Contributions of microtubule dynamic instability and rotational diffusion to kinetochore capture

R. Blackwell, O. Sweezy-Schindler, C. Edelmaier, Z. R. Gergely, P. J. Flynn, S. Montes, A. Crapo, A. Doostan, J. R. McIntosh, M. A. Glaser, and M. D. Betterton
Abstract

Microtubule dynamic instability allows search and capture of kinetochores during spindle formation, an important process for accurate chromosome segregation during cell division. Recent work has found that microtubule rotational diffusion about minus-end attachment points contributes to kinetochore capture in fission yeast, but the relative contributions of dynamic instability and rotational diffusion are not well understood. We have developed a biophysical model of kinetochore capture in small fission-yeast nuclei using hybrid Brownian dynamics/kinetic Monte Carlo simulation techniques. With this model, we have studied the importance of dynamic instability and microtubule rotational diffusion for kinetochore capture, both to the lateral surface of a microtubule and at or near its end. Over a range of biologically relevant parameters, microtubule rotational diffusion decreased capture time, but made a relatively small contribution compared to dynamic instability. At most, rotational diffusion reduced capture time by 25%. Our results suggest that while microtubule rotational diffusion can speed up kinetochore capture, it is unlikely to be the dominant physical mechanism. In addition, we found that when microtubules undergo dynamic instability, lateral captures predominate even in the absence of rotational diffusion. Counterintuitively, adding rotational diffusion to a dynamic microtubule increases the probability of end-on capture.

Key words: mitosis; cytoskeleton; microtubules; kinetochores; kinetochore capture
Mechanisms of kinetochore capture

Introduction

Cell division is essential to the propagation of life. For a cell to divide successfully, each daughter cell must inherit the correct genetic material. In eukaryotes, segregation of duplicated chromosomes is performed by the mitotic spindle, a cellular machine composed of microtubules (MTs) and their associated proteins (1). Specialized sites on the chromosomes called kinetochores (KCs) attach to spindle MTs, and these KC-MT attachments are necessary for proper chromosome segregation. Understanding KC capture by spindle MTs and the subsequent chromosome movements is challenging because the process depends on multiple overlapping mechanisms (2–4), including the action of multiple KC-associated motors and highly dynamic MTs that maintain KC attachment during significant MT turnover. Numerous proteins localize to MT plus ends and KCs, but the roles of these different proteins are not yet clear (5–7). Problems in kinetochore-MT attachment and chromosome segregation can lead to aneuploidy, which is associated with birth defects and cancer progression (8).

The discovery of MT dynamic instability 30 years ago (9) led to the proposal that MT search and capture is the primary mechanism of initial KC-MT attachment in mitosis. In this picture, dynamic MTs grow in different directions from centrosomes and make end-on attachments with KCs (10–13). Perturbations to MT dynamics are predicted to have significant effects on KC capture (12), suggesting that dynamic instability is a key component of any KC capture model. Search and capture has been directly observed in large cells (14). However, the simplest search-and-capture mechanism does not appear rapid enough to capture multiple chromosomes quickly enough to match measured time in mitosis. Extensions to the search-and-capture mechanism that can make KC capture more rapid in large cells or cell extracts include KC diffusion (12, 15), MT growth that is spatially biased toward chromosomes (16–18), chromosome spatial arrangements and rotation (15, 19, 20) and KC-initiated MTs that can interact with searching MTs (15, 21, 22). KCs in human cells change size and shape during mitosis, which can both speed up capture and minimize errors (20).

KC capture may occur differently in smaller cells. Lateral KC contacts has been directly observed both in budding yeast (23) and fission yeast (24). Recent work on the small cells of fission yeast found that lateral KC attachment to MTs that rotationally diffuse about their attachment points at the spindle-pole bodies (SPBs, the yeast centrosomes) enabled rapid KC capture, even for relatively less dynamic MTs (24). Based on experiments and a biophysical model, this work concluded that MT rotational diffusion was the primary determinant of the time to capture lost KCs in these cells. This important finding suggests that MT rotational diffusion may significantly contribute to efficient KC capture, an effect that has been neglected previously. Since initial KC captures are typically lateral rather than end-on (14, 19, 20, 23, 25), MT rotational diffusion about minus-end attachment points could be an important determinant of the capture time.

We have sought to evaluate the relative importance of MT dynamic instability versus MT rotational diffusion to KC capture. Previous theoretical work has focused either on MT dynamic instability (11, 12, 15, 17, 20, 25) or rotational diffusion (24); as a result, this work has been unable to compare the two mechanisms and determine their relative importance. One model examined the relative contributions of dynamic instability and rotational diffusion for a single parameter set and found that turning off rotational diffusion caused a modest increase in the mean capturer time (27). Because MT rotational diffusion can lead to significant increases in the effective volume searched by a single MT, search-and-capture models that neglect MT rotational diffusion could lead to incorrect conclusions.

We extended the Kalinina et al. model of fission-yeast KC capture to include both MT rotational diffusion and dynamic instability in order to gauge their relative importance to KC capture. We
modeled both relatively slow dynamic instability (using measured parameters (24)) and faster dynamic instability based on our measurements of single mitotic MTs in fission yeast. Rotational diffusion typically gave only a modest speed-up of KC capture in our model. For slow dynamics, rotational diffusion decreased the KC capture time by up to 25%, while for faster MT dynamics, rotational diffusion caused at most a 16% decrease in the capture time. Our results suggest that the capture time in fission yeast is primarily determined by MT search and capture. We also found that while lateral captures are typical even in the model with no rotational diffusion, including MT diffusion made end-on attachments more likely. This occurred because rotational diffusion sweeps the tip of the MT through space, increasing the volume searched by the tip. Our findings suggest that associating search and capture with end-on attachment and rotational diffusion with lateral attachment is oversimplified.
Materials and methods

Kinetochore capture model

We developed a computational model of KC capture in fission yeast that includes the key physical effects of MT dynamic instability and rotational diffusion. Capture occurs within the spherical nucleus of radius $R = 1.5 \ \mu$m. A dynamic MT has its minus end attached to the SPB (which is fixed for this study), rotationally diffuses, and has a length $L(t)$ that changes with time due to dynamic instability (fig. 1, S1). Simultaneously, a spherical KC of radius $a = 100 \ \text{nm}$ diffuses in the nucleus (24). We assumed the fission yeast KC size remained constant during mitosis, in contrast to recent work on human KCs (20). KC capture occurs when the KC contacts the MT, either at its end or along its lateral wall.

The simulations used a hybrid Brownian dynamics-kinetic Monte Carlo scheme approach based on our previous work (28-31). Brownian dynamics model the diffusive random motion of MTs and KCs; kinetic Monte Carlo models the stochastic MT dynamic instability (Supporting Material).

Slow and fast microtubule dynamics

We studied two MT dynamic instability models that represent relatively slow and fast dynamics (Supporting Material, fig. 1, table 1). Kalinina et al. found that MT dynamics were relatively slow and MTs spent most of their time paused (24). Therefore, we modeled MTs with growing, shrinking, or paused states; the fixed-length paused state is an intermediate between the growing and shrinking states (Supporting Material). In other work on single fission yeast mitotic (32) and meiotic (27) MTs and our measurements, MT dynamics were faster and pausing was rarely seen. We modeled this with growing and shrinking states only, where catastrophe is the transition from growing to shrinking, and rescue the transition from shrinking to growing.

In our model, MTs in the growing or shrinking state increase or decrease in length at the constant speed $v_g$ or $v_s$. For MTs that are not interacting with the nuclear envelope boundaries, the state switching frequencies are constant in time. Any shrinking MTs that reach the minimum length of $4\sigma_{MT} = 100 \ \text{nm}$ switch to the growing state (Supporting Material).

Microtubule interactions with the nuclear envelope

MTs that touch the nuclear envelope experience steric forces and torques from the interaction of the MT tip with the envelope (Supporting Material). The torque can cause the MT tip to slip along the edge of the envelope, reorienting the MT, as has been measured and modeled previously for MTs interacting with microchamber boundaries (33-35). In addition, MTs that grow into a boundary exhibit increased catastrophe frequency (36). The force component along the MT long axis increases the catastrophe frequency, as measured previously (37, 38). By combining these previous measurements of the force dependence of MT growth speed with the growth speed dependence of the catastrophe time, we wrote the catastrophe frequency $f_c(F_{||}) = f_c \exp(\alpha F_{||})$, where $F_{||}$ is the component of the steric force along the MT long axis, and $f_c$ is the zero-force catastrophe frequency (or the analogous grow-to-pause frequency in the model with pausing).

Initial conditions and measurements

We began simulations with a single MT of length $4\sigma_{MT}$ (100 nm) placed at a random angle subject to the requirement that the MT was not initially interacting with the nuclear envelope. We inserted a KC at a random position uniformly sampled within the simulation volume, with the requirement
that the KC was not initially interacting with either the MT or the nuclear envelope. We ran the
simulation until the KC collided with the MT (either laterally or end-on), which defined a capture.
For each parameter set, we repeated simulations 2000-5000 times to determine the distribution of
capture times, shown as the fraction of lost KCs as a function of time in figs. 1D, S2. From these
data, we computed the mean capture time $\langle \tau_c \rangle$.

We defined reference parameter sets and wide parameter ranges around the reference for both
the slow and fast dynamic instability models (table 1). For both slow and fast models, we performed
simulations of dynamic instability with no MT rotational diffusion, and dynamic instability plus
MT rotational diffusion. To connect to previous search-and-capture models, MTs that shrink to
the minimum length re-enter the growing state with a new random orientation.

**Experimental methods**

To understand the difference between previous measurements of fission-yeast mitotic MT dynamics
that were relatively slow (24) or fast (32), we measured MT dynamic instability in *S. pombe*. To
facilitate these measurements, we used a strain with temperature-sensitive inactivation of kinesin-5
motors (*cut7-24* in fission yeast) and low-level fluorescent tagging with *mCherry-atb2* (39) (Sup-
porting Material, table S1). Cells carrying the *cut7-ts* allele are unable to form bipolar mitotic
spindles at restrictive temperature (36-37°C) (40). The cells instead form monopolar mitotic spin-
dles, in which individual fluorescently labeled mitotic MTs can be imaged (fig. 2) (41).

**Measurement of labeled tubulin fraction**

We performed immunoblots using the TAT-1 tubulin antibody on *S. pombe* cell lysate with serial
dilutions ranging from 100%-10% of the original cell suspension concentration (Supporting Material)
and scanned the bands for analysis. The two lower, darker bands corresponded to $\alpha$-tubulin-1
(nda2) at 51 kDa and $\alpha$-tubulin-2 (atb2) at 50 kDa, while the fainter third band corresponded to
mCherry-atb2 at 79 kDa (fig. 2). To analyze the scanned images, we inverted the images so that
labeled regions corresponded to high intensity, drew equally-sized regions of interest around each
band, and determined the average pixel intensity in each region. From this, we determined the
fraction of intensity in the mCherry-atb2 band relative to the total. Each lane had similar ratios
(data not shown), and we averaged the results for each lane.

**Cell preparation and confocal imaging**

We grew cells using standard techniques (Supporting Material) and cultured them on glass-bottomed
dishes at 36°C for 2–4 hrs to allow monopolar spindles to form. The dishes were transferred to the
microscope in less than 60 sec to prevent the monopolar spindles from becoming bipolar. Images
were taken on an spinning disk (Yokogawa, Musashino, Japan) Nikon Eclipse Ti inverted confocal
microscope (Nikon, Tokyo, Japan) with a 100X, 1.4 NA Plan Apo oil-immersion objective, an iXon
Ultra 897 EM-CCD camera (Andor, Belfast, United Kingdom) and a TIZSH Stage Top incubator
(Tokai Hit, Fujinomiya, Japan) warmed to 36°C. Three-dimensional images were obtained with an
EM Gain of 300, and an exposure time per plane of 40 to 150 msec with 595 nm laser illumination
for each of 5 focal planes separated by 500 nm in z, and subsequent stacks are separated by 4–6
sec. Images of fig. 2A are displayed as pixel-interpolated maximum-intensity projections.
Mechanisms of kinetochore capture

Figure 2: Measurements of single MT dynamics and length in monopolar mitotic spindles. (A) Image sequences. Images are pixel-interpolated maximum intensity projections of monopolar spindles with mCherry-labeled MTs. Red arrowheads indicate single MTs, time is shown in sec, and the scale bar is 1 µm. (B) Example raw maximum-intensity-projected image (top) and analyzed image (lower). The red cross indicates the center of brightness of the SPB, green boxes the regions analyzed near MT tips, blue lines the fits to the MT backbone positions, and blue dots the fits to the MT tip positions. The scale bar is 1 µm. (C) Example traces of MT length versus time (points) and fits to growing and shrinking events (lines). (D) Western blot used to quantify fraction of fluorescently labeled tubulin. (E) Histograms of growth speed and shrinking speed. (F) Histograms of growth time and MT length.

Image and data analysis

In our images of monopolar spindles, we were able to observe dynamic MTs (fig. 2). We quantified the dynamic instability of mitotic MTs by determining the location of the SPBs and the tip of each emanating MT with image analysis software adapted from TipTracker. To identify the center of the monopolar spindle, we noted that near the two spindle-pole bodies (SPBs), many short and overlapping MTs produce a bright fluorescent region. When using this software, one selects a rectangle around this region, and our program then computes the center of intensity within that region to estimate the SPB location and determines the length of each MT in a given frame by assuming the MT formed a line between the estimated SPB location and the MT tip position. Visual inspection of each frame confirmed that MTs in the monopolar spindles formed in these cells were typically straight lines emanating from the bright spindle center region (fig. 2). Each frame is analyzed separately, and the MT length data stored for analysis. The software allows us to make MT length and angle measurements with subpixel resolution and quantify the lengths and dynamics of mitotic MTs in monopolar spindles.

We identified growth and shrinking events by comparing the movies and plots of MT length
Mechanisms of kinetochore capture

| Parameter                        | Symbol | Reference value | Range              | Notes                                                                 |
|----------------------------------|--------|-----------------|--------------------|----------------------------------------------------------------------|
| Nuclear envelope radius          | R      | 1.5 µm          | –                  | Kalinina et al. (24)                                                  |
| KC diameter                      | σ_{KC} | 200 nm          | –                  | Ding et al. (44)                                                      |
| MT diameter                      | σ_{MT} | 25 nm           | –                  | Alberts et al. (45)                                                  |
| MT angular diffusion coefficient | D_{θ}  | –               | Varies with MT length | Kalinina et al. (24), Supporting Material Dogterom and Yurke (37) and Janson et al. (38) |
| Force-induced catastrophe constant| α      | 0.5 pN^{-1}     | –                  |                                                                      |

**Slow dynamic instability model**
- Growth speed: \( v_g = 2.7 \, \mu m \, min^{-1} \) (0.7–11 \, \mu m \, min^{-1} )
- Shrinking speed: \( v_s = 3.8 \, \mu m \, min^{-1} \) (1–16 \, \mu m \, min^{-1} )
- Grow-to-pause frequency: \( f_{+0} = 1.8 \, min^{-1} \) (0.4–7.1 \, min^{-1} )
- Shrink-to-pause frequency: \( f_{-0} = 2.53 \, min^{-1} \) (1.2–5 \, min^{-1} )
- Pause-to-shrink frequency: \( f_{0-} = 0.49 \, min^{-1} \) (0.2–1 \, min^{-1} )
- Pause-to-grow frequency: \( f_{0+} = 0 \, min^{-1} \) Transitions from pausing to growing appeared infrequent in Kalinina et al. (24)

**Fast dynamic instability model**
- Growth speed: \( v_g = 4.1 \, \mu m \, min^{-1} \) (1–10 \, \mu m \, min^{-1} )
- Shrinking speed: \( v_s = 6.7 \, \mu m \, min^{-1} \) (5–25 \, \mu m \, min^{-1} )
- Catastrophe frequency: \( f_{cat} = 3.7 \, min^{-1} \) (1–8 \, min^{-1} )
- Rescue frequency: \( f_{res} = 0.175 \, min^{-1} \) (0–8 \, min^{-1} )

Table 1: Model parameter values.

versus time to identify starting and ending times of events. We then performed weighted least-squares linear fits to the MT length versus time during each event (fig. 2). Growth and shrinking speeds were the slopes determined from the fits, and the catastrophe and rescue times were the duration of the events before a switch.

**Results and discussion**

**Experimental results**

Kalinina et al. (24) found that fission yeast mitotic MTs on average spent 75% of their time in a paused state and had lifetimes of 3 min, growth speed \( v_g = 2.7 \, \mu m \, min^{-1} \), and shrinking speed \( v_s = 3.8 \, \mu m \, min^{-1} \). These results differed from the results of Sagolla et al. (32), who observed highly dynamic polar MTs in early mitosis (before spindle formation), with lifetimes of seconds and a shrinking speed \( v_s = 20 \, \mu m \, min^{-1} \), and more recent work on meiotic MTs (27). These differences in lifetime and dynamics could be related to the stage of mitosis (before and after spindle formation), the number of MTs per bundle, and/or to the fraction of fluorescent tubulin in the cells (which affects MT dynamics (46)). The differences are most likely due to MT bundling, which alters MT dynamics (47, 48). Since KC capture could occur either by single MTs that are more dynamic or bundled MTs that are more stable, we undertook additional measurements of mitotic MT dynamic instability in S. pombe.

We adapted the strategy of Costa et al. (41), who used temperature-sensitive inactivation of the kinesin-5 motor (cut7-24) to obtain cells stably arrested in a monopolar state. Fission yeast carrying the cut7-ts allele arrest in early mitosis at restrictive temperature (36-37°C), because bipolar spindles cannot form when cut7p is inactive (40). These cells instead form monopolar
mitotic spindles, in which individual fluorescently labeled mitotic MTs can be imaged and tracked (fig. 2A, B) (41).

To measure MT length, we adapted the TipTracker algorithm (42, 43) to measure MT lengths in monopolar spindles (Methods, fig. 2B). Brighter MTs showed lengths that were more stable in time, and dimmer MTs showed more rapid dynamics (fig. 2A). We identified the dimmer, more dynamic MTs as single MTs, as in previous work (41). We analyzed 20 MTs from 15 cells that showed low intensity compared to other MTs in the same cell and relatively fast dynamics. We determined growth and shrinking events and their associated speeds and times (fig. 2C, Methods).

Fluorescent-protein fusions to tubulin in fission yeast can alter MT dynamics (46). We studied fission yeast carrying mCherry-atb2 that is an additional copy of this α-tubulin gene under a weak promoter (39). We used Western blotting to determine the fraction of tubulin our cells that was fluorescently tagged (fig. 2D, Methods) and found a low fraction of 8.8% ± 0.5% labeled α-tubulin. These MTs had a median growth speed $v_g = 4.1 \pm 1.5 \, \mu m \, min^{-1}$ and shrinking speed $v_s = 6.7 \pm 1.4 \, \mu m \, min^{-1}$ (fig. 2E). Measuring the rescue frequency was challenging, because it was difficult to distinguish rescue from complete shrinkage followed by regrowth of a different MT in the same area. We saw one possible rescue event, which gave a bound $f_r \leq 0.175 \, min^{-1}$.

Our data were collected at 37°C, and MT dynamic instability is sensitive to temperature (49). However, Kalinina et al. found relatively little change in fission-yeast MT dynamic instability parameters between 24°C and 32°C (24), suggesting that S. pombe mitotic MT dynamic instability may not vary markedly with temperature in this regime.

Pooled length measurements of MTs we identified as single MTs (including all measurements, not just points identified as growing/shrinking events) had a median $\langle L \rangle = 1.31 \pm 0.02 \, \mu m$ (fig. 2F). We compared this to the predicted mean length for dynamic instability in an infinite volume (neglecting boundary effects): in the bounded growth regime $\langle L \rangle = v_g v_s / (v_s f_c - v_g f_r)$ (50). If we estimate the mean length using our median dynamic instability parameters with the upper bound on the rescue frequency of $f_r \leq 0.175 \, min^{-1}$, we predict $\langle L \rangle = 1.14 \, \mu m$, while using $f_r = 0$ gives $\langle L \rangle = 1.11 \, \mu m$. There is little difference between these values, showing that the mean MT length is primarily determined by the growth speed and catastrophe frequency in this low-rescue-frequency regime. This typical MT length estimated from the dynamic instability parameters is consistent with our direct measurements of MT length.

**Model results**

**Kinetochoore capture by single microtubules with fast or slow dynamic instability**

Since KC capture could occur either by single MTs that are more dynamic or bundled MTs that are more stable, we sought to understand how these different MT arrangements affect KC capture in fission yeast. We studied two reference parameter sets that we denote slow (based on the Kalinina et al. measurements) and fast (based on our measurements). In the slow model, MTs can be growing, shrinking, or paused. The reference parameter values (table 1) were taken from Kalinina et al., (24) assuming that transitions from pausing to growing did not occur. In the fast model, we considered only growing and shrinking states with reference parameter values determined from our measurements. Examples of the resulting MT length as a function of time and the dynamics of KC capture are shown in figure 1. For each model, we varied parameters around the reference values by factors of 4-20 (table 1) so that we could study the dependence of the capture time on parameter values. We then studied both slow and fast models with and without MT rotational
Mechanisms of kinetochore capture

Figure 3: Dependence of capture time on MT length and rotational diffusion. (A) Slow model. (B) Fast model. Points are results of simulations with all dynamics parameters varied for search and capture only (blue) and search and capture with microtubule rotational diffusion (orange). Lines are fits to $\langle \tau_c \rangle = A\langle L \rangle^{-1} + B$. Insets, fractional decrease in capture time when rotational diffusion is added to the model.

Kalinina et al. found that 3 polar MTs were typically visible during their KC capture experiments, and that the time of KC capture was sensitive to MT number (24). Here we determined capture times for a single MT. Our model results for capture by a single MT and slow dynamic instability agree well with those of Kalinina et al. (fig. S2). Studying capture by single MTs allowed us to focus on the effect of dynamic instability parameters and rotational diffusion.

**Parametric study using polynomial chaos expansion**

To understand how the mean capture time $\langle \tau_c \rangle$ and the mean MT length $\langle L \rangle$ depend on model parameters (table 1), we used polynomial chaos (PC) expansion (51, 52), a widely used technique for uncertainty quantification. PC expansion is a type of spectral method in which we represent $\langle \tau_c \rangle$ and $\langle L \rangle$ as functions of the dynamic instability parameters in a high-order, multivariate, orthogonal polynomial basis, here of Legendre type. This allowed us to use a relatively small number of simulations, corresponding to random samples of input parameters generated uniformly over their allowed ranges (table 1), to accurately approximate $\langle \tau_c \rangle$ and $\langle L \rangle$ over the full multidimensional parameter space (53–55). We then used this expansion to perform global and local sensitivity analysis to determine which dynamic instability parameters are of most importance to $\langle \tau_c \rangle$ and $\langle L \rangle$. The global sensitivity analysis provides information on the importance of each parameter in terms of its contribution to the overall solution variability, while the local analysis identifies the dependence of the solution on each parameter in the small neighborhood of its nominal value. To construct the PC expansions, here of total degree three, we used a regression approach based on $\ell_1$-minimization (53, 55) using 250 randomly sampled parameter sets, and the corresponding realizations of $\langle \tau_c \rangle$ and $\langle L \rangle$. In figs. 3 and 5–7 below, each point corresponds to one of the 250 parameter sets.
Figure 4: Dependence of capture time on growth speed and effective catastrophe frequency for search and capture model determined from polynomial chaos expansion. (A) Slow model. (B) Fast model. Color shows capture time in minutes. White arrows show the magnitude and direction of most rapid change in $\langle \tau_c \rangle$ (the gradient). The white line is the curve that follows the gradient and passes through the reference parameter set.

Effects of varying mean MT length

The rotational diffusion coefficient of a rod varies as $L^{-3}$, making MT length important for KC capture by a fixed-length MT (24). We found that the MT mean length was also important for KC capture with dynamic instability. Indeed, the primary determinant of the capture time was the mean MT length (fig. 3), for all models studied. The capture time decreased by approximately a factor of 10 as $\langle L \rangle$ increased from short ($\lesssim 0.3 \mu m$) to long ($\gtrsim 1.5 \mu m$, half the nuclear diameter). A longer MT increases the effective number of binding sites for a KC, lowering the capture time. If the number of binding sites were the sole factor determining the rate of KC capture, we would expect $\langle \tau_c \rangle \sim \langle L \rangle^{-1}$. The dependence in our model is more complex, because the MT dynamics also change with $\langle L \rangle$. Nevertheless, fitting the capture time as a function of the mean length to the form $\langle \tau_c \rangle = A(L)^{-1} + B$ (solid lines in fig. 3) gave reasonable agreement with our simulation results.

For all MT lengths and both slow and fast dynamic instability, adding MT rotational diffusion to a model with only dynamic instability reduced the capture time. The contribution of rotational diffusion depended on the MT dynamics: $\langle \tau_c \rangle$ decreased by 23% on average (25% at most) for the slow model, and by 9% on average (16% at most) for the fast model. The speed up due to rotational diffusion was larger for slower MT dynamics and shorter MTs (fig. 3). For the most relevant MT lengths in fission yeast of around 1 $\mu m$, rotational diffusion shortened the capture time by at most 5%. This suggests that while rotational diffusion does speed up KC capture, it makes a relatively small quantitative contribution, consistent with recent work (27).

Sensitivity analysis

We performed sensitivity analysis to check how the capture time and MT mean length vary with model parameters. For dynamic instability with no boundary effects, we would expect a mean length of $\langle L \rangle = v_g/f_{+0}$ in the slow model and $\langle L \rangle = v_gv_s/(v_s f_c - v_g f_r) \approx v_g/f_c$ in the fast model ([52] and Supporting Material). While interactions of MTs with the nuclear envelope alter this relationship, we expected that the MT mean length and therefore the capture time depend primarily on the MT growth speed and the catastrophe frequency (or its analogue in the slow model, the...
To test these relationships, we performed a global sensitivity analysis of the mean capture time and MT length to the dynamic instability parameters. To quantify this sensitivity, we relied on the analysis of the variance of $\langle \tau_c \rangle$ and $\langle L \rangle$ based on the so-called Sobol’ decomposition (56), which we computed directly using the PC expansion (57). The dynamic instability parameters were treated as random variables uniformly distributed over the ranges in table 1. The overall contribution of each parameter to the solution variance was quantified using its total Sobol’ index (57); a larger value indicates a higher degree of sensitivity of the model to that parameter (table S2). As expected, $\langle \tau_c \rangle$ and $\langle L \rangle$ are most sensitive to the growth speed and effective catastrophe frequency, and this dependence is not altered significantly by the addition of rotational diffusion to the model.

We also determined the local sensitivity of the capture time to these parameters by computing the gradient (the local direction of steepest change) of $\langle \tau_c \rangle$ as a function of the growth speed and catastrophe frequency from the PC expansion, with other parameters fixed at their reference values (fig. 4; here white line follows the local gradient and passes through the reference parameters).
Variation with individual parameters

We used both our individual simulations and the PC expansion to study how the MT mean length and capture time depend on variation of individual parameters (figs. 5, S3, S4). The solid lines are the predicted dependence of $\langle L \rangle$ for the reference parameter set on the varied single parameter from the PC expansion, while the dashed line is the analytic prediction for $\langle L \rangle$ with unconstrained dynamic instability. As expected, the analytic solution that neglects boundary interactions matches the data and PC expansion well when $\langle L \rangle$ is relatively small, but differs significantly where $\langle L \rangle \gtrsim 1 \mu m$.

The variation of the capture time with individual parameters show qualitatively similar dependence (figs. 6, S3, S4). Parameter sets with higher growth speed and lower catastrophe frequency lead to shorter capture times. Growth speed is the main parameter affecting capture time, causing it to vary by up to a factor of five, consistent with its total Sobol’ index (table S2). MTs with higher growth speeds search a given direction more quickly than slower MTs with the same orientation, resulting in lower capture time; fast-growing MTs have also typically longer mean lengths. Catastrophe frequency also affects the capture time, because high catastrophe frequency tends to reduce the mean length of MTs. MTs with low catastrophe frequency typically reach the nuclear...
envelope and undergo force-induced catastrophe, which limits the advantage gained by lowering the catastrophe frequency. The shrinking speed and rescue frequency have little effect on the capture time, because they don’t significantly affect the mean MT length (figs. S3, S4). For simulations both with and without MT rotational diffusion, varying these parameters alone varies the capture time by $\lesssim 25\%$.

Lateral versus end-on capture

In fission yeast, KC capture occurs primarily via lateral attachment, with about 75% of captures occurring $>500$ nm away from the MT tip (24), consistent with other work both in large and small cells (14, 19, 20, 23, 25). In our model, we classified attachments as end-on if the capture occurred within $4.5\sigma_{MT}$ ($\approx 113$ nm) from the MT tip, and lateral otherwise. We found that lateral attachments are more likely in our model, but the fraction of captures that occur laterally depends on the mean MT length (fig. 7). Increasing $\langle L \rangle$ increased the probability of lateral capture, because the available MT surface area for binding laterally increases.

Remarkably, we found that lateral captures predominated even in the model with dynamic instability only (no rotational diffusion). This occurred for two reasons: first, interactions of MTs with the nuclear envelope caused some MT reorientation in the absence of thermal diffusion. However, we observed significant lateral attachment even for relatively short MTs that didn’t interact with the nuclear envelope, because KC diffusion allowed lateral attachment even for fixed-orientation MTs.

Surprisingly, adding rotational diffusion to the model made end-on attachments more likely: diffusion decreases the probability of lateral capture, an effect that was more noticeable for shorter MTs. Rotational diffusion allows the MT to sweep through space, increasing the volume searched by the MT. This increase in effective volume searched is largest at the MT tip because the tip is furthest from the pivot point at the SPB. This effect was more important for shorter MTs where end-on attachments were more likely.
Conclusions

The search-and-capture model has been considered the main mechanism by which KCs are captured since the discovery of MT dynamic instability \([9, 11, 13]\). The original picture of search and capture posited that MTs undergo dynamic instability nucleated from centrosomes until they form end-on attachments with KCs. Additional effects such as diffusion of KCs, \([12, 15]\), a bias in MT growth toward chromosomes \([16, 18]\), MT nucleation from KCs \([15, 21, 22]\), spatial distribution and rotation of chromosomes \([15, 19, 20]\), and KC size decreases after capture \([20]\) can make search and capture more rapid. MT rotational diffusion and lateral capture were found to be important mechanisms in fission yeast \([24]\). This work suggested that MT rotational diffusion about centrosomes is important, but because the model assumed fixed-length MTs it was unable to directly evaluate the relative importance of rotational diffusion and dynamic instability to KC capture.

We developed a biophysical model of KC capture in fission yeast that includes MT dynamic instability and rotational diffusion, KC diffusion, and interactions with the nuclear envelope (fig. 1). We used the model to compare the time course of KC capture in models with and without MT rotational diffusion. The mitotic MTs measured by Kalinina et al. \(24\) were primarily paused \(24\), and the measured dynamics appeared slow compared to previous measurements \(32\). Therefore, we performed further measurements of the dynamics of MTs in fission yeast monopolar spindles with low-level fluorescent tubulin labeling. We found more rapid dynamic instability of single MTs, with little pausing (fig. 2). This motivated us to consider two dynamic instability models: a slow model with dynamics measured by Kalinina et al., and a fast model with dynamics measured in this work (table 1).

We studied KC capture by single MTs in our model both for the reference parameter sets and for broad ranges of parameters around the reference sets. In all cases, KC capture occurred more quickly when rotational diffusion about SPBs was added to a model with only dynamic instability (fig. 3): \(\langle \tau_c \rangle\) decreased by 23% on average for the slow model, and by 9% on average for the fast model, similar to recent work \(27\). We found that the primary determinant of the capture time was the mean MT length. Longer MTs have a larger surface on which the KC can bind, which suggests that the capture time \(\langle \tau_c \rangle \sim \langle L \rangle^{-1}\), as we observed. Since the MT growth speed and catastrophe frequency controlled the mean length in the parameter regimes we studied, these parameters had the biggest effect on the capture time (table S2), fig. 4. For the experimentally measured mitotic polar MT lengths of \(\sim 1 \mu m\) in fission yeast, adding rotational diffusion to our model shortened the capture time by at most 5%. This suggests that rotational diffusion causes relatively small changes to the capture time.

We further examined how MT length (fig. 5) and capture time (fig. 6) varied with the growth speed and catastrophe frequency. The mean length varied qualitatively as expected from the predictions of analytic theory that neglects MT interactions with the nuclear envelope. In the computational model, changes from this theory became significant for longer MTs. Parameter sets with higher growth speed and lower catastrophe frequency that lead to longer mean MT lengths had shorter capture times.

The original search-and-capture model supposed that KC attachments to MTs are end on, but Kalinina et al. \(24\) and others \(14, 19, 20, 23, 25\) observed primarily lateral attachments. Therefore, we studied how the probability of lateral or end-on attachment varied with MT length and the presence or absence of rotational diffusion in the model (fig. 7). Lateral captures predominated, even in the absence of MT rotational diffusion. Somewhat counterintuitively, we found that adding rotational diffusion to the model made end-on attachments more likely. A diffusing MT searches a larger volume of space than a rotationally constrained MT, an effect which is largest for the MT
tip. Therefore, rotational diffusion can decrease lateral attachment.

Recently, Magidson et al. proposed that initial lateral contacts are important to the timing and low error rates of KC-spindle attachment in human cells (20). Although many proteins are known to contribute to KC-MT attachment, including motors and non-motor MT-binding proteins (the Ndc80 complex, other components of the KMN network, Ska or Dam1, and others) (58), the contributions of end-on versus lateral KC-MT attachment pathways are not fully understood. Our work suggests that lateral captures are enhanced when MT rotational diffusion about SPBs is decreased or eliminated, as would be the case in large spindles with many long MTs. Future work dissecting contributions of lateral and end-on attachment mechanisms may contribute interesting additional insights into this biologically important problem.

Author contributions
RB, OS-S, CE, ZRG, AD, JRM, MAG, and MDB designed research; RB, OS-S, CE, ZRG, PJF, SM, AC, and MDB performed research; RB, OS-S, CE, ZRG, PJF, AD, MAG, and MDB contributed analytic tools; RB, OS-S, CE, ZRG, PJF, SM, AC, and MDB analyzed data; RB, CE, ZRG, PJF, and MDB wrote the manuscript.

Acknowledgements
We thank Iain Hagan and Jonathan Millar for providing fission yeast strains, Keith Gull for providing the TAT-1 antibody, and Nenad Pavin for useful discussions. This work was supported by NSF grants DMR-0847685 and DMR-1551095 to MDB, MRSEC DMR-0820579 and DMR-1420736 to MAG, and CMMI-1454601 to AD; and NIH grants K25 GM110486 to MDB and R01 GM033787 to JRM. This work utilized the Janus supercomputer, which is supported by the National Science Foundation (CNS-0821794), the University of Colorado Boulder, the University of Colorado Denver, and the National Center for Atmospheric Research. The Janus supercomputer is operated by the University of Colorado Boulder.

References
1. McIntosh, J. R., M. I. Molodtsov, and F. I. Ataullakhanov, 2012. Biophysics of mitosis. Quarterly Reviews of Biophysics 45:147–207.
2. Cottingham, F. R., and M. A. Hoyt, 1997. Mitotic spindle positioning in Saccharomyces cerevisiae is accomplished by antagonistically acting microtubule motor proteins. The Journal of Cell Biology 138:1041–1053.
3. Goshima, G., and R. D. Vale, 2003. The roles of microtubule-based motor proteins in mitosis. The Journal of Cell Biology 162:1003–1016.
4. Grishchuk, E. L., and J. R. McIntosh, 2006. Microtubule depolymerization can drive poleward chromosome motion in fission yeast. The EMBO Journal 25:4888–4896.
5. Schroer, T. A., 2001. Microtubules don and doff their caps: dynamic attachments at plus and minus ends. Current Opinion in Cell Biology 13:92–96.
6. Garcia, M. A., N. Koonrugsa, and T. Toda, 2002. Spindle–kinetochore attachment requires
the combined action of Kin I-like Klp5/6 and Alp14/Dis1-MAPs in fission yeast. The EMBO
Journal 21:6015.

7. Akhmanova, A., and M. O. Steinmetz, 2008. Tracking the ends: a dynamic protein network
controls the fate of microtubule tips. Nature Reviews Molecular Cell Biology 9:309–322.

8. Duesberg, P., R. Li, A. Fabarius, and R. Hehlmann, 2006. Aneuploidy and cancer: from
correlation to causation. In T. Dittmar, K. S. Zaenker, and A. Schmidt, editors, Infection and
Inflammation: Impacts on Oncogenesis, Karger, Basel, volume 13 of Contrib Microbiol, 16–44.

9. Mitchison, T., and M. Kirschner, 1984. Dynamic instability of microtubule growth. Nature
312:237–242.

10. Mitchison, T. J., and M. W. Kirschner, 1985. Properties of the kinetochore in vitro. II. Micro-
tubule capture and ATP-dependent translocation. The Journal of Cell Biology 101:766–777.

11. Hill, T. L., 1985. Theoretical problems related to the attachment of microtubules to kineto-
chores. Proceedings of the National Academy of Sciences 82:4404–4408.

12. Holy, T. E., and S. Leibler, 1994. Dynamic instability of microtubules as an efficient way to
search in space. Proceedings of the National Academy of Sciences 91:5682–5685.

13. Heald, R., and A. Khodjakov, 2015. Thirty years of search and capture: The complex simplicity
of mitotic spindle assembly. The Journal of Cell Biology 211:1103–1111.

14. Rieder, C. L., and S. P. Alexander, 1990. Kinetochores are transported poleward along a
single astral microtubule during chromosome attachment to the spindle in newt lung cells. The
Journal of Cell Biology 110:81–95.

15. Paul, R., R. Wollman, W. T. Silkworth, I. K. Nardi, D. Cimini, and A. Mogilner, 2009. Com-
puter simulations predict that chromosome movements and rotations accelerate mitotic spindle
assembly without compromising accuracy. Proceedings of the National Academy of Sciences
106:15708–15713.

16. Carazo-Salas, R. E., G. Guarguaglini, O. J. Gruss, A. Segref, E. Karsenti, and I. W. Mattaj,
1999. Generation of GTP-bound Ran by RCC1 is required for chromatin-induced mitotic
spindle formation. Nature 400:178–181.

17. Wollman, R., E. Cytrynbaum, J. Jones, T. Meyer, J. Scholey, and A. Mogilner, 2005. Efficient
Chromosome Capture Requires a Bias in the ‘Search-and-Capture’ Process during Mitotic-
Spindle Assembly. Current Biology 15:828–832.

18. O’Connell, C. B., J. Lončarek, P. Kaláb, and A. Khodjakov, 2009. Relative contributions of
chromatin and kinetochores to mitotic spindle assembly. The Journal of Cell Biology 187:43–51.

19. Magidson, V., C. B. O’Connell, J. Lončarek, R. Paul, A. Mogilner, and A. Khodjakov, 2011.
The Spatial Arrangement of Chromosomes during Prometaphase Facilitates Spindle Assembly.
Cell 146:555–567.

20. Magidson, V., R. Paul, N. Yang, J. G. Ault, C. B. O’Connell, I. Tikhonenko, B. F. McEwen,
A. Mogilner, and A. Khodjakov, 2015. Adaptive changes in the kinetochore architecture facil-
itate proper spindle assembly. Nature Cell Biology .
21. Witt, P. L., H. Ris, and G. G. Borisy, 1980. Origin of kinetochore microtubules in Chinese hamster ovary cells. *Chromosoma* 81:483–505.

22. Kitamura, E., K. Tanaka, S. Komoto, Y. Kitamura, C. Antony, and T. U. Tanaka, 2010. Kinetochores Generate Microtubules with Distal Plus Ends: Their Roles and Limited Lifetime in Mitosis. *Developmental Cell* 18:248–259.

23. Tanaka, K., N. Mukae, H. Dewar, M. van Breugel, E. K. James, A. R. Prescott, C. Antony, and T. U. Tanaka, 2005. Molecular mechanisms of kinetochore capture by spindle microtubules. *Nature* 434:987–994.

24. Kalinina, I., A. Nandi, P. Delivani, M. R. Chacón, A. H. Klemm, D. Ramunno-Johnson, A. Krull, B. Lindner, N. Pavin, and I. M. Tolić-Norrelykke, 2012. Pivoting of microtubules around the spindle pole accelerates kinetochore capture. *Nature Cell Biology*.

25. Kitajima, T. S., M. Ohsugi, and J. Ellenberg, 2011. Complete Kinetochore Tracking Reveals Error-Prone Homologous Chromosome Biorientation in Mammalian Oocytes. *Cell* 146:568–581.

26. Gopalakrishnan, M., and B. S. Govindan, 2011. A First-Passage-Time Theory for Search and Capture of Chromosomes by Microtubules in Mitosis. *Bulletin of Mathematical Biology* 73:2483–2506.

27. Cojoc, G., A.-M. Florescu, A. Krull, A. H. Klemm, N. Pavin, F. Jülicher, and I. M. Tolić, 2016. Paired arrangement of kinetochores together with microtubule pivoting and dynamics drive kinetochore capture in meiosis I. *Scientific Reports* 6:25736.

28. Gao, T., R. Blackwell, M. A. Glaser, M. D. Betterton, and M. J. Shelley, 2015. Multiscale modeling and simulation of microtubule-motor-protein assemblies. *Physical Review E* 92:062709.

29. Gao, T., R. Blackwell, M. A. Glaser, M. Betterton, and M. J. Shelley, 2015. Multiscale Polar Theory of Microtubule and Motor-Protein Assemblies. *Physical Review Letters* 114:048101.

30. Kuan, H.-S., R. Blackwell, L. E. Hough, M. A. Glaser, and M. D. Betterton, 2015. Hysteresis, reentrance, and glassy dynamics in systems of self-propelled rods. *Physical Review E* 92:060501.

31. Blackwell, R., O. Sweezy-Schindler, C. Baldwin, L. E. Hough, M. A. Glaser, and M. D. Betterton, 2016. Microscopic origins of anisotropic active stress in motor-driven nematic liquid crystals. *Soft Matter*.

32. Sagolla, M. J., S. Uzawa, and W. Z. Cande, 2003. Individual microtubule dynamics contribute to the function of mitotic and cytoplasmic arrays in fission yeast. *Journal of Cell Science* 116:4891–4903.

33. Laan, L., N. Pavin, J. Husson, G. Romet-Lemonne, M. van Duijn, M. P. López, R. D. Vale, F. Jülicher, S. L. Reck-Peterson, and M. Dogterom, 2012. Cortical Dynein Controls Microtubule Dynamics to Generate Pulling Forces that Position Microtubule Asters. *Cell* 148:502–514.

34. Pavin, N., L. Laan, R. Ma, M. Dogterom, and F. Jülicher, 2012. Positioning of microtubule organizing centers by cortical pushing and pulling forces. *New Journal of Physics* 14:105025.

35. Ma, R., L. Laan, M. Dogterom, N. Pavin, and F. Jülicher, 2014. General theory for the mechanics of confined microtubule asters. *New Journal of Physics* 16:013018.
36. Tischer, C., D. Brunner, and M. Dogterom, 2009. Force- and kinesin-8-dependent effects in the spatial regulation of fission yeast microtubule dynamics. Molecular Systems Biology 5:1–10.

37. Dogterom, M., and B. Yurke, 1997. Measurement of the Force-Velocity Relation for Growing Microtubules. Science 278:856–860.

38. Janson, M. E., M. E. de Dood, and M. Dogterom, 2003. Dynamic instability of microtubules is regulated by force. The Journal of Cell Biology 161:1029–1034.

39. Yamagishi, Y., C. H. Yang, Y. Tanno, and Y. Watanabe, 2012. MPS1/Mph1 phosphorylates the kinetochore protein KNL1/Spc7 to recruit SAC components. Nature Cell Biology 14:746–752.

40. Hagan, I., and M. Yanagida, 1990. Novel potential mitotic motor protein encoded by the fission yeast cut7+ gene. Nature 347:563–566.

41. Costa, J., C. Fu, V. Syrovatkina, and P. T. Tran, 2013. Chapter 24 - Imaging Individual Spindle Microtubule Dynamics in Fission Yeast. In J. J. C. Wilson, and Leslie, editors, Methods in Cell Biology, Academic Press, volume 115 of Microtubules, in Vitro, 385–394.

42. Demchouk, A. O., M. K. Gardner, and D. J. Odde, 2011. Microtubule Tip Tracking and Tip Structures at the Nanometer Scale Using Digital Fluorescence Microscopy. Cellular and Molecular Bioengineering 4:192–204.

43. Prahl, L. S., B. T. Castle, M. K. Gardner, and D. J. Odde, 2014. Chapter Three - Quantitative Analysis of Microtubule Self-assembly Kinetics and Tip Structure. In R. D. Vale, editor, Methods in Enzymology, Academic Press, volume 540 of Reconstituting the Cytoskeleton, 35–52.

44. Ding, R., K. L. McDonald, and J. R. McIntosh, 1993. Three-dimensional reconstruction and analysis of mitotic spindles from the yeast, Schizosaccharomyces pombe. The Journal of Cell Biology 120:141–151.

45. Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, 2008. Molecular Biology of the Cell. Garland, New York, 5th edition.

46. Snaith, H. A., A. Anders, I. Samejima, and K. E. Sawin, 2010. Chapter 9 - New and Old Reagents for Fluorescent Protein Tagging of Microtubules in Fission Yeast: Experimental and Critical Evaluation. In Lynne Cassimeris and Phong Tran, editor, Methods in Cell Biology, Academic Press, volume Volume 97, 147–172.

47. Bratman, S. V., and F. Chang, 2007. Stabilization of Overlapping Microtubules by Fission Yeast CLASP. Developmental Cell 13:812–827.

48. Bratman, S. V., and F. Chang, 2008. Mechanisms for maintaining microtubule bundles. Trends in Cell Biology 18:580–586.

49. Fygenson, D. K., E. Braun, and A. Libchaber, 1994. Phase diagram of microtubules. Physical Review E 50:1579.

50. Dogterom, M., and S. Leibler, 1993. Physical aspects of the growth and regulation of microtubule structures. Physical Review Letters 70:1347–1350.

51. Ghanem, R., and P. Spanos, 2002. Stochastic Finite Elements: A Spectral Approach. Dover.
52. Xiu, D., and G. Karniadakis, 2002. The Wiener-Askey polynomial chaos for stochastic differential equations. *SIAM Journal on Scientific Computing* 24:619–644.

53. Doostan, A., and H. Owhadi, 2011. A non-adapted sparse approximation of PDEs with stochastic inputs. *Journal of Computational Physics* 230:3015–3034.

54. Hampton, J., and A. Doostan, 2015. Compressive sampling of polynomial chaos expansions: Convergence analysis and sampling strategies. *Journal of Computational Physics* 280:363–386.

55. Hadigol, M., K. Maute, and A. Doostan, 2015. On uncertainty quantification of lithium-ion batteries: Application to an LiC6/LiCoO2 cell. *Journal of Power Sources* 300:507–524.

56. Sobol’, I., 1990. On sensitivity estimation for nonlinear mathematical models. *Matematicheskoe Modelirovanie* 2:112–118.

57. Sudret, B., 2008. Global sensitivity analysis using polynomial chaos expansions. *Reliability Engineering and System Safety* 93:964–979.

58. Cheeseman, I. M., and A. Desai, 2008. Molecular architecture of the kinetochore–microtubule interface. *Nature Reviews Molecular Cell Biology* 9:33–46.