure S3.3-1, right). The average tip separation for stiff-coupled pairs after 300 s was 0.25 ± 0.06 μm (mean $\pm$ SEM from $N = 10$ pairs) (Figure 3.3b, right), significantly lower than for soft-coupled pairs ($p = 0.03$ based on a Wilcoxon-Mann-Whitney two sample rank test).

Because the plus-ends of microtubules within k-fiber bundles are rarely separated by more than 0.8 μm from each other in vivo (O'Toole et al., 2020), we used Kaplan Meier survival analysis to ask how often the pairs of coupled microtubule tips in our experiments exceeded this threshold separation. Consistent with their lower average tip separation, stiff-coupled microtubule pairs were much less likely to exceed a separation of 0.8 μm than their soft-coupled counterparts ($p = 0.01$, log-rank test) (Figure 3.3c), which sometimes separated by 0.8 μm in as little as 60 s. Even after 400 s, more than 80% of the stiff-coupled pairs had tip separations that remained below 0.8 μm , while fewer than half of the soft-coupled pairs had tip separations below 0.8 μm . (Similar survival analyses using thresholds of 0.4, 0.6, and 1.2 μm are provided in Figure S3.6-2.)

Even at shorter time periods, the average behavior of soft- and stiff-coupled microtubule pairs was distinct. During the first 100 s of growth, the average rate of tip separation for soft-coupled pairs was 0.23 ± 0.04 $\mu\text{m}\cdot\text{min}^{-1}$ (mean $\pm$ SEM from $N = 23$ pairs), while the average rate of tip separation for stiff-coupled pairs was only 0.14 ± 0.02 $\mu\text{m}\cdot\text{min}^{-1}$ (mean $\pm$ SEM from $N = 26$ pairs; $p = 0.03$ based on a Wilcoxon-Mann-Whitney two sample rank test). Notably, the average rate of tip separation for stiff-coupled microtubule pairs decreased as their average tip separation increased, suggesting they were approaching a steady-state level of separation (Figure 3.3b, right). In contrast, the average rate of tip separation for soft-coupled microtubule pairs remained approximately constant throughout the 400 s measurement, suggesting that soft-coupled pairs would require more time to approach a steady state. Altogether, these observations show that the growth of microtubule pairs can be tightly coordinated, even over relatively short (< 100 s) timescales, by mechanically coupling their tips to a shared load through couplers of sufficient stiffness.

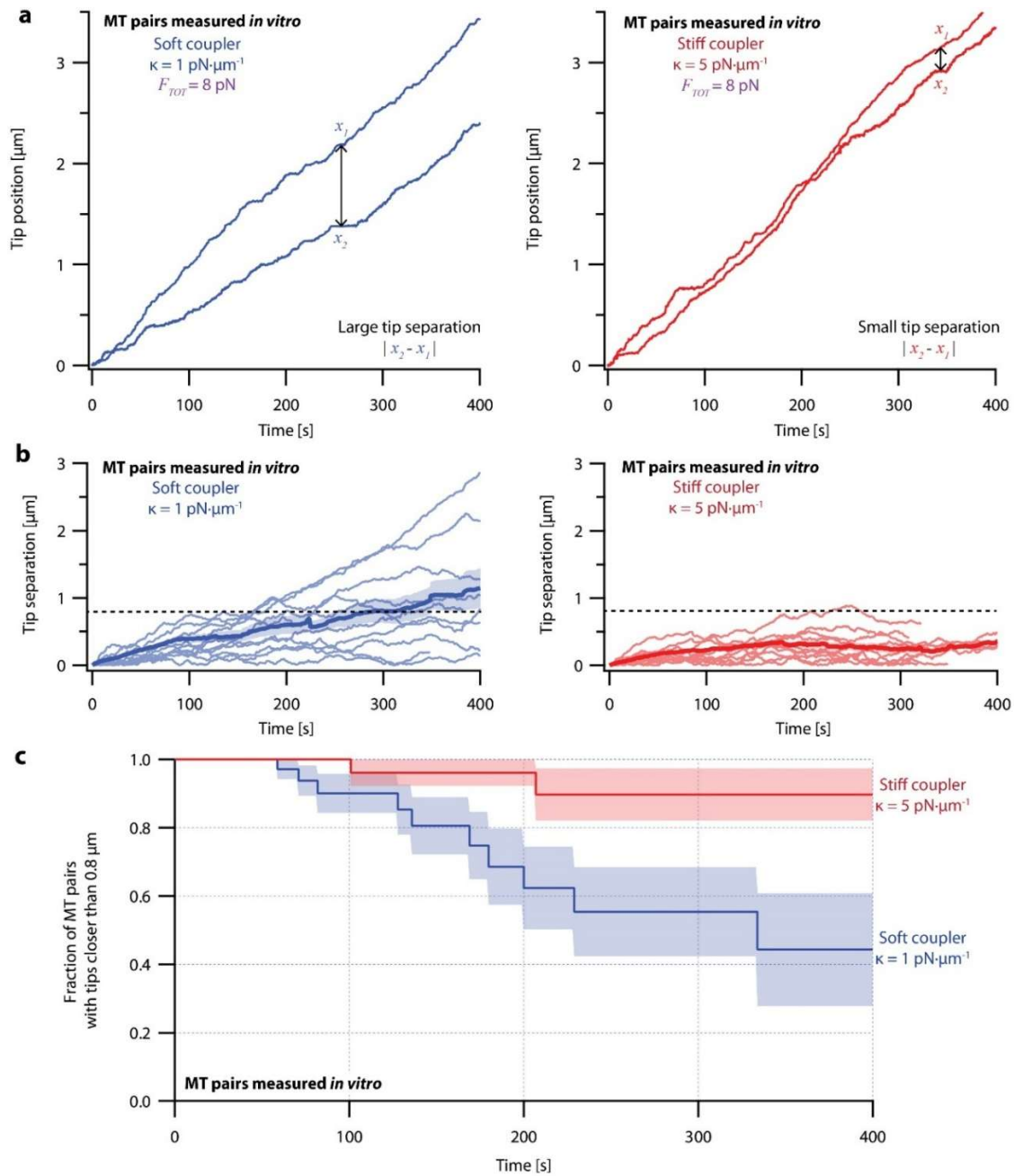


Figure 3.3. Microtubule pairs growing in vitro are coordinated by mechanical coupling. (a) Example dual-trap assay recordings showing real microtubule (MT) plus-end positions over time for representative microtubule pairs coupled via soft ($1 \text{ pN}\cdot\mu\text{m}^{-1}$, left) or stiff ($5 \text{ pN}\cdot\mu\text{m}^{-1}$, right) simulated spring-couplers. All dual-trap data was recorded with $F_{TOT} = 8 \text{ pN}$. (b) Tip separation ($|x_1 - x_2|$) between microtubule plus-end pairs that were coupled with either soft (left) or stiff (right) simulated spring-couplers over 400 s of growth. Light colors show tip separations for 10 individual microtubule pairs with each coupler stiffness, while dark colors show mean tip separation for all recorded pairs ($N = 50$ and $N = 43$ recordings with soft and stiff couplers, respectively). (c) Fraction of microtubule pairs whose plus-ends remained within $0.8 \mu\text{m}$ of each other over time. (In other words, the fraction of pairs at any given time whose tip separation had not exceeded the dashed line in (b).) Shaded regions in (b) and (c) show SEMs from $N = 50$ and $N = 43$ recordings with soft and stiff couplers, respectively. See Figure 4.3 for the updated version of Figure 3.3b.

Models of microtubule growth variability and force sensitivity can be derived from recordings of individual microtubules

Coordination of microtubule growth can be thought of as the suppression of variability in microtubule growth speed. Understanding how mechanical coupling suppresses this variability requires a realistic model of how the growth of individual microtubules varies over time and between microtubules, as well as how growth behaviors are influenced by force. Previous simulations of microtubule bundles ([Banigan et al., 2015](#); [Schwietert & Kierfeld, 2020](#)), based on force-clamp measurements of individual microtubules ([Akiyoshi et al., 2010](#)), have suggested that mechanical coupling might coordinate the stochastic switching of microtubules between shortening and growth, such that sister k-fibers are nearly always kept in opposite states. However, these models assumed identical growth speeds for all microtubules under the same force, and therefore cannot address how mechanical coupling might coordinate differences in growth speed. We sought to create a model of microtubule growth that explains both the variability in microtubule growth speeds and how that variability is suppressed by sufficiently stiff mechanical coupling, as observed in the dual-trap assay.

To characterize the variability and force dependence of microtubule growth, we re-examined the force-clamp recordings by Akiyoshi et al., 2010. This large dataset ($N = 356$ recordings) was recorded with an identical setup to our dual-trap experiments, except that each microtubule was grown individually with a single laser trap exerting a constant level of tension (between 0.5 and 18 pN). Using this dataset, we created two models derived from the natural variability of individual kinetochore-attached microtubules and their average behaviors as a function of force. Below, we begin by briefly describing a simple non-pausing model, which was developed first and served as a foundation for our second, more successful pausing model. The pausing model reasonably explains our dual-trap results by incorporating both stochastic pausing and intrinsic differences in microtubule growth speed.

A simple non-pausing model predicts larger tip separations than measured in vitro

When averaged across time and across many individual microtubules, mean growth speed increases exponentially with applied tension ([Akiyoshi et al., 2010](#)), such that

$$v_g = v_o * \exp\left(\frac{F}{F_g}\right) \quad (3.1)$$

where v_o represents the unloaded growth speed, F represents the applied tension, and F_g represents the sensitivity with which the growth speed increases with tension. However, individual microtubules show considerable variability around this mean behavior, with some microtubules remaining persistently slower than others for many minutes ([Figure 3.1a](#)). In our first model, we adopted the simple assumptions that this variability stems entirely from intrinsic differences in unloaded microtubule growth speed, v_o , and that all microtubules have identical

force-sensitivity (with $F_g = 8.4$ pN, based on the exponential curve that best fit the mean growth speed-vs-force data (Akiyoshi et al., 2010)). In principle, such static heterogeneity in growth speed might arise from persistent differences in microtubule tip structure (Cleary & Hancock, 2021; Mahserejian et al., 2022; Rai et al., 2021) or from variation in the numbers of protofilaments per microtubule (Chretien et al., 1992; Rai et al., 2021).

For each simulated microtubule, we randomly chose an individual growth speed measurement from the force-clamp dataset (Akiyoshi et al., 2010), then extrapolated the microtubule's intrinsic unloaded speed (v_o) from the chosen point (v_g, F) using equation (3.1) with $F_g = 8.4$ pN. The growth speed of this simulated microtubule then followed the extrapolated curve as a function of force. To simulate a pair of coupled microtubules, we made two such random draws from the dataset, defining two distinct exponential (v_g -versus- F) relations for the two microtubules. (Bundles of three or more microtubules could be simulated in the same manner by making additional draws.) We used a simple Monte Carlo-based method (Gardner & Odde, 2010) to simulate traces of tip position versus time for the pair. As in our dual-trap experiments, the simulated tip pairs were coupled with either soft or stiff elastic springs to a constant shared load, $F_{TOT} = 8$ pN. Both the variability and force-dependence of growth in the model were derived directly from the experimental force-clamp data, with no adjustable ('free') parameters.

Despite its direct derivation from force-clamp data, the non-pausing model could not explain the behavior of microtubule pairs measured in the dual-trap assay (Figure 3.4). Simulated microtubule pairs developed much larger tip separations than real microtubule pairs over time, implying that this model overestimates real microtubule variability. The simulated microtubule pairs showed especially poor agreement with real microtubule pairs under the stiff-coupled condition (Figure 3.4c, red curves), indicating that the non-pausing model also does not capture the degree to which real microtubules are coordinated by mechanical coupling.

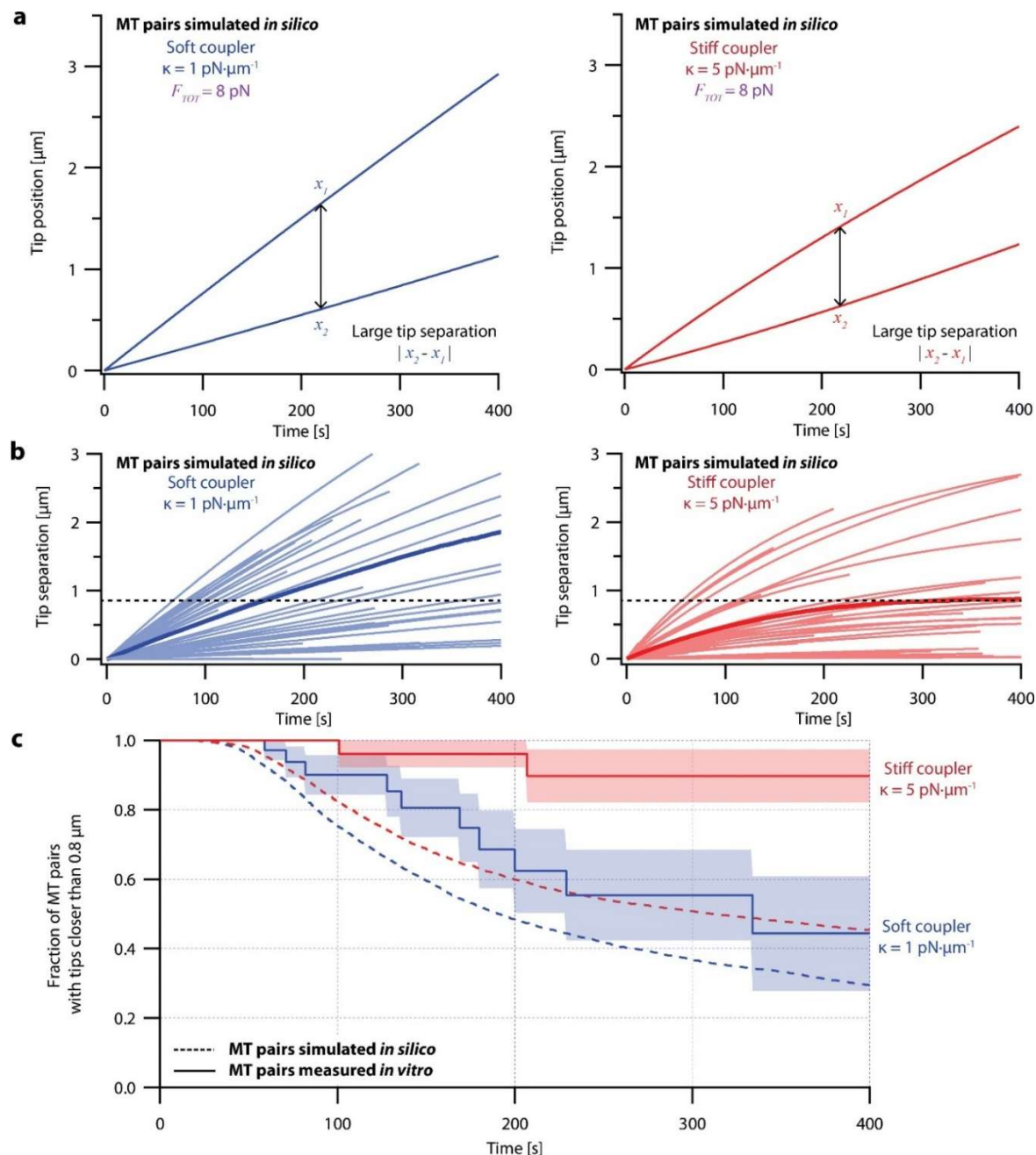


Figure 3.4. Simple non-pausing simulations fail to recapitulate the coordinated growth of mechanically coupled microtubule pairs. (a) Example simulations of microtubule (MT) plus-end positions over time using the non-pausing model. Simulated microtubule pairs were coupled via soft or stiff spring-couplers and shared a total load $F_{TOT} = 8 \text{ pN}$ to match dual-trap assay conditions. (b) Tip separations between microtubule plus-end pairs that were simulated using the non-pausing model and coupled with either soft (left) or stiff (right) spring-couplers. Light colors show tip separations for $N = 40$ individual simulated microtubule pairs, while dark colors show mean tip separation for $N = 10,000$ simulated pairs. (c) Comparison between the fraction of *in silico* (simulated, dashed curves) and *in vitro* (real, solid curves) microtubule pairs whose plus-ends remained within $0.8 \mu\text{m}$ of each other over time. Shaded regions show SEMs from $N = 50$ and $N = 43$ *in vitro* recordings with soft and stiff couplers, respectively. Soft- and stiff-coupled microtubule pairs were each simulated $N = 10,000$ times over 400 s of growth in both (b) and (c). Simulation parameters for (a), (b), and (c) can be found in Table 3.1.

Microtubule growth is interrupted by stochastic, force-dependent pauses

Our simple non-pausing model lacked a notable behavior exhibited by microtubules that could influence their response to tension: Microtubule growth is frequently interrupted by stochastic pauses of varying durations, both in cells (Gaudenz et al., 2010; Sousa et al., 2007) and in purified tubulin systems (Mahserejian et al., 2022; Stumpff et al., 2012). Indeed, pauses recorded during our force-clamp (Figures 3.1a and S3.4-1b) and dual-trap assays (Figures 3.3a and S3.3-1) were frequent and often lasted tens of seconds. Presumably, these pauses occur because the configuration of the growing microtubule tip fluctuates and sometimes becomes transiently refractory to the incorporation of new tubulin subunits (Cleary & Hancock, 2021; Mahserejian et al., 2022). We hypothesized that entry and exit into the pause state might be force-dependent processes, and that pausing might therefore be a key aspect of microtubule force-sensitivity.

To test whether pause entry and exit rates vary with force, we reanalyzed the force-clamp dataset (Akiyoshi et al., 2010), applying a 2 s sliding window to identify intervals during each recording ($N = 356$) when the instantaneous growth speed fell to near-zero, indicative of a pause (Figure S3.5-1; see Materials and Methods for details about our pause marking procedure). The intervals in-between these pauses were designated as ‘runs.’ Distributions of run and pause durations were approximately exponential (Figure S3.5-2), consistent with a relatively simple two-state kinetic model of microtubule growth (Figure S3.5-1a), in which pause entry and exit are considered Poisson processes. For each force level, we estimated the pause entry and exit rates, k_{EN} and k_{EX} , by counting the total number of observed entrances into and exits out of pause and dividing by the total time spent in run and pause, respectively (Figure S3.5-1a).

This analysis revealed that tension delays pause entry and accelerates pause exit (Figure 3.5a). Both effects tend to decrease the time spent in the pause state for growing microtubule tips under high tension. In addition, the average growth speed during runs was accelerated by tension (Figure 3.5b). All three rates, pause entry, pause exit, and average growth speed during runs, were well-fit by single exponential functions of force (Figure 3.5; see Table 3.1 for fit parameters). These observations show that stochastic pausing during microtubule growth is force-dependent and imply that pausing kinetics will strongly influence the response of growing microtubules to mechanical coupling.

A pausing model can explain the variability between paired microtubule and their coordination by mechanical coupling

To examine how force-dependent pausing might affect the behavior of coupled microtubules, we created a second model in which growing microtubules switch stochastically between states of pause and run. Pause entry and exit were considered Poisson processes with kinetic rates dictated by the exponential fits of k_{EX} and k_{EN} versus force estimated from the

force-clamp dataset (Figure 3.5a, dashed curves; Table 3.1). Intrinsic heterogeneity among microtubules was incorporated by randomly choosing an individual run speed measurement for each simulated microtubule from the force-clamp dataset (i.e., by randomly choosing one point in Figure 3.5b). We then extrapolated the microtubule's intrinsic unloaded run speed (v_{RUN}^0) from the chosen point (v_{RUN}, F) by assuming an exponential force-dependence with sensitivity $F_{RUN} = 14.8$ pN (based on the dashed curve in Figure 3.5b; Table 3.1). The growth speed during runs for this simulated microtubule then followed the extrapolated curve as a function of force. We used a simple Monte Carlo-based method (again) to simulate traces of microtubule tip position versus time. All model parameters were derived directly from the experimental force-clamp data, with no adjustable ('free') parameters. As in our dual-trap experiments, simulated microtubule tip pairs were coupled with either soft or stiff elastic springs to a constant shared load, $F_{TOT} = 8$ pN. Simulations of individual microtubules growing under constant force closely resembled force-clamp recordings of real microtubules in vitro. (Compare Figure S3.6-1 to Figure 3.1a.)

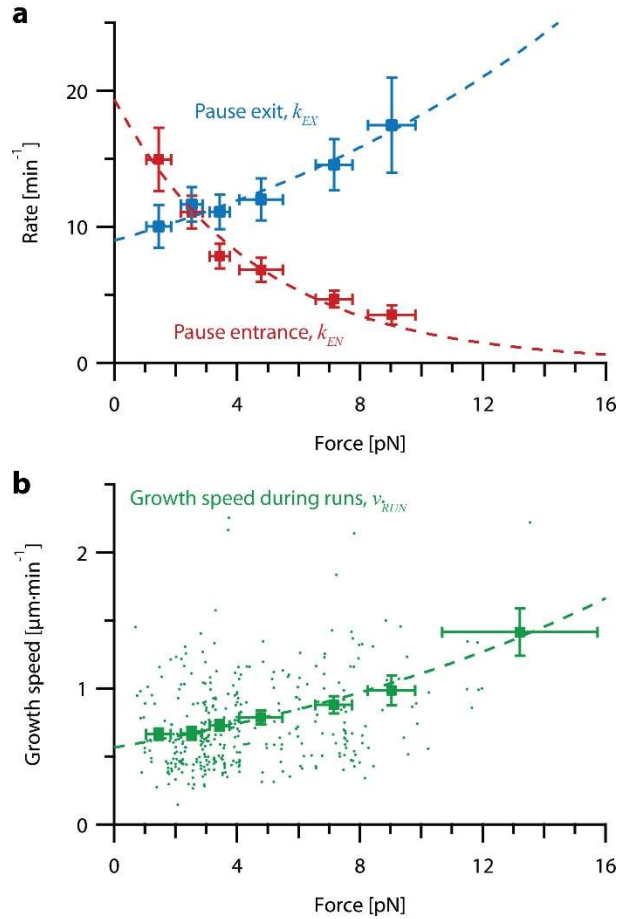


Figure 3.5. Increasing tension on individual growing microtubules suppresses pausing and accelerates growth during runs. (a) As tension on a growing microtubule tip increases, the rate of pause entrance decreases, while the rate of pause exit increases. Mean pause entrance (red) and exit (blue) rates (k_{EN} and k_{EX}) were estimated as described in Figure S3.5-1 and are plotted as functions of force. Error bars show counting uncertainties from $N = 25$ to 85 individual microtubule recordings and dashed curves show least squares exponential fits. (b) As tension on a microtubule tip increases, the growth during runs (i.e., in-between pauses) is accelerated. Points show run speeds measured from recordings of individual microtubules subject to constant tensile forces (from (Akiyoshi et al., 2010)). Symbols (green) represent mean run speeds (v_{RUN}) grouped according to the applied tensile force. Error bars show SEMs from $N = 9$ to 85 individual microtubule recordings and dashed curve shows the least squares exponential fit. See Figure S3.5-1 and Materials and Methods for details and Table 3.1 for fit parameters.

The pausing model of microtubule growth provided significantly better fits to our dual-trap data than the non-pausing model. Like real microtubules in the dual-trap experiments, the simulated pairs of tips tended to drift apart under soft-coupled conditions, while under stiff-coupled conditions they stayed closer together (Figure 3.6a). After 300 s, the average tip separation for simulated soft-coupled pairs grew to $0.79 \pm 0.01 \mu\text{m}$ (mean $\pm$ SEM from $N = 10,000$ simulations), while simulated stiff-coupled pairs had a lower average tip separation of $0.43 \pm 0.005 \mu\text{m}$ (mean $\pm$ SEM from $N = 10,000$ simulations) (Figure 3.6b), similar to the corresponding separations measured in dual-trap experiments ($0.8 \pm 0.2 \mu\text{m}$ and 0.25 ± 0.06

μm , respectively). Simulated pairs of microtubule tips under stiff-coupled conditions were also less likely to exceed a threshold separation of $0.8\ \mu\text{m}$ (Figure 3.6c, dashed curves), similar to the survival analyses of microtubule pairs measured with the dual-trap assay (Figure 3.6c, solid curves). (Survival analyses of simulated microtubule pairs with threshold separations of 0.4 , 0.6 , and $1.2\ \mu\text{m}$ also resembled the survival analyses of microtubule pairs measured with the dual-trap assay. See Figure S3.6-2.) In addition, the pausing model scored better than the non-pausing model when we used probability to compare simulated to in vitro measurements (Figure S3.6-4). (To quantify the variability due to stochastic pausing alone, we also compared the pausing model to a version in which we assumed that microtubules were intrinsically identical ('ergodic'). The low variability predicted by this model (Figure S3.6-3), particularly under the soft-coupled condition, indicates that stochastic pausing alone is insufficient to generate the large and persistent differences in growth speed exhibited by real microtubules). Overall, the strong agreement between the pausing model and the dual-trap recordings suggests that mechanical coupling coordinates microtubule growth in at least two distinct ways: first, by counteracting persistent (intrinsic) differences in microtubule growth speed, and second, by selectively suppressing stochastic pausing in the lagging tip relative to the leading tip.

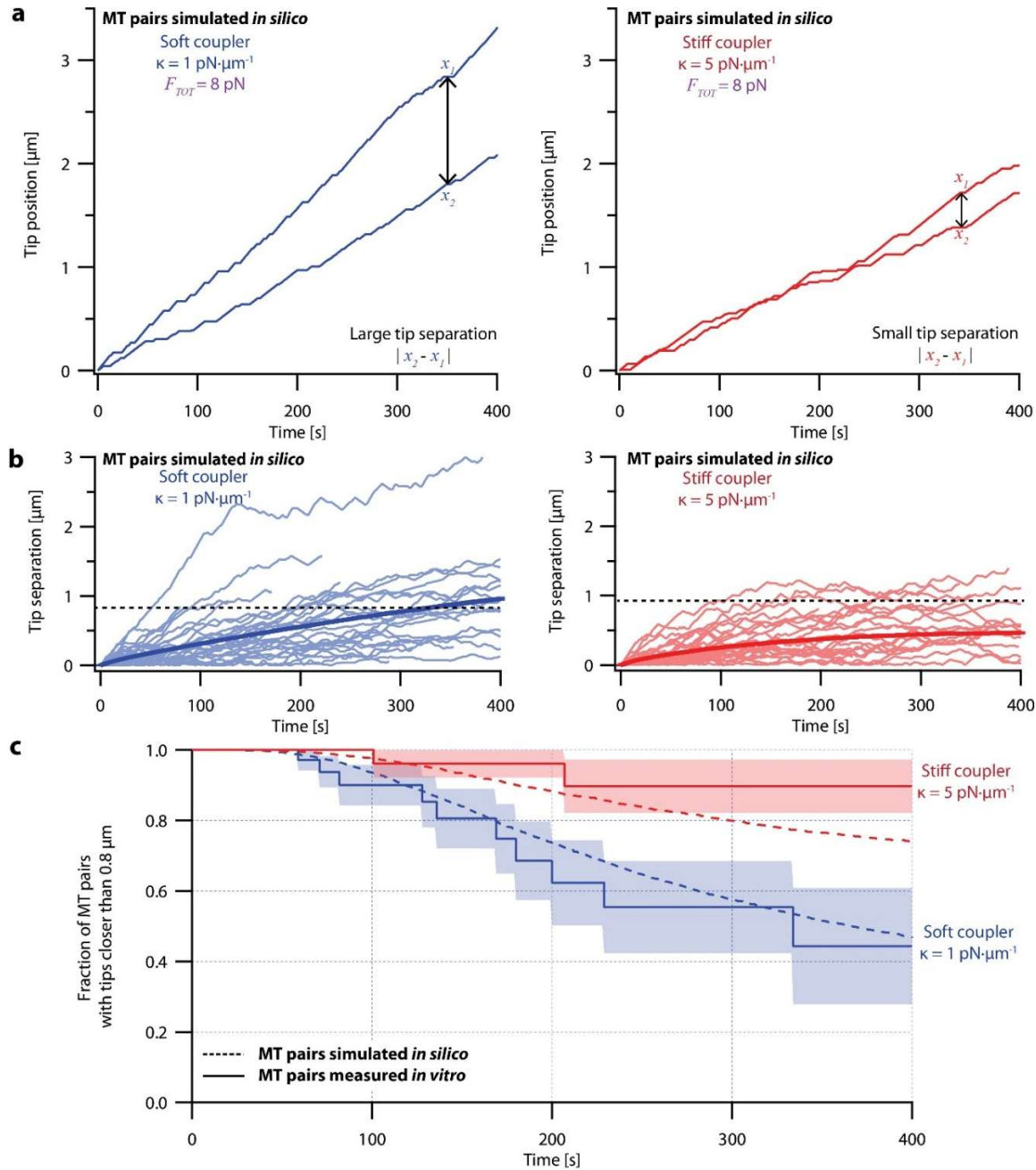


Figure 3.6. Simulations recapitulate the coordinated growth of mechanically coupled microtubule pairs. (a)

Example simulations of microtubule (MT) plus-end positions over time using the pausing model. Simulated microtubule pairs were coupled via soft or stiff spring-couplers and shared a total load $F_{TOT} = 8 \text{ pN}$ to match dual-trap assay conditions. **(b)** Tip separations between microtubule plus-end pairs that were simulated using the pausing model and coupled with either soft (left) or stiff (right) spring-couplers. Light colors show tip separations for $N = 40$ individual simulated microtubule pairs, while dark curves show mean tip separation for $N = 10,000$ simulated pairs. **(c)** Comparison between the fraction of *in silico* (simulated, dashed curves) and *in vitro* (real, solid curves) microtubule pairs whose plus-ends remained within $0.8 \mu\text{m}$ of each other over time. Shaded regions show SEMs from $N = 50$ and $N = 43$ *in vitro* recordings with soft and stiff couplers, respectively. Soft- and stiff-coupled microtubule pairs were each simulated $N = 10,000$ times over 400 s of growth in both **(b)** and **(c)**. Simulation parameters for **(a)**, **(b)**, and **(c)** are listed in Table 3.1. See Figure 4.4 for the updated version of Figure 3.6b.

Discussion

Our work demonstrates the effectiveness of mechanical coupling for coordinating the growth of microtubules and helps explain how numerous filaments within a k-fiber bundle can be tightly regulated to grow in near-perfect unison. Using our dual-trap assay, we found that pairs of microtubule tips growing in vitro are kept in close proximity when sharing a single load through a coupling material of sufficient stiffness. Tip pairs in our experiments were kept within a fraction of a micrometer by couplers of only $\kappa = 5 \text{ pN}\cdot\mu\text{m}^{-1}$, a modest stiffness within the ranges reported for elements of the kinetochore ([Schwietert et al., 2022](#); [Volkov et al., 2018](#)), pericentromeric chromatin ([Chacon et al., 2014](#)), and mitotic chromosomes ([Nicklas, 1983](#); [Poirier et al., 2002](#)). Because the kinetochore and its underlying centromeric chromatin both appear to deform together to maintain close contacts with k-fiber plus-ends in vivo ([O'Toole et al., 2020](#)), the plus-ends presumably exert forces on each other through a composite material composed of both kinetochore and chromatin. We propose that this mechanical coupling is a major coordinating influence on plus-end growth dynamics within k-fiber bundles.

Our dual-trap approach provides information that prior single-trap experiments, and theoretical work based solely on these prior measurements, cannot provide. Microtubules in real k-fiber bundles presumably adjust their growth speeds dynamically in response to the forces they exert on each other. The dual-trap assay uniquely allows this kind of mutual dynamic influence to be examined. Because each microtubule in the pair grows simultaneously in the same preparation of buffer, any differences in growth speed represent true biological variability, without the potentially confounding influences of prep-to-prep or sample-to-sample variability. While previous experiments with individual microtubules implied that a shared load should tend to coordinate growing microtubules, the dual-trap experiments are a direct, model-independent way to examine the extent to which load sharing can suppress the intrinsic variability of microtubules growing under identical conditions. With the dual-trap assay, we can test models and assumptions about microtubule tension-dependence and variability derived from recordings of individual microtubules, as we did with the pausing and non-pausing models presented here.

Since most known eukaryotes have k-fibers of more than two microtubules, an important area of future work will be to study how effectively mechanical coupling can coordinate k-fibers with more microtubules. While extending our in vitro laser trap methods to more than two microtubules is impractical, our model can be easily extended to explore mechanical coordination within k-fibers of three or more microtubules. Simulation parameters, such as microtubule growth speeds, can also be changed to explore how well mechanical coupling coordinates microtubules in different cell types or cell cycle stages with varying microtubule dynamics ([Cassimeris et al., 1988](#); [Gaudenz et al., 2010](#); [Yamashita et al., 2015](#); [Rusan et al., 2001](#)). Similarly, simulations can be used to study how the addition of microtubule

polymerases or depolymerases with known effects on microtubule dynamics influences coordination.

A variety of microtubule-modifying proteins are implicated in regulating k-fiber dynamics (reviewed in (Ou & Scholey, 2022) and (Amin et al., 2019)). These biochemical factors must work in concert with mechanical effects, and we suggest that the two classes of mechanisms probably operate over different length- and timescales. Certain tip-tracking proteins capable of influencing microtubule dynamics are enriched at growing k-fiber ends specifically near kinetochores that are moving anti-poleward (Amaro et al., 2010; Castrogiovanni et al., 2021; Tirnauer et al., 2002). Various kinesins are known to regulate the size of the metaphase spindle (Goshima et al., 2005; Neahring et al., 2021), to influence k-fiber dynamics (Maiato, Fairley, et al., 2003; Rizk et al., 2009; Stumpff et al., 2012; Wordeman et al., 2007), and to alter microtubule dynamics in vitro in a length-dependent manner (Varga et al., 2006). However, it is unclear whether the specific enrichment of such microtubule-modulators on longer or faster-growing tips could occur quickly enough and with enough selectivity to slow the growth of a single tip that has grown ahead of its neighbors by only a fraction of a micrometer, as required for tight coordination within a k-fiber. On the other hand, mechanical forces would be transmitted essentially instantaneously between neighboring tips through local deformations of the material through which the tips are coupled. Therefore, mechanical coupling could account for the rapid, sub-micron responses required to coordinate microtubule tips within a k-fiber, while microtubule-modifiers and spindle-wide phenomena, like polar ejection forces and poleward flux, are likely to help set overall k-fiber length (Risteski et al., 2021).

We found that tension accelerates microtubule growth not only by directly increasing growth speed, but also by decreasing time spent in pause. More specifically, tension reduced the rate at which growing tips entered a pause state. This finding implies that pause entry is associated with movement of tension-bearing elements at the tip toward the minus end, opposite the direction of applied force and therefore inhibited by the force. Tension also increased the rate at which paused tips resumed growth, which implies that pause exit is associated with movement toward the plus-end, in the same direction as the applied force and therefore assisted by the force. These tension-dependent dynamics may reflect mechanical deformations of the microtubule tip; perhaps pauses occur when newly added tubulin dimers at a growing tip adopt curved structures that disfavor lateral bonding (McIntosh et al., 2018). Tension could help straighten these curved structures, thereby accelerating lateral bond formation and favoring growth (Cleary & Hancock, 2021).

We derived a model of microtubule growth with no free parameters, based on constant-force recordings of individual microtubules (from (Akiyoshi et al., 2010)), and found that a combination of stochastic, force-dependent pausing and intrinsic differences in microtubule growth speed explained both the variability and the coordination measured in our dual laser trap assay. The necessity of including microtubules with different intrinsic growth speeds for our

model's success implies structural heterogeneity in the growing tips that must persist for many minutes, even as thousands of tubulin subunits are being added. In principle, these persistent differences might arise from different or mismatched protofilament numbers ([Chretien et al., 1992](#); [Rai et al., 2021](#)) or the accumulation of other lattice defects ([Wieczorek et al., 2015](#)) that result in varying degrees of tip taper ([Coombes et al., 2013](#)) (or tip “raggedness” ([Brouhard & Rice, 2018](#))) which are more or less conducive to microtubule growth.

For technical reasons, we focused exclusively here on microtubule tips in a state of net growth. However, coordination of k-fiber microtubules in vivo presumably also requires tight regulation of tip shortening speeds and rates of switching between growth and shortening (i.e., ‘catastrophe’ and ‘rescue’ rates) ([Banigan et al., 2015](#); [Schwietert & Kierfeld, 2020](#)). These will be more difficult to study with our dual-trap assay because switch events are stochastic and episodes of shortening are observed less frequently than episodes of growth under our assay conditions. Nevertheless, the dual-trap approach can be used in the future (potentially with a cutting laser to sever the microtubule tips to induce shortening ([Driver et al., 2017](#); [Murray et al., 2022](#))) to study how mechanical coupling affects microtubule pairs in which one or both filaments are shortening, as well as switch rates between shortening and growth. Our dual-trap approach can also be adapted to examine mechanical coupling through materials with complex mechanical properties, such as viscoelasticity and strain-stiffening ([Cojoc et al., 2016](#); [Schwietert et al., 2022](#)). In principle, the effect of mechanical coupling on pairs of processive motor proteins ([Holzbaur & Goldman, 2010](#)) or on pairs of dynamic actin filaments linked to a shared load ([Garner & Theriot, 2022](#)) can also be studied with the dual-trap approach. Overall, our work demonstrates that mechanical coupling is a feasible mechanism that could coordinate k-fiber microtubules and maintain their synchronous growth in the mitotic spindle.

Supplemental Figures

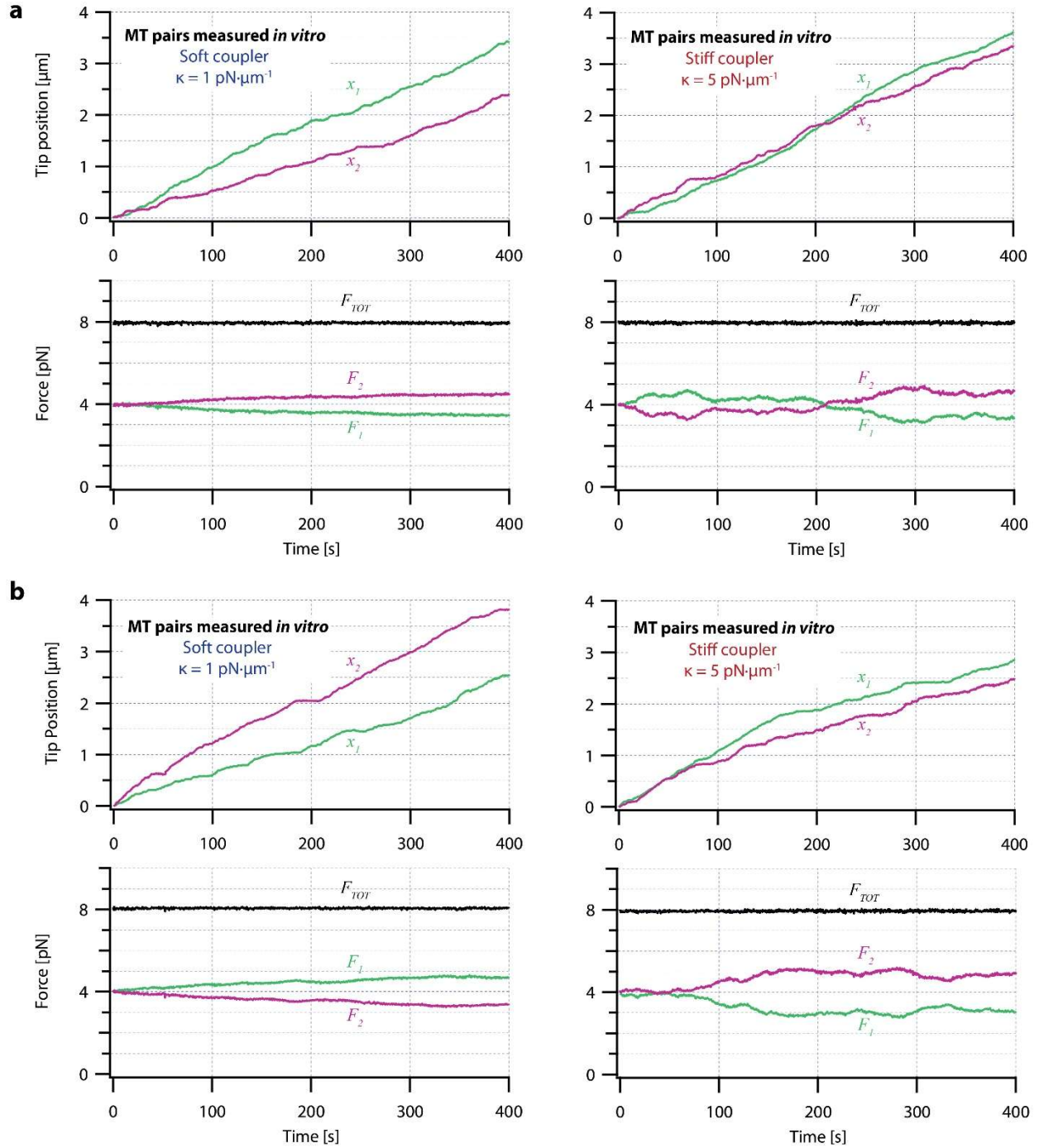


Figure S3.3-1. Additional dual-trap assay recordings showing tip positions and forces on pairs of growing microtubules. (a) Example dual-trap assay recordings showing real microtubule (MT) plus-end positions (top) and forces (bottom) plotted against time for representative microtubule pairs coupled via soft or stiff simulated spring-couplers. Plus-end position data in the top graphs is recopied from Figure 3.3a. Black traces in the bottom graphs show total force on the two microtubules (F_{TOT}) and demonstrate that a constant total force of $F_{TOT} = 8 \text{ pN}$ was maintained by the feedback-control. (b) Two additional examples of dual-trap assay recordings, formatted as in (a).

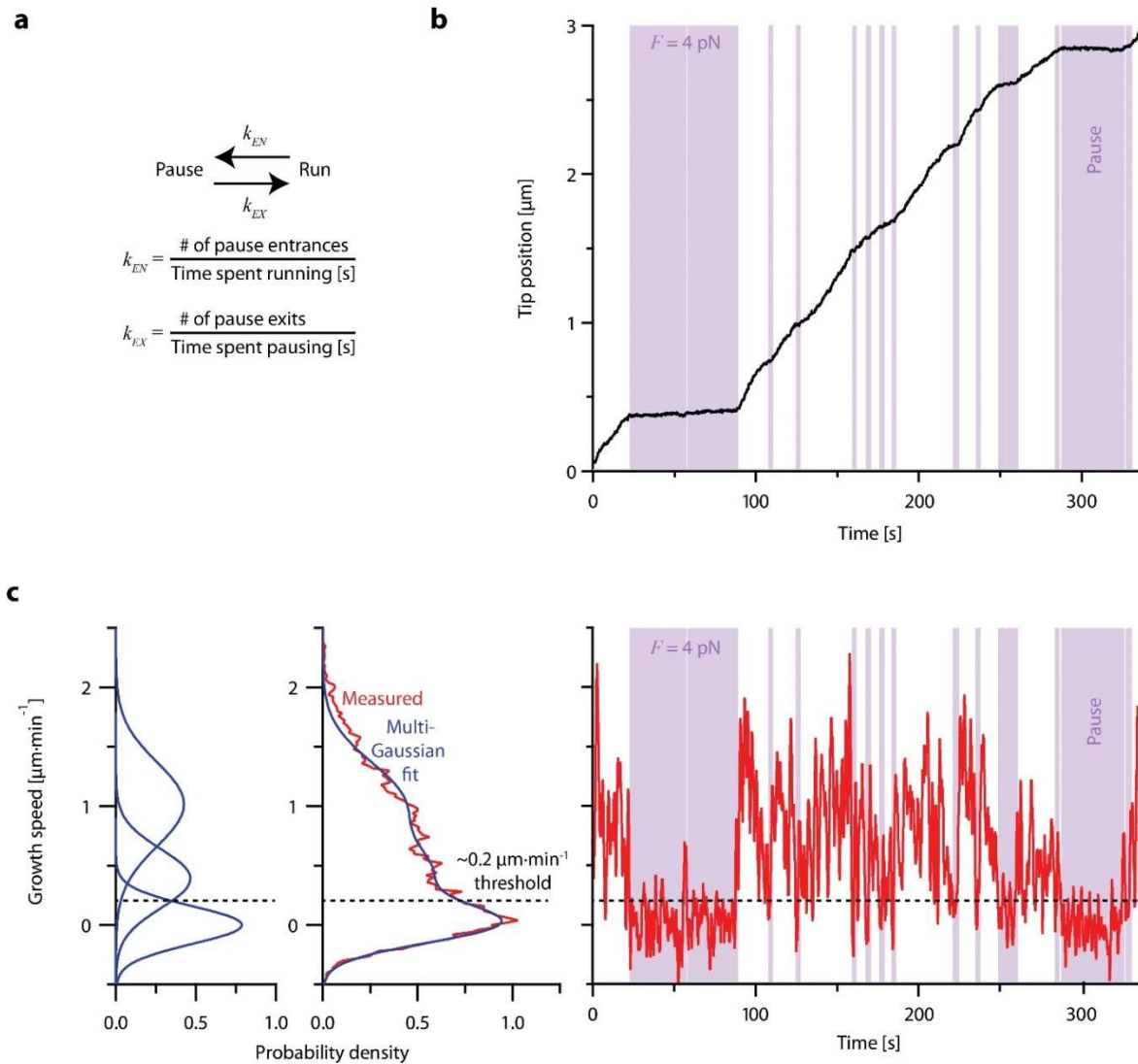


Figure S3.5-1. Individual microtubules frequently pause during periods of overall growth. (a) Growing microtubules switch between states of ‘pause,’ when plus-end growth speed is nearly zero, and ‘run,’ when the plus-end is clearly growing. Pause entrance and exit rates, k_{EN} and k_{EX} , were quantified by counting the numbers of entrances or exits and dividing by the total amount of time spent in the run or pause states, respectively. (b) Example recording showing plus-end position versus time for an individual microtubule subject to a constant tensile force of 4 pN and exhibiting frequent pauses (denoted by purple shaded regions). This example recording is one of $N = 356$ force-clamp traces (from (Akiyoshi et al., 2010)) used to estimate the pause entrance and exit rates and growth speeds during runs as functions of force (as shown in Figure 3.5). (c) Classification of runs and pauses. Instantaneous growth speed was computed as a function of time (and plotted at right) from the recording shown in (b), using a 2 s sliding window, and then assembled into a histogram (middle plot, red) and fit with a sum of multiple Gaussian curves (middle and left plots, blue). One Gaussian was always centered at $0 \mu\text{m}\cdot\text{min}^{-1}$ and defined as the ‘pause’ peak. The intersection of the pause peak and the Gaussian having the next highest mean growth speed was identified for each individual recording and used as a threshold (dashed horizontal line) to distinguish pauses and runs for that recording. Pauses were defined as periods longer than 1 s when the instantaneous growth speed fell below the threshold, while runs were defined as periods longer than 1 s when growth speed was above the threshold (see Materials and Methods for details).

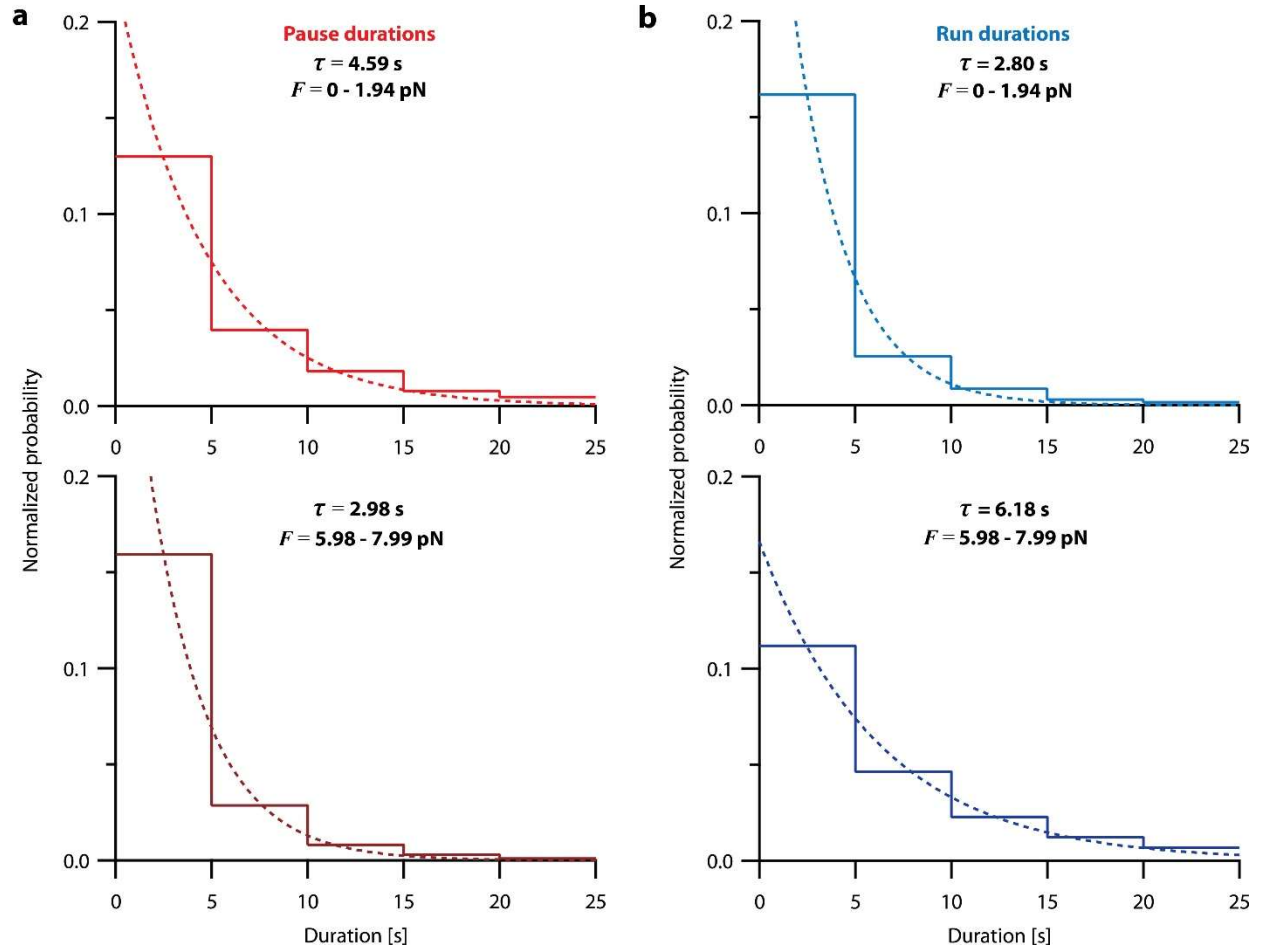


Figure S3.5-2. Pause and run durations are exponentially distributed. (a) Normalized histograms showing pause durations measured from recordings of individual microtubules growing under constant levels of tension (force-clamp data from (Akiyoshi et al., 2010)), either between 0 and 2 pN (top), or between 6 and 8 pN (bottom), corresponding to the lowest and third-highest force bins in Table 3.2, respectively. (b) Normalized histograms showing run durations measured from recordings of individual microtubules growing under constant levels of tension (force-clamp data from (Akiyoshi et al., 2010)), as in (a). For both (a) and (b), least-squares regressions are shown as dashed curves. The indicated time constants (τ) were derived from these fits.

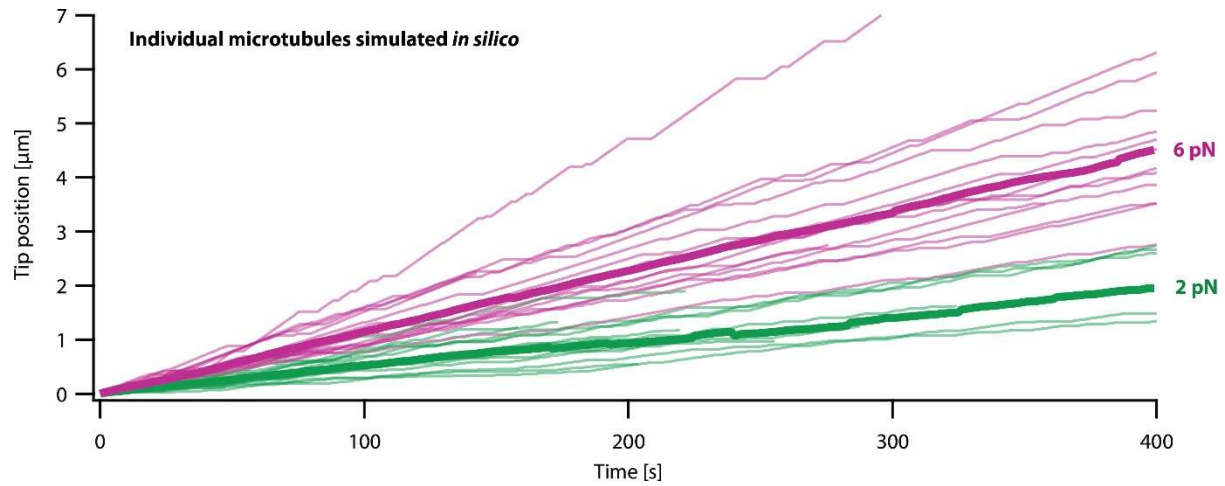


Figure S3.6-1. Simulations of microtubule growth under constant force using the pausing model resemble real microtubule recordings. Examples using the pausing model to simulate plus-end position versus time for individual growing microtubules subject to a constant tensile force of either 2 or 6 pN (light green or magenta traces, respectively). At each force, $N = 16$ example simulations are shown, together with the corresponding mean positions versus time (thick traces, computed from the $N = 16$ individual simulations).

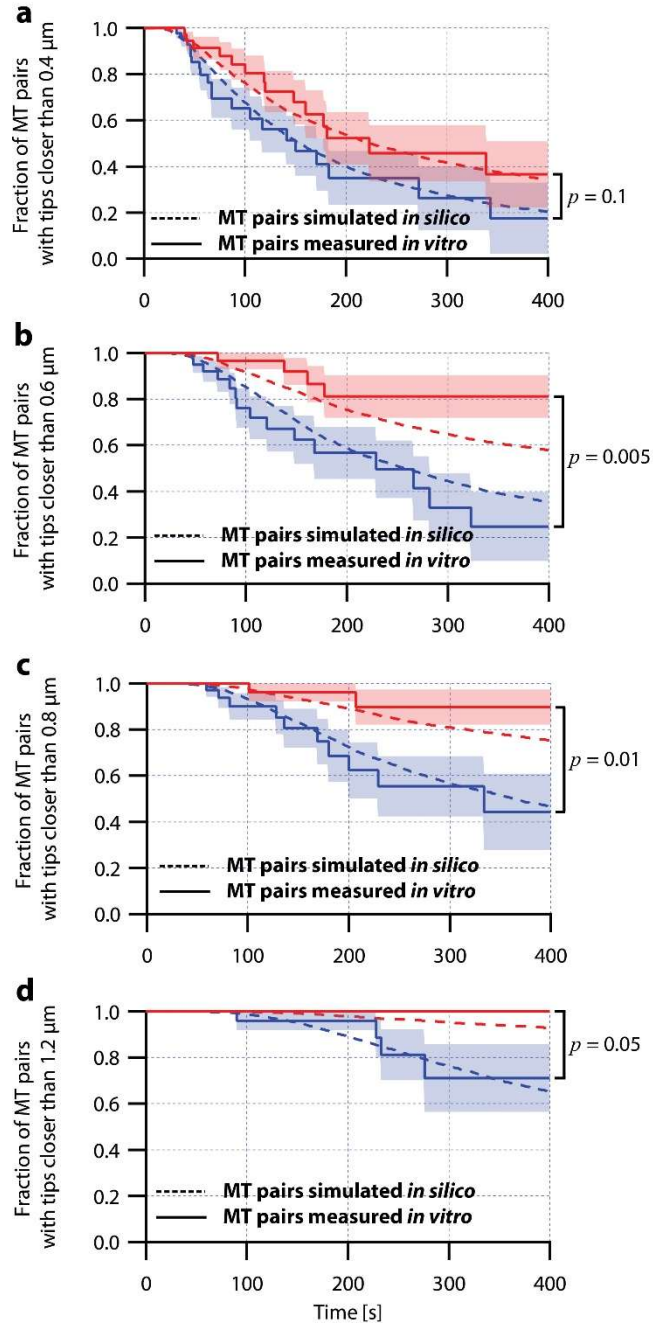


Figure S3.6-2. Simulations of microtubule pairs using the pausing model maintain tip separations similar to real microtubule pairs. Comparison between the fraction of in silico (simulated, dashed curves) and in vitro (real, solid curves) microtubule (MT) pairs whose plus-ends remained within (a) 0.4 μm, (b) 0.6 μm, (c) 0.8 μm, and (d) 1.2 μm of each other over time. Microtubule pairs in silico and in vitro shared a total load of $F_{TOT} = 8$ pN and were coupled via soft or stiff spring couplers. Shaded regions show SEM from $N = 50$ and $N = 43$ in vitro recordings with soft and stiff couplers, respectively. Soft- and stiff-coupled microtubule pairs were each simulated $N = 10,000$ times over 400 s of growth. Simulation parameters can be found in Table 3.1. P -values indicate comparisons between soft- and stiff-coupled microtubule pairs in vitro using a log-rank test. See Figure 4.1 for the updated version of Figure S3.6-2.

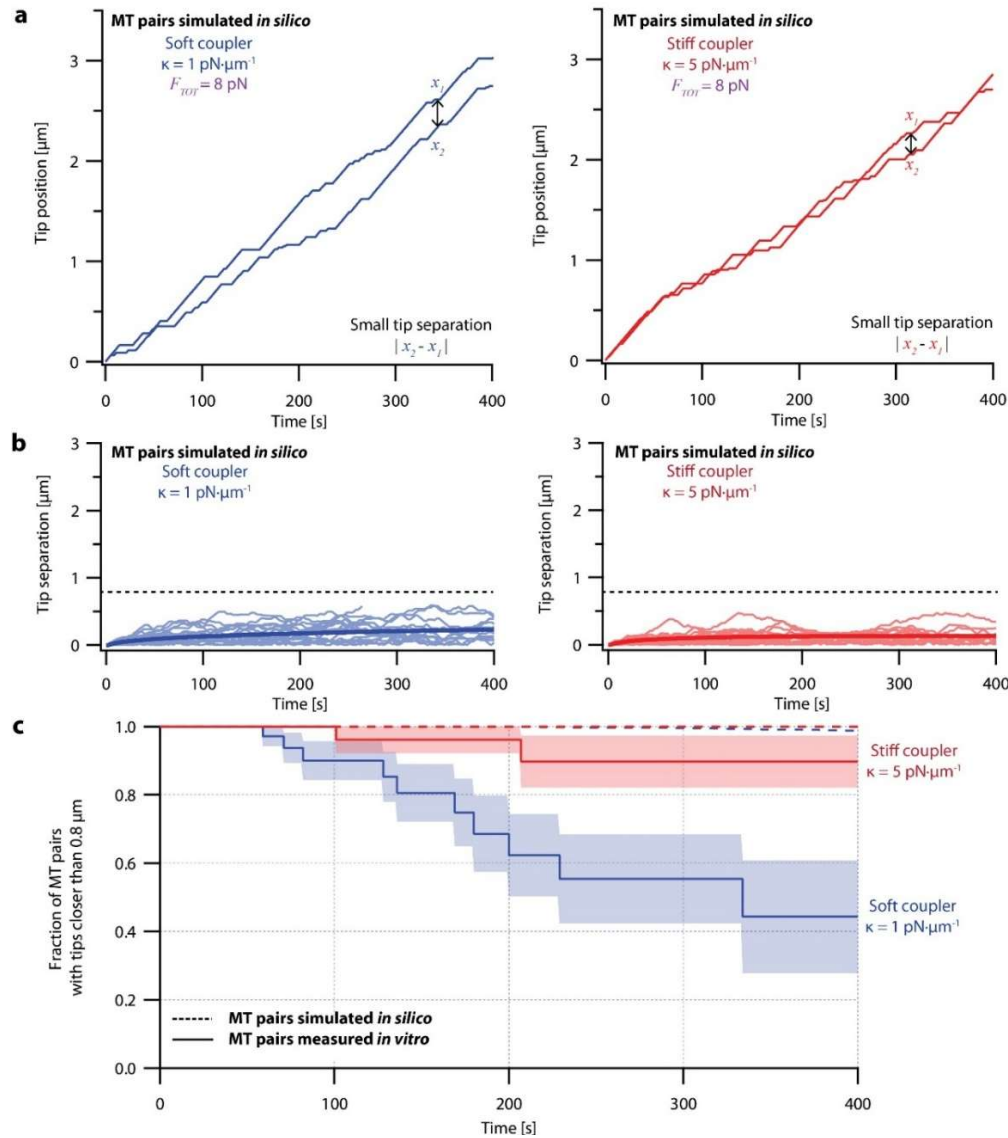


Figure S3.6-3. Simulations using an ergodic version of the pausing model, without intrinsic heterogeneity, fail to recapitulate the coordinated growth of mechanically coupled microtubules pairs. (a) Example simulations of microtubule (MT) plus-end positions over time using the ergodic pausing model. Simulated microtubule pairs were coupled via soft or stiff spring-couplers and shared a total load $F_{TOT} = 8 \text{ pN}$ to match dual-trap assay conditions. (b) Tip separations between microtubule plus-end pairs that were simulated using the ergodic pausing model and coupled with either soft (left) or stiff (right) spring-couplers. Light colors show tip separations for $N = 40$ individual simulated microtubule pairs while dark colors show mean tip separation for $N = 10,000$ simulated pairs. (c) Comparison between the fraction of *in silico* (simulated, dashed curves) and *in vitro* (real, solid curves) microtubule pairs whose plus-ends remained within $0.8 \mu\text{m}$ of each other over time. Shaded regions show SEMs from $N = 50$ and $N = 43$ *in vitro* recordings with soft and stiff couplers, respectively. Soft- and stiff-coupled microtubule pairs were each simulated $N = 10,000$ times over 400 s of growth in both (b) and (c). Simulation parameters for (a), (b), and (c) can be found in Table 3.1. As expected, microtubule pairs simulated using this ergodic model remained much closer together than real microtubule pairs, rarely separating by more than $0.5 \mu\text{m}$ irrespective of coupler stiffness. After 300 s , the average tip separation for soft-coupled pairs was only $0.202 \pm 0.002 \mu\text{m}$ (mean $\pm$ SEM from $N = 10,000$ simulations), barely different from the average tip separation for stiff-coupled pairs of $0.134 \pm 0.002 \mu\text{m}$ (mean $\pm$ SEM from $N = 10,000$ simulations). Combined with the success of the non-ergodic pausing model in fitting our dual-trap data, this supports the idea that microtubule growth can be considered non-ergodic during our 400 s experiments.

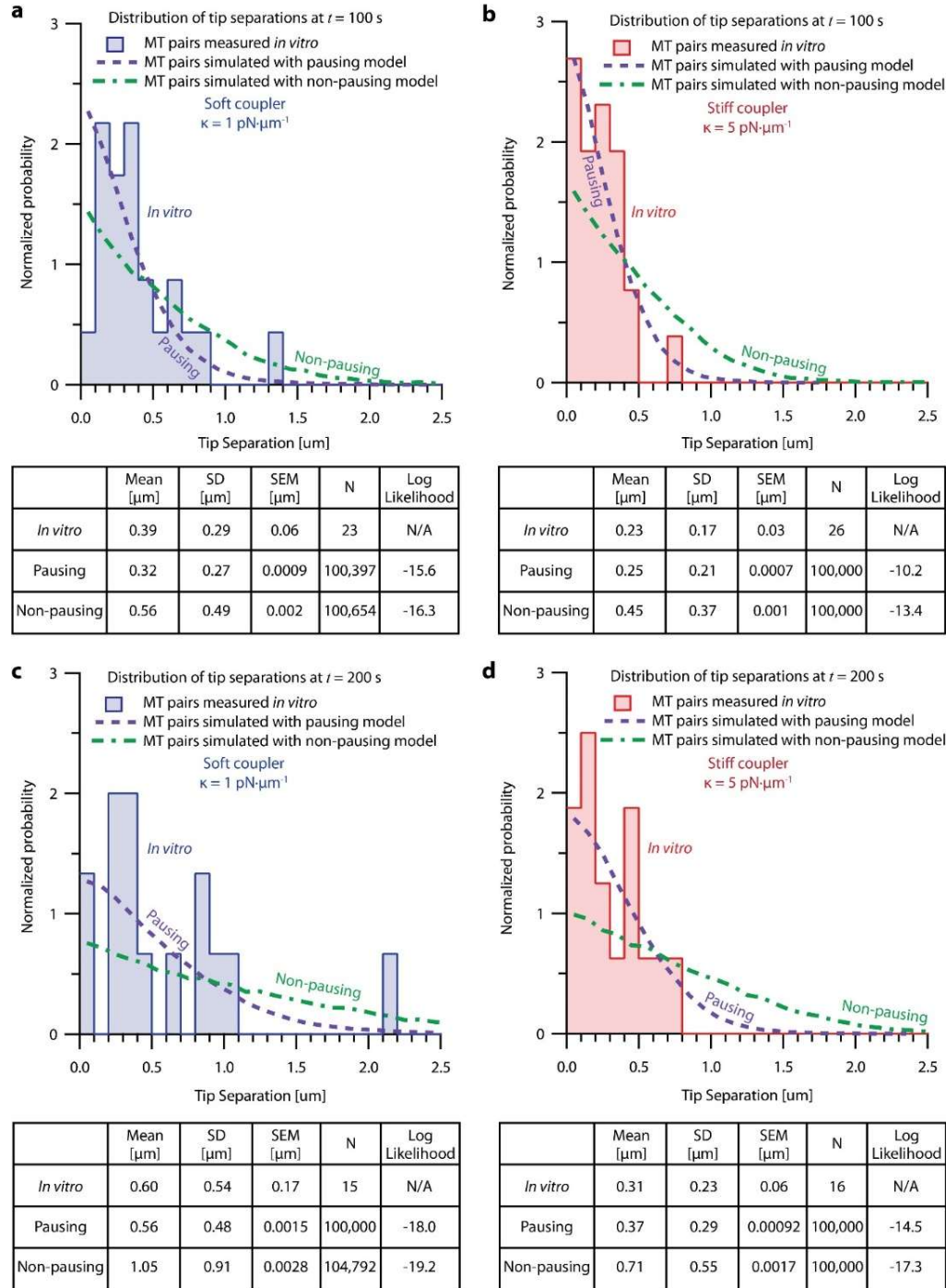


Figure S3.6-4. Probability analysis shows pausing model fits dual-trap recordings better than the non-pausing model. (Top of each panel) Histograms of tip separation between microtubule pairs measured in the dual-trap assay and simulated using the pausing and non-pausing model. Soft-coupled pairs are shown on the left ((a) and (c)), while stiff-coupled pairs are shown on the right ((b) and (d)). Histograms of tip separations measured 100 s after the start of the recording or simulation are shown on the top ((a) and (b)), while tip separations measured 200 s after the start of the recording or simulation are shown on the bottom ((c) and (d)). Tip separation histograms produced by the pausing (purple) and non-pausing model (green) are represented with dashed lines, while dual-trap data is represented by bars. (Bottom of each panel) Tables comparing the means, standard deviations (SD),

standard errors of the mean (SEM) and numbers of recordings (N) for in vitro microtubule pairs and microtubule pairs simulated using the pausing and non-pausing models. The log likelihood probability for each model given the in vitro data is shown in the righthand column. The greater the log likelihood, the greater the probability that the given model could reproduce the measured in vitro data. At both 100 s and 200s, for soft and stiff couplers, the pausing model scores higher than the non-pausing model, indicating that the pausing model is a better fit to our in vitro data.

Parameter name	Parameter symbol	Relevant equation	Parameter estimate	Applicable models	Reference
Growth speed force sensitivity	F_g	$v_g = v_{g0} \exp(F/F_g)$	8.4 pN	non-pausing	Akiyoshi et al., 2010 Figure 4e
Unloaded pause entrance rate	k_{EN0}	$k_{EN} = k_{EN0} \exp(F/F_{EN})$	19.4 min ⁻¹	pausing	Figure 5a, red
Pause entrance rate force sensitivity	F_{EN}	$k_{EN} = k_{EN0} \exp(F/F_{EN})$	-4.6 pN	pausing	Figure 5a, red
Unloaded pause exit rate	k_{EX0}	$k_{EX} = k_{EX0} \exp(F/F_{EX})$	9.0 min ⁻¹	pausing	Figure 5a, blue
Pause exit rate force sensitivity	F_{EX}	$k_{EX} = k_{EX0} \exp(F/F_{EX})$	14.1 pN	pausing	Figure 5a, blue
Unloaded run speed	v_0	$v_{RUN} = v_0 \exp(F/F_{RUN})$	0.52 $\mu\text{m} \cdot \text{min}^{-1}$	neither	Figure 5b
Run speed force sensitivity	F_{RUN}	$v_{RUN} = v_0 \exp(F/F_{RUN})$	14.8 pN	pausing	Figure 5b

Table 3.1. Parameter table for coupled microtubule pairs simulated for non-pausing and pausing models.

Relevant parameters measured from constant force recordings from (Akiyoshi et al., 2010). These parameters were used to simulate coupled microtubule pairs using each of the models described in the main text. The equation in which each parameter is used, as well as which models of microtubule growth use that equation, are indicated.

Bin number	N	Lower bound [pN]	Upper bound [pN]	Pause entrances	Time in pause [s]	Pause exits	Time in run [s]
1	41	0.69	1.94	1834	10957	1830	7364
2	85	1.94	2.97	3502	18047	3501	18952
3	77	2.97	3.999	2376	12842	2374	18159
4	60	3.999	5.98	1937	9661	1932	16993
5	60	5.98	7.99	1439	5930	1438	18465
6	25	7.99	10.3	387	1298	378	6559
7	9	10.3	18	23	93	23	712

Table 3.2. Number of recordings, bounds, and pause quantification from force-clamp dataset, binned by force.

Table describing constant force recordings in (Akiyoshi et al., 2010) and the pause quantification performed in the current work. N indicates the number of recordings in a particular bin. For consistency, these force bins are identical to those used in (Akiyoshi et al., 2010).

Methods

Spring-coupler model

For both in vitro and in silico experiments, the force on each microtubule plus-end was dictated by a model in which plus-ends were coupled to a shared “kinetochore” via simple Hookean springs of identical stiffnesses (Figure 3.2b). Because k-fibers grow relatively slowly in cells (Khodjakov et al., 1996; Skibbens et al., 1993; Stumpff et al., 2012), we neglect viscous drag such that the sum of the forces on individual k-fiber tips equals F_{TOT} . Microtubule pairs were considered to grow along a shared 1D axis. Microtubules were assumed to be incompressible because microtubules have high axial stiffness (Howard, 2001; Kis et al., 2002; Pampaloni et al., 2006) and are cross-linked in bundles (O'Toole et al., 2020), thus forming the structural support required to maintain mitotic spindle integrity. Microtubules were considered stationary except at the plus-end, so that only growth or shortening at the plus-end affected plus-end position.

Kinetochore purification

Native kinetochore particles were purified from asynchronously grown *S. cerevisiae* SBY8253 cells (Genotype: MATa DSN1-6His-3Flag:URA3) grown in YPD medium (2% glucose) by modifying previous protocols (Akiyoshi et al., 2010; Miller et al., 2016). Protein lysates were prepared using a Freezer Mill (SPEX SamplePrep) submerged in liquid nitrogen. Lysed cells were resuspended in buffer H (25 mM Hepes, pH 8.0, 2 mM MgCl₂, 0.1 mM EDTA, 0.5 mM EGTA, 0.1% NP-40, 15% glycerol, and 150 mM KCl) containing phosphatase inhibitors (0.1 mM Na-orthovanadate, 0.2 μM microcystin, 2 mM β-glycerophosphate, 1 mM Na pyrophosphate, and 5 mM NaF) and protease inhibitors (20 μg/ml leupeptin, 20 μg/ml pepstatin A, 20 μg/ml chymostatin, and 200 μM PMSF). Lysates were ultracentrifuged at 98,500 g for 90 min at 4°C. Protein G Dynabeads (Invitrogen) were conjugated with an α-FLAG antibody (Sigma-Aldrich), and immunoprecipitation of Dsn1-6His-3Flag was performed at 4°C for 3 h. Beads were washed once with lysis buffer containing 2 mM DTT and protease inhibitors, three times with lysis buffer with protease inhibitors, and once in lysis buffer without inhibitors, and kinetochore particles were eluted by gentle agitation of beads in elution buffer (buffer H + 0.5 mg/ml 3FLAG Peptide [Sigma-Aldrich]) for 30 min at room temperature.

Bead functionalization and slide preparation for laser trap experiments

To functionalize laser trapping beads with native kinetochore particles, streptavidin-coated polystyrene beads (0.44 μm in diameter, Spherotech) were incubated with biotinylated anti-5His antibodies (Qiagen) and stored for up to 6 months with continuous rotation at 4°C in BRB80 (80 mM Pipes, 1 mM MgCl₂, and 1 mM EGTA, pH 6.9) supplemented with 8 mg·mL⁻¹ bovine serum albumin and 1 mM DTT. Before each experiment, functionalized beads were sonicated for two minutes and decorated with kinetochore particles by incubating 6 pM anti-

5His beads for 1-2 hours at 4°C with purified kinetochore material, corresponding to concentrations of Dsn1-His-Flag between 1.5 and 4 nM, well within the single-kinetochore-particle condition described in (Akiyoshi et al., 2010).

Each flow chamber (~5-10 μL in volume) was constructed by placing two parallel strips of double-stick tape between a glass slide and a KOH- or plasma-cleaned coverslip. Immediately before every experiment, two flow chambers were each functionalized as follows. First, flow chambers were incubated with 10 μL of 10 $\text{mg}\cdot\text{mL}^{-1}$ biotinylated bovine serum albumin (Vector Laboratories) for 15 minutes at room temperature. Chambers were then washed with 100 μL BRB80. Next, 20 μL of 333 $\text{mg}\cdot\text{mL}^{-1}$ avidin DN (Vector Laboratories) in BRB80 was added to the chamber and incubated for 3 minutes before washing again with 100 μL BRB80. Chambers were then incubated for 5 minutes with GMPCPP-stabilized biotinylated microtubule seeds warmed for 5-10 minutes in BRB80. Next, chambers were washed with 100 μL of growth buffer (1 mM GTP, 1 $\text{mg}\cdot\text{mL}^{-1}$ K-casein, and 0.08 $\text{mg}\cdot\text{mL}^{-1}$ biotinylated bovine serum albumin in BRB80). Finally, chambers were incubated with kinetochore-decorated beads diluted approximately 8-fold into growth buffer augmented with 1.5 $\text{mg}\cdot\text{mL}^{-1}$ purified bovine brain tubulin and an oxygen scavenging system (1 mM DTT, 500 $\mu\text{g}\cdot\text{mL}^{-1}$ glucose oxidase, 60 $\mu\text{g}\cdot\text{mL}^{-1}$ catalase, and 25 mM glucose). The flow chamber exit and entrance were then sealed with nail polish and used for experiments at 23°C for up to 2 hours.

Performing the dual-trap assay

Laser trap experiments were performed using two custom-built instruments based on commercial inverted microscopes (Nikon; TE2000U) modified to each incorporate a trapping laser (Spectra-Physics; J20I-BL-106C-NSI), a computer-controlled piezo specimen stage (Physik Instrumente; P-517.3CL), and a position-sensitive photodetector (Pacific Silicon Sensor; DL100-7-PCBA2). Experiments were performed at 22° C with a trap stiffnesses of 0.35 – 0.91 $\text{pN}\cdot\text{nm}^{-1}$. See (Franck et al., 2010) for details regarding instrument setup and calibration.

The dual-trap assay was modified from previous force-clamp protocols (Akiyoshi et al., 2010; Franck et al., 2010; Miller et al., 2016) and maintained constant total force on two bead-laser trap systems, rather than one (Figure 3.2a). Both lasers were controlled by a single computer, which adjusted the force on each bead to maintain force equilibrium on the modeled coupler given the measured bead positions (Figure 3.2b). The bead positions served as accurate proxies for the microtubule tip positions, because kinetochore-decorated beads are known to track with microtubule plus-ends under tensions between 0.5 and 18 pN (Akiyoshi et al., 2010; Asbury et al., 2006; Franck et al., 2010; Miller et al., 2016; Powers et al., 2009; Tien et al., 2010). In addition, the yeast kinetochores used to link the beads to the microtubule tips are very small, only ~130 nm in length (Gonen et al., 2012), compared to the relevant micrometer-scale tip displacements. The small size of the yeast kinetochore, as well as the slow change in force on

each tip (Figure S3.3-1), ensures that the kinetochore's (visco)elastic properties did not significantly affect experimental results, because any force-dependent changes in its length had a negligible effect compared to tip growth.

To calibrate the laser trap's zero-force position, we recorded the position of a free bead using each laser-trap at the start of each 2-hour experiment, and as necessary between recordings. Feedback to exert precisely the amount of force dictated by the relative bead positions and the spring-coupler model was implemented with custom LabView software (National Instruments). Bead-trap separation was sampled at 40 kHz, while stage position to maintain the desired bead-trap separation (and thus force) was updated at 50 Hz. Bead and stage position data were decimated to 200 Hz before storing to disk, a temporal frequency which is more than sufficient to capture pauses and runs of many seconds.

LabView software to perform force-clamp experiments is available at <https://github.com/casbury69/laser-trap-control-and-data-acquisition> and the LabView software to perform dual-trap experiments is available at <https://github.com/casbury69/dual-trap-control-and-data-acquisition>.

Analyzing dual-trap recordings

We analyzed recordings from the dual-trap assay using custom software made in Igor Pro 9 (Wavemetrics). First, by hand, we marked the beginning and end of recorded segments in which both microtubules were growing and separated these episodes into individual recordings. We then calculated the tip separation between coupled microtubules for each excised recording by taking the absolute value of the difference in position at 1 s intervals from 0 to 400 s. For each recording, total force on the microtubule pair was computed by adding the force on both microtubules together at 0.005 s intervals (Figure S3.3-1).

To calculate the speed of tip separation, we fit the first 100 s to a line with the Levenberg-Marquardt method. Uncertainties in tip separation speed were estimated from residuals as described in (Press et al.).

SEMs for microtubule pair tip separation measured using the dual-trap assay (Figure 3.3b) were calculated at 1 s intervals after the recording began using the following equation:

$$SEM_{tip\ sep}(t) = \frac{\sigma_t}{\sqrt{N_t}}$$

where σ_t is the standard deviation of the tip separations at t seconds and N_t is the number of microtubule pairs which were still growing and tip-attached to a bead at t seconds.

Download files at <https://doi.org/10.5061/dryad.xksn02vnb> to view position- and force-versus-time curves for each of the dual-trap recordings analyzed in this work. See also 'Dual-trap analysis.txt' at <https://doi.org/10.5281/zenodo.8433109> for the software used to analyze dual-trap recordings.

Creating Kaplan-Meier survival curves

Kaplan-Meier survival curves for both simulated and in vitro microtubule pairs (Figures 3.3c, 3.4c, 3.6c, S3.6-2, and S3.6-3c) were calculated at 1 s intervals after the recording began using the following equation:

$$S(t) = \prod_{j=0}^t \frac{n_j - d_j}{n_j}$$

where $S(t)$ is the Kaplan-Meier survival probability at t seconds, t is the number of Kaplan-Meier estimates made between time 0 and t seconds, n_j is the number of microtubule pairs still growing, tip-attached to a bead, and within the threshold tip separation at j seconds, and d_j is the number of microtubule pairs that exceeded the threshold tip separation (0.4, 0.6, 0.8, or 1.2 μm) between time $j - 1$ and j seconds.

SEMs for Kaplan-Meier survival plots estimated using the dual-trap assay (Figures 3.3c and S6-2) were calculated at 1 s intervals after the recording began using the following equation:

$$SEM_{\text{survival}}(t) = S(t) * \sqrt{\sum_{j=1}^t \frac{d_j}{n_j(n_j - d_j)}}$$

where $S(t)$ is the Kaplan-Meier survival probability at t seconds, t is the number of Kaplan-Meier estimates made between time 0 and t seconds, d_j is the number of microtubule pairs that exceeded the threshold tip separation (0.4, 0.6, 0.8, or 1.2 μm) between time $j - 1$ and j seconds, and n_j is the number of microtubule pairs still growing, tip-attached to a bead, and within the threshold tip separation at j seconds.

Segmenting constant force traces into periods of run and pause

To estimate pause entrance and exit rates as a function of force, we segmented $N = 356$ recordings of growing microtubules under constant force (from (Akiyoshi et al., 2010)) into pauses and fast growth ('runs,' Figures S3.5-1a and b). First, we estimated the instantaneous growth speed over the entirety of each recording to be the slope of the best fit line for a sliding 2 s window (Figure S3.5-1c). Next, we defined a unique threshold between pause and run for each recording by assembling a histogram of growth speeds throughout the recording and fitting that histogram with a sum of between two and six Gaussian peaks. We centered one Gaussian at or near 0 $\mu\text{m} \cdot \text{min}^{-1}$ and defined it as the 'pause' peak. Fitting the growth speed histograms to Gaussian peaks was performed using the 'Multipeak fitting' package in Igor Pro 9. The intersection of the pause peak and the Gaussian with the next highest mean growth speed

was identified for each individual recording and defined as the threshold to distinguish pauses and runs for that recording.

To reduce the impact of high-frequency noise, we excluded pauses and runs that lasted less than 1 s. If the instantaneous growth speed fell below that recording's predefined threshold for at least 1 s, the microtubule was considered to have paused. If the instantaneous growth speed fell below the threshold for less than 1 s, the short interval was combined with the run intervals before and after to make a single run. Likewise, if the instantaneous growth speed exceeded the threshold for at least 1 s, the microtubule was considered to be in run. If the instantaneous growth speed exceeded the threshold for less than 1 s, the short interval was combined with the pause intervals before and after to make a single pause. If the defined threshold was never crossed, the recording was considered a single run interval.

The overall trends of tension suppressing pause entrance and promoting pause exit were maintained irrespective of our choices of minimum event duration and smoothing window size. While the 1 s minimum event duration decreased the overall frequency of pause entrances and exits, it did not significantly affect the measured force sensitivities of pause entrance (F_{EN}) and exit (F_{EX}). Re-analyzing the constant force recordings with only a 0.01 s minimum event duration, we measured force sensitivities of $F_{EN} = -5.1$ pN and $F_{EX} = 14.3$ pN, compared to $F_{EN} = -4.6$ pN and $F_{EX} = 14.1$ pN with the 1 s minimum event duration. The size of the smoothing window had a significant effect on measured pause exit force sensitivity, but not pause entrance force sensitivity—re-analyzing the constant force recordings with a 5 s smoothing window, we measured force sensitivities of $F_{EN} = -4.4$ pN and $F_{EX} = 24.8$ pN. Ultimately, we chose the 2 s smoothing window and 1 s minimum duration threshold because these choices resulted in recording segmentation that agreed best with how we would differentiate pauses and runs by eye.

Download files at <https://doi.org/10.5061/dryad.6djh9w16v> to view position- and velocity-versus time curves for each force-clamp recording analyzed in this work, as well as how pauses were marked for each recording. This file also includes additional shortening events which were not studied here. See also 'Force-clamp viewer.txt' at <https://doi.org/10.5281/zenodo.8433062> for the software used to distinguish pauses from runs.

Quantifying growth speed during runs for individual recordings

For each pause-based model that we considered, microtubule growth speed during runs was assumed to be time-invariant under constant force. Thus, we made the same assumption when we quantified the growth speed during runs of individual microtubules in vitro, and calculated a unique growth speed during runs for each recording (dots in Figure 3.5b). The growth speed during each run interval was calculated by averaging together the instantaneous growth speeds comprising that interval, measured with the 2 s sliding window described above.

We calculated the overall growth speed during runs for a single microtubule by averaging together the growth speed of all contained run intervals, weighted by the duration of that interval, such that:

$$v_{run} = \frac{\sum v_i * t_i}{T}$$

where v_{run} is the overall growth speed during runs for a particular recording, v_i is the growth speed for the i^{th} run interval, t_i is the duration of the i^{th} run interval, and T is the total time spent in the run state.

Quantifying the force dependence of pause entrance rates, exit rates and growth speed

To calculate average pause exit rates, entrance rates, and growth speeds as a function of force we binned each recording by the constant tensile force to which the microtubule was subjected (Figure 3.5). For consistency, we used the same seven force bins used by (Akiyoshi et al., 2010) (see Table 3.2 for bin boundaries). For both the pause entrance and exit rate, we excluded the highest force bin from our analysis because of low statistics. For each force bin, we calculated the mean pause entrance and exit rate by counting the number of times microtubules in that force bin entered and exited pause and dividing by the total time microtubules in that bin spent in run and pause, respectively (Figure S3.5-1a). Error bars in the y-direction represent counting uncertainties, calculated with the equation:

$$SEM_{rate}(i) = \frac{k_i}{\sqrt{N_i}}$$

where k_i is the rate of pause entrance or exit for the i^{th} force bin, and N_i is the number of pause entrances or exits in the recordings in the i^{th} bin ($N = 387$ to 3502 pause entrances and $N = 378$ to 3501 pause exits per bin, see Table 3.2 for the number of pause entrances and exits by bin). Error bars in the x-direction represent standard deviations of forces for the recordings in each bin.

We found the mean growth speed during runs for each force bin by averaging together the growth speed during runs for all the recordings in that bin. Error bars in the y-direction represent the SEMs of growth speeds, calculated with the equation:

$$SEM_{run\ speed}(i) = \frac{\sigma_i}{\sqrt{N_i}}$$

where σ_i is the standard deviation of the growth speeds during runs, averaged over each recording, for the i^{th} force bin, and N_i is the number of recordings in the i^{th} bin ($N = 9$ to 85 recordings per bin, see Table 3.2 for the number of recordings by bin). Once again, error bars in the x-direction represent the standard deviations of forces for the recordings in each bin. Fit lines for pause exit and entrance rates and growth speed were calculated with Levenberg-Marquardt least-squares method (Press et al.).

Simulating pairs of growing microtubules

Simulated recordings using each model followed the same general procedure. For simulations in which the growth or run speed was different between microtubules (both non-ergodic models), the ‘unloaded’ growth or run speed was drawn from a distribution of measured growth or run speeds as described in the results section. For the non-ergodic pausing model, intrinsic unloaded run speeds ($v_{intrinsic}$) of more than 30 nm/s were considered outliers and excluded from the distribution. For microtubules simulated with the ergodic pausing model, both microtubules had the same growth speed during runs, based on the equation and parameters for v_{run} in Table 3.1. Each simulation began with both microtubules growing (in run, for pause-based models) from the same position under the same tensile force. As microtubules stochastically grew or paused, the tensile force on each microtubule evolved according to the spring-coupler model, just like they did in the dual-trap assay. As the force changed, depending on the model type, the growth or run speed changed according to exponential relationships with force estimated from (Akiyoshi et al., 2010) (Table 3.1).

A modified Gillespie algorithm was used to implement stochastic microtubule events: pause entrance and exit, detachment from the kinetochore, switches into shortening (catastrophe), and other event interruptions. Each of these event rates were assumed independent Poisson processes and were derived directly from the data summarized in (Akiyoshi et al., 2010). Each microtubule was assumed to have identical rates for all stochastic events, given the same external force. Detachment and catastrophe rates were calculated using the exponential relationships as a function of force estimated by (Akiyoshi et al., 2010). The rate of interruption by other events was also estimated from these force-clamp recordings but was force-independent. For simulations that included pausing, rates of pause entrance and exit were calculated from the fit equations in Table 3.1 (fit lines in Figure 3.5).

Because detachment, catastrophe, and pause rates are force-dependent, they can only be approximated as Poisson (time-invariant) rates for short time intervals over which the force on the tip is relatively constant. Consequently, we imposed a maximum timestep of no greater than 1 s for each simulation. If the timestep chosen by our Gillespie algorithm exceeded the maximum timestep, no event occurred and the microtubule continued growing during that 1 s interval. All simulations were performed using custom software in Igor Pro 9 (Wavemetrics).

Download ‘Dual-trap simulations.pxp’ at <https://github.com/casbury69/simulations-of-coupled-microtubules> to run dual-trap simulations as described in the results and see ‘Dual-trap simulator.txt’ at <https://github.com/casbury69/simulations-of-coupled-microtubules> for the code used to run these simulations.

Comparing pausing and non-pausing models with simple probability

To evaluate whether the pausing or non-pausing model was more likely given our dual-trap measurements, we compared histograms of tip separations at 100 and 200 s after the start of the recording or simulation (Figure S3.6-4). We used 0.1 μm bins for both simulated and dual-trap data and normalized all histograms to have an area of 1.

We calculated the likelihood that simulations performed using the pausing and non-pausing models would produce each in vitro tip separation:

$$L_{\text{measurement}} = w_{\text{bin}} * P_{\text{normalized}}$$

where w_{bin} is the bin width of the tip separation histogram (0.1 μm) and $P_{\text{normalized}}$ is the normalized probability of the given model at the measured tip separation (y-values of the dashed lines in Figure S3.6-4).

To compare the overall likelihood that the pausing and non-pausing simulations would produce all the tip separations measured in vitro, we multiplied together the likelihoods of each measurement for a given model at a given coupler stiffness and at a given time point. We calculated the log likelihood by taking the base 10 logarithm of each probability (reported in the right-hand column of each table in Figure S3.6-4).

Statistical analysis

All statistical analysis was performed using custom software written in Igor Pro 9 (Wavemetrics).

We calculated p -values to determine whether survival curves of stiff-coupled and soft-coupled in vitro microtubule pairs were statistically different for each threshold tip separation using a log-rank test. For each survival curve, at 1 s intervals after the recording began, we calculated an expected value using the following equation:

$$E(t) = \frac{n_t}{n_{\text{tot}}} (d_{\text{tot}})$$

where n_t is the number of microtubule pairs comprising one survival curve that are still growing, tip-attached to a bead, and within the threshold tip separation at t seconds, n_{tot} is the total number of microtubule pairs comprising both survival curves being compared that are still growing, tip-attached to a bead, and within the threshold tip separation at t seconds, and d_{tot} is the total number of microtubule pairs that exceeded the threshold tip separation between time $t - 1$ and t seconds for both survival curves being compared. Expected values for each pair of survival curves were used to calculate a chi-square value for each tip separation threshold using the following equation:

$$\chi^2 = \sum_{j=1}^2 \frac{(\sum_{t=1}^T S_j(t) - \sum_{t=1}^T E_j(t))^2}{\sum_{t=1}^T E_j(t)}$$

where T is the total number of timepoints (400, in all cases), $S_j(t)$ is the Kaplan-Meier survival probability of curve j at time t , and $E_j(t)$ is the expected value of Kaplan-Meier survival curve j

at time t . A chi-square table was then used to estimate the p -value for each tip separation threshold.

To determine whether tip separation and tip separation speed between soft and stiff couplers were statistically significant, we calculated p -values with a Wilcoxon-Mann-Whitney two sample rank test using built-in Igor Pro software (Cheung & Klotz, 1997; Klotz, 2006; Streitberg & Rohmel, 1986; Zar, 1999).

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Chapter 4: Extended work for “Mechanical coupling coordinates microtubule growth.”

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Correction for stiffness in laser trap system and software update

Summary

In this update, we provide a more nuanced analysis of our existing dual-trap data by adjusting our reported microtubule tip positions to account for small changes in force and springiness in our microtubule-bead-trap system. We measured system compliance by abruptly changing the force on a microtubule tip and measuring the displacement. We then adjusted the positions of each microtubule pair recorded using the dual-trap assay to account for this springiness. Overall, the effect was slight, increasing average tip separation at 400 s by 3% for soft-coupled pairs and 17% for stiff-coupled pairs. We have uploaded the adjusted tip positions as .csv files and included a new dual-trap viewer .pxp file to automatically display the adjusted tip positions. We updated the in vitro survival curves in the dual-trap simulations .pxp file at <https://github.com/casbury69/simulations-of-coupled-microtubules> using the adjusted tip positions.

We also address a minor bug affecting the code that runs the dual-trap simulations, causing simulated tip separations based on the pausing model to be assigned to timepoints about 19% higher than the timepoints at which they were simulated. The effect of this error was slight, increasing simulated tip separation for soft-coupled microtubule pairs by 12% and stiff-coupled microtubule pairs by 4% at 400 s. We have uploaded a new version of Dual-trap simulator.txt and updated the code in Dual-trap simulations.pxp at <https://github.com/casbury69/simulations-of-coupled-microtubules> to address this error.

The changes to in silico and in vitro data were minor and compensatory, as both adjusting for system stiffness and correcting the error in simulations slightly increased tip separation. An updated version of [Figure S3.6-2](#) is displayed below, showing the strong agreement between in vitro microtubule pairs and those simulated using the pausing model. As in our original publication, stiff-coupled pairs in vitro maintained significantly lower tip separations ($p < 0.05$) for all threshold tip separations higher than 0.4 μm . Therefore, our conclusions, that mechanical coupling significantly coordinates microtubule pairs and that microtubule growth and coordination in vitro can be well-described by our pausing model, are not affected.

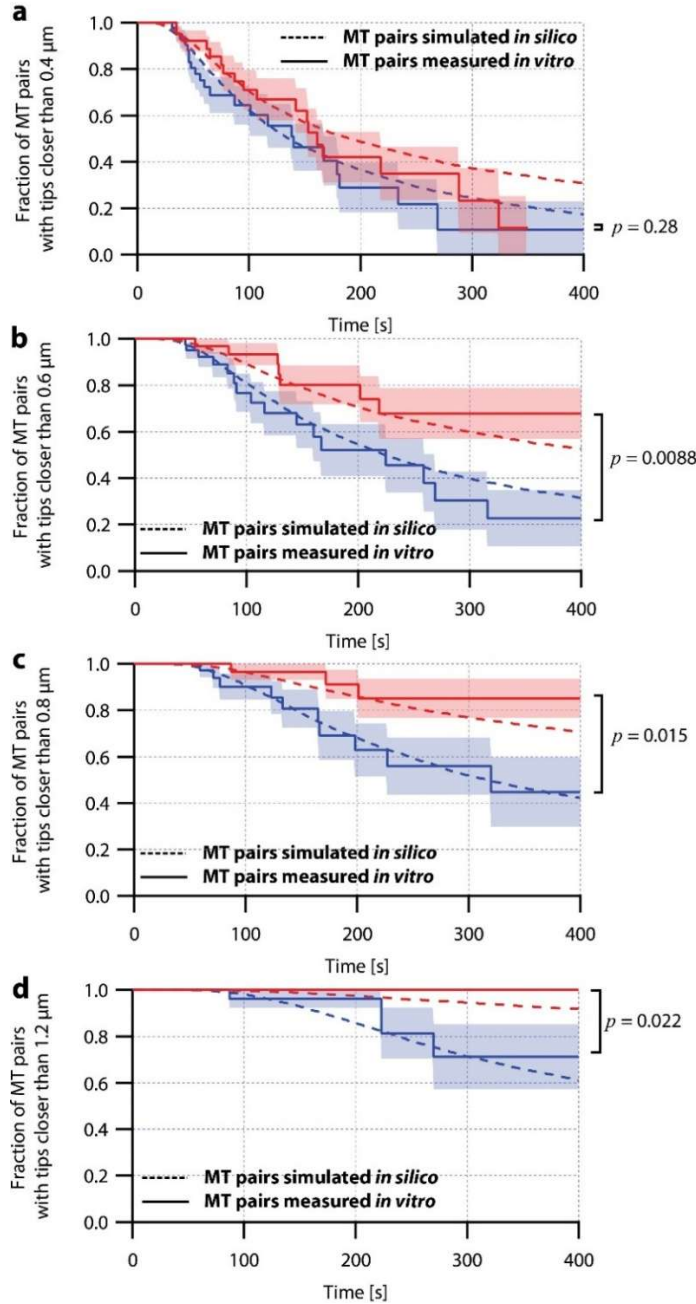


Figure 4.1. Updated version of Figure S3.6-2. Simulations of microtubule pairs using the pausing model maintain tip separations similar to real microtubule pairs. Comparison between the fraction of in silico (simulated, dashed curves) and in vitro (real, solid curves) microtubule (MT) pairs whose plus-ends remained within (a) 0.4 μm , (b) 0.6 μm , (c) 0.8 μm , and (d) 1.2 μm of each other over time. Microtubule pairs in silico and in vitro shared a total load of $F_{TOT} = 8$ pN and were coupled via soft (blue) or stiff (red) spring couplers. Shaded regions show SEM from $N = 50$ and $N = 43$ in vitro recordings with soft and stiff couplers, respectively. Soft- and stiff-coupled microtubule pairs were each simulated $N = 10,000$ times over 400 s of growth. P -values indicate comparisons between soft- and stiff-coupled microtubule pairs *in vitro* using a log-rank test. In this updated version, the experimental data has been adjusted slightly compared to the original version to account for the series elastic compliance of the components connecting the trap center to the coverslip, and the simulated curves were re-generated after a minor bug in the code was fixed. Both changes are described in more detail below.

Adjustment of in vitro recordings for system stiffness

As we argued in the methods section, the small size of the yeast kinetochore and the slow-changing force in our dual-trap experiments means that we expect kinetochore stretching behavior under increasing force to be negligible compared to our overall measurements of tip position. However, the kinetochore is not the only source of springiness in our experiments. A major source of compliance in our system is the laser trap itself. The biotin-avidin attachment between the microtubule seeds and the coverslip, as well as springiness in the microtubule itself (both from curling protofilament ends and slight curvature in the microtubule from thermal noise) also contribute to system compliance. We were concerned that these sources of springiness might affect our measurements of tip separation over time and make microtubule pairs, especially stiff-coupled pairs, appear more coordinated than they truly were.

To understand why, consider that in the beginning of an experiment, at both stiffnesses, both tips are under 4 pN tension. As one microtubule grows faster than the other, it bears less of the overall force, which causes the springy elements in our system to contract. Conversely, the slower microtubule bears more of the force, causing the springy elements in our system to stretch. Thus, the faster microtubule appears to be growing slightly slower, while the slower microtubule appears to be growing slightly faster, artificially reducing tip separations. This is especially true for stiff-coupled pairs, since the force changes more quickly with increasing tip separation.

To account for system compliance in our tip separation measurements, we first had to approximate system compliance. We did this by abruptly increasing or decreasing the force on a growing microtubule tip and measuring the change in tip position. We calculated the system compliance with the equation:

$$k_{sys} = \frac{F_{final} - F_{initial}}{x_{final} - x_{initial}}$$

We measured system compliance in this way for $N = 17$ and $N = 16$ microtubules for Trap 1 and 2, respectively, since trap stiffness differs slightly on our two instruments (Trap 1: 43.8 pN/ μ m, Trap 2, 34.7 pN/ μ m). We found an average system compliance of 29.8 ± 1.8 pN/ μ m using Trap 1 and 29.4 ± 2.3 pN/ μ m using Trap 2 (Mean $\pm$ SEM). We also found that system stiffness is approximately Hookean (not strain-stiffening or softening) within the force ranges we measured (~ 2 -6 pN) since the measured stiffness does not appear to change as a function of force.

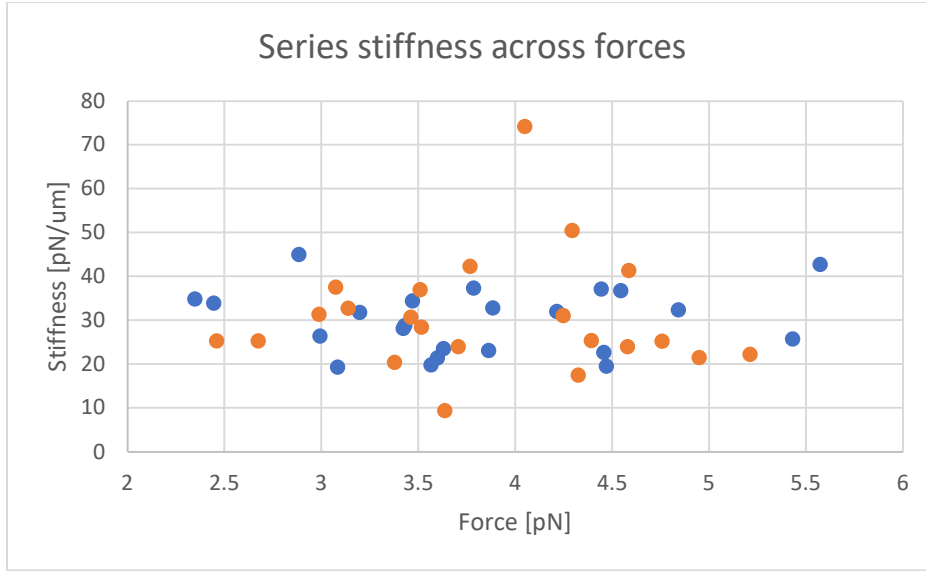


Figure 4.2. Series stiffness is Hookean between 2 and 6 pN tension. Series stiffnesses ($\text{pN} \cdot \mu\text{m}^{-1}$) measured from $N = 17$ and $N = 16$ individual microtubules using Trap 1 (blue) and Trap 2 (orange), respectively. Series stiffness is plotted versus the average of the forces (pN) just before and just after we abruptly changed the requested trap force. Series stiffness was sometimes measured twice for the same microtubule.

We then adjusted our measured tip positions at each timepoint based on the mean measured system compliances using the equation:

$$x_{adjusted}(t) = x_{measured}(t) + \frac{F_{initial} - F(t)}{k_{sys}}$$

Finally, we calculated tip separation by taking the absolute value of the difference between adjusted tip positions. The new graphs of tip separation over time after adjustment for series compliance are shown below.

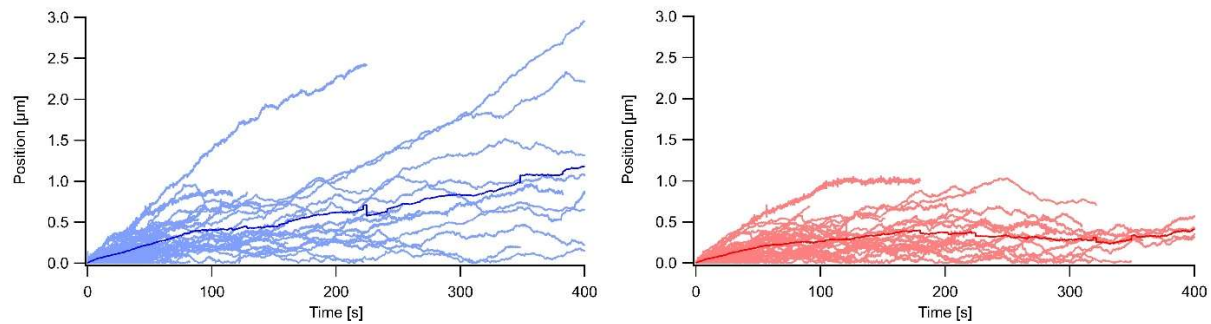


Figure 4.3. Updated version of Figure 3.3b. Microtubule pairs growing in vitro are coordinated by mechanical coupling. Tip separation ($|x_1 - x_2|$) between microtubule plus-end pairs that were coupled with either soft ($1 \text{ pN} \cdot \mu\text{m}^{-1}$, left) or stiff ($5 \text{ pN} \cdot \mu\text{m}^{-1}$, right) simulated spring-couplers over 400 s of growth. Light colors show tip separations for individual microtubule pairs, while dark colors show mean tip separation for all recorded pairs ($N = 50$ and $N = 43$ recordings with soft and stiff couplers, respectively). In this updated version, the experimental data has been adjusted slightly compared to the original version to account for the series elastic compliance of the components connecting the trap center to the coverslip.

The difference between the adjusted and unadjusted tip separations was slight, with an average increased tip separation at 400 s of 3% for soft-coupled pairs and 17% for stiff-coupled pairs. The effects are slight because even using the stiffer coupler, the force at 0 s and 400 s are usually similar (usually <2 pN difference). Thus, our conclusion that mechanical coupling can significantly coordinate a pair of growing microtubules is still supported. This method of correcting tip position in variable-force experiments will be used for future experiments focusing on disassembling microtubules.

Error-correction in code affecting the pausing model only

Our code analyzing the tip separations over time for our simulations assumed that each timestep taken was the maximum timestep of 1 s. However, for the pausing model, this was not the case, since the Gillespie algorithm we used took timesteps that were less than 1 s if the microtubule entered or exited the pause state before the end of the 1 s maximum step. Therefore, the simulated tip separations reported at 400 s using the pausing model were in fact the simulated tip separations at ~330-340 s for both soft- and stiff-coupled microtubule. The simulated tip separations at 400 s were somewhat larger than those at ~330-340 s, shifting both simulated survival curves down. This was especially true for soft-coupled pairs, which were 12% more separated at 400 s compared to what was reported, while the stiff-coupled microtubules were only 4% more separated at 400 s compared to what was reported. These differences are slight relative to the overall tip separation and this error does not affect our conclusions.

The new graphs of simulated tip separation over time after fixing this timestep error are below.

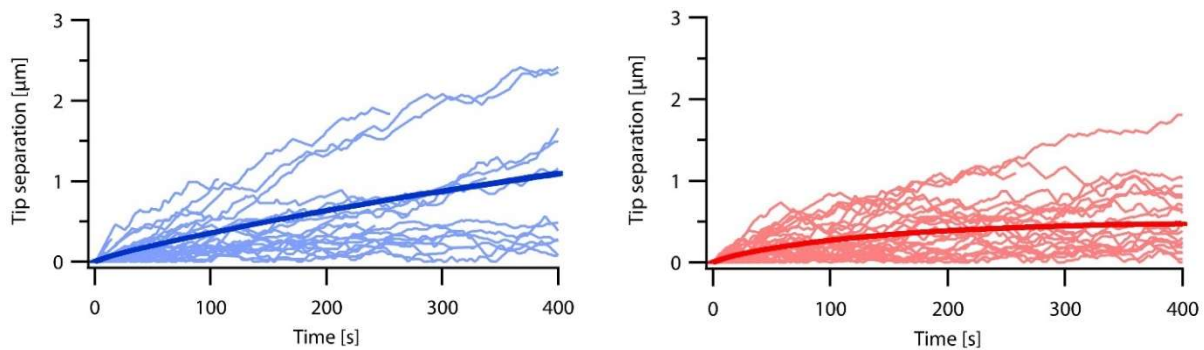


Figure 4.4. Updated version of Figure 3.6b. Simulations recapitulate the coordinated growth of mechanically coupled microtubule pairs. Tip separations between microtubule plus-end pairs that were simulated using the pausing model and coupled with either soft ($1 \text{ pN} \cdot \mu\text{m}^{-1}$, left) or stiff ($5 \text{ pN} \cdot \mu\text{m}^{-1}$, right) spring-couplers. Light colors show tip separations for $N = 40$ individual simulated microtubule pairs, while dark curves show mean tip separation for $N = 10,000$ simulated pairs. In this updated version, the simulated curves were re-generated after a minor bug in the code was fixed, as described in more detail above.

In conclusion, our findings still show that mechanical coupling significantly coordinates microtubule pairs and that microtubule growth and coordination in vitro can be well-described by our pausing model.

Faster microtubules have shorter pauses and longer, faster runs

In [Chapter 3](#), we assumed that kinetochore-microtubules at the same force have the same rate of pause entry and exit, varying only in their speed of growth during runs. However, it appears that contrary to this assumption, faster-growing kinetochore-microtubules are faster not only because they grow faster during runs, but also because they have shorter pauses and longer runs ([Figure 4.5](#)). This implies that kinetochore-microtubules with tips compatible with faster tubulin addition (or slower tubulin loss) are also less vulnerable to enter the pause state and more likely to recover quickly from that pause state. This applies to kinetochore-microtubules accelerated by either force or other factors, because run speed, pause entry rate, and pause exit rate all follow the same trends of increasing or decreasing with growth speed, even at similar forces.

It would be interesting to adapt the previous pausing model to include the covariation of run speed, pause entry, and pause exit. Unfortunately, variable pause entry and exit speeds are difficult to incorporate into simulations because the high variability in pause exit rates between constant force measurements overinflates the variability in kinetochore-microtubules growth. High variability may be in part because there are insufficient pauses during a single measurement to get accurate rates for that microtubule. A pausing model with variable pause entry and exit rates for microtubules at the same force would likely need to restrict the variability in pause exit so that overall microtubule growth speed variability still matches constant force and dual-trap measurements.

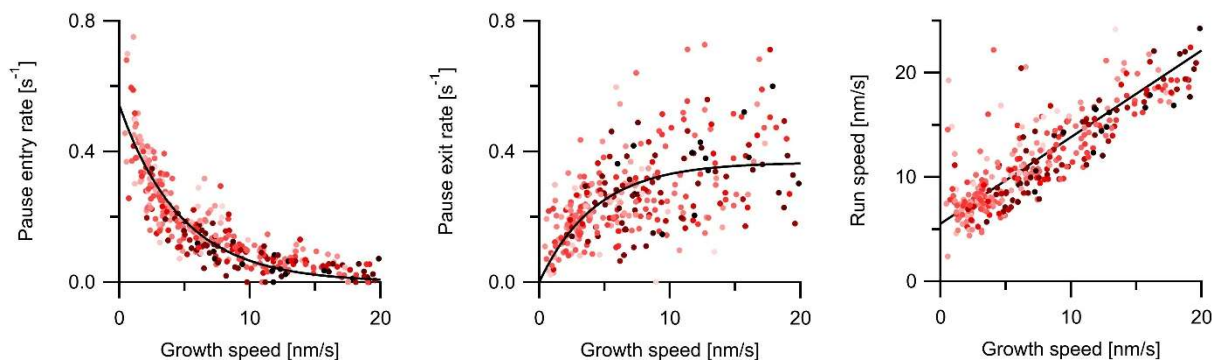


Figure 4.5: Faster microtubules have shorter pauses and longer, faster runs. Rate of pause entry (left), pause exit (middle), and run speed (right) as a function of average growth speed. Circles represent individual constant-force recording from ([Akiyoshi et al., 2010](#)), colored so that the darker the circle, the higher the force ($N = 356$ run speeds and pause entry rates, $N = 338$ pause exit rates). All rates and speeds were calculated as described in [Chapter 3](#).

References for Chapter 4

Akiyoshi, B., K.K. Sarangapani, A.F. Powers, C.R. Nelson, S.L. Reichow, H. Arellano-Santoyo, T. Gonen, J.A. Ranish, C.L. Asbury, and S. Biggins. 2010. Tension directly stabilizes reconstituted kinetochore-microtubule attachments. *Nature*. 468:576-579.

Chapter 5: Future directions: How are microtubules in large kinetochore-fiber bundles coordinated?

My thesis work has shown how kinetochores may consistently maintain correct attachments in a sea of incorrect attachments and coordinate pairs of microtubules in growth. These behaviors illustrate two ways that cells in general, and kinetochores in particular, can organize the stochastic chaos of microtubules into robust subprocesses of the enormous and complicated task of dividing genetic information equally between progeny.

I chose to study simplified scenarios, the budding yeast spindle with only one microtubule per kinetochore in [Chapter 2](#) and a pair of growing microtubules in [Chapter 3](#). However, mammalian k-fibers consist of 10 or more microtubules ([Kiewisz et al., 2022](#); [McEwen et al., 1997](#); [O'Toole et al., 2020](#)), each of which can be growing or shortening at any moment, as the entire bundle also can be growing or shortening at any moment. One next fascinating direction for this project will be to fully embrace this k-fiber chaos.

How do microtubule shortening and switching affect k-fiber coordination? How do behaviors seen with pairs of microtubules extend to bundles of many microtubules in heterogeneous states of growth and shortening? I began to explore these questions using both simulations and laser trap experiments with the three undergraduate researchers I recruited and trained, Katelyn Kostello, Yuna Liu, and Rhea Kulkarni. Some of this work is described below. I do not intend to publish the work presented in the following sections in any other format. Instead, I hope that this chapter, in addition to other summative reports I will leave with the Asbury lab, can serve as a starting place for future works by myself or others.

Shortening microtubules have force-dependent slow and fast phases

To understand how microtubules in k-fibers grow and shorten as a single unit, it is critical to study variability in microtubule shortening as well as how tension-dependent shortening and switch rates can coordinate mechanically-coupled tips. The following describes my work starting to explore these subjects with Rhea Kulkarni, who performed the following analyses under my guidance.

To understand variability in microtubule shortening, we once again examined traces of kinetochore-microtubule attachments under constant forces from ([Akiyoshi et al., 2010](#)). As in growth, microtubules shorten at highly variable rates ([Figure 5.1](#)). On average, microtubules under higher tension shorten more slowly, as previously reported ([Akiyoshi et al., 2010](#)).

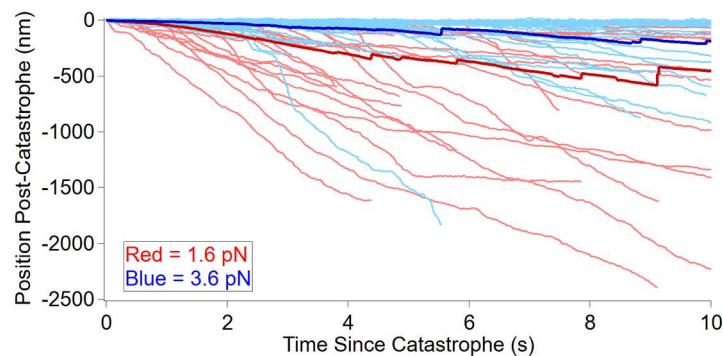


Figure 5.1. Microtubules shorten at highly variable rates that generally decrease with tension. Tip position for shortening microtubules under low (red) and high (blue) constant tension. 0 s is the time of catastrophe. Pale curves show individual traces, and darker curves show the average position at each time point for low and high forces.

We calculated the instantaneous velocities for all recordings of microtubule shortening from (Akiyoshi et al., 2010) with a 1 s sliding window (Figure 5.2). The corresponding shortening speed histograms were bimodal at both high and low forces. At high forces, the fast peak was shifted to slower shortening speeds, but the slow peak had similar speeds at both high and low forces. In addition, microtubules at higher forces spent more time shortening at slower speeds. This data is consistent with a model similar to the pausing model of Chapter 3, with distinct fast and slow phases that microtubules switch between stochastically. In this model, tension decreases shortening speed in the fast phase and increases the probability of microtubules being in the slow phase. This model is supported by visual inspection, as individual traces often exhibit a step-like appearance (Figure 5.1) and many have an initial or final slow phase (Figure 5.3).

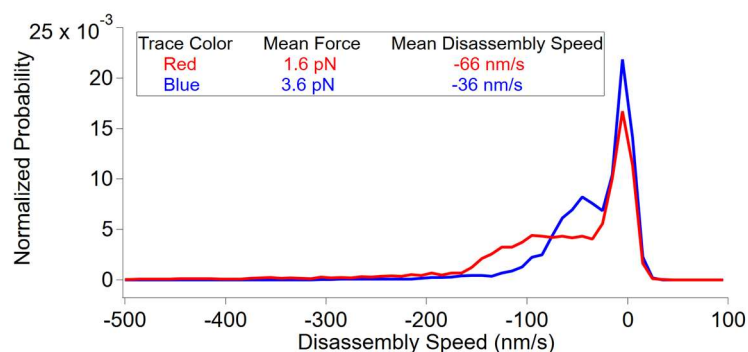


Figure 5.2. Distributions of instantaneous shortening velocities are bimodal at high and low forces. Probability of instantaneous microtubule shortening speeds at low (red) and high (blue) forces normalized such that the area under the curve was 1 nm/s at each force.

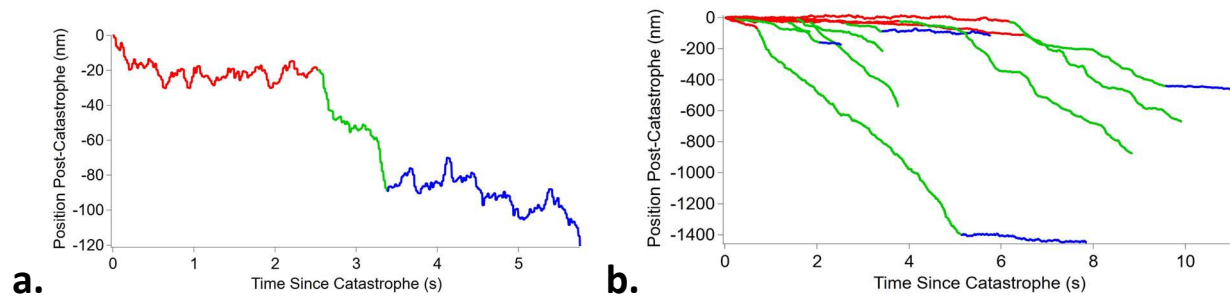


Figure 5.3. Kinetochore-microtubule shortening is often slow at the beginning and end of constant force experiments. **a.** Example position of a microtubule tip shortening under constant force after catastrophe (0 s). Initial (red) and final (blue) slow phases are highlighted. The middle (green) phase is generally characterized by faster shortening, but can also have periods of slow shortening. **b.** Position of $N = 8$ microtubule tips shortening under constant force, displayed and colored as in panel a. Note that the slow phase is not present at the beginning and end of every shortening event.

In order to parameterize this two-state model of shortening, we began to segment constant force traces into slow and fast phases, as was done for pausing in [Chapter 3](#). This analysis proved challenging and is still incomplete, primarily because the semi-automated algorithm to differentiate slow and fast phases did not perform well on longer traces. Slow- and fast-phases will probably need to be hand-marked for these traces, and it will require significant work to ensure that differentiation between phases is consistent between hand- and automatic-marking. Once complete, the rate of entry and exit into the slow phase, as well as mean speeds in the slow and fast phase for microtubules shortening under high and low forces, can be estimated. These parameters can be used to make a model of microtubule shortening similar to our pausing model of microtubule growth which might then be tested against measurements of real kinetochore-microtubules shortening under variable forces. While I have performed some in vitro measurements of kinetochore-microtubule shortening under variable force, I will compile them elsewhere for the Asbury lab as there is not yet sufficient data to make meaningful conclusions or provide strong support for a specific shortening model.

Preliminary analyses suggest that slow microtubule shortening may favor rescue compared to detachment independent of force. When shortening events ended with a slow phase, they were much more likely to rescue than detach, while shortening events that ended without a slow phase had nearly equal likelihood of rescue or detachment ([Table 5.1](#)). Once traces have been fully segmented into slow and fast phases, rescue and detachment rates during fast and slow phases at both high and low forces can be measured, then fit using single exponentials as a function of force to confirm this trend.

	Slow ending absent	Slow ending present
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Mean Force	% Rescue	N Disassembly Events	% Rescue	N Disassembly Events
1.3 pN	54.8%	31	100%	8
4.4 pN	60%	25	86.7%	15

Table 5.1. Rescue is more likely when microtubule shortening ends with a final slow phase. Table displaying percent of kinetochore-microtubule shortening events at high (4.4 pN) and low (1.3 pN) forces ending with rescue. The alternative option was detachment or other interruptions, which were quite rare.

Ideally, these fits could be incorporated into a two-phase model where microtubules switch between fast and slow phases of shortening with force-dependent rates. Rescue or detachment would occur at rates based on whether they are currently shortening quickly or slowly. If corroborated, these observations suggest that microtubules may have distinct modes of shortening—one slow in which the microtubule is more likely to switch back to growth, and one fast in which the microtubule either continues to shorten or detaches. Because the slow phase is favored by high force, microtubules under high tension are likely to not only shorten more slowly but also rescue more quickly. A microtubule in a bundle that shortens too far and falls behind may feel higher force, biasing it toward the slow phase which is more likely to rescue and allowing the lagging to catch up to the rest of the bundle. It will be fascinating to see how this effect helps to coordinate microtubules in k-fibers with mixed populations of growing and shortening microtubules, as well as in shortening k-fibers with predominantly shortening tips.

References for Chapter 5

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