Tension-dependent Regulation of Microtubule Dynamics at Kinetochores Can Explain Metaphase Congression in Yeast

Melissa K. Gardner,* Chad G. Pearson,† Brian L. Sprague,‡ Ted R. Zarzar,† Kerry Bloom,† E. D. Salmon,† and David J. Odde*

*Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455; †Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599; and ‡Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, Bethesda, MD 20892

Submitted April 3, 2005; Revised May 20, 2005; Accepted May 23, 2005
Monitoring Editor: Orna Cohen-Fix

During metaphase in budding yeast mitosis, sister kinetochores are tethered to opposite poles and separated, stretching their intervening chromatin, by singly attached kinetochore microtubules (kMTs). Kinetochore movements are coupled to single microtubule plus-end polymerization/depolymerization at kinetochore attachment sites. Here, we use computer modeling to test possible mechanisms controlling chromosome alignment during yeast metaphase by simulating experiments that determine the 1) mean positions of kinetochore Cse4-GFP, 2) extent of oscillation of kinetochores during metaphase as measured by fluorescence recovery after photobleaching (FRAP) of kinetochore Cse4-GFP, 3) dynamics of kMTs as measured by FRAP of GFP-tubulin, and 4) mean positions of unreplicated chromosome kinetochores that lack pulling forces from a sister kinetochore. We rule out a number of possible models and find the best fit between theory and experiment when it is assumed that kinetochores sense both a spatial gradient that suppresses kMT catastrophe near the poles and attachment site tension that promotes kMT rescue at higher amounts of chromatin stretch.

INTRODUCTION

During mitosis, a dynamic array of kinetochore microtubules (kMTs) serve to accurately segregate a duplicated genome into two complete sets of chromosomes (Inoue and Salmon, 1995; Rieder and Salmon, 1998; Nasmyth, 2002; Howard and Hyman, 2003; Scholey et al., 2003). Budding yeast offers an attractive system for answering fundamental questions about the regulation of kMT dynamics, because each kinetochore is thought to be attached to only one kMT plus-end (Peterson and Ris, 1976; Winey et al., 1995; O’Toole et al., 1999). The relative simplicity of the yeast spindle, with ~16 kMT minus-ends anchored at each pole, makes this an excellent system for computational modeling. Although the dynamics of individual kMTs have not been directly observed in vivo, kMT-plus ends seem to exhibit dynamic instability, switching stochastically between extended periods of polymerization and depolymerization (Maddox et al., 2000; Pearson et al., 2001). In general, regulation of microtubule (MT) dynamic instability involves control of four parameters: the rates of polymerization and depolymerization, and the frequencies of catastrophe (transition from growing to shortening) and rescue (transition from shortening to growing) events. In budding yeast, kinetochore movement during metaphase is coupled to individual kMT growth and shortening, which likely occurs solely by polymerization and depolymerization at the kinetochore-attached kMT plus-ends (Maddox et al., 2000; Pearson et al., 2003).

Labeling of single centromere proximal markers in yeast indicates that sister centromeres separate toward opposite sides of the spindle during metaphase and exhibit abrupt transitions in their direction of movement, as would be expected for dynamic instability of kMTs (He et al., 2000; Tanaka et al., 2000; Goshima and Yanagida, 2001; Pearson et al., 2001). Fluorescently labeled kinetochores persist in clusters midway between each spindle pole body and the spindle equator during yeast metaphase, and therefore the oscillations of fluorescent probes on chromosome arms suggest that dynamic kMT plus-ends coordinate congression of kinetochores to a steady-state, bilobed metaphase configuration in yeast (Pearson et al., 2001; Krishnan et al., 2004).

Green fluorescent protein (GFP) kinetochore fusions, such as Cse4-GFP, allow for live cell imaging of kinetochores in yeast spindles (Meluh et al., 1998; Chen et al., 2000; Pearson et al., 2001). In our previous work, a stochastic model of kMT plus-end dynamics in the budding yeast metaphase spindle was developed and then evaluated by simulating images of kinetochore-associated fluorescent probes (Sprague et al., 2003). Although individual kMT dynamics cannot be resolved, computer simulations of kMT dynamics combined with statistical measures of how well the simulation data predict experimental fluorescence kinetochore distributions recorded by live cell imaging can be used to build an understanding of budding yeast mitotic spindle kMT dynamics (Sprague et al., 2003). Through this analysis, it was demonstrated that a model based on any set of constant dynamic instability parameters was insufficient to explain how kinet-
ochores tend to cluster midway between the poles and the equator in yeast metaphase spindles (Sprague et al., 2003). However, reasonable agreement between simulated and experimental data for the distribution of kinetochores was found using a model with a temporally stable spatial gradient between the spindle poles in either catastrophe or rescue frequency combined with constant values for the other frequency (Sprague et al., 2003). For example, in the spatial gradient models, higher frequencies of catastrophe in the middle of the spindle relative to the poles promoted kinetochore movement poleward, or higher frequencies of rescue near the poles relative to the middle of the spindle promoted kinetochore movement away from the poles. It has been proposed for higher eukaryotes that mechanical tension on the kinetochore could modulate MT stability, acting as a key regulator of kMT dynamics (Nicklas, 1988; Skibbens et al., 1993, 1995; Rieder and Salmon, 1994, 1998; Inoue and Salmon, 1995; Skibbens and Salmon, 1997). Recent evidence in Xenopus extract spindles indicated that mechanical stress regulates MT dynamics locally at the kinetochore–MT attachment site, such that tension between sister kinetochores may promote MT polymerization (Maddox et al., 2003; Cimini et al., 2004). In addition, tension between sister kinetochores is important for the stability of kMT attachments and for turning off the spindle checkpoint that regulates anaphase onset in yeast (Dewar et al., 2004). Due to the significant spacing between sister kinetochores in yeast metaphase (~700 nm), communication between sister kinetochores is likely facilitated via mechanical tension through the intervening chromatin, because chemical signaling over such a distance would be improbable.

Here, we have used computer simulation to explore how mechanical tension at the kinetochore might contribute to metaphase chromosome alignment in budding yeast. First, we established that spatial gradient models similar to those described by Sprague et al. (2003) do not predict the low incidence of kinetochores crossing the equator, as observed experimentally by measurements of fluorescence recovery after photobleaching (FRAP) of the kinetochore-associated protein Cse4-GFP (Pearson et al., 2004). We then tested four various ways that kinetochore tension alone or in combination with catastrophe or rescue gradients between the poles would predict the extent of kinetochore movements as measured by the Cse4-green fluorescent protein (GFP) FRAP data. The best fit to the experimental data was achieved by kinetochores sensing a stable gradient between the poles to spatially control kMT plus-end catastrophe frequency and by sensing tension generated via chromatin stretching between sister kinetochores to control kMT plus-end rescue frequency. This model also quantitatively reproduces metaphase kinetochore distributions and kMT dynamics as measured by GFP-Tubulin FRAP experiments without parameter value adjustment between different experimental data sets. In addition, by eliminating simulated tension between sister kinetochores, the model quantitatively reproduces the kinetochore distribution in yeast mutants (cdc6) that enter mitosis with unreplicated chromosomes. In these cells, chromosomes in mitosis have single kinetochores and thus lack the tension generated via chromatin stretching from a sister kinetochore. Kinetochores in cdc6 mutant spindles achieve average positions up close to the poles, positions not predicted by the rescue gradient model.

All of the simulations were based on the explicit assumptions that 1) kMT dynamics are at steady state during metaphase (Figure S1 and Supplemental Material), 2) there is one kinetochore attached per MT, 3) MT assembly dynamics occur only at the kinetochore, 4) kinetochores do not detach from MTs during steady-state metaphase, and 5) the kinetochore marker Cse4-GFP closely tracks the plus-end dynamics of kMTs (see Figure S2). In addition, spindle length was held constant during each simulation, although the exact distribution of experimentally observed spindle lengths was reproduced in both wild-type and cdc6 simulations such that spindle length was allowed to vary between each simulation. A number of alternate models were considered and failed to reproduce one or more of the four different experimental results (Table S1 and Supplemental Material). In this way, we show that a model in which the kinetochore regulates kMT dynamics by sensing both distance from its sister kinetochore (via tension) and spindle position relative to the middle of the spindle (via a catastrophe gradient) is able to reproduce experimentally observed kinetochore dynamics and congression in yeast metaphase.

**MATERIALS AND METHODS**

**Yeast Strains and Media**

The yeast strains used for this study were KBY2125 (MATa cdc6GAL-CDC6: URA cdc55-2 PDS1 myc:LEU2 pKk1 cse4::HB SPC29CFPKAN), KBY2100 (MATa trpl-63, leu2-1, ura3-52, his3-200, lys2-801 cse4::HYG pKK1) and KBY2012 (MATa trpl-63, leu2-1, ura3-52, his3-200, lys2-801 cse4::HYG SPC29-CFP Kan pKk1). Fluorescent constructs to generate GFP-labeled kinetochores (Cse4-GFP) and cyan fluorescent protein (CFP)-labeled centromeres (Spc29-CFP) were described previously (Pearson et al., 2001).

Cell growth techniques and conditions were described previously (Sprague et al., 2003). However, KBY2125 was grown in galactose media for expression of Cdc6p. Unreplicated chromosomes were generated by arresting an asynchronous culture in S phase with 200 mM hydroxyurea (HU) for 2 h. Cells were then washed into glucose media to repress Cdc6p expression with HU for 1 h, released from HU into glucose media, and allowed to complete replication and progress through mitosis. Cells were then allowed to progress into a second mitosis with unreplicated chromosomes. Control cells were created by repeating the S-phase arrest protocol in galactose only to maintain Cdc6p expression.

**Fluorescence Microscopy**

All cell imaging was performed as reported previously (Pearson et al., 2001; Sprague et al., 2003).

**Simulation of MT Dynamics**

A Monte Carlo technique was used to simulate individual kMTs undergoing dynamic instability using MATLAB (version 6.0; Mathworks, Natick, MA) as described previously (Sprague et al., 2003). Additional details provided in Supplemental Material.

**Simulation of Image Formation by Fluorescence Microscopy**

Simulated kinetochore positions were compared with experimentally obtained images of kinetochore-bound fluorescence by simulation of the image formation process in fluorescence microscopy (Sprague et al., 2003). Briefly, it was assumed that each kinetochore remained attached to the tip of its kMT for the duration of the simulated experiments. At specified time points in each simulated experiment, a simulated fluorescence image of the spindle was generated by convolving the three-dimensional point spread function of the microscope with the kinetochore and spindle pole position matrices (Sprague et al., 2003).

**Simulation of Cse4-GFP and GFP-Tubulin FRAP Experiments**

FRAP experiments were simulated by modeling experimental fluorescence bleaching events and quantifying recovery over time. Detailed simulation methods are described in Supplemental Materials.

**Simulation of Kinetochore Distribution in cdc6 (Replication-deficient) Spindles**

As in the simulation of wild-type spindles, the spindle length and relative background noise for each cdc6 replication-deficient experimental image were matched to create a simulated fluorescence image of each mutant spindle. All remaining aspects of the MT dynamics simulations were identical to wild-type cells, with the exception that eight kMTs were modeled per spindle pole rather than 16 for replicated chromosomes. It was assumed that, on average, the 16 single kinetochores were distributed in equal numbers to each pole.
RESULTS

Model Assumptions

As in previous work (Sprague et al., 2003), we have used a simplified model of the yeast metaphase spindle to test mechanisms for the congression of kinetochores to a metaphase configuration. All models considered assumed that kMTs exhibit dynamic instability, as observed in yeast cytoplasmic MTs (Carminati and Stearns, 1997; Shaw et al., 1997; Tirnauer et al., 1999; Kosco et al., 2001; Gupta et al., 2002) (see Supplemental Material for further review).

In terms of spindle structure, the model was constructed to be consistent with electron micrographs of the yeast spindle (Winey et al., 1995), where kMT tips were only allowed to grow straight from the spindle pole (i.e., no microtubule curving or splaying was allowed). In addition, any kMT that grew the entire length of the spindle to the opposite pole immediately switched to a shortening state, whereas any kMT that completely shortened to its spindle pole immediately switched to a growth state.

In the simulation, all growth and shortening was assumed to take place at the kMT plus-ends. Minus-end depolymerization (i.e., poleward flux) was not required to reproduce results in any of the simulations. Rates of kMT polymerization and depolymerization were assumed to be constant over the length of the spindle, and unaffected by force, as suggested by recent measurements in Xenopus extract spindles (Tirnauer et al., 2004).

In all cases, kinetochores were assumed to remain attached to the plus-end tip of the kMT throughout the simulation, an assumption supported by lack of recovery in Cse4-GFP FRAP experiments (Pearson et al., 2004). In addition, possible lateral interactions between chromosomes and kMTs are ignored in the simple Hookean spring model for tension between sister kinetochores. For example, chromosomes could become transiently associated with interpolar MTs so that the forces on the two kinetochores would not be equal and opposite, as we assumed. In the interest of parsimony, we opted for the simplest possible model that explains all of the data analyzed.

Models without Tension-dependent Dynamic Instability Parameters Allow Equator Crossing

To determine the extent of kinetochore equator crossing in yeast metaphase spindles, kinetochore-associated Cse4-GFP

Figure 1. Position-dependent gradient models for the regulation of kMT dynamics fail to reproduce Cse4-GFP FRAP experimental results. (A) The catastrophe gradient model: kMT plus-end catastrophe frequency peaks at the spindle equator, whereas plus-end rescue frequency remains constant. (B) Representative simulated image before the bleach event using the catastrophe gradient model.

Figure 1 (cont). (kinetochore-associated Cse4-GFP, green; spindle pole body-associated Spc29-CFP, red). (C) Significant fluorescence recovery of kinetochore-associated Cse4-GFP fluorescence using the spatial catastrophe gradient model does not reproduce experimental results. (D) Representative Cse4-GFP FRAP experimental and simulated time series of fluorescence recovery. Kinetochore-associated markers in one-half-spindle were bleached and then observed over time to quantify fluorescence recovery. Because Cse4-GFP is stably bound at the kinetochore, fluorescence recovery results exclusively from redistribution of kinetochores between spindle halves. The lack of recovery observed experimentally indicates that kinetochores remain constrained to their own half-spindle throughout the experiment. Models with position-dependent catastrophe frequencies only (i.e., no tension dependence) do not limit spindle-equator crossing sufficiently to reproduce experimental results. (E) Typical simulated plus-end kMT positions at steady state using the spatial catastrophe gradient model for regulation of MT dynamics. The representative trace shows a pair of sister kMT plus ends and their movements relative to the spindle poles and the equator. Although individual kinetochores separate and oscillate on either side of the spindle equator, kinetochores frequently move into the opposite half-spindle for extended periods of time.
fluorescence in one-half-spindle was photobleached and
spindles observed for 10 min. A mean Cse4-GFP recovery percentage of 4.5 ± 7.3% (n = 9; Figure 1D) was observed in
10-min recovery time experiments (Pearson et al., 2004). This
low level of fluorescence recovery indicates that kineto-
chores are highly constrained to their respective half-spindle
(Pearson et al., 2004). Because cells proceed normally into
anaphase after photobleaching of Cse4-GFP, it is not likely
that photodamage has affected normal kinetochore dynam-
ic.In addition, FRAP experiments performed using centro-
mere-proximal GFP-lacI/lacO markers indicated that these
markers were stably oriented to their respective bud or
mother cell, supporting a low incidence of kinetochores
switching attachment to their respective poles in yeast meta-
phase spindles (Pearson et al., 2004). Sister centromeres
rarely reassociate after separation during metaphase, such
that switching from one spindle-half to another would be
unlikely (Goshima and Yanagida, 2001; Pearson et al., 2001).

A limitation of this experiment and of yeast spindles in
general is that individual kinetochores cannot be directly
tracked. For this reason, simulation is extremely helpful in
understanding how the dynamics of individual kinetochores
could be regulated to reproduce the low level of fluores-
cence recovery, as observed in the Cse4-GFP FRAP exper-
iments.

KMT dynamics were simulated using models with spatial
gradients in catastrophe and rescue frequency for plus-ends
as a function of position along the spindle axis (Sprague et
al., 2003) (Figure 1A). Cse4-GFP FRAP simulations were run
for each model using parameter values that were optimized
to best reproduce the experimentally observed kinetochore
distributions for wild-type metaphase spindles (a constraint
not imposed in previous work; Pearson et al., 2004). Position-
dependent models for regulation of kMT plus-end catastro-
phe frequency (Figure 1A) or rescue frequency performed
poorly in the Cse4-GFP FRAP simulations (Table 1 and
Figure 1, B–D), meaning that these models do not effectively
constrain kinetochores to the correct half-spindle (Figure
1E). Here, we calculate a “probability of fit” (p value) to
evaluate statistically how well the mean experimental Cse4-
GFP fluorescence recovery percentage would fit into a set of
50 mean simulated recovery percentages (n = 13, each sim-
ulation; see Supplemental Material for details). Models with
calculated p values < 0.05 on any single test were consid-
ered to be unacceptable, whereas models with p > 0.05
could not be statistically ruled out. In general, both the

<table>
<thead>
<tr>
<th>Experimental results</th>
<th>Simulated mean Cse4-GFP recovery %a</th>
<th>Probability of fit to experimental results (p value)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental results</td>
<td>4.5 ± 7.3c</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Position-dependent regulation of kMT catastrophe frequencya</td>
<td>13.8 ± 2.5</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Position-dependent regulation of kMT rescue frequencya</td>
<td>19.2 ± 10.1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Position-dependent catastrophe + tension-dependent rescuea</td>
<td>6.5 ± 1.7</td>
<td>0.24</td>
</tr>
<tr>
<td>Position-dependent rescue + tension-dependent catastrophea</td>
<td>8.3 ± 2.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

All mean recovery percentages are reported for model parameter sets that are optimized to qualitatively reproduce steady-state Cse4-GFP kinetochore clustering in yeast metaphase spindles.

The probability of fit (p value) was calculated through comparison of experimental mean recovery values to the range of simulated recovery values over 50 experiments (see Supplemental Material for calculation details). The 10-min time point data was used for comparison with simulation, because the simulation was allowed to run for a simulated recovery time of 10 min before evaluation of simulated recovery.

Experimental results reported for 10-min recovery times (n = 9) (Pearson et al., 2004).

Because both the catastrophe and rescue gradient models failed, we then considered models in which the parameters of kMT plus-end dynamic instability depended not only on position but also on tension generated by the stretch of chromatin between sister kinetochores. In this model, sister kinetochores are assigned such that the position of each kinetochore in the spindle has a direct impact on the dynamics of its sister kinetochore in the opposite half-spindle based on the amount of centromere stretch. High tension is proposed to promote rescue and thus kinetochore movement away from the kMT-attached pole, or alternatively low tension is proposed to promote catastrophe and therefore kinetochore movement toward the pole.

Two models that included position-dependent gradients and tension to control catastrophe or rescue frequencies at kMT plus-ends successfully reproduced the lack of Cse4-GFP FRAP observed experimentally (Table 1 and Figure 2, A–E). Therefore, both of these models effectively control kinetochore dynamics by keeping centromeres in their respective half-spindle (Figure 2F) and thus act to minimize fluorescence recovery in the simulated Cse4-FRAP experiment. The reason these models succeed is because as a simulated kinetochore enters the opposite half-spindle during plus-end assembly, it tends to approach its sister kinetochore. This reduces tension, destabilizing the kMT plus-end, so that the kinetochore rapidly returns to its own half-spindle (Figure 2F).

Ranges of acceptable parameter values across all wild-
type kMT models and experiments are listed in Table 2. The ranges of values for growth and shrinking velocities are similar to those measured for cytoplasmic MTs during meta-

Vol. 16, August 2005
3767
phase, and catastrophe and rescue frequency model values are somewhat higher than values reported for cytoplasmic MTs (Table 2). The "spring constant" determines the magnitude of the tension effect. Although a lower tension effect can be somewhat offset by increasing the gradient in catastrophe frequency, a minimum value for the spring constant is required. Figure 2. Models where kinetochores sense both spindle position to regulate kMT plus-end catastrophe frequency and tension due to chromatin stretch to regulate kMT plus-end rescue frequency successfully reproduce Cse4-GFP FRAP experimental results. Excursions of kMT plus-ends into the opposite spindle half are limited, and therefore these models quantitatively reproduce Cse4-GFP FRAP experimental results. (A) The spatial model for regulation of kMT plus-end catastrophe frequency with kMT rescue frequency regulated by tension generated via chromatin stretch between separated sister kinetochores: rescue frequencies shown are mean values calculated for a given spindle position during the simulation, because rescue frequency is directly dependent on the sister kinetochore separation distance. Dependence of kMT plus-end rescue frequency on tension between sister kinetochores is directional, such that mean rescue frequencies tend to decrease as kMTs lengthen, due to decreased separation between sister kinetochores. For this distribution, $V_g = V_s = 2.0 \mu m/min$ and the spring constant is $k = 0.9 \mu m^{-1}$. Gold arrows correspond to the spindle locations of predicted peaks in kinetochore-associated Cse4-GFP fluorescence. (B) The spatial model for regulation of kMT plus-end rescue frequency with kMT catastrophe frequency regulated by tension between sister kinetochores. (C) Representative simulated images for the Cse4-GFP FRAP experiment using a model where the kinetochore senses spindle position to regulate kMT plus-end catastrophe frequency and senses tension generated via chromatin stretch to regulate kMT plus-end rescue frequency. For the model shown in A, there is negligible visible recovery in Cse4-FRAP experiment simulations, reproducing experimental results. (D) Simulated Cse4-GFP FRAP images for the model shown in B. (E) Representative Cse4-GFP FRAP experimental and simulated time series of fluorescence recovery. Models that include regulation of kMT plus-end switching frequencies based on tension between sister kinetochores reproduce experimental results. (F) Typical simulated plus-end kMT positions at steady state for the models shown in A and B. Here, equator crossing is limited, because kMT plus-ends are less likely to experience rescue events as kinetochores move closer to their sisters. Kinetochores rarely cross the equator, but remain dynamic, moving toward the spindle equator and back to the poles.
plus-end positions were recorded at the conclusion of each parameter sets were tested and optimized over a wide range of parameters, similar to previous work (Sprague et al., 2003).

**Table 2.** Wild-type model parameter value ranges and constraints

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Symbol</th>
<th>Range of acceptable values (all wild-type models)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth velocity</td>
<td>$V_g$</td>
<td>$1.0$–$2.0$ $\mu$m/min$^a$</td>
</tr>
<tr>
<td>Shortening velocity</td>
<td>$V_s$</td>
<td>$1.0$–$2.0$ $\mu$m/min$^b$</td>
</tr>
<tr>
<td>Spring constant$^c$</td>
<td>$\rho$</td>
<td>$0.8$–$1.5$ $\mu$m$^{-1}$</td>
</tr>
<tr>
<td>Catastrophe frequency</td>
<td>$k_c$</td>
<td>$0.25$–$35$ min$^{-1}$$^d$</td>
</tr>
<tr>
<td>Rescue frequency</td>
<td>$k_r$</td>
<td>$2.0$–$25$ min$^{-1}$$^e$</td>
</tr>
</tbody>
</table>

$^a$ Cytoplasmic MT measurements, $V_g = 0.5$ $\mu$m/min (Carminati and Stearns, 1997).

$^b$ Cytoplasmic MT measurements, $V_s = 1.35$ $\mu$m/min (Carminati and Stearns, 1997).

$^c$ Applies to models with tension-dependent switching frequencies only.

$^d$ Longer $cdc6$ mutant spindles result in a higher calculated catastrophe frequency at the spindle equator, as shown in Figure 4A.

$^e$ Cytoplasmic MT measurements, $k_r = 0.36$ min$^{-1}$ (Carminati and Stearns, 1997).

(0.8 $\mu$m$^{-1}$) is required to impart directionality to the model, thus maintaining kinetochores in the correct half-spindle throughout the simulation. To reject a given model, parameter sets were tested and optimized over a wide range of parameters, similar to previous work (Sprague et al., 2003).

**Experimental Metaphase Kinetochore Distributions Are Correctly Predicted by Models Including Tension-dependent Dynamic Instability Parameters**

To further assess models combining stable spatial gradients in catastrophe or rescue frequency with tension-dependent parameters, simulations of kMT dynamics were run and the plus-end positions were recorded at the conclusion of each simulation. These positions were used to generate simulated fluorescence images of kinetochore-bound Cse4-GFP (Figure 3, A and B). Statistical comparison of simulated Cse4-GFP images to the experimentally observed steady-state metaphase kinetochore fluorescence distribution was then used as a computational screen for selection of valid models (see Figure S1 and Supplemental Material for analysis of steady-state metaphase). The two models where catastrophe and rescue frequency depend on both a spatial gradient and kinetochore tension were successful in predicting the average distribution of kinetochores between the poles at metaphase (Figure 3, A–D, and Table S1). Spatial gradient models with no tension-dependent parameters qualitatively reproduced experimentally observed metaphase kinetochore clustering but resulted in low calculated $p$ values (Table S1). By quantitatively analyzing experimental spindle images to include steady-state metaphase spindles only (see Supplemental Material), models for regulation of kMT dynamics are more tightly constrained compared with our previous work (Sprague et al., 2003).

**Kinetochore Distributions in Metaphase Spindles Lacking Tension Are Correctly Predicted by the Catastrophe Gradient with Tension-dependent Rescue Model**

To test the hypothesis that tension between sister kinetochores regulates kMT dynamics in yeast, we performed experiments using a replication deficient $cdc6$ mutant that is incapable of developing tension between sister kinetochores (Stern and Murray, 2001). Here, chromosomes in mitosis have single kinetochores and lack the tension generated via chromatin stretching from a sister kinetochore.

Kinetochore positions at metaphase in $cdc6$ mutants were quantified using the Cse4-GFP fluorescence distribution ($n = 27$ cells, 54 spindle halves). The $cdc6$ mutant cells generally had spindles with kinetochore clusters very near to each spindle pole body, as shown in Figure 4C. The peak in mean kinetochore-associated fluorescence relative to the pole was ~0.21 $\mu$m in $cdc6$ mutants, compared with ~0.39 $\mu$m in control metaphase cells, a ~46% reduction in mean

**Figure 3.** The models shown in Figure 2, A and B, reproduce experimental metaphase kinetochore clustering. (A) Experimental metaphase spindle image of Cse4-GFP–labeled kinetochores (green) relative to Spc29-CFP–labeled spindle poles (red). (B) Representative simulated image using the model as shown in Figure 2B. Tight kinetochore-associated fluorescence clusters are comparable with the experimental image. Bar, 1000 nm. (C) Representative simulated image using the model as shown in Figure 2B. (D) Quantitative analysis of average simulated kinetochore clustering observed via Cse4-GFP compared with mean experimental results. Simulation results reproduce experimental results for both the model shown in Figure 2A and the model in Figure 2B.
kMT length. These experimental results indicate that kMT dynamics are altered in spindles lacking tension between sister kinetochores, resulting in net shortening of average kMT lengths.

Before simulation of the cdc6 mutant data, parameter value sets for each model to be tested were adjusted to reproduce the Cse4-GFP fluorescence distribution for GALCDC6 control cells grown in galactose media (with replication) and arrested in HU (p = 0.10). Specifically, \( V_g \) and \( V_s \) were reduced by \( 15\% \) in all models compared with previous yeast strain simulations, although values remained well within the range of values given in Table 1. Tension-dependent catastrophe and tension-dependent rescue models were then used to simulate kinetochore movements in the cdc6 mutants by reducing the spring constant in each model to zero (Figure 4, A and B). This reduction effectively eliminated any tension effect on kMT dynamics. A catastrophe frequency gradient together with a tension-dependent rescue frequency model modified such that the chromatin spring constant was reduced to zero was successful in quantitatively reproducing the kinetochore distribution in tension deficient spindles (p = 0.11; Figure 4, A, D, and F). In contrast, a model based on a position-dependent rescue gradient with the spring constant for a tension-based catastrophe frequency model reduced to zero performed very poorly in predicting kinetochore positions in cdc6 mutants (p ≤ 0.01; Figure 4, B, E, and F). The position-dependent rescue gradient model relies on a high rescue frequency at the poles, which decreases toward the spindle equator. This tends to push kinetochore clusters toward the equator, such that rescue gradient models are ineffective in reproducing kinetochore clusters very near to the poles, as was experimentally observed in the cdc6 mutant phenotype. These results argue against a spatial rescue gradient model for regulation of budding yeast kMT dynamics during metaphase.

Thus, a model where the kinetochore senses spindle position to regulate kMT plus-end catastrophe frequency and senses tension generated from the stretch of chromatin between sister kinetochores to regulate kMT plus-end rescue frequency was the single model that was able to reproduce all experimental results (see Table S1 and Supplemental Material for further details).
Simulated GFP-Tub1 FRAP Experiment

Figure 5. Simulation of GFP-Tub1 FRAP experiments. Experimentally, GFP-Tub1 labeled spindles are imaged, and half-spindles are photobleached at time t = 0 (Maddox et al., 2000). FRAP of the bleached half-spindle and loss-of-fluorescence in the unbleached half-spindle are quantified experimentally and in simulations. Simulated results are compared with live cell experimental data from Maddox et al. (2000). For the simulation results shown, Vg = Vs = 2.0 μm/min and the spring constant is k = 0.9 μm⁻¹. Catastrophe and rescue frequency are modeled as shown in Figure 2A. GFP-Tub1 recovery profiles for simulated kMT dynamics qualitatively and quantitatively reproduce experimental results (p = 0.96). The average experimental time to half-maximal recovery was 52 ± 24 s (n = 6; Maddox et al., 2000), compared with a simulated half-maximal recovery time of 53 ± 15 s (n = 6).

GFP-Tub1 FRAP Experiments Are Correctly Predicted by Simulated kMT Dynamics

The final step in our analysis was to ask how well simulated kinetochore dynamics predict metaphase kMT dynamics. GFP-Tub1 FRAP experiments were simulated using the parameter value sets as defined above (Table 1) and then compared with published results (Maddox et al., 2000). Interpolar MTs were assumed to be stable and nondynamic in the simulation, such that all recovery was the result of kMT plus-end dynamics, as in Figure 2F. GFP-Tub1 recovery profiles for simulated kMT dynamics qualitatively and quantitatively reproduced experimental results (Figure 5) for the parameter values of dynamic instability listed in Table 1. The average experimental time to half-maximal recovery was 52 ± 24 s (n = 6; Maddox et al., 2000), compared with a simulated half-maximal recovery time of 53 ± 15 s (n = 6). Growth and shortening velocities were highly constrained in simulations of the GFP-Tub1 FRAP data. Rapid velocities (>2 μm/min) generally resulted in faster MT turnover than is experimentally observed.

DISCUSSION

The Extent of Kinetochore Oscillation Is Limited in Models with kMT Plus-End Dynamic Instability Regulated by Tension, Thus Reproducing Cse4-GFP FRAP Results

Regulation of kMT dynamics in a local tension-dependent manner results in a “self-correcting” system, in which the range of chromatin stretching, and therefore the mean sister kinetochore separation distances, are tightly controlled. In a model where catastrophe is regulated by a spatial gradient and rescue is regulated by tension between sister kinetochores, kMT lengths will tend to be most stable at moderate tension values (Figures 2A and 6A, gold arrows), away from the high catastrophe zone at the spindle equator. In this way, congression of kinetochores to a metaphase configuration can be accurately reproduced regardless of the initial positions of the MT-attached kinetochores, as shown in Figure 6B.

Tension-based control of kMT dynamics is particularly efficient in limiting excursions of kMTs across the spindle equator. Because Cse4-GFP protein is stably bound to the kinetochore during metaphase (Pearson et al., 2004), recovery of bleached Cse4-GFP in FRAP experiments results exclusively from unbleached kinetochores shifting from the unbleached half-spindle to the bleached half-spindle after the bleach event. Lack of any significant recovery in Cse4-GFP FRAP experiments implies that kinetochores in metaphase spindles remain constrained to the half-spindle in which their kMT is anchored. This half-spindle fidelity is reproduced effectively in models where the kinetochore senses tension developed from chromatin stretch to regulate kMT plus-end dynamics (Figure 2). As a kMT grows across the spindle equator, its attached kinetochore moves closer to its sister, reducing tension (Figure 6A, 1). After a catastrophe event near the spindle equator, low tension ensures that the kMT will undergo steady depolymerization to return the kinetochore to its correct half-spindle. This tension-dependent rescue effect is consistent with studies on animal cell kinetochores (Skibbens et al., 1995; Skibbens and Salmon, 1997) and with experimental fluorescence speckle microscopy experiments by Maddox et al. (2003), in which Xenopus MTs switched from polymerization to depolymerization when there was loss of tension at the kinetochore. Modeling of the Cse4-GFP FRAP experiment indicates that recovery is highly sensitive to even a small percentage of MT plus-ends growing across the spindle equator, because significant recoveries (~14%) were predicted in simulations using a position-dependent catastrophe gradient model (no tension-based regulation), in which approximately three kMTs (of 32 total) were present in the wrong half-spindle at any given time during the simulation. Accurate replication of GFP-Tub1 FRAP experiments indicates that simulated kMT dynamics approximate the kMT turnover in yeast metaphase spindles, such that although kinetochores rarely cross the equator, they remain dynamic, oscillating back and forth between the spindle equator and their attached pole (Figure 2F).

Lack of Tension at the Kinetochore in cdc6 Mutants Results in Net kMT Depolymerization, Both Experimentally and in Simulation

The model with a spatial gradient in kMT plus-end catastrophe frequency and with kMT plus-end rescue frequency dependent on tension at the kinetochore provided an excellent fit to the cdc6 mutant experimental data, where lack of tension results in clustering of kinetochore-associated fluorescence very near to each spindle pole body (Figure 6C). In this model, loss of tension at the kinetochore significantly reduces overall kMT rescue frequency, allowing kinetochores to move on average closer to the spindle pole bodies (Figure 4). Peak catastrophe frequencies increase at the equator as a natural consequence of increasing spindle length in the spatial catastrophe gradient model.

A model based on a spatial rescue gradient with catastrophe frequency at the kMT plus-end dependent on the stretch...
on chromatin between sister kinetochores performed very poorly in reproducing tension-deficient cdc6 mutants (Figure 4, E and F). In this model, rescue frequencies are high at the poles, and decrease with distance from each pole, similar to polar ejection forces in vertebrate spindles. A high rescue frequency at the spindle pole moves simulated kinetochore clusters in tension-deficient spindles toward the equator, thus failing to reproduce the experimentally observed average kinetochore positions for cdc6 mutants. In the above-mentioned assays, we have identified a model where kinetochores sense both a stable gradient between the poles to control kMT plus-end catastrophe frequency, and tension developed from chromatin stretch to control kMT plus-end rescue frequency. This is the best model, because it successfully reproduces all four simulated experiments with an overall probability of \( p > 0.10 \).

**Implications of Tension-dependent kMT Plus-End Dynamic Instability for Budding Yeast Mitosis**

Tension-based regulation of kMT dynamics provides a mechanism for kinetochores under high tension to relieve this tension by switching from poleward movement, which builds tension, to an away from pole movement, which relieves tension (Figure 6A, 3) (Maddox et al., 2003). Without a means to relieve tension, kinetochores could possibly detach from kMTs due to high mechanical stress on the MT–kinetochore attachment (Skibbens et al., 1995; Skibbens and Salmon, 1997; Maddox et al., 2003). If not resolved before anaphase, such detachments would lead to aneuploidy.

Low tension at the kinetochore may have critical consequences for the fidelity of chromosome segregation as well.

---

**Figure 6.** Model for metaphase congression in budding yeast. For clarity, the right kinetochore is fixed at its mean position, whereas the left kinetochore moves, although both kinetochores are dynamic in simulation, each affecting the relative tension experienced by its assigned sister. Kinetochores are green, spindle pole bodies red, kMTs blue, and the cohesin/chromatin “spring” is gray. Gold arrows indicate spindle locations of predicted peaks in kinetochore-associated fluorescence. (A, 1) The left kinetochore is near to the spindle equator, under low tension, resulting in a high catastrophe and low rescue frequency for the kMT plus-end originating from the left pole. This biases the left kMT plus-end toward net depolymerization. (A, 2) The left kinetochore in the quarter spindle area with “proper” sister separation and moderate tension has equal probabilities of catastrophe and rescue at the left kMT plus-end. Therefore, the left kMT is not biased toward either growing or shrinking. (A, 3) The left kinetochore is near the left kMT spindle pole body and under high tension, resulting in a low catastrophe and high rescue frequency at the kMT plus-end. This biases the left kMT plus-end toward net polymerization. (B) Simulation of congression: from an initially random distribution of kinetochore localization at the initiation of the simulation (t = 0), the simulation results in alignment of kinetochores into a metaphase configuration within a few minutes. (C) Simulation of anaphase via loss of tension: after normal metaphase alignment, a sudden loss of tension results in simulated kinetochore movement to average positions close to the spindle poles. This is observed experimentally for Cdc6p-depleted cells and during anaphase A (Guacci et al., 1997; Straight, 1997; Pearson et al., 2001). Simulated spindle lengths were increased to match experimentally observed Cdc6p-depleted spindles. (D) A representative simulated image in which a theoretical catastrophe gradient mediator molecule is depleted. Bar, 1000 nm. A threefold decrease in peak catastrophe frequency at the equator results in one focused cluster of kinetochore-associated fluorescence that stochastically moves from one spindle-half to another and transiently separates into two closely spaced clusters. Thus, a gradient in catastrophe frequency drives the separation of sister kinetochores to generate chromatin stretch.
Spindle assembly checkpoint signaling requires tension between sister kinetochores (Nicklas and Ward, 1994; Biggins and Murray, 2001; Stern and Murray, 2001; Zhou et al., 2002; Biggins and Walczak, 2003; Cleveland et al., 2003), and low tension could act to destabilize attachment of MTs to kinetochores (Nicklas et al., 2001; Biggins and Walczak, 2003; Dewar et al., 2004).

Thus, a model where the kinetochore senses both spindle position to regulate kMT plus-end catastrophe frequency and tension generated via chromatin stretch to regulate kMT plus-end rescue frequency has the overall effect of limiting the range of tensions experienced at the kinetochore compared with a model with position-dependent switching frequencies only. Limiting the range of tensions to intermediate levels (Figure 6A, 2) may help the spindle avoid MT detachment by reducing high forces on the kinetochore and allow the checkpoint to be turned off by limiting low tension. The net result is that both high and low tension on kinetochores are unfavorable, resulting in a congressed state of approximately uniform separation distance between sister kinetochores.

In contrast to Cdc6p-depleted spindles, in which kinetochore localization near the poles suggests that lack of tension results in net depolymerization of kMTs, loss of tension and kMT attachment in ndc10 kinetochore mutants does not result in significant MT depolymerization (Pearson et al., 2003). Therefore, it may be that the kinetochore itself acts to depolymerize kMTs via a catastrophe gradient, an effect that could be antagonized by tension. Loss of attachment could thus allow for net polymerization of MTs, as is observed for interpolar MTs.

A Mechanism for Tension-dependent Regulation of kMT Dynamics

Our analysis shows that tension promotes kMT assembly by increasing rescue. What could be a mechanism by which tension promotes rescue? One possibility for tension-dependent regulation of kMT dynamics is a purely physical effect that could be mediated by the kinetochore. For example, recent work with the purified components of the Dam1p/DASH complex shows that the complex forms rings around microtubules in vitro (Miranda et al., 2005; Westermann et al., 2005). This type of structure could form a sleeve that surrounds the kMT tip and links to other kinetochore components (reviewed by Cheeseman et al., 2002), although the existence of rings in vivo remains an open question (McIntosh, 2005). As shown schematically in Figure 7A, the kinetochore-associated sleeve could move toward the kMT minus-end during depolymerization via protofilament splaying and peeling. As a kinetochore moves away from its sister, tension will build in the intervening chromatin (green spring), and in the kinetochore itself (blue spring), advancing the sleeve toward the kMT plus-end. This would in turn force kMT protofilaments to straighten (Figure 7B). The straightening of protofilaments could suppress tubulin departures from the kMT tip and thereby promote rescue (Figure 7C).

What would be the force required to promote rescue? The answer hinges on how much energy is required to straighten a curled GDP-tubulin dimer. Previous analyses estimate the mechanical energy stored in the lattice upon GTP hydrolysis to be \( \sim 2.1-2.5 \ k_B T \) (Caplow and Shanks, 1996; VanBuren et al., 2002). This amount of energy is equal to \( \sim 10 \) pN nm, so that the force required to straighten one GDP dimer of length 8 nm would be \( F = 10 \ \text{pN nm}/8 \ \text{nm} = 1.25 \ \text{pN} \). Because there are 13 protofilaments, there would a requirement of \( F_{\text{total}} = 13 \times 1.25 \ \text{pN} = 16 \ \text{pN} \). Is this characteristic force plausible? Previous analysis of chromatin stretching in budding yeast metaphase showed that the centromere proximal chromatin is highly stretched, to the point where individual nucleosomes are almost certainly forced off the chromatin (Pearson et al., 2001). Studies with laser tweezers in vitro show that \( \sim 15-20 \) pN is required to force nucleosomes off of double-stranded DNA (Brower-Toland et al., 2002). Thus, the typical tension generated via chromatin stretch during yeast metaphase is approximately equal to that estimated as necessary for kMT protofilament straightening.

Models Lacking a Spatial Gradient in Catastrophe Frequency Result in Loss of Sister Kinetochore Separation at Metaphase

The catastrophe frequency gradient shown in Figure 2A is an essential model element. In a model that includes a spatial gradient in catastrophe frequency, kMT plus-ends experience a peak in catastrophe frequency at the spindle equator. This has the effect of destabilizing kMT plus-ends located at the spindle equator, such that kinetochores tend to cluster on either side of the equator in a bilobed metaphase configuration.

A model in which kinetochores sense both a spatial gradient in catastrophe frequency and attachment site tension to promote rescue results in specific predictions for spindles with a reduced catastrophe gradient, as might be observed in mutants depleted of a theoretical catastrophe gradient mediator molecule (Figure 6D). In simulations with the catastrophe gradient modified such that the peak catastrophe frequency at the equator is threefold less than in wild-type...
simulations, whereas catastrophe frequency at the poles remains unchanged, metaphase kinetochore clusters collapse into a single, focused cluster that stochastically moves between spindle halves and transiently separates into closely spaced separated clusters (representative simulated image, Figure 6D).

What could be the origin of a position-dependent catastrophe gradient? A spatially segregated antagonistic kinase/phosphatase pair could establish a stable gradient in phosphorylation state (Brown and Kholodenko, 1999; Sprague et al., 2003). If the substrate is capable of promoting kMT catastrophe in a manner dependent on its phosphorylation state, then there will be a position-dependent catastrophe gradient over the length of the spindle. In yeast, Stu2p acts at kMT plus-ends to promote dynamics (Kosco et al., 2001; Pearson et al., 2003; Van Breugel et al., 2003) and may be required to promote transient sister chromatid separation (He et al., 2001). Given that Stu2p is known to affect kMT dynamics, it seems to be a likely candidate for the gradient, although it is not clear how phosphorylation might be involved. Another possibility is Gsp1 (Ran), which could modulate kMT dynamics via a gradient of Ran-GTP, an MT stabilizer (Kalab et al., 2002). Other microtubule-associated proteins implicated in regulating MT dynamics, such as Kip3p (an MT depolymerase), Kar3p, Cin8p, or Dam1p could mediate a gradient in kMT plus-end catastrophe as well.

In conclusion, by using computer simulations that account for both presumed kMT dynamics and the imaging of those dynamics, a method we call “model-convolution,” we have identified a model for kMT dynamics in the yeast metaphase spindle. Certainly, the mitotic spindle is complex, but our analysis shows that a number of simple models, and even some relatively sophisticated models, ultimately fail to describe the observed behavior. Through a process of continual model scrutiny via integrated modeling and experiment, we expect that alternative plausible scenarios of similar complexity can be tested and key experimental predictions made (e.g., as with Cse4-GFP distribution in the cdc6 mutant). Because individual kMT dynamics have not been resolved in live cells, the ability to simulate kinetochore dynamics in wild-type and genetically manipulated spindles will provide a useful tool for future studies aimed at understanding the complex mechanisms underlying mitosis.

ACKNOWLEDGMENTS

We thank Bodo Stern and Andrew Murray for providing the cdc6 strain. We also thank Mark Winney for providing yeast microscopy data and for helpful suggestions on the manuscript. This study was funded by National Science Foundation Career Award BES 9984953 (to D.J.O.), National Institutes of Health Grant GM-24364 (to E.D.S.), and National Institutes of Health Grant GM-32238 (to K.S.B.).

REFERENCES


Table S1: Screening of Computational Models for kMT Dynamic Instability in Yeast

<table>
<thead>
<tr>
<th>Model</th>
<th>Castastrophe Gradient Model</th>
<th>Rescue Gradient Model</th>
<th>Tension Between Sister Kinetochore-Dependent Rescue Model</th>
<th>Tension Between Sister Kinetochore-Dependent Catastrophe Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catastrophe Gradient Model</strong></td>
<td>(single model)</td>
<td></td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>Exp 1^a: p^d&lt;0.01</td>
<td></td>
<td></td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>Exp 2^b: p&lt;0.02</td>
<td>Exp 3^c: p&lt;0.01</td>
<td></td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td><strong>Rescue Gradient Model</strong></td>
<td>Exp 1: p=0.04</td>
<td>(single model)</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>Exp 2: p=0.08</td>
<td>Exp 3: p&lt;0.01</td>
<td>Exp 1: p&lt;&lt;0.01</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td><strong>Tension Between Sister Kinetochore-Dependent Rescue Model</strong></td>
<td>Exp 1: p=0.55</td>
<td>Exp 2: p=0.24</td>
<td>Exp 1: p&lt;&lt;0.01</td>
<td>xxx</td>
</tr>
<tr>
<td>Exp 3: p= 0.11</td>
<td>Exp 1: p&lt;&lt;0.01</td>
<td>Exp 2: p=0.05</td>
<td>Exp 1: p&lt;&lt;0.01</td>
<td>xxx</td>
</tr>
<tr>
<td><strong>Tension Between Sister Kinetochore-Dependent Catastrophe Model</strong></td>
<td>Exp 1: p=0.25</td>
<td>Exp 1: p= 0.27</td>
<td>Exp 1: p&lt;&lt;0.01</td>
<td>Exp 1: p&lt;&lt;0.01</td>
</tr>
<tr>
<td>Exp 2: p=0.26</td>
<td>Exp 3: p&lt;.01</td>
<td>Exp 3: p&lt;&lt;.01</td>
<td>Exp 1: p&lt;&lt;0.01</td>
<td>Exp 1: p&lt;&lt;0.01</td>
</tr>
</tbody>
</table>

^aExp 1: Experiment #1 is a comparison of the simulated mean wild-type kinetochore-associated metaphase Cse4-GFP fluorescence distribution to the experimental mean wild-type kinetochore-associated fluorescence distribution via statistical analysis of fluorescence distribution curves.

^bExp 2: Experiment #2 is a comparison of the simulated mean Cse4-GFP FRAP percentage to the experimental mean Cse4-GFP FRAP.

^cExp 3: Experiment #3 is a comparison of the simulated mean cdc6 mutant kinetochore-associated metaphase Cse4-GFP fluorescence distribution to the experimental mean cdc6 mutant kinetochore-associated Cse4-GFP fluorescence distribution via statistical analysis of fluorescence distribution curves. No parameter value adjustments are allowed in simulating the cdc6 mutant spindles from the wild-type parameter values, with the exception of reducing the tension spring constant to 0.

^dP-values = probability of fit of simulated results to experimental results, calculated as described in supplemental materials and methods for each experiment.

^eModels that were unable to qualitatively reproduce metaphase kinetochore clustering were immediately ruled out and therefore not run through additional simulated experiments.
SUPPLEMENTAL RESULTS: ANALYSIS OF MODEL ASSUMPTIONS

Steady-state metaphase as determined experimentally by kinetochore-associated fluorescence imaging

In comparing yeast kinetochore-associated Cse4-GFP fluorescence images at different spindle lengths, it was qualitatively observed that shorter spindles exhibited kinetochore clustering distinct from longer metaphase spindles. The shorter spindles had a disorganized band of kinetochore-associated fluorescence across the spindle equator (Fig. S1 (A)), whereas the longer metaphase spindles had relatively tight kinetochore-associated fluorescence clusters (green) on either side of the spindle equator (Fig. S1(B)).

Quantitative statistical analysis relating kinetochore distributions to spindle length supported qualitative observations (Fig. S1(C)). In this analysis, it was found that spindles of lengths 1.6-1.9 μm had a consistent pattern of separated kinetochore clustering (probability of fit >0.25, Fig. S1(B, C)), and therefore represented a steady-state metaphase configuration. Spindles with lengths outside of this window had kinetochore clustering patterns which were not consistent with the putative metaphase steady-state data set (probability of fit <0.10, Fig. S1(A, C)). We therefore considered spindles having lengths 1.6-1.9 μm as being in “metaphase,” with the shorter spindles being in “prometaphase.”

The data set used to calculate the metaphase distribution of kinetochore clustering was therefore redefined from earlier work (Sprague et al., 2003) to limit metaphase spindle lengths to the range of 1.6-1.9 μm. The mean distribution of kinetochore-associated fluorescence (n=56 spindles, n=112 spindle halves) for the steady state
metaphase spindles is shown in Fig. S1(D). For comparison, the distribution of kinetochore-associated fluorescence in the elongating (and therefore unsteady-state) prometaphase spindles is shown as well (Fig. S1(D)). The peak in kinetochore clustering midway between the pole and equator (i.e. at the quarter-spindle location) was more prominent using the redefined metaphase data set, with distinctly reduced kinetochore-associated fluorescence at the spindle equator. By redefining the metaphase experimental data set, models for regulation of kMT dynamics are more tightly constrained as compared to our previous work.

**Analysis of the kinetochore marker Cse4-GFP for use in characterizing kMT plus-end dynamics**

In order to test the model assumption that the kinetochore marker Cse4-GFP provides a reasonable approximation of kMT plus-end locations in the yeast metaphase spindle, quantitative electron microscopy data was forward-convolved to create simulated fluorescence images. Here, simulated green fluorescent markers were placed at the plus-end location of each putative kMT analyzed in electron micrograph reconstructions (Winey et al., 1995) and simulated red fluorescent markers were placed in the locations of the kMT minus ends. These images were then convolved with the measured microscope point spread function and typical background and noise levels in Cse4-GFP fluorescence images. Representative simulated images generated from electron microscopy data are shown in Fig. S2(A), with typical experimental Cse4-GFP fluorescence images shown in Fig. S2(B). A fluorescence distribution curve comparing the mean simulated plus-end fluorescence generated from electron microscopy data (n=4)
to mean kinetochore-associated Cse4-GFP fluorescence (n=56) is shown in Fig. S2(C).

Reasonable agreement between the locations of simulated peak kMT plus-end associated fluorescence and peak Cse4-GFP kinetochore-associated fluorescence supports the assumption that Cse4-GFP locations can reasonably predict kMT plus-end locations in yeast metaphase spindles.

**Analysis of the assumption of kMT dynamic instability**

All models considered assumed that kMTs exhibit dynamic instability, as observed in yeast cytoplasmic microtubules (Carminati and Stearns, 1997). Specifically, kMTs are assumed to be in either one of two states: growing or shortening, as produced by polymerization or depolymerization at kMT plus ends, respectively. Is it possible that the microtubules do not conform to a dynamic instability model? Lack of dynamic instability behavior has been previously observed in living cells (Vorobjev et al., 1999). However, this does not seem to be especially problematic as a more generalized model for assembly would be a “diffusion with drift” model as put forward by (Maly, 2002). In this work, Maly showed that dynamic instability can be modeled as a special case of diffusion with drift in the limit of rapid switching, and that the two parameters of the diffusion with drift model (i.e. the drift velocity and the diffusion coefficient) can be calculated directly from the four parameters of dynamic instability. Therefore, yeast kMT self-assembly behavior via diffusion with drift can be modeled by the dynamic instability model with very high catastrophe and rescue frequencies.

Previous studies have documented a possible “pause” state in dynamic instability, which represents a third assembly state where there is no change in length during the
pause phase (Cassimeris et al., 1988). Could pausing be regulated spatially, rather than catastrophe or rescue, to explain the experimental observations? Certainly if pausing were extensive near the quarter-spindle location, then there would be clustering of kinetochore fluorescence as observed experimentally. However, such a model would allow long-lived equator crossing to occur, in conflict with the Cse4-GFP FRAP experiment. Finally, such an effect would not give rise to the phenotype observed in the cdc6 mutant cells. Therefore, spatially-dependent pausing would not explain the experimental results.

The only other documented phenomenon that could in principle give rise to the experimentally observed metaphase kinetochore clustering is that of “history-dependent” switching (Dogterom et al., 1996; Howell et al., 1997; Odde et al., 1995). However our previous analysis documented that this model produces only qualitative agreement and cannot explain Cse4-GFP distribution quantitatively (Sprague et al., 2003).
SUPPLEMENTAL RESULTS: MODEL SCREENING

A number of models with varying complexity were considered and failed to reproduce one or more of the four experiments, as shown in Table S1. As the Tubulin-GFP experiment did not invalidate any models, it is not included in Table S1. However, this experiment did serve to constrain the values for $V_g$ and $V_s$ (see text). In this table, all tested individual models as well as model combinations are summarized. All models were first screened for their ability to correctly predict wild-type experimental metaphase kinetochore clustering (Exp 1). If a model or model combination failed to reproduce wild-type metaphase kinetochore clustering correctly (i.e. $p<.05$), it was ruled out, and therefore not tested through further simulations. Models and associated parameter value sets that qualitatively reproduced metaphase kinetochore clustering were then run through the Cse4-GFP FRAP simulation (Exp 2) and the simulation results compared to experimental Cse4-GFP FRAP percentages. Finally, all models that correctly reproduced both wild-type kinetochore fluorescence distributions and Cse4-GFP FRAP experiments were used to simulate tension-deficient $cdc6$ mutant spindles by setting the mechanical spring constant in the simulation to 0 (Exp 3). Probabilities of fit ($p$-values) were calculated for all simulations through quantitative comparison of simulated and experimental data as described below. This approach effectively implemented a computer-based combinatorial screen of models having complexity similar to the catastrophe gradient with tension-dependent rescue model that was ultimately found to satisfactorily explain all of the experimental results.
SUPPLEMENTAL MATERIALS AND METHODS

Statistical Analysis of Kinetochore-Associated Fluorescence

Image analysis was completed by integrating the total kinetochore Cse4-GFP fluorescence over a 15 pixel wide band along the length of the spindle and then calculating the percentage of spindle fluorescence at each spindle position as a function of relative distance along the spindle, as described previously (Sprague et al., 2003). Integrated Cse4-GFP fluorescence along the spindle for cdc6 mutant cells was binned into 42 equal bins rather than the 24 equal intervals used for wild-type spindles, in order to account for the experimentally observed longer spindle lengths. For the Cse4-GFP FRAP and GFP-tubulin FRAP experiments, the total integrated fluorescence intensity was calculated for each half-spindle. Statistical comparison between simulated images and experimental images was completed as previously described (Sprague et al., 2003).

Statistical Comparison of Simulated and Experimental FRAP Results

The simulated Cse4-GFP FRAP results were compared to the experimental results by running 50 simulated experiment sets (n=13 cells/experiment), and then calculating the mean recovery over all 50 simulated experiments. The sum-of-squares error (SSE) between each simulated experiment set and the 50-set mean was then calculated. The p value was calculated by ranking the sum-of-squares error between the mean experimental recovery and the 50-set simulated mean in the list of 50 simulated SSEs.

For each simulated GFP-Tub1 FRAP experiment, recovery half-times were calculated as described in (Maddox et al., 2000). P values for fit of simulated results to
published experimental results were then calculated as described above, with n=6 cells/experiment.

Statistical Determination of Steady-State Metaphase Kinetochore Distribution

Simulated images of yeast metaphase spindles were generated only after a warm-up time was allowed (~10 minutes), such that the system was allowed to settle into a steady-state configuration prior to construction of simulated images. In order to ensure that experimental images represented steady state metaphase we performed a statistical analysis of the experimental Cse4-GFP fluorescence image data set, such that only steady-state metaphase experimental images were used for quantitative comparison to simulated images. To compare kinetochore-associated fluorescence distributions at various spindle lengths, experimental fluorescence image data was sorted by spindle length (in pixels, 1 pixel = 0.066 µm), and a mean fluorescence distribution calculated for each spindle length. By comparing the similarity of these fit curves, it was possible to build a combined “steady-state” mean fluorescence distribution curve for metaphase spindles. For example, starting with the data set for a 26 pixel spindle length, we statistically compared the 26 pixel length mean fit fluorescence distribution curve to the 25 pixel mean fit curve by performing a sum-of-squares error (SSE) comparison of fluorescence distribution curves between the individual 26 pixel data set and a randomly reshuffled 25-26 pixel merged data set. In each case, integrated kinetochore-associated fluorescence was calculated for each of 24 equal bins in the fluorescence distribution curve. If these curves were the "same" (p>0.01, calculated based on ranking of the merged 25-26 pixel data set mean SSE value in the individual 26 pixel data set SSE
values), they would be combined into a "steady-state" distribution. Then, this new "steady-state" distribution was compared to the 24 pixel fluorescence mean fit curve. Again, if the fit curves were not statistically distinct, the 24 pixel set would be added to the "steady-state" distribution. Once we came to a spindle length in which the probability of fit of its mean fit curve to the steady state mean fit curve was low (p<0.01), this spindle length and all spindle lengths shorter (or longer) than this were excluded from the steady-state data set.

Simulation of microtubule dynamics
A Monte Carlo technique was used to simulate individual kMTs undergoing dynamic instability using MATLAB (Version 6.0, The MathWorks, Natick, MA) as previously described (Sprague et al., 2003), with the following exception. In previous work, a spindle length of 1500 nm was assumed for all simulated spindles. The current model has been updated to allow for flexibility in simulated spindle lengths. Rather than using a mean experimentally observed spindle length, the measured spindle length of each of the experimental spindles was used in the simulation. This spindle length was then held constant for the duration of the simulation for each cell.

Estimation of point spread function and background fluorescence
The point spread function of the microscope imaging system was determined as previously described (Sprague et al., 2003). The method for estimating background fluorescence was determined in a manner similar to previous work, with the following exception. Rather than determining the mean background fluorescence in 10 random
cells, the mean and standard deviation of the background fluorescence was measured on each experimental image, and then reproduced in a simulation for that particular spindle. As background fluorescence was found to vary by location within the cell, the mean background fluorescence was calculated along the spindle length for each cell (15 pixel wide band 7 pixels away from the mid-line of the spindle) on both sides of the spindle. The background noise was then calculated based on the standard deviation of the background fluorescence and reproduced for each of the experimental images.

Models for MT dynamics

Models for a rescue gradient mediated by a polar ejection force and a catastrophe gradient mediated by a chemical gradient model were used as described by Sprague et al. (2003). Briefly, the rescue gradient model was defined such that the kMT rescue frequency was high at the spindle pole bodies and decreased rapidly as the kMT plus ends approached the spindle equator. The catastrophe gradient model, based on the theoretical gradient of a catastrophe promoting molecule, resulted in low kMT catastrophe frequencies at the spindle pole bodies, with a maximum in catastrophe frequencies at the spindle equator.

The tension-dependent catastrophe frequency and tension-dependent rescue frequency models were used as described by Sprague et al. Briefly, the tensile force between sister kMTs was modeled as a Hookean spring with a rest length between sister kinetochores of 200 nm,

$$F_{\text{tensile}} = \rho(s - s_{r}),$$

(2)
where $s$ is the separation distance between the tips of sister kMTs (units of m), $s_r$ is the rest length between sister kMT tips (units of m), and $\rho$ is the spring constant (units of N/m). The dependence of rescue frequency on tension was assumed to obey an exponential relationship, given by

$$k_r = k_{r,0}e^{F_{\text{tensile}}/F_o}$$  \hspace{1cm} (3)

where $k_{r,0}$ is the rescue frequency in the absence of tensional force (units of m$^{-1}$), $F_{\text{tensile}}$ is the tensional force between sister kinetochores (units of N), and $F_o$ is the characteristic force at which the rescue increases e-fold (units of N). Combining Eqs. 2 and 3 yields that

$$k_r = k_{r,0}e^{(\rho^*(s-s_r))}$$  \hspace{1cm} (4)

where $\rho^* = \rho/F_o$, which has units of m$^{-1}$ (note: this parameter was termed $\rho$ in Sprague et al., 2003).

**Simulation of the Cse4-GFP FRAP experiment**

The FRAP of Cse4-GFP was recently reported by Pearson et al. (Pearson et al., 2004). In this study, one half of a metaphase spindle was bleached and the recovery in the bleached zone quantitatively measured over time. It was found that recovery of the spindles was barely detectable, even over long periods of time (\textgreater{}10 min). To simulate the Cse4-GFP FRAP experiment, a standard simulation of microtubule dynamics was allowed to run for 350 seconds (700 time steps) prior to simulation of Cse4-GFP laser photobleaching, in order to allow the system to first reach a steady-state. At the time of the bleach event (350 s), an array was generated with 32 elements, one element corresponding to each simulated kMT tip, to record the bleach status of each simulated
kinetochore. If the length of a kMT attached to the left SPB (at 350 seconds) was less than or equal to \( \frac{1}{2} \) of the total spindle length, or if the length of a kMT attached to the right SPB was greater than or equal to \( \frac{1}{2} \) of the spindle length, the element in the bleach status array corresponding to that kMT tip was assigned as 0, indicating that the kinetochore Cse4-GFP attached to the plus-end of that kMT was bleached. All other kMT tips were assigned as 1 in the bleach status array, indicating that the kinetochore Cse4-GFP was not bleached. Thus, any kinetochores in the left half of the spindle were considered bleached, regardless of which pole their respective kMT was anchored to. This array was then stored, remaining unchanged, for the duration of the simulation.

Simulated microtubule dynamics were allowed to continue for an additional 650 seconds after the bleach event. Simulated fluorescence images were then generated as described above, with the following exception. Prior to generating images of the kinetochore position data array, each element in the bleach status array was multiplied by its corresponding element in the kinetochore position data array. In this way, all bleached kMT tips were assigned 0 values in the kinetochore position array, rendering bleached kinetochores nonfluorescent during the image simulation process.

In modeling of the Cse4-GFP FRAP experiment, it was assumed that one entire half-spindle was bleached during the experimental bleaching event. In addition, the model does not account for Cse4-GFP turnover on the kinetochore or diffusion of free (unbleached) Cse4-GFP into or out of the bleached spindle half. The observed lack of recovery in Cse4-GFP FRAP experiments supports this assumption. A spindle length of 1.72 \( \mu \text{m} \) was used for all of the Cse4-FRAP simulations.
Analysis of the Cse4-GFP FRAP experiment and simulation

The calculation of Cse4-GFP FRAP was identical for both the experimental work as well as the simulation (Pearson et al., 2004). In each case, the baseline fluorescence bleach amount was established by measuring the integrated fluorescence intensity of the spindle half prior to bleaching, and then again immediately after laser photobleaching. Another total fluorescence intensity measurement was then obtained for the bleached spindle half after 10 minutes of simulation and/or experimental time had elapsed. The fluorescence recovery in each case was calculated according to the following formula:

\[
\text{\% Recovery} = \left( \frac{F_{\text{final}} - F_{t=0}}{F_{\text{pre}} - F_{t=0}} \right) \times 100
\]  

(1)

where \(F_{\text{final}}\) = integrated fluorescence intensity of bleached spindle half after 10 minutes, \(F_{\text{pre}}\) = pre-bleach fluorescence intensity of spindle half to be bleached, and \(F_{t=0}\) = bleached spindle half fluorescence intensity immediately post-bleach. Any negative recoveries (due to stochastic fluctuations) were recorded as zero for the experiment as well as the simulation.

Simulation of the GFP-Tub1 FRAP experiment

GFP-Tub1 FRAP experiments were simulated using a method similar to the FRAP of Cse4-GFP, as described above. In the case of GFP-Tub1 FRAP, each tubulin subunit in a given kMT is simulated. During the bleach event, every incorporated tubulin subunit in the bleached half-spindle is assigned a value of zero in a bleach status array. Tubulin subunits are then exchanged with a free pool as kMTs depolymerize and then polymerize. A given percentage of the free tubulin pool is bleached during the bleaching event, as occurs in live cells (Maddox et al., 2000), and the percentage of unbleached free
units is updated as bleached or unbleached tubulin dimers are released from shortening kMTs and consumed by growing kMTs. It is assumed that free tubulin subunits can diffuse rapidly throughout the nucleus relative to the speed of MT polymerization, i.e. when bleached subunits are released from kMTs in the bleached half they are well mixed into the nuclear free subunit pool so that kMTs anywhere in the nucleus have an equal probability of picking up a bleached subunit. This assumption is justified, as the rate of polymerization is slow relative to diffusion over the distance of the nucleus (Odde, 1997). The probability of adding an unbleached subunit to a polymerizing kMT is equal to the fraction of unbleached subunits remaining in the free tubulin pool. Calculation of half-spindle integrated fluorescence is completed as described above for every time step in the simulation.

In the GFP-tubulin FRAP simulation, it is assumed that all incorporated GFP-tubulin subunits in the bleached half-spindle are rendered permanently nonfluorescent during the bleach event. In addition, 50% of the free GFP-tubulin pool, estimated to be $3.4 \times 10^4$ molecules (Sprague, 2002), is bleached. All dynamics are assumed to occur at kMT plus-ends, with no minus end flux. The simulation is not corrected for experimental photobleaching due to image acquisition, as the Maddox et al. (2000) experimental data is already corrected to compensate for this effect.
SUPPLEMENTAL REFERENCES


SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1: Metaphase congression is correlated with spindle length. (A) Shorter spindle length “prometaphase” experimental image with indistinct kinetochore clustering (green; labeled with Cse4-GFP). Spindle poles are labeled with Spc29-CFP (red). (B) Metaphase spindle experimental image with distinct kinetochore clustering on either side of the spindle equator. Scale bar, 1000 nm (C) Statistical comparison of kinetochore localization by spindle length. Spindles between 1.6-1.9 μm had consistent kinetochore distributions (p>0.25), whereas spindle lengths outside of this range had decreased probabilities of fit. Kinetochores exhibit a “prometaphase” configuration at short spindle lengths (1.1 to 1.5 μm) and congress to a metaphase configuration at spindle lengths from 1.6 to 1.9 μm. (D) Quantitative comparison of experimental kinetochore clustering in prometaphase and metaphase spindles. Relative fluorescence at each point is given as mean ± standard error of the mean (SEM). Normalized spindle position equals 0.5 at the spindle equator. Prometaphase spindles have less organized kinetochore clusters, whereas metaphase spindles have a distinct peak in kinetochore-associated fluorescence at the “quarter spindle” location, with a definite reduction of fluorescence at the spindle equator.

FIGURE S2: Kinetochore-associated Cse4-GFP clustering provides a reasonable approximation for kMT plus-end locations. (A) Representative simulated images generated by placing green fluorescent markers at putative kMT plus-ends as measured in electron micrograph reconstructions (Winey et al., 1995), and red fluorescent markers at
kMT minus ends (Scale bars, 1000 nm). Absolute kMT lengths are increased by ~30 nm in the simulated images to account for the distance between the kMT minus ends as measured in electron micrograph reconstructions and the Spc29-CFP spindle pole marker used in experimental fluorescence images (Adams and Kilmartin, 1999). All simulated images have been convolved with the measured microscope point spread function and typical noise levels in the Cse4-GFP fluorescence images. (B) Typical Cse4-GFP experimental fluorescence images (Scale bar, 1000 nm) (C) Quantitative analysis of average simulated kMT plus-end fluorescence clustering with comparison to mean experimental Cse4-GFP kinetochore-associated fluorescence clustering. Thus, Cse4-GFP fluorescence provides a good approximation of the kMT plus-end positions as evaluated by electron micrograph reconstructions.