Chromosome Congression by Kinesin-5 Motor-Mediated Disassembly of Longer Kinetochore Microtubules

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SUMMARY
During mitosis, sister chromatids congress to the spindle equator and are subsequently segregated via attachment to dynamic kinetochore microtubule (kMT) plus ends. A major question is how kMT plus-end assembly is spatially regulated to achieve chromosome congression. Here we find in budding yeast that the widely conserved kinesin-5 sliding motor proteins, Cin8p and Kip1p, mediate chromosome congression by suppressing kMT plus-end assembly of longer kMTs. Of the two, Cin8p is the major effector and its activity requires a functional motor domain. In contrast, the depolymerizing kinesin-8 motor Kip3p plays a minor role in spatial regulation of yeast kMT assembly. Our analysis identified a model where kinesin-5 motors bind to kMTs, move to kMT plus ends, and upon arrival at a growing plus end promote net kMT plus-end disassembly. In conclusion, we find that length-dependent control of net kMT assembly by kinesin-5 motors yields a simple and stable self-organizing mechanism for chromosome congression.

INTRODUCTION
A central question in biology is how replicated chromosomes are properly segregated during mitosis, such that exactly one copy of each chromosome moves to each of the two daughter cells (Nicklas, 1997). In eukaryotes, chromosome-associated kinetochores attach to dynamic microtubule (MT) plus ends, while MT minus ends in turn generally attach to spindle poles (Inoue and Salmon, 1995). Once properly bioriented, so that one kinetochore is mechanically linked via one or more MTs to one pole and its sister kinetochore linked to the opposite pole, the sister chromosomes move toward the equator of the mitotic spindle, a process known as congression (Figure 1A). In budding yeast, congression of bioriented sister chromosomes requires that the associated kinetochore microtubule (kMT) plus ends add tubulin subunits efficiently when they are near the poles (i.e., when kMTs are short) but inefficiently when near the equator (i.e., when kMTs are relatively long; Gardner et al., 2005; Pearson et al., 2006; Sprague et al., 2003; Figure 1A). The origin of this spatial gradient in net kMT plus-end assembly is currently unknown.

One possible explanation for the spatial gradient of kMT net assembly derives from studies with the budding yeast kinesin-8 molecular motor Kip3p, which showed that processive plus-end-directed depolymerizing motors could result in length-dependent disassembly of MTs in vitro (Howard and Hyman, 2007; Varga et al., 2006). However, to date there has not been an in vivo observation of length-dependent MT regulation via Kip3p or any other motor. Recent in vivo studies of Kip3p showed that cytoplasmic microtubule dynamics are altered by KIP3 deletion results in spindle positioning defects (Gupta et al., 2006). Whether these in vivo effects resulted from the length-dependent control of MT assembly as proposed by Varga et al. (2006) was not clear. As a result, it is not clear at this point whether this interesting phenomenon identified in vitro by Varga et al. plays an important role in a biological process.

We now find that the kinesin-5 molecular motors, mainly Cin8p and, to a lesser extent, Kip1p, exhibit length-dependent control of MT assembly and so mediate the spatial gradient in kMT plus-end net assembly that drives congression during mitosis. Since their discovery, kinesin-5 motors have been viewed as mitosis-specific sliding motors that crosslink antiparallel MTs and exert outward extensional forces on the poles (Hoyt et al., 1992; Kapitein et al., 2005; Roof et al., 1992; Saunders and Hoyt, 1992; Tao et al., 2006). The results were surprising to us because kinesin-5 (Eg5 in vertebrates) is not known to affect microtubule assembly. Surprisingly, the disassembly-promoting activity that we now report is not specific to kMTs since we found that Cin8p promotes disassembly of cytoplasmic astral MTs (aMTs) as well.

Because the kinesin-5-mediated length-dependent kMT disassembly is similar to that described by Varga et al. for Kip3p on aMTs in vitro, we examined the role of Kip3p in controlling kMT assembly in vivo. We found that Kip3p only weakly affects kMT dynamics and is not a major mediator of congression. Our computer simulations show that the difference in kinesin-5 and kinesin-8 behavior can be accounted for simply by the bipolar ability of tetrameric kinesin-5 to crosslink microtubules, a
Figure 1. Cin8p Organizes Metaphase Yeast Kinetochores in a Manner Consistent with Length-Dependent Suppression of Net kMT Plus-End Assembly

(A) A spatial gradient in net kMT plus-end assembly mediates kinetochore congression in yeast. Kinetochores (cyan) congress to attractor zones (yellow arrows) on either side of the spindle equator (dotted line) during yeast metaphase via the plus-end assembly dynamics of kMTs (black). The kMT plus-end assembly dynamics are spatially regulated such that plus-end assembly is favored near the poles (gray) when kMTs are relatively short (favorable assembly zone shown as green gradient) and suppressed near the spindle equator (dotted line) when kMTs are relatively long (assembly suppression zone shown as red gradient).

(B) A computational model of yeast metaphase kMT plus-end dynamics predicts that deletion of the length-dependent promoter of kMT disassembly will result in longer kMTs and disorganized kinetochores. Conversely, overexpression will result in shorter kMTs and focused kinetochore clusters. All model parameters as in Table S2.

(C) CIN8 deletion results in kinetochore disorganization and the net shifting of kinetochores toward the spindle equator, suggesting that kMTs are on average longer than they are in WT cells (red, Spc29-CFP pole marker; green, Cse4-GFP kinetochore marker) (scale bar 1000 nm, error bars, SEM).

(D) Cin8p overexpression results in clusters of kinetochores near the SPBs, suggesting that kMTs are shorter than in WT cells (error bars, SEM).
property lacking in dimeric kinesin-8. This naturally biases kinesin-5 toward kMT plus ends and kinesin-8 toward interpolar MT plus ends. Together, the results presented here suggest a simple explanation for metaphase chromosome congression in budding yeast by identifying a microtubule length-dependent disassembly-promoting activity in vivo that is associated with kinesin-5 molecular motors.

RESULTS

Simulated Phenotypes of a Disrupted Gradient in Net kMT Plus-End Assembly
MTs self-assemble from αβ-tubulin heterodimers via an unusual process called “dynamic instability” where MTs grow at a roughly constant rate, then abruptly and stochastically switch to shortening at a roughly constant rate, then switch back to growth again, and so forth. The switch from growth to shortening is called “catastrophe,” and the switch from shortening to growth is called “rescue” (Desai and Mitchison, 1997). Together, the four parameters of dynamic instability, growth rate (Vg = \( \frac{1}{t} \) μm/min), shortening rate (Vs = \( \frac{1}{t} \) μm/min), catastrophe frequency (kC = \( \frac{1}{t} \) 1/min), and rescue frequency (kr = \( \frac{1}{t} \) 1/min), define the net assembly state of MTs. If the mean length added during a growth phase (Lg = Vg/kC) exceeds the mean length lost during shortening (Ls = Vs/kr), then there will be net growth; otherwise there will be net shortening. If the parameters depend upon position in the cell, then there can exist net growth in one part of the cell and net shortening in another part of the cell. At the transition between these regions there will be no net growth, and this will create an attractor for plus ends, provided net growth is favored for short MTs and net shortening favored for long MTs (Figure 1A).

Our previous studies showed that net kMT plus-end assembly in budding yeast metaphase spindles is favored for plus ends located near the spindle pole bodies (SPBs) (i.e., for short kMTs) and inhibited for plus ends near the equator (i.e., for longer kMTs) (Figure 1A; Gardner et al., 2005; Pearson et al., 2006; Pearson et al., 2004; Sprague et al., 2003). This spatial control over net kMT assembly was most readily explained using a catastrophe gradient in kMT plus-end assembly that creates two attractor points where there is no net assembly, one attractor in each half-spindle (Figure 1B, left, dotted line denotes the location of the attractor point for one half-spindle). These two attractors establish the bilobed distribution of kinetochores into the two distinct clusters that are characteristic of the congressed metaphase spindle (Figure 1A).

We were interested in identifying the molecules responsible for this net assembly gradient, and so we simulated the expected phenotypes for changes in expression level of a putative spatial regulator of net kMT plus-end assembly. Figure 1B (left) shows a simulation of a wild-type (WT) budding yeast metaphase spindle where kMT plus-end assembly is favored near the SPB (where kMTs are short) and suppressed near the spindle equator (where kMTs are longer). Note that in the haploid budding yeast there are 16 kMTs emanating from each SPB, for a total of 32 kMTs, and ~8 longer interpolar MTs (iMTs), for a total of 40 MTs in the metaphase spindle (for the sake of clarity the iMTs are omitted from the animation in Figure 1). If a molecule promoted disassembly of longer kMTs in WT cells and was then deleted, the predicted phenotype would be longer kMTs with kinetochores more broadly distributed along the spindle, as depicted in Figure 1B, center. Conversely, overexpression of a spatial assembly regulator would produce very short kMTs with highly focused clusters of kinetochores near the SPBs, as depicted in Figure 1B, right. These model predictions establish specific requirements for experimental identification of a spatial kMT plus-end assembly regulator.

The Yeast Kinesin-5 Motors, Cin8p and Kip1p, Control Kinetochore Positions
While studying various deletion mutants, we observed that cin8Δ mutants lost the clustering of kinetochores within each half-spindle (measured by labeling of a kinetochore component, Cse4-GFP, in live cells), as shown in Figure 1C, consistent with an earlier report (Tytell and Sorger, 2006). Quantitative analysis of experimental Cse4-GFP fluorescence revealed that the peak fluorescence intensity also shifted toward the spindle equator, as predicted by simulations used to model deletion of a molecule that promotes net kMT plus-end disassembly of longer kMTs (Figure 1C, p = 0.82, where p is the probability that the experimental Cse4-GFP fluorescence distribution curve is consistent with the simulated curve; see Experimental Procedures for calculation procedure). The simulated Cse4-GFP fluorescence distribution was obtained by convolution of the simulated fluorophore positions with the imaging system point spread function and noise, a computational process we call “model-convolution” (Gardner et al., 2007; Sprague et al., 2003). Deletion of the other yeast kinesin-5 motor, KIP1, had a similar, but weaker, phenotype to cin8Δ, with a moderate shift of kinetochores toward the spindle equator (Figure S1A available online). We note that the effects of CIN8 deletion were not due to the well-known moderate decrease in steady-state spindle length (Hildebrandt and Hoyt, 2000; Hoyt et al., 1992; Saunders et al., 1997; Saunders and Hoyt, 1992) since we selected spindle lengths that were equal for both WT and cin8Δ cells (although results were similar regardless of the spindle length population analyzed; Figure S2). To further establish that the effects of CIN8 deletion were not due to changes in metaphase spindle lengths, we performed separate experiments using histone H3 repression mutants, which make centromeric chromatin more compliant and thus increase average spindle length (Bouck and Bloom, 2007). In these longer spindles, WT kinetochores were still bilobed while cin8Δ kinetochores were still disorganized (Figure S1B), showing an insensitivity of the disorganization phenotype to spindle length. In addition, bim1Δ mutant spindles with short spindle lengths (similar to cin8Δ mutant spindle lengths, Figure S3) did not result in spindle disorganization (Figure S3). We conclude that kinetochores are declustered in kinesin-5 deletion mutants, independent of the spindle length.

If Cin8p mediates net kMT plus-end disassembly, then Cin8p overexpression will result in short kMTs with focused clusters of kinetochores, one near to each SPB (Figure 1B, right). As shown in Figure 1D, kinetochore clusters were indeed tightly focused within each half-spindle and much closer to each SPB. Spindles overexpressing Cin8p also have increased length due to increased motor sliding between oppositely oriented central
spindle non-kMTs (also known as interpool MTs) (Saunders et al., 1997) (Figure S4). Despite the spindles being longer, kinetochores in Cin8p overexpressing cells were still ~50% closer to SPBs than WT controls (Figure 1D), consistent with Cin8p overexpression resulting in shorter kMTs (Figure S4). We conclude that Cin8p and, to a lesser extent, Kip1p promote net kMT plus-end disassembly as judged by kinetochore position.

### GFP-Tubulin Fluorescence Confirms that kMTs Are Longer in Cin8Δ Mutants

If Cin8p promotes net kMT plus-end disassembly, then CIN8 deletion will result in longer kMTs, producing a continuous “bar” of fluorescent tubulin along the length of the spindle (Figure 2A, right), rather than the WT fluorescent tubulin “tufts” that emanate from each of the two SPBs (Figure 2A, left). In experiments with GFP-Tub1, quantitative analysis of tubulin fluorescence in cin8Δ mutants revealed a shift in fluorescence toward the spindle equator, indicating that kMT length was increased (Figure 2A, bottom). The distribution of GFP-Tub1 was quantitatively predicted in simulations using the same parameter set used to model kinetochore organization in cin8Δ mutants (Figure 2A, p = 0.22, Table S2). In addition, we found that the ratio of spindle tubulin polymer signal to free tubulin signal outside of the spindle area is 2.0:1 in WT spindles (n = 27) and 3.2:1 in cin8Δ mutant spindles (n = 35), which represents an increase in tubulin polymer relative to free tubulin of ~62% in cin8Δ mutants as compared to WT cells (p < 10⁻⁵). This suggests that the increased kMT length in cin8Δ spindles is not the result of an overall increase of tubulin level but rather reflects a thermodynamic shift toward increased net kMT assembly.

### Cryo-electron Tomography Confirms that kMTs Are Longer in cin8Δ Mutants

To directly visualize individual spindle MTs, we used cryo-electron tomography to reconstruct complete mitotic spindles from WT and cin8Δ mutant spindles (Figure 2B; Movies S1 and S2). To control for the moderate spindle length shortening in cin8Δ mutants, we selected spindles of similar length in the WT and mutant cell populations. Consistent with model predictions, we found a substantial increase in mean MT length in cin8Δ mutant spindles as compared to WT spindles (Figure 2B, 41% increase in mean overall length, p = 0.0007, statistical consistency between cells confirmed by ANOVA in Table S1). Total non-kMT number also increased in cin8Δ as compared to WT spindles (42% increase in total MT number, p = 0.02), demonstrating that the total polymer level in the cin8Δ cells is increased relative to WT cells. Interestingly, the mean length of the eight longest MTs in each spindle, presumably iMTs, is not statistically different between WT and cin8Δ mutant cells (WT = 931 ± 81 nm [mean ± SEM, n = 33 MTs]; cin8Δ = 1141 ± 25 nm [n = 41 MTs], p = 0.05). This result suggests that deletion of CIN8 most significantly affects the kMT length rather than iMT length.

In summary, the electron microscopy results independently confirm the model predictions and the light microscopy studies by demonstrating that kMTs are indeed longer in cin8Δ cells. We conclude that Cin8p participates in a process that promotes net kMT disassembly.

### A Cin8p Motor-Domain Mutant Has Increased kMT Length

To test whether kMT length regulation requires the Cin8p motor domain, the organization of fluorescent tubulin was examined in the cin8-F467A mutant (Gheber et al., 1999). Previous studies found that the cin8-F467A mutation reduced the binding of Cin8p to microtubules in vitro by 10-fold (Gheber et al., 1999). Similar to the cin8Δ mutants, analysis of GFP-Tub1 distribution in the cin8-F467A mutants produced a continuous bar of fluorescent tubulin along the length of the spindle (Figure 2C, right), rather than the WT fluorescent tubulin tufts (Figure 2C, left). The similarity between the cin8Δ and the cin8-F467A mutant phenotypes suggests that the kMT disassembly promoting activity of Cin8p requires motor binding to kMTs. Furthermore, since our previous studies showed that net kMT assembly is promoted when kMTs are short and suppressed when kMTs are long (i.e., when kMT plus ends extend into the equatorial region, Figure 1B), we also conclude that Cin8p-mediated suppression of kMT assembly is specific to longer kMTs.

### Cin8p Mediates the Gradient in Net kMT Assembly as Measured by GFP-Tubulin FRAP

To further test whether Cin8p specifically suppresses assembly of longer kMTs and thus mediates a gradient in net kMT assembly, we measured the spatial gradient in tubulin turnover within the mitotic spindle. In our previous work, we found that tubulin turnover, as measured by spatially resolved GFP-tubulin fluorescence recovery after photobleaching (FRAP), is most rapid where kMT plus ends are clustered in WT cells (Pearson et al., 2006). If Cin8p mediates a gradient in net kMT assembly, then its deletion is predicted to result in loss of the gradient in FRAP half-time. As shown in Figure 2D, deletion of Cin8p results in loss of the tubulin turnover gradient, as predicted by the model (Figure 1B, center). In general, the kMTs remain dynamic (overall t₁/₂ = 46 ± 15 s integrated over the half-spindle in cin8Δ [n = 11], compared to t₁/₂ = 63 ± 30 s for WT [Pearson et al., 2006], [n = 22; p = 0.03; Figure S5]) and have a high fractional recovery (~90% for cin8Δ, compared to ~70% for WT [Maddox et al., 2000; Pearson et al., 2006]).

In simulating the cin8Δ GFP-tubulin FRAP experiment, the best fit between theory and experiment is achieved with a flattened spatial gradient in net kMT plus-end assembly (e.g., a flattened catastrophe gradient) and with values for MT plus-end growth and shortening rates that are slightly higher than in WT simulations (Table S2). This result explains why FRAP is relatively rapid along the entire length of the half-spindle in the cin8Δ mutants and is consistent with recent studies that find increased chromosome oscillation velocities upon deletion of the human kinesin-8 motor Kif18A (Stumpf et al., 2008; reviewed in Gardner et al., 2008). In summary, we find that the tubulin-FRAP studies confirm that Cin8p mediates a spatial gradient in kMT assembly dynamics.

### Deletion of the Kinesin-8 Motor Kip3p Does Not Perturb Congression

Recent studies found that the kinesin-8 molecular motor Kip3p acts as a length-dependent depolymerase in vitro (Varga et al., 2008), and further studies have implicated the human kinesin-8...
Figure 2. Cin8p Promotes kMT Disassembly

(A) CIN8 deletion results in flattening of the GFP-tubulin fluorescence distribution and shifting of fluorescence toward the spindle equator, suggesting that kMT lengths are increased in the mutant (red, Spc29-CFP SPB marker; green, GFP-Tub1 MT marker) (scale bar 1000 nm, error bars, SEM). All model parameters as in Table S2.

(B) Cryo-electron tomography reveals increased mean MT length and number in cin8Δ spindles (n = 5 spindles, mean spindle length = 1387 nm), relative to WT spindles (n = 4 spindles, mean spindle length = 1265 nm) (error bars, SEM).

(C) Similar to the CIN8 deletion mutant, the motor-domain mutant, cin8-F467A, which has reduced affinity for MTs, results in flattening of the GFP-tubulin fluorescence distribution and shifting of fluorescence toward the spindle equator (error bars, SEM).

(D) CIN8 deletion eliminates the characteristic gradient in GFP-tubulin FRAP recovery half-time, consistent with disruption of the gradient in net kMT assembly (error bars, SEM).

(E) In contrast to the cin8Δ mutants, deletion of the kinesin-8 depolymerase KIP3 does not significantly perturb kinetochore microtubule (kMT) organization (error bars, SEM).
molecular motor Kif18A in the regulation of chromosome oscillation amplitude (Stumpff et al., 2008) and chromosome congression (Mayr et al., 2007). Thus, we asked whether Kip3p deletion would perturb kMT length organization in the yeast mitotic spindle. Interestingly, deletion of KIP3 does not produce the continuous bar of fluorescent tubulin along the length of the spindle that is characteristic of cin8Δ spindles but rather shows WT fluorescent tubulin tufts (Figure 2E). Although microtubule organization appears WT, spindle lengths are moderately increased in kip3Δ cells, as previously reported (Straight et al., 1998) (mean spindle lengths in Figure 2E, WT: 1.60 ± 0.22; kip3Δ: 1.97 ± 0.15 μm). Thus, Kip3p regulates spindle length, possibly through regulation of iMT lengths, while Cin8p regulates kMT length without a measurable effect on iMT lengths. Regardless of effects on iMTs, the effect of Kip3p on kMTs is relatively weak compared to the effect of Cin8p and is insufficient to establish congression in the absence of Cin8p.

Cin8p Promotes Shortening of Astral MTs in the Cytoplasm

MTs are densely packed in the yeast mitotic spindle (Figure 2B), and so it is difficult to resolve individual spindle MTs via fluorescence microscopy. In contrast, yeast aMTs are normally much fewer in number (1–3) and splayed apart (Figure 3A) (Gupta et al., 2002; Shaw et al., 1997). Because Kip3p is known to affect aMT dynamics (Cottingham and Hoyt, 1997; Gupta et al., 2006; Miller et al., 1998), but the effect of Cin8p is not known, we then examined the effect of motor deletion on aMTs outside of the mitotic spindle. Indeed, we found that aMT lengths were statistically longer in kip3Δ mutants as compared to WT cells (Figures 3B and 3C), consistent with previous studies (Cottingham and Hoyt, 1997; Gupta et al., 2006). A recent report suggests that Cin8p plays a role in spindle positioning through an aMT-dependent mechanism, and so, coupled with our results for kMTs, we hypothesized that Cin8p also suppresses aMT assembly (de Gramont et al., 2007; Geiser et al., 1997). Using GFP-tubulin fluorescence microscopy, we measured the length of individual aMTs in the cytoplasm (Figure 3B) (Carminati and Stearns, 1997; Gupta et al., 2002; Shaw et al., 1997). Consistent with the behavior of kMTs, aMT lengths were longer in cin8Δ mutants relative to WT cells (Figures 3B, 3C and S6). In addition, a previous study showed that aMT numbers are not decreased in cin8Δ mutants (de Gramont et al., 2007), consistent with an overall increase in both kMT and aMT polymer in cin8Δ mutants. Interestingly, aMT lengths in the cin8Δ-F467A mutants with reduced binding of Cin8p to microtubules were also statistically longer than WT aMTs (Figure 3C). Conversely, overexpression of Cin8p resulted in shorter aMTs (Figures 3B, 3C, and S6).

Since the tubulin polymer level in the cin8Δ cells increases in both the nucleus and the cytoplasm, these results argue against a simple repartitioning of tubulin (or some other Cin8p-dependent assembly-promoting factor) from the cytoplasm into the nucleus in response to CIN8 deletion. Conversely, MT polymer levels decrease in both compartments upon Cin8p overexpression.

One possible reason why both kMTs and aMTs are longer in cin8Δ cells is that CIN8 deletion indirectly promotes MT assembly globally. To test this hypothesis, we shifted Cin8p from the nucleus to the cytoplasm while keeping the overall Cin8p-dependent assembly-promoting factor in the cytoplasm into the nucleus in response to CIN8 deletion. Conversely, MT polymer levels decrease in both compartments upon Cin8p overexpression.
the nuclear Cin8p concentration and increases the cytoplasmic Cin8p concentration (Hildebrandt and Hoyt, 2001). We found that cin8-nls Δ spindle MTs had a flat GFP-Tub1 fluorescence distribution, similar to cin8 Δ cells (i.e., no tufts, Figure S7A). This result is consistent with net kMT assembly in the absence of Cin8p locally in the nucleus (Figures 3B and S7A). Importantly, and in contrast to cin8 Δ cells, aMT lengths in cin8-nls Δ cells were shorter than in WT cells (Figures 3B and 3C, p < 0.001, Figure S7C), consistent with elevation of Cin8p concentration locally in the cytoplasm. These results indicate that Cin8p acts locally in a given cellular compartment, rather than globally throughout the entire cell, to influence the local MT assembly state.

Together, these results suggest that Cin8p and Kip3p act similarly to suppress assembly of aMTs outside of the mitotic spindle. We then asked how Cin8p could act to promote kinetochore congression through suppression of kMT plus-end assembly, while Kip3p appears to regulate spindle length, possibly through suppression of IMT plus-end assembly.

A Model for Cin8p Motor Interaction with kMTs

Because Cin8p requires microtubule binding to promote length-dependent kMT plus-end disassembly, we hypothesized that Cin8p acts directly on kMT plus ends, either by itself or with a binding partner. To test the direct-interaction hypothesis, we first predicted the spatial distribution of Cin8p motors on kMTs via computational modeling. As a starting point, we extended our previous model for individual kMT plus-end dynamics (Gardner et al., 2005) to also include the dynamics of microtubule-associated kinesin-5 molecular motors. This motor model assumes that motors reversibly attach and detach, crosslink MTs, and move toward plus ends (Movies S3 and S4; Figures S8 and S9; Tables S3–S6) (Gheber et al., 1999; Kapitein et al., 2005; Kashina et al., 1996; Valentine et al., 2006).

Cin8-GFP Is Distributed in a Gradient along kMTs

As shown in Figure 4A, this simple model for motor dynamics predicts that, at steady-state, a substantial fraction of the motors crosslink parallel kMTs emanating from the same SPB (green). In the simulation, these motors walk to a kMT plus end and follow the end as it grows until catastrophe occurs, at which point the motor head detaches (Movie S4). By repetitive photobleaching of Cin8-3XGFP-labeled spindles, we were able to experimentally observe spindle-associated Cin8p motor dynamics in the “speckle” regime. Similar to the motor model simulation, we observed motor movement in the spindle with a mean velocity of 58 ± 24 nm/s (mean ± standard deviation [SD], n = 10) (Figure 4B; see Figure S8 and Supplemental Data).

To test the motor model, we predicted the distribution of Cin8-GFP fluorescence in live cells as a function of spindle position and then measured it experimentally. As shown in Figure 4C, the motor model is able to quantitatively reproduce the experimentally observed Cin8-GFP motor fluorescence distribution (green) relative to kinetochores as measured by Ndc80-cherry fluorescence (red) (p = 0.35). We found that in order to correctly reproduce the Cin8-GFP fluorescence distribution, simulated motors are required to track growing but not shortening kMT plus ends, resulting in a slight, but detectable, shift in the peak of motor-associated fluorescence away from kinetochores and toward SPBs (see Supplemental Data for analysis of various alternative models, Figure S9). In the motor model, this shift is the result of the increased motor off-rate imposed by shortening kMT plus ends within the kinetochore clusters (Figure 4C, red). In benomyl-treated spindles with stabilized microtubules, this shift is decreased, such that the motor-associated fluorescence is nearly coincident with kinetochores (Figure S10). Thus, the results with benomyl further support the hypothesis that kMT plus-end assembly dynamics control motor dynamics, in particular motor detachment from kMT plus ends.

The Kip1–GFP fluorescence distribution was qualitatively similar to the Cin8-GFP fluorescence distribution but was relatively less focused into two clusters, consistent with a weaker affinity of Kip1p for MTs (Figure S11).

By normalizing the Cin8-GFP fluorescence to the local kMT density, we then calculated the Cin8-GFP density on a per kMT basis (see Experimental Procedures for details). As shown in Figure 4D, Cin8-GFP concentration per kMT gradually increases with increasing distance from the SPB, as predicted by the motor model. We conclude that αβ-tubulins at the plus ends of longer kMTs are more likely to be associated with Cin8-GFP than αβ-tubulins at the plus ends of shorter kMTs.

Cin8-GFP Turnover Is Most Rapid near kMT Plus Ends

If motors rapidly detach from shortening kMT plus ends, then the rate of Cin8-GFP turnover on the spindle should be fastest in the vicinity of shortening kMT plus ends. Specifically, the motor model predicts there will be a spatial gradient in Cin8-GFP FRAP half-time that is very similar to the GFP-tubulin FRAP half-time gradient that we reported previously (Pearson et al., 2006) (Figure 4C). Alternatively, the Cin8-GFP FRAP half-time could be longest where kMT plus ends are clustered, indicative of high-affinity binding to the kinetochore (Tytell and Sorger, 2006), which would also in principle explain the gradient in Cin8-GFP intensity observed experimentally in Figure 4C. We performed kinesin-5-GFP FRAP experiments and found that motor turnover is most rapid in the location of kMT plus-end clustering (Figures 4E and S12; p < 1 × 10−10 as compared to half-times near the SPBs), suggesting that Cin8p motors rapidly detach from shortening kMT plus ends. Mean Kip1-GFP FRAP half-times were ~50% faster than Cin8-GFP recovery half-times (Figure S12), again suggesting that Kip1p has a lower affinity for microtubules than Cin8p. The Cin8-GFP FRAP results are all consistent with a model in which motors frequently interact with kMT plus ends in a length-dependent manner.

To summarize our studies of spindle-bound kinesin-5 dynamics, we find that kMT-bound kinesin-5 motors are distributed in a spatial gradient along kMTs, with their concentration increasing with increasing distance from the SPB. Consistent with the observed gradient, we also find that kinesin-5 motors frequently interact with, and are controlled in their detachment from the MT lattice by, dynamic kMT plus ends. Because Cin8p suppresses assembly of longer kMTs to mediate kinetochore congression, while Kip3p appears to act primarily to regulate metaphase spindle length, we then asked how the behavior of the kinesin-5 motor Cin8p compares to the behavior of the kinesin-8 motor Kip3p in the mitotic spindle environment.
Cin8-GFP Concentrates near kMT Plus Ends, while Kip3-GFP Distribution Is Diffuse

As shown in Figure 5A and described above, Cin8-GFP concentrates near kMT plus ends. This localization is reproduced in simulation because a substantial fraction of the homotetrameric Cin8 motors crosslink parallel kMTs emanating from the same SPB and then walk efficiently toward the plus ends of both cross-linked microtubules. In contrast, motors crosslinking antiparallel microtubules in the simulation (i.e., microtubules attached to opposite SPBs, Figure 4A, magenta) are not able to make progress.
toward the plus ends of either microtubule to which they are attached, as the motor heads walk in opposite directions. These antiparallel attached motors quickly stall and remain stationary until the detachment of one or both heads. Interestingly, since iMTs run nearly the full length of the spindle, their plus ends reside in a highly antiparallel environment, with ~4 iMT plus ends surrounded by ~20 antiparallel MTs attached to the opposite SPB. Thus, the processivity of the tetrameric kinesin-5 motor is naturally limited near to iMT plus ends due to the bipolar cross-linking properties of the motor.

In contrast, dimeric Kip3p motors are not bipolar, and thus their processivity is not hindered by crosslinking to antiparallel microtubules. In this case, the longer iMTs act as larger antennae, attracting larger numbers of plus-end-directed Kip3p motors to their ends (Varga et al., 2006). As shown in Figure 5A, while Cin8-GFP is specifically concentrated near to kMT plus ends, Kip3-GFP is more diffusely distributed along the length of the spindle. Importantly, the relative concentration of Kip3-GFP is higher near to where the iMT plus ends are located (Figure 5A) as compared to Cin8-GFP, whose relative concentration is higher near to where kMT plus ends are located (Figure 5A).

To test this interpretation of the localization data, simulations were run that reproduced both Cin8-GFP and Kip3-GFP localization in the spindle (Figure 5A). In these simulations, Cin8-GFP is a bipolar MT crosslinking motor with simulation parameters as described above, and Kip3p is a slightly faster monopolar non-crosslinking motor (Kip3p motor velocity = 75 nm/s; Gupta et al., 2006) with otherwise identical simulation parameters. In these simulations, ~90% of Cin8p motor visits to all MT plus ends were to kMT plus ends specifically, while the majority of Kip3p plus-end visits were to iMT plus ends (~60%, Figure 5B). Thus, Cin8p molecular motors mediate congression by suppressing the assembly of kMT plus ends, while Kip3p appears to act primarily to limit assembly of iMT plus ends. The two different outcomes can be modeled simply by either including bipolar crosslinking (Cin8p) or not (Kip3p).

Cin8-GFP Motors on aMTs
Because Cin8p promotes aMT disassembly (Figure 3), we considered whether the motor model applies to cytoplasmic aMTs as well. In contrast to the densely packed kMTs, 1–3 individual aMTs can be readily observed in the cytoplasm so that individual motor interaction with aMTs should also be readily observable. Using timelapse microscopy we were able to visualize Cin8-NLSΔ-3XGFP interacting with 1–3 individual aMTs. As shown in Figure 6A (left), Cin8-NLSΔ-3XGFP moves persistently in the plus-end direction along stationary mcherry-Tub1-labeled aMTs (Movie S6). Motors did not move in the minus-end direction, consistent with the motor model and the inferred motor behavior on kMTs. The mean motor velocity was 15 ± 3.3 nm/s (mean ± SD, n = 8), which is similar to previously reported aMT plus-end growth rates of 8 ± 23 nm/s (Carminati and Stearns, 1997; Gupta et al., 2002, 2006; Huang and Huffaker, 2006; Shaw et al., 1997), suggesting that motors track growing aMT plus ends. Since Cin8-GFP was distributed along kMTs in a gradient of increasing motor with increasing distance from the SPB, we were interested to see whether a similar motor gradient occurs on aMTs. As expected from the motor model and similar to the behavior of motors on kMTs, we found that Cin8-NLSΔ-GFP fluorescence is distributed along the length of aMTs with a peak fluorescence shifted slightly away from aMT plus ends (Figures 6B and 6C). We then normalized the motor fluorescence to the aMT density and, as shown in Figure 6D, found a spatial gradient of Cin8-NLSΔ-GFP that is very similar to the gradient observed on kMTs and to the gradient predicted by the motor model. An interesting feature of both the motor and the experiment is a slight dip in the motor concentration at the peak aMT plus-end location (gray arrow in Figure 5D). This dip was also apparent in the kMT data and simulation and again suggests that dynamic MT plus ends mediate motor detachment from the MT lattice. We conclude that Cin8p interacts frequently with aMT plus ends in a manner similar to its interaction with kMT plus ends and consistent with the motor model. We then asked if the spatially regulated interaction of Cin8p with kMT plus

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Figure 5. Relative Distribution of Cin8-GFP and Kip3-GFP
(A) The experimental and simulated distribution of Cin8-GFP and Kip3-GFP (error bars, SEM).
(B) In simulations, the crosslinking properties of Cin8p frustrate its processivity toward the plus ends of interpolar (iMT) plus ends, such that Cin8p visits to iMT plus ends are rare relative to kMT plus-end visits.

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ends, together with the disassembly-promoting activities of Cin8p, could act to mediate chromosome congression in the yeast metaphase spindle.

Cin8p-Mediated “Self-Organized” Model for Metaphase Kinetochore Organization

The model for kinesin-5 motor dynamics that best agrees with the experimental data described above is one in which motors bind randomly to kMTs (Figure 7A, top) and then walk toward kMT plus ends, where they act to promote net kMT disassembly (Figure 7A, middle). The longer the MT, the more sites there are for motors to attach, which results in more motors at the plus end, and the more that assembly will be disrupted. Once net disassembly occurs (e.g., via a catastrophe), then motors detach from the shortening kMT plus end (Figure 7A, bottom). Importantly, because simulated motors bind randomly to microtubules and are plus end directed, the motor model predicts that the number of motor interactions at kMT plus ends will increase with increasing kMT length, as shown recently for kinesin-8 molecular motors in vitro (Mayr et al., 2007; Varga et al., 2006) (Figures 4D and 6D).

To determine if simulated Cin8p motors could mediate chromosome congression via their disassembly-promoting activity at kMT plus ends, we allowed the Cin8p motors to promote catastrophe as they concentrate on kMT plus ends in the motor model simulation as described above. In this self-organized model, there is no externally imposed “catastrophe gradient” to regulate kMT lengths (as in Figure 1B), but rather the catastrophe-promoting motors naturally concentrate on kMT plus ends in a length-dependent manner. Thus, by starting the simulation with a random organization of motors and kMT lengths (Figure 7B, top), we found that self-organization of kinetochores into a bilobed metaphase configuration arises naturally when Cin8p motors promote catastrophe at kMT plus ends (Figure 7B, bottom; Movie S5). Here, the catastrophe-promoting Cin8p motors self-organize a simulated bilobed spindle in which both Cin8-GFP and Ndc80-cherry fluorescence distributions are qualitatively consistent with experimental results (Figure 7C). By allowing motor presence at the kinetochore to directly promote catastrophe, the theoretical kMT plus-end catastrophe gradient model (Figure 1B) is closely reproduced (Figure 7D). As in Figure 1B, this gradient serves to establish the bilobed distribution of kinetochores into the two distinct clusters that are characteristic of the congressed metaphase spindle (Figure 1A). Similar to experimental results in cin4 mutants, reducing the motor number in the self-organized simulation disrupts the gradient in kMT assembly and thus leads to disorganization of the spindle (Figure S13).

DISCUSSION

Our results demonstrate that kinesin-5 motors, in particular Cin8p, promote net kMT and aMT disassembly in vivo. Since net kMT assembly is specifically suppressed for longer kMTs (i.e., whose plus ends are near the equator) during yeast metaphase, kinesin-5 motors must be mediating their effect, either alone or with binding partners, most strongly on longer kMTs. It is this length-dependent regulation of net kMT plus-end
assembly that establishes the congressed state of chromosomes that is characteristic of metaphase. These results are surprising to us since the only established activity of kinesin-5 is its well-known antiparallel MT sliding activity, and, to our knowledge, no effect on MT assembly has been reported previously.

We also found that Cin8-GFP interacts frequently with MT plus ends in vivo and exists in a spatial gradient on MTs. The close correspondence of the gradient in net kMT assembly and the gradient in motor distribution strongly suggests that Cin8p, either alone or with a binding partner, directly promotes kMT disassembly via its presence at the kMT plus end. In addition, this conclusion is strengthened by the close similarity between the cin8Δ mutant and the cin8 motor-domain mutant (F467A) phenotypes.

It is interesting to consider the consequences of the disassembly promotion for spindle length regulation. It is well established that kinesin-5 promotes spindle pole separation by generating an outward extensional force, presumably via crosslinking of antiparallel MTs. The newly identified disassembly-promoting activity shortens kMTs and thereby should generate an inward pulling force on the spindle poles via stretching of the intervening chromatin between the sister kMT plus ends. Thus, the two activities of Cin8p antagonize each other and are expected to result in stable spindle pole separation during yeast metaphase.

As described above, the model for kinesin-5 motor dynamics in the metaphase budding yeast mitotic spindle that best agrees with experimental data is one in which motors bind randomly to kMTs (Figure 7A, top) and then walk toward kMT plus ends, where they act to promote net kMT disassembly (Figure 7A, middle). Here, because longer kMTs have a larger number of possible motor-binding sites, the motor model predicts that the number of motor interactions at kMT plus ends will increase with increasing kMT length, as shown recently for kinesin-8 molecular motors in vitro (Mayr et al., 2007; Varga et al., 2006). Interestingly, the plus ends of iMTs, which are the longest MTs in the yeast mitotic spindle, may attract fewer Cin8p motors than the plus ends of kMTs simply because iMT plus ends are surrounded by potential antiparallel motor attachments, frustrating the plus-end motility of bipolar crosslinking Cin8p motors. This explanation is supported by results for the monopolar depolymerizing

Figure 7. A Self-Organized Model for Cin8p Motor-Mediated Spindle Organization

(A) A model for interaction of Cin8p motors with kMT plus ends: Cin8p motors that crosslink parallel microtubules are plus end directed (top). Cin8p concentrates on longer kMT plus ends to directly promote kMT disassembly (middle). kMT depolymerization then promotes motor detachment (bottom).

(B) Starting with a random distribution of kinetochores and motors in the spindle (top), motor-mediated promotion of kMT plus-end disassembly can organize the spindle into a typical metaphase bilobed kinetochore configuration as motors concentrate in a length-dependent fashion onto kMT plus ends (bottom and Movie S5).

(C) Simulated images of Cin8-GFP (green) and Ndc80-cherry (red). At simulation start (t = 0), motor and kinetochore fluorescence is randomly distributed in the spindle (left). After t = 135 s, simulated fluorescence distributions qualitatively reproduce experimental results (top right, simulated image; bottom, quantitative comparison to experimental data). Model parameters are in Table S7. (Error bars, SEM.)

(D) The self-organized model results in a gradient of catastrophe frequency (red triangles) that is similar to the theoretical catastrophe gradient depicted in Figure 1B (red line).
motor Kip3p, whose localization and deletion phenotypes are distinct from those of Cin8p. We found that Kip3-GFP behavior could be "reproduced" by taking the Cin8-GFP simulation and turning "off" the crosslinking. In this case, the noncrosslinking motor tends to accumulate more onto iMTs and less so onto kMTs. The simple physical feature of bipolar MT crosslinking, enabled in the tetrameric Cin8p and disabled in the dimeric Kip3p, is sufficient to explain all the experimental results.

The simplest molecular mechanism for length-dependent MT disassembly is that the kinesin-5 motor itself acts directly to promote MT plus-end disassembly. We speculate that mechanical stress between walking motor head domains would stress tubulin-tubulin bonds to destabilize the lattice and promote MT disassembly. Alternatively, kinesin-5 could carry a disassembly-promoting binding partner to promote net disassembly at MT plus ends, although to our knowledge there are no known cargoes that are transported by kinesin-5 motors.

Either way, the role of kinesin-5 motors in regulating kMT assembly dynamics is a new property that we have now identified for a motor previously known only as a sliding motor that acts between antiparallel MTs. Because of the potent effect that Cin8 deletion has on kinetochore organization, it seems unlikely that a significant Cin8p-independent pathway will be found to also promote length-dependent disassembly. The effects of kinesin-5 on MT assembly will be important to consider as anticancer drugs directed toward inhibiting kinesin-5 sliding activity are presently in clinical trials (Sudakin and Yen, 2007).

EXPERIMENTAL PROCEDURES

Yeast Strains and Cell Culture
All relevant genotypic information can be found in Table S6. Genes of fusion proteins remained under control of their endogenous promoters. Cell growth techniques and conditions were performed as previously described (Bouck and Bloom, 2007; Gardner et al., 2003, 2004). The Cin8p NLS deletion was performed as previously described (Hildebrandt and Hoyt, 2001). Overexpression of Cin8p from the Pgal1 promoter was performed as previously described (Saunders et al., 1997). The Cin8p-3XGFP was made by PCR amplification of 3XGFP from a plasmid, and then the linear PCR product was integrated at the endogenous Cin8 gene. The cin8-F467A strain was made and verified as previously described (Gheber et al., 1999). Benomyl treatment for stabilization of kMT plus-end dynamics was performed as previously described (Pearson et al., 2003).

Fluorescence Imaging and Photobleaching
Fluorescence imaging and photobleaching experiments were performed as previously described (Pearson et al., 2001, 2006). Astral MT lengths were assessed by measuring the lengths of GFP-Tub1- or mcherry-Tub1-labeled aMTs in which both the plus and minus ends were clearly visible within one focal plane. Counting of Cin8p and Kip1p on the mitotic spindle was completed as previously described (Joglekar et al., 2006).

Image Analysis
Average fluorescence distributions calculated over normalized spindle lengths were obtained as previously described (Gardner et al., 2005).

In FRAP experiments, FRAP half-times were resolved according to spindle position, as previously described (Maddox et al., 2000; Pearson et al., 2006). Reported half-spindle FRAP half-times were calculated by averaging over all spindle positions in the photobleached half-spindle.

Cin8-GFP fluorescence normalized to the number of tubulin-binding sites (Figures 4D and 6D) is calculated as follows. For Figure 4D, the tubulin decay function was calculated by assuming a tubulin-binding site fraction of 1.3 at the SPBs, which decays inversely with increasing Ndc80-cherry fluorescence such that there remains a fraction of 0.3 binding sites at the spindle equator (representing interpolar MTs). Then, Cin8-GFP fluorescence was normalized to the number of tubulin-binding sites by dividing Cin8-GFP signal minus background by the tubulin decay function at each spindle position. A similar method was used for Figure 6D, except that the tubulin decay function was calculated directly from the distribution of aMT plus ends as is shown in Figure 6C (red). All p values for relative comparisons of experimental data sets were calculated using Student’s t test, unless otherwise noted. Quantitative comparisons of simulations to experimental results were completed as previously described (Sorague et al., 2003).

Simulation Methods
All simulations were run using MATLAB R7.1 (Natick, MA, USA). Detailed simulation methods are provided in the Supplemental Data.

Tomography Methods
Cells were prepared for electron microscopy using high-pressure freezing followed by freeze substitution as previously described (Winey et al., 1995). Dual axis electron tomography was carried out as described previously (O’Toole et al., 2002).

In total, we recorded four WT spindles and five cin8Δ spindles. Individual microtubules and the position of the SPB central plaque were modeled from the tomographic volumes. A projection of the three-dimensional (3D) model was then displayed and rotated to study its 3D geometry. Microtubule lengths were extracted from the model contour data using the program IMODINFO.

SUPPLEMENTAL DATA
Supplemental Data include Supplemental Results, thirteen figures, eight tables, and six movies and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(08)01239-7.

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REFERENCES


Supplemental Data

Chromosome Congression by Kinesin-5

Motor-Mediated Disassembly

of Longer Kinetochore Microtubules

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Introduction

During metaphase in the budding yeast mitotic spindle (Fig. 1A) sister mitotic chromosomes (blue) are still physically attached to one another. Each chromosome is mechanically linked via a single kinetochore (cyan) to the plus end of a single kinetochore microtubule (kMT, black) whose minus end is in turn linked to a spindle pole body (SPB, grey) (Goshima and Yanagida, 2000; He et al., 2000; Joglekar et al., 2006; O'Toole et al., 1999; Pearson et al., 2001; Pearson et al., 2004; Winey et al., 1995). The sister chromosome is similarly linked to the opposite SPB. Kinetochores move as their associated kMT plus ends dynamically exchange αβ-tubulin subunits with the soluble pool in the nucleus, while kMT minus end assembly remains non-dynamic (Maddox et al., 2000; Pearson et al., 2006; Tanaka et al., 2005). In all, there are 16 sister chromosome pairs in haploid cells, for a total of 32 kinetochores and 32 kMTs, although for schematic simplicity we show only a single pair in Fig. 1A.

The mechanical linkage that runs from pole to pole via the kMTs and kinetochores is almost always under tension in metaphase (Goshima and Yanagida, 2000; Goshima and Yanagida, 2001; He et al., 2000; Pearson et al., 2001). As a result, kinetochores constrain chromosomes to congress at the equator, provided each kinetochore remains in its respective half of the spindle. Since each kinetochore is persistently attached to a single kMT plus end during metaphase in budding yeast (Gardner et al., 2005; Pearson et al., 2004), kMT plus end assembly must be limited so that each kMT plus end generally remains in its own half-spindle. If subunit exchange at the kMT plus end were unfavorable everywhere in the spindle, then kinetochores would tend to cluster around their respective poles, as the pole would be the most probable position for a kMT plus end. In fact, kinetochores do not cluster near their poles, but instead cluster about midway between their pole and the equator (Gardner et al., 2005; He et al., 2000; Pearson et al., 2006; Pearson et al., 2001; Shimogawa et al., 2006; Sprague et al., 2003; Tytell and Sorger, 2006). Therefore, kMT plus ends should have net assembly favored near the SPBs, but net disassembly near the equator, which together establish a gradient in net kMT assembly.

Through a series of fluorescence and electron microscopy studies, we confirmed the existence of the gradient in net kMT plus end assembly (Gardner et al., 2005; Pearson et al., 2006; Pearson et al., 2004; Shimogawa et al., 2006; Sprague et al., 2003). This gradient is depicted in Fig. 1A as a region near the SPB where net assembly is favorable (green, i.e. when kMTs are relatively short), and another region near the equator where assembly is unfavorable (red, i.e. when kMTs are relatively long). As a result of this gradient in net assembly, kMT plus ends grow efficiently away from poles, but rarely cross the equator. In between these two regions is an intermediate region where net assembly is close to zero, and this region constitutes an attractor zone for kMT plus ends and their associated kinetochores (Fig. 1A, yellow arrows). A key question, that we now address, is how the kMT plus end net assembly gradient is established.
Supplemental Results

**Kinetochore Position Distribution: Wild-type, cin8Δ, and kip1Δ**

There are two kinesin-5 motors in budding yeast, Cin8p and Kip1p. Kip1p is considered the less important of these two motors (Hildebrandt and Hoyt, 2000; Roof et al., 1992; Saunders et al., 1997; Tytell and Sorger, 2006), as kip1Δ phenotypes are less severe than those of cin8Δ cells. Nevertheless, we quantified kinetochore organization in kip1Δ mutants via Cse4-GFP fluorescence analysis, similar to the analysis for cin8Δ mutants. Here, kip1Δ mutants had a similar, but moderated, effect on kinetochore organization as compared to cin8Δ mutants (Fig. S1A). The peak in kinetochore-associated fluorescence is shifted towards the spindle equator, indicating that kMTs are longer in kip1Δ mutants as compared to wild-type spindles. This shift is less significant than in cin8Δ mutants, though, suggesting that Kip1p ultimately has a more moderate effect on kMT assembly than Cin8p (Fig. S1A).

**Kinetochore Position Distribution Histone H3-repressed Cells: Wild-type, cin8Δ and kip1Δ**

To further ensure that reduced spindle length was not responsible for the observed kinetochore organization changes in cin8Δ mutants, we repeated the kinetochore localization experiments in histone H3 repression mutants. As reported previously, histone H3 repression results in longer metaphase spindles, apparently by making the chromatin between sister kinetochores more compliant (Bouck and Bloom, 2007). Thus, cin8Δ spindles in a histone H3 repressed mutant have lengths typical of wild-type cells (Bouck and Bloom, 2007). In these cells, although the entire range of spindle lengths is within the typical wild-type range, loss of kinetochore clustering and shifting of kinetochores toward the equator are still apparent, similar to cin8Δ spindles in a wild-type background (Fig. S1B). Thus, deletion of Cin8p affects metaphase kinetochore organization independent of its role in regulating spindle length.

Although spindle lengths are increased in the histone H3 repressed spindles, kip1Δ mutants again show a moderate shift in kinetochore-associated fluorescence towards the spindle equator (Fig. S1B).
Figure S1: kip1Δ mutants have a similar, but attenuated, kinetochore disorganization phenotype as compared to cin8Δ mutants. (A) As shown in experimental images (left), kinetochore organization is mildly disrupted in kip1Δ mutant spindles (Cse4-GFP, green; Spc29-RFP pole markers, red). By quantifying Cse4-GFP fluorescence over a large number of spindles (right) mean kinetochore positions suggest that kMTs in kip1Δ mutants are longer than in wild-type spindles, but shorter than in cin8Δ mutant spindles. (scale bar, 500 nm; error bars s.e.m.) (B) Similar results are obtained using histone-repression mutants, where the spindle length is longer than in wild-type spindles.
In this study, we focused on the regulation of kMT plus-end microtubule dynamics by kinesin-5 molecular motors, independent of their role in generating outward extensional sliding forces on the poles to establish a steady-state metaphase spindle length. As Cin8p and Kip1p are redundant for the microtubule sliding function in yeast, deletion of either molecular motor by itself results in metaphase spindles with either a modest reduction in spindle length (cin8Δ) or little change in spindle length (kip1Δ) (Fig. S2A). To ensure that our analysis of kMT plus-end dynamics was not affected by differences in spindle lengths between wild-type and mutant cells, we selected a subset of the total distribution of wild-type and cin8Δ spindle lengths for analysis (Fig. S2B). This selection was made by selecting all wild-type and mutant spindles within the range of 1.5-1.7 μm for analysis, such that the mean and standard deviation of both wild-type and mutant spindle lengths were similar. This sub-selection represents a substantial fraction of all the spindles (~30-40%), and results in a mean spindle length only slightly different than the mean of the parent population (~5-15% different). A similar process was used for both Cse4-GFP and GFP-Tub1 fluorescence analysis experiments.

For comparison, the Cse4-GFP kinetochore localization analysis was also performed on the entire wild-type and cin8Δ spindle length populations (Fig. S2C, left). Although results in the main text are sub-selected to eliminate any spindle length bias in the analysis, nearly identical results were achieved using the entire spindle length population (Fig. S2C, left). In addition, the longest 50% of spindle lengths for both populations were analyzed, with similarly indistinguishable results (Fig. S2C, right).

As an added control, spindle microtubule organization was evaluated in a strain with reduced spindle lengths. In bim1Δ mutants, spindle lengths are shorter than in wild-type spindles, with a spindle length distribution that is similar to cin8Δ mutant cells (Fig. S3A). However, in contrast to cin8Δ mutants, spindle microtubule organization is not disrupted in bim1Δ mutants, but rather is similar to the wild-type distribution (Fig. S3B). Thus, we conclude that the disrupted spindle organization in cin8Δ mutant cells is not simply a consequence of reduced cin8Δ spindle lengths.
Figure S2: Mutant spindle lengths are moderately shorter than wild-type spindle lengths, although kinetochore organization results are similar regardless of spindle length. (A) Spindle length distributions for wild-type, cinΔ, and kip1Δ spindles. cin8Δ spindle lengths are moderately shorter than wild-type spindle lengths. (B) To control for spindle length, a subset of the total distribution of spindles is selected for analysis of kMT dynamics, as shown. (C) Cse4-GFP kinetochore organization analysis of alternate spindle length populations produced similar results.
Figure S3: A bim1Δ mutant with similar spindle lengths to cin8Δ mutants does not have disrupted spindle microtubule organization. (A) bim1Δ spindle lengths are shorter than in wild-type cells, but similar to cin8Δ spindle lengths. (B) GFP-Tub1 organization is similar in wild-type and bim1Δ spindles, but disrupted in cin8Δ spindles.
ANOVA Analysis of MT Length Distributions Measured via Cryo-Electron Microscopy

A single-factor ANOVA analysis of MT length distributions between cells was performed for the wild-type and cin8Δ spindles to ensure that all MT lengths could be analyzed as independent entities (Table S1, below). All cells were statistically indistinguishable from each other.

Table S1: Single-Factor ANOVA Analysis: Wild-type and cin8Δ Mutant Spindles

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SUMMARY: Wild-type

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ANOVA: Wild-type

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</table>
"Cin8p Overexpression: Spindle Statistics"

Similar to kinesin-5 deletion experiments, overexpression of kinesin-5 sliding motors has an effect on spindle length (Saunders et al., 1997). Here, an increased concentration of sliding motors in the nucleus results in longer spindle lengths (Fig. S4A). Because the Cin8p overexpression assay had a substantial effect on spindle length (Fig. S4A), it was not possible to compare equal spindle lengths between wild-type and Cin8p-overexpression in considering kinetochore organization. Thus, quantitative analysis was performed such that kinetochore fluorescence was recorded as a function of absolute distance from the pole, regardless of spindle length (Fig. S4B). It was found that although spindle lengths were increased, the peak in kinetochore-associated fluorescence was ~50% closer to the poles in spindles with Cin8p overexpression than in control spindles. This result suggests that kMT length is substantially reduced in the presence of high concentrations of Cin8p. The kMT shortening occurs despite the increase in chromatin stretching between sister kinetochores, which by itself promotes kMT assembly (Franck et al., 2007; Gardner et al., 2005; King and Nicklas, 2000). Thus, the effect of Cin8p overexpression and its associated promotion of kMT disassembly is dominant over the tension-dependent promotion of assembly, and so net kMT shortening occurs.

Although the overexpression experiment results in increased Cin8p expression as evidenced by longer spindle lengths and shorter kMTs, it is difficult to predict the expression level quantitatively, and large fluctuations in expression level are likely (Bloom et al., 1998). For this reason, simulation results, although qualitatively predicted in Fig. 1A, are not quantitatively fit to the Cin8p overexpression experiment.
A  **Cin8p Overexpression: Spindle Statistics**

<table>
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*a*mean ± s.d.

B  **Cse4-GFP Fluorescence in Cin8p Overexpression**

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**Figure S4: Results for Cin8p overexpression studies.** (A) Spindles are longer in Cin8p overexpression cells than in wild-type cells, although the estimated kMT length is reduced. (B) The Cse4-GFP fluorescence distribution as a function of absolute distance from one spindle pole body is similar to normalized results (Fig 1D).
GFP-Tubulin Fluorescence Recovery After Photobleaching: Half-Spindle Time Series Recovery

GFP-Tubulin FRAP is robust in both wild-type and cin8Δ cells, as shown by the representative time series in Fig. S5. Recovery obeys a single exponential, and summary statistics are as shown.

**Figure S5: GFP-Tubulin FRAP in wild-type and cin8Δ spindles.** As shown by the FRAP time series, half-spindle FRAP recovery rate is similar in both wild-type and cin8Δ cells (p=0.03), indicating that kMT self-assembly remains dynamic in cin8Δ spindles. The extents of recovery are also similar.
Astral Microtubule Length Distributions

In Fig. S6 is shown the distribution of astral microtubule lengths for varying cytoplasmic concentrations of Cin8p. Although the distributions are broad, as expected, the distribution shifts toward increasing aMT length with decreasing Cin8p expression.

Figure S6: Astral MT length distributions. Astral microtubule lengths are broadly distributed, although there is a shift in mean length depending on the Cin8p expression level. The mean aMT length correlates inversely with Cin8p expression level.
Experimental results for cin8-nlsΔ cells

We performed experiments using a mutant yeast strain in which the Cin8p nuclear localization signal (NLS) is deleted (Hildebrandt and Hoyt, 2001). This mutation reduces the concentration of Cin8p in the nucleus, while increasing the cytoplasmic concentration of Cin8p. Fluorescent mCherry-tubulin images of metaphase spindles with associated aMTs were collected. Analysis of mcherry-tubulin distribution in the spindle produced results similar to cin8Δ cells (Fig. S7A). Wild-type spindles had characteristic tubulin “tufts”, with a peak in mcherry-tubulin fluorescence roughly midway between each pole and the spindle equator. In contrast, mcherry-tubulin fluorescence in cin8-nlsΔ spindles was shifted towards the spindle equator, suggesting that kMT lengths were increased (Fig. S7A). Spindle lengths were reduced in the mutation, similar to cin8Δ spindles, although comparable spindle lengths were used between wild-type and mutant cells for the mcherry-Tub1 spindle fluorescence analysis (Fig. S7B). Importantly, and in contrast to cin8Δ spindles, aMT lengths in cin8-nlsΔ cells were shorter than in wild-type cells (Fig. S7C). In general, the lengths of the MTs in a given compartment, either the nucleus or the cytoplasm, depended inversely on the relative amount of Cin8p in that compartment. This analysis indicates that the effect of Cin8p is mediated locally in a given cellular compartment, rather than globally.
Figure S7: **Spindle lengths, kMT lengths, and aMT lengths in cin8-nlsΔ spindles.** (A) A comparison of mcherry-tubulin fluorescence distributions in wild-type and cin8-nlsΔ spindles. As shown in the images on the left, wild-type spindles have characteristic mcherry-Tub1 “tufts” (green) on either side of the spindle equator, while cin8-nlsΔ spindles have a single bar of mcherry-Tub1 fluorescence, similar to cin8Δ spindles. This result indicates that kMTs are longer in the cin8-nlsΔ cells. Quantification over many images produces similar results (right). (scale bar 500 nm, error bars s.e.m.) (B) Similar to cin8Δ mutants, spindle length is reduced in cin8-nlsΔ mutants, presumably due to the lower concentrations of anti-parallel-attached Cin8p motors that act to exert an outwardly directed sliding force to separate the SPBs. (C) In contrast to cin8Δ mutants, overexpression of Cin8p in the cytoplasm of cin8-nlsΔ mutants results in shorter aMTs, rather than the longer aMTs observed in cin8Δ mutants.
Experimental Evidence for ATP-Driven Motor Activity

Numerous studies have demonstrated that kinesin-5 motors, including Cin8p, have plus-end directed motor activity both in vitro and in vivo (Gheber et al., 1999; Gordon and Roof, 1999; Hildebrandt and Hoyt, 2000; Kapitein et al., 2005; Kashina et al., 1996; Tao et al., 2006; Valentine et al., 2006). In addition, because of its bipolarity, the kinesin-5 motor Eg5 is capable of cross-linking adjacent MTs and motoring toward the plus ends of each MT, independent of the relative orientation angle between the two MTs (Kapitein et al., 2005). Because of the results of (Kapitein et al., 2005) and the widely recognized torsional compliance of kinesins (Hancock and Howard, 1998; Hunt and Howard, 1993; Kuo et al., 1991), it seems likely that kinesin-5 motors, which are largely regarded as sliding motors that push antiparallel MTs apart, could just as easily cross-link parallel MTs and motor toward the MT plus ends without generating sliding force between the parallel MTs.

To test the simulation assumption that motors walk toward MT plus-ends on parallel microtubules, we performed experiments to provide evidence for ATP-driven motility of kinesin-5 motors in the yeast spindle. As shown in Fig. S8A (left), both simulated and experimental kymographs of spindle Cin8-GFP were generated to examine spindle-associated motor motility over time. Due to the density of motors in the spindle, it was not possible to detect individual Cin8p motility without repeatedly photobleaching to reduce fluorescent motor numbers. However, motor dynamics on the spindle can be inferred from changes in half-spindle Cin8-GFP fluorescence intensity over time. As motors move along the spindle, they would eventually reach the ends of parallel kMTs and soon dissociate. The expected result is that the number of Cin8-GFP molecules will fluctuate over time due to motor-based turnover and movement on the spindle.

If the motor activity were inhibited, then the turnover would be less dynamic, and thus the fluctuations in number would be smaller. To assess whether the Cin8-GFP dynamics on the spindle were affected by inhibition of motor activity, Cin8-GFP spindle kymographs were then obtained for cells treated with azide (Pearson et al., 2003), which will stall motor motility. Quantitative analysis showing the half-spindle standard deviation in Cin8-GFP fluorescence over time for both experiments is shown in Fig. S8B. The variation in Cin8-GFP fluorescence intensity is reduced in azide-containing media as compared to control media, suggesting that motor motility is reduced. Experiments in low-dose benomyl had variability in half-spindle Cin8-GFP fluorescence that was intermediate between control spindles and azide media spindles, suggesting that kMT plus-end dynamics also contribute to motor exchange on the spindle. We conclude that ATP is required for normal Cin8p dynamics, and that the fluctuations in Cin8p on the spindle are consistent with a model that assumes plus-end directed motility along parallel spindle MTs.

As the density of Cin8-GFP motors in the spindle precluded identification of individual motor spindle movements, we then performed experiments in which we repeatedly photobleached spindles labeled with Cin8-3XGFP and then collected rapid time-lapse images of the spindle after Cin8-3XGFP fluorescence redistribution. This method reduced the fluorescent motor number, allowing for detection of individual Cin8-3XGFP movements. As is experimentally observed and is predicted via simulation (Fig.
S8C), motor movements in the spindle are apparent in both directions, as predicted by a model in which with motors walk along parallel kMTs and ipMTs. We observed a bimodal distribution of motor velocities that is consistent with faster intrinsic motor velocities (Fig. S8 C, left, and D), and slower MT polymerization-limited motor velocities (Fig. S8 D, right, and D). The slower velocity values were similar to those observed on astral MTs where aMT assembly rate presumably limits motor velocity at the growing plus end (Fig. S8, D).
Figure S8: Experimental evidence for ATP-driven motor motility in the spindle. (A) Simulated and experimental kymographs of Cin8-GFP (green) in metaphase spindles over time show clear qualitative differences between Cin8p activity in control media (left) vs azide (right; horizontal scale bar, 1 µm, vertical scale bar, 4 sec) (B) Cin8-GFP kymographs are quantitatively compared by calculating the standard deviation in half-spindle fluorescence over time. The p-value (calculated via F-test) reflects a significant difference in the variation of Cin8-GFP fluorescence intensity over time in control conditions vs. azide. (C) Repetitive photobleaching of Cin8-3XGFP labeled spindles reveals Cin8p motor motility in the spindle. Rapid excursions (left, horizontal scale bar, 1 µm, vertical scale bar, 20 sec) are consistent with intrinsic motor motility on MTs, while slower excursions are consistent with MT-polymerization limited motor motility rates. (right) (D) The measured distribution of motor velocities is approximately bimodal. Slower spindle velocity measurements are consistent with motor motility rates as measured on astral MTs, and faster spindle velocities are consistent with previously measured in vitro motility assays for Cin8p.
Alternate Models

The best fit between experiment and simulation was achieved with a model in which motors randomly attach to microtubules. In this model, motors cross-linking parallel-oriented microtubules walk towards and frequently interact with kMT plus-ends. As shown in Fig. S9, the evidence for motor motility is further supported by simulation results with stationary motors. In this alternate model, motors stochastically attach to and detach from microtubules, without any plus-end directed motion. Quantitative analysis of Cin8-GFP simulation results from the stationary Cin8p motor model (Fig. S9, green line) reveals a predicted Cin8-GFP fluorescence distribution that is inconsistent with the observed Cin8-GFP distribution. Rather, the predicted Cin8-GFP distribution for the stationary motor model is roughly similar to the experimentally observed fluorescence distribution for GFP-Tub1 (main text, Fig. 2A). The similarity in the predicted Cin8-GFP and the GFP-Tub1 fluorescence distributions arises because microtubule polymer density is a major factor in determining motor attachment positions on the spindle. However, the quantitative difference between the stationary motor model Cin8-GFP positions (Fig. S9, green line) and the experimentally measured Cin8-GFP positions (Fig. S9, indigo line) can be accounted for by assuming that Cin8-GFP has plus-end motor activity once attached. Note that if the motor were diffusing on the lattice, rather than actively motoring, then the motor fluorescence distribution would also appear to be very similar to GFP-tubulin fluorescence distribution (not shown).

An alternate model could be one in which motors are stably associated with the kinetochore, acting as “markers” of yeast kMT plus-ends, much like Cse4-GFP or Nuf2-GFP (or any other kinetochore component). In simulations where motors are stably associated with both polymerizing and depolymerizing kMT plus-ends, Cin8-GFP fluorescence is shifted, such that it is essentially coincident with kinetochore-associated fluorescence (Fig. S9, blue line). Thus, the experimentally observed off-set between the peak in kinetochore-associated fluorescence and the peak in motor-associated fluorescence (main text, Fig. 4C) is inconsistent with Cin8p being a stable kinetochore component. Rather, the off-set suggests that dynamic kMT plus-ends increase motor dissociation near to the kMT tip so that the peak of Cin8-GFP fluorescence is shifted toward the pole relative to kinetochore fluorescence.

Another way in which the motor could associate with MTs is via copolymerization with tubulin, and then dissociation subsequent to incorporation into the microtubule lattice. This so-called “tip-tracking” mechanism is hypothesized to be used by EB-1 and other plus-end tip-interacting proteins (+TIPs). However, in this model the Cin8-GFP is again coincident with the kinetochore (results not shown), much like the distribution predicted by the kinetochore-associated model described above, and inconsistent with experimental observation.

The best fit between simulation and experiment is achieved when motors bind to and cross-link kMTs of the same polarity, move towards the kMT plus-ends, and track growing but not shortening plus-ends (Fig. S9, red line). This model results in a steady increase in the Cin8-GFP concentration along the kMT, but a decrease in Cin8-GFP concentration near to where the dynamic kMT plus ends are located (main text, Fig. 4C,D), thus quantitatively predicting the fluorescence distribution of Cin8-GFP using reasonable parameters for the motor (see values below).
Note that in this model Cin8p is allowed to randomly crosslink MTs, with no inherent preference for either parallel or anti-parallel binding. This assumption is consistent with the experimentally observed distribution of Cin8-GFP on the spindle, because if the motor had a preference for crosslinking anti-parallel oriented MTs, then the majority of the Cin8-GFP fluorescence would be located near the spindle equator. Since this is inconsistent with the observed Cin8-GFP localization, we conclude that there is no preference for antiparallel MTs. Instead, the model predicts that a substantial majority of cross-links will be between parallel MTs. This is because in the regions where the polymeric tubulin concentration is highest (i.e. in the vicinity of the pole), there is also a strong parallel alignment (>90% parallel). Near the equator the polymeric tubulin level is lower and alignment is mixed (50% parallel). Thus, the majority of Cin8p is expected to be bound to MTs somewhere between the pole and the kinetochores, and of these a substantial majority will likely cross-link parallel kMTs emanating from the nearby pole. This explanation is entirely consistent with both the Cin8-GFP distribution and the Cin8-GFP FRAP, including FRAP resolved by position (see below).

Kinesin-5 motor crosslinking of MTs only affects the model via the effect that crosslinking has on the ability of the motor to consistently interact with the MT plus-ends. Thus, in the kMT-dense spindle, even motors simulated with relatively low individual head processivity will tend to rebind to a nearby MT while the second head remains attached, so that the motor is less likely to completely dissociate from the spindle. Therefore, the “effective” motor processivity is increased due to motor crosslinking (Valentine et al., 2006), ensuring that Cin8p motors frequently interact with the plus-ends of kMTs. A non-crosslinking motor would require a higher intrinsic processivity to consistently arrive at the plus-ends of longer MTs. So, although crosslinking will affect the motor interaction with kMT plus ends, it is not an essential component of the model and can be compensated for by changing other parameters within reasonable limits.
Figure S9: Alternate models for Cin8p dynamics. Alternate models were considered for Cin8p behavior relative to dynamic spindle microtubules. For example, simulated motors that were non-motile (green line), or those that tracked both polymerizing and depolymerizing plus-ends (blue line) had distinct simulation results that differed from the experimentally observed Cin8-GFP fluorescence distribution. The only model considered that was consistent with the experimental Cin8-GFP distribution was one in which motors tracked polymerizing but not depolymerizing kMT plus-ends (red line) (scale bar 1000 nm).
Experimental testing of the motor model: Experimental Benomyl Treatment

In the motor model, motor detachment is largely mediated by disassembling kMTs. If the kMT disassembly rate were reduced, then motors should, on average, be bunched closer toward kMT plus ends and their turnover rate on the spindle should decrease. To test the first prediction, low-dose benomyl was used to experimentally stabilize kMT plus-end dynamics (Pearson et al., 2003), and then the Cin8-GFP distribution was measured relative to kinetochores. As shown in Fig. S10A, Cin8-GFP clusters are nearly coincident with kinetochores in conditions of low-dose benomyl. To quantitatively measure the mean positions of motor and kinetochore clusters, fluorescence centroids were calculated as previously described (Odde and Hawkins, 1997) for Cin8-GFP and Ndc80-Cherry in each half-spindle. The distance between Cin8-GFP and Ndc80-Cherry centroids in control media was 54 ±2 nm (mean ± s.e.m., n=154 half-spindles), while the centroid separation was 19 ±3 nm in benomyl (n=78 half-spindles, p<10^-37; distribution shown in Fig. S10A).

To test the second prediction, we measured the Cin8-GFP FRAP half-time in control and benomyl-treated cells. As predicted by the motor model and shown in Fig. S10B, the t_{1/2} for Cin8-GFP FRAP is increased where kMT plus-ends are located in benomyl-treated cells as compared to controls. We conclude that the experimentally observed Cin8-GFP distribution and dynamics are sensitive to kMT plus end dynamics, both of which are explained in the motor model by the assumption that motors fail to remain attached to shortening kMT plus ends. Furthermore, the observed motor sensitivity to kMT plus end assembly dynamics indicates that motors interact frequently with kMT plus ends.
Figure S10: Cin8-GFP localization and dynamics in benomyl-treated cells (A) Cin8-GFP is nearly co-localized with kinetochores in spindles with benomyl-stabilized kMT plus-ends, as shown by the distribution of centroid spacing between Cin8-GFP and Ndc80-Cherry in spindles with dynamic kMT plus-ends (control media, indigo), and stabilized plus-ends (Benomyl, cyan). Modeling suggests that the observed off-set between kinetochore fluorescence and Cin8-GFP in conditions with dynamic kMT plus-ends is due to increased motor off rates from depolymerizing kMT plus-ends. (scale bar 500 nm) (B) Benomyl-stabilized kMT plus-ends results in Cin8-GFP FRAP half-times that are increased in the location of plus-end clustering relative to control cells. (error bars, s.e.m.)
**Kip1-GFP Spindle Localization**

As shown in Fig. S11, Kip1-GFP localization in the spindle is qualitatively similar to Cin8-GFP. However, Kip1-GFP is more diffuse than Cin8-GFP, suggesting that Kip1p affinity for MTs is lower than for Cin8p.

![Figure S11: Experimental Kip1-GFP fluorescence distribution in the spindle.](image)

The Cin8-GFP spindle fluorescence distribution (indigo line) is more tightly focused than Kip1-GFP distribution (maroon line). This result suggests that Cin8p has a higher affinity for microtubules than Kip1p, such that Cin8p has longer run lengths and thus concentrates more efficiently at the growing kMT plus-ends. Low affinity of Kip1p for MTs may result in short motor run lengths that decrease the likelihood of motor concentration at kMT plus-ends. (scale bar 1000 nm)
Kinesin-5 Motor Turnover on the Spindle

By measuring Cin8-GFP FRAP half-time as a function of spindle position (Fig. S12, green line, we find that Cin8-GFP turns over most rapidly in the position of kMT plus-end clustering, suggesting that dynamic kMT plus-ends mediate motor off-rates. Kip1-GFP turnover (Fig S12, violet line) is also most rapid where kMT plus ends are located. In addition, Kip1-GFP recovery is on average more rapid than Cin8-GFP turnover, again suggesting that Kip1p has a lower affinity for microtubules than Cin8p.

Figure S12: Cin8-GFP and Kip1-GFP FRAP, resolved by spindle position. As was previously reported, FRAP half-time can be resolved by spindle position for GFP-tubulin(Pearson et al., 2006). There is a gradient in GFP-tubulin recovery half-times, such that recovery is most rapid in the location of kMT plus-end clustering (position 0.25). There is a similar gradient in Cin8-GFP FRAP half-times (green line), suggesting that kMT plus-end dynamics mediate Cin8p motor off-rates. Kip1-GFP FRAP half-time obeys a similar gradient as well. However, Kip1-GFP turns over more rapidly than Cin8-GFP, consistent with a model where Kip1p has a lower affinity for MTs, and thus interacts less frequently with kMT plus-ends. (error bars, s.e.m.)
A "Self-Organized" Model: Kinetochore Organization

In previous work, (Gardner et al., 2005; Sprague et al., 2003) kMT dynamics were modeled using an imposed “catastrophe gradient”. Although the kMT dynamics simulations in Figures 1-6 were performed as described previously, the results in Fig. 7 and Fig. S13 are the result of a “self-organized” model (see also supplemental movie 5), in which the presence of kinesin-5 motors on kMT plus ends is assumed to increase the catastrophe frequency proportionally to their number at the plus end (see detailed description of model below in the methods section). We have used this self-organized model to test our hypothesis that, by this mechanism, kinetochores could self-organize into the characteristic bi-lobed metaphase configuration of kinetochores.

Typical results from the “self-organized” model are shown in Fig. S13(A). In this model, both experimental wild-type Cin8-GFP localization as well as the experimentally observed bi-lobed Cse4-GFP kinetochore organization are qualitatively reproduced in simulation. In addition, by reducing the number of motors, such as in a cin8Δ mutant in which only Kip1p remains, the gradient in motor localization at the kinetochore is disrupted (Fig. S13B), and thus the experimentally observed disruption of kinetochore disorganization is qualitatively reproduced by the model (Fig. S13C). Similarly, by increasing the number of motors in the simulation, such as in the Cin8p-overexpression experiment, the experimentally observed depolymerization of kMTs can be qualitatively reproduced by the model (not shown). Therefore, it is no longer necessary to externally impose a catastrophe gradient on the system, but rather the catastrophe gradient is now a natural consequence of a model where kinesin-5 motors walk to plus ends and promote net disassembly upon arrival. Note that the model makes no assumption about whether the motor itself promotes disassembly, or whether it requires a cargo.
Figure S13: Self-Organized model (A) Experimental Cse4-GFP localization as well as the experimental Cin8-GFP distribution is qualitatively reproduced via a “self-organized” model in which Cin8p promotes net disassembly at the kMT plus end. (scale bar, 1000 nm) (B) By altering the number of simulated motors, the gradient in motor presence at the kinetochore is reduced (indigo line, simulating cin8Δ mutants), and (C) kinetochore organization is disrupted, similar to experimental results (scale bar, 1000 nm).
Simulation Methods

A stochastic simulation was developed to account both for dynamic kMT plus-ends in the yeast metaphase spindle as well as for dynamic plus-end directed kinesin-5 motors that interact with MTs, as described below.

Microtubule Dynamics: Model Description

As was previously described (Gardner et al., 2005; Sprague et al., 2003), a Monte Carlo simulation was developed and run in MATLAB to simulate kMT plus-end dynamics. The model assumes that kMT plus-ends remain in one of two states, growing or shortening, at all times. Thus, their dynamics can be fully described via the four parameters of dynamic instability, where $V_g$ and $V_s$ describe rates of kMT plus-end polymerization and depolymerization, respectively, and $k_c$ and $k_r$ describe the probability of switching between these two states. Here, $k_c$ is defined as catastrophe frequency, which is the rate at which a kMT plus-end will switch from a growing state to a shortening state, and $k_r$ is defined as rescue frequency, which is the rate at which a kMT plus-end will switch from a shortening state to a growing state. Previously, we found that there does not exist a constant set of dynamic instability parameters that is able to reproduce kMT plus-end localization in the yeast metaphase spindle (Sprague et al., 2003). Rather, catastrophe and/or rescue frequency vary spatially along the length of the spindle in the model, with net assembly promoted for kMT plus-ends near the pole, i.e. when kMTs are relatively short, and net disassembly promoted for kMT plus-ends near the equator, where kMTs are relatively long (Gardner et al., 2005; Sprague et al., 2003).

All wild-type simulations of kMT plus-end dynamics were run with models and parameter values as previously described (Gardner et al., 2005), and as shown in Table S2. Here, rescue frequency is regulated by tension between sister kinetochores, as measured by the separation distance along the spindle axis between the tips of sister kMTs. Thus, this tensile force, $F_{tensile}$, is defined as:

$$F_{tensile} = \rho^* (s - s_0)$$  \hspace{1cm} (1) (Sprague et al., 2003)

Where $\rho^*$ is a Hookean spring constant for chromatin connecting sister kMTs with units $\mu m^{-1}$, $s$ is the separation distance between the tips of sister kMTs, and $s_0$ is the rest length of the chromatin. Using this equation, rescue frequency is calculated as a function of tension between sister kinetochores at every time step using the equation:

$$k_r = k_{r,0} \exp(F_{tensile})$$  \hspace{1cm} (2) (Sprague et al., 2003)

In addition, catastrophe frequency in all simulations (except the final “self-organized” model) was calculated using a theoretical “chemical gradient” model, where $k_c$ varies according to:

$$k_c = k_{c,0} + \beta c_B$$  \hspace{1cm} (3) (Sprague et al., 2003)

Where $\beta$ is an adjustable parameter that describes the magnitude of the catastrophe promoter’s effect, and $c_B$ is the concentration of a theoretical catastrophe promoter. In the model, $c_B$ varies by spindle position via a reaction-diffusion process, in which the
concentration of the theoretical catastrophe promoter is highest at the spindle equator and lower elsewhere (Sprague et al., 2003).

kMT dynamics in CIN8 deletion experiments were simulated by modifying the catastrophe gradient model to decrease the parameter $\beta$, with model parameters as listed in Table S2. The best fit between experiment and theory was achieved with values for MT plus-end growth and shortening rates that were slightly higher than in wild-type simulations (Fig. 2E and Table S2), which decreases FRAP half-times at all spindle positions in simulations of the cin8Δ GFP-Tub1 FRAP experiment. These results suggest that Cin8p may also have a slight effect on the suppression of plus-end growth and shortening velocities.

The parameters in Table S2 were used for simulations across all experiments as shown in Figures 1-6.

Non-kMTs (ipMTs) were modeled both using non-dynamic plus-ends, and using a model in which $k_r >> k_c$, with constant values of $V_g$ and $V_s$. The model for ipMT dynamics did not have a significant effect on simulation results where kMT density is high (not shown, but reviewed in (Pearson et al., 2006)(supplemental material)).
Table S2: Model for kMT Dynamics: Simulation Parameters

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^a In all simulations, growth and shortening rates are assumed to be equal. GFP-Tub1 FRAP experiments constrain growth and shortening values in both wild-type and cin8Δ experiments.
^b Catastrophe frequency depends on spindle position of kinetochore, as described in equation (3) and (Sprague et al., 2003)
^c Rescue frequency is dependent on distance between sister kinetochores, such that high tension at the kinetochore promotes rescue, as described in equations (1) and (2) and (Gardner et al., 2005).
^d In the cin8Δ simulation, tension-dependent rescue frequency decreases near to basal (no tension) values as a natural consequence of reduced sister kinetochore separation distances.

Kinesin-5 Motor Dynamics: Model Description

A simulation was developed to explicitly model individual kMT dynamics as well as the dynamics of individual MT-associated homotetrameric kinesin-5 molecular motors, based on known properties of the motors (Kapitein et al., 2005; Kashina et al., 1996; Tao et al., 2006; Valentine et al., 2006). Each kinesin-5 homotetramer is modeled as a bipolar motor that has two motor “heads”, one at each end of the complex, with each “head” consisting of a pair of motor head domains. As shown schematically in Table S3, one motor head stochastically attaches to an individual MT (at rate k_{on} *), and subsequently crosslinks via the other head to an adjacent MT (at rate α k_{on} *). Once bound, motor heads move toward their respective MT plus ends (at velocity v), and eventually detach (each head detaching at rate k_{off}). The motor velocity is slowed by opposing force (Table S3) (Gheber et al., 1999; Valentine et al., 2006), and motor off-rate increases with increasing force (Korneev et al., 2007; Valentine et al., 2006). Additional model assumptions are reviewed in Table S4, with variables and parameter values specified in Tables S5 and S6.

Load-dependent motor velocity results in two distinct motor behaviors depending on whether motors are crosslinking parallel oriented or anti-parallel oriented MTs. As shown in Table S3 (top), motor heads crosslinking anti-parallel oriented MTs walk in opposite directions, stretching the motor and quickly increasing load. Thus, these motors are stationary on the spindle, and tend to concentrate in locations with increased density of anti-parallel MTs, such as at the spindle equator (supplemental movies 3,4). Because
of the increasing load, motors bound to antiparallel MTs tend to detach more rapidly than motors bound to parallel MTs. In contrast, motor heads crosslinking parallel-oriented microtubules walk together towards the plus-ends of both microtubules (Table S3 (top), supplemental movies 3,4). In this case, these motors do not stretch or generate load, but rather walk along the spindle at the unloaded motor velocity. In simulation, it is this behavior that results in the experimentally observed concentration of motors near the plus-ends of kMTs in the yeast mitotic spindle. Once one of the motor heads reaches the plus end, the head is assumed to travel at the rate of MT plus end growth, which is likely to be slower than the unloaded motor velocity (Carminati and Stearns, 1997; Gheber et al., 1999; Gupta et al., 2002; Gupta et al., 2006). The differing rates for each of the two heads results in motor stretching and thus increases the detachment rate. If a plus end undergoes catastrophe, then any motor heads that are at the plus end are assumed to detach. Motor heads that encounter shortening plus ends are also assumed to detach.
**Table S3: Model for Kinesin-5 Motor Behavior**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Model Equation</th>
<th>Variables</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor Head</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attachment</td>
<td>$k_{on}^* = k_{on} [\text{Tubulin}]$</td>
<td>$k_{on}^*$ = motor on-rate constant</td>
<td>[Tubulin] = polymer concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\alpha$ = second head on-rate correction</td>
</tr>
<tr>
<td>Motor Head</td>
<td>$v_i = v_u(1 - F_{L,i}/F_{\text{stall}})$</td>
<td>$v_i$ = motor velocity $F_{L,i}$ = motor tension</td>
<td>$F_{\text{stall}}$ = stall force $v_u$ = unloaded velocity</td>
</tr>
<tr>
<td>Movement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Stretching</td>
<td>$F_{L,i} = \rho_{\text{motor}} \Delta x_m$</td>
<td>$\Delta x_m$ = stretch distance</td>
<td>$\rho_{\text{motor}}$ = spring constant</td>
</tr>
<tr>
<td>Motor Head</td>
<td>$k_{off,i}^* = k_{off} e^{(F_{L,i}/F_c)}$</td>
<td>$k_{off,i}^*$ = motor off-rate constant (force dependent)</td>
<td>$F_c$ = critical force $k_{off}$ = unloaded off-rate constant</td>
</tr>
<tr>
<td>Detachment</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Kinesin-5 Motor Dynamics: Model Assumptions**

Model assumptions for simulation of kinesin-5 motor dynamics are summarized in Table S4, along with references and explanations.

**Table S4: Model Assumption Summary**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Model Assumption</th>
<th>Reference or Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Head Motor Attachment</td>
<td>Weighted random attachment of first head along spindle according to local MT density</td>
<td>Diffusion in the nucleoplasm is expected to be fast relative to motor rebinding time. In simulation, ( x_{\text{rms}} \sim 2 \mu \text{m} ) prior to motor rebinding, length of spindle ( \sim 1.5 \mu \text{m} ).</td>
</tr>
<tr>
<td>Second Head Motor Attachment</td>
<td>Random attachment of second head within a 40 nm radius of first head due to torsional flexibility of motor</td>
<td>(Kashina et al., 1996) (Kapitein et al., 2005) (Tao et al., 2006)</td>
</tr>
<tr>
<td>Motor Attachment and MT Polarity</td>
<td>No preference of motor attachment to parallel vs anti-parallel attachments, or to kMTs vs the non-kMTs that generally run the length of the spindle (interpolar MTs)</td>
<td>(Kapitein et al., 2005) In general, the metaphase Cin8p motor distribution roughly mirrors MT density rather than enrichment of motors at the midzone</td>
</tr>
<tr>
<td>Motor Movement: Force</td>
<td>Motor heads move according to a force-velocity relationship</td>
<td>(Valentine et al., 2006)</td>
</tr>
<tr>
<td>Motor Movement: Compliance</td>
<td>Motors act as Hookean springs</td>
<td>(Lee et al., 2006)</td>
</tr>
<tr>
<td>Motor Movement: Velocity</td>
<td>Singly-attached motors move at unloaded velocity</td>
<td>By definition</td>
</tr>
<tr>
<td>Motor Detachment: Both heads</td>
<td>Motor detachment is force dependent</td>
<td>(Korneev et al., 2007) (Valentine et al., 2006) (Bell, 1978) (for review, see (Evans and Calderwood, 2007))</td>
</tr>
<tr>
<td>Attached to Adjacent MTs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Detachment: Single</td>
<td>Singly-attached motors detach at unloaded off-rate</td>
<td>By definition</td>
</tr>
<tr>
<td>Attachments</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Kinesin-5 Motor Dynamics: Parameter Value Summary

All simulated parameter values are summarized in Table S5, along with references and explanations. Average numbers of kinesin-5 motors on the mitotic spindle were experimentally determined for this study via analysis of fluorescence intensity (as described in (Joglekar et al., 2006)). We found that ~50 Cin8p motors and ~20 Kip1p motors are bound at any given time to the spindle.

In addition, Cin8p FRAP studies generally constrained motor off-rates in the simulation. Here, the model value for $k_{\text{off}}$ was a ‘fitting parameter’ that was adjusted to reproduce Cin8-GFP spindle localization and FRAP half-times. For Cin8p movement along astral microtubules, the motor appears to processively walk along astral microtubules for 30-60 seconds (500-1000 nm), while the single-head $k_{\text{off}}$ modeling parameter would predict single-head run lengths of 3-4 seconds. Because Cin8p is a homotetrameric crosslinking motor, though, motors with a relatively low individual head processivity may tend to rebind to a nearby MT while the second head remains attached, increasing the observed effective processivity of the motor (Valentine et al., 2006). Thus, it may be that motors observed walking along astral microtubules are in fact crosslinking two microtubules. Consistent with this interpretation of the results, recent in vitro studies measured a $k_{\text{off}}$ of 12 sec$^{-1}$ for Eg5, which is larger than the Cin8p simulated value of 0.3 sec$^{-1}$, even though the measured $k_{\text{off}}$ on astral microtubules is ~0.02 sec$^{-1}$ (Valentine et al., 2006). Therefore, the “effective” motor processivity may be increased due to either motor crosslinking or differing biochemical conditions in the various intracellular compartments/in vitro assays.

A free parameter ($\alpha$) was defined to account for the diffusion and attachment of a second Kinesin-5 motor head once the first head has attached. The value of this parameter depends on the density of microtubules near the first-head attachment site, and is calculated as follows:

$$\alpha = \frac{\rho_M}{3}$$

(4)

where $\alpha$ is the correction factor, and $\rho_M$ equals the number of neighboring MTs (within a 40 nm radius in the $yz$ plane) that are of sufficient length to allow for crosslinking of the motor at the first head location. In this way, the second head on-rate constant is moderately increased as compared to the first head on-rate constant, depending on the number of MTs in the vicinity of the first head attachment point.

A velocity of 50 nm/sec was used for Cin8p in simulations, to be consistent with in vitro measurements of Cin8p motility (Gheber et al., 1999). We hypothesize that the observed motor motility on astral MTs may be limited by MT polymerization, an interpretation that is consistent with the in vivo data obtained at rapid temporal resolution on Cin8p motors in the spindle (Fig. S8). The value used in simulation is similar to the measured values for motor motility in the spindle (Fig. S8).

All remaining parameter values are based on previous studies, as described in Table S5.
**Kinesin-5 Motor Dynamics: Variable Summary**

Typical values for model variables, resulting from the equations in Table S3 and the parameter values described in Table S5, are listed in Table S6. In general, modeling results were highly sensitive to the rules for motor behavior at kMT plus-ends, and it was necessary that parallel-attached motors were motile in order to reproduce experimentally observed motor localization. Simulation of motor FRAP experiments constrained motor off-rates, although on-rates are high enough that many of the simulated motors are attached throughout the simulation. Thus, parameter values and the resulting variable values were not highly constrained to narrow ranges, and it was possible to reproduce both motor localization and FRAP experiments with physically reasonable numbers.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Typical Value</th>
<th>Reference or Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_u$</td>
<td>Unloaded Motor Velocity</td>
<td>50 nm/sec</td>
<td>(Gheber et al., 1999) 57 nm/sec</td>
</tr>
<tr>
<td>$F_{stall}$</td>
<td>Motor Stall Force</td>
<td>6 pN</td>
<td>(Valentine et al., 2006), 5-7 pN</td>
</tr>
<tr>
<td>$\rho_{motor}$</td>
<td>Motor Spring Constant</td>
<td>0.5 pN/nm</td>
<td>(Kawaguchi and Ishiwata, 2001)</td>
</tr>
<tr>
<td>$k_{off}$</td>
<td>Unloaded Motor Off-rate Constant</td>
<td>0.3 sec$^{-1}$</td>
<td>Model Fit Parameter (via FRAP)</td>
</tr>
<tr>
<td>$k_{on}$</td>
<td>Motor On-rate Constant</td>
<td>1 $\mu$M$^{-1}$sec$^{-1}$</td>
<td>(Northrup and Erickson, 1992)</td>
</tr>
<tr>
<td>$N_m$</td>
<td>Number of Cin8p Spindle Motors</td>
<td>50</td>
<td>This study (via counting)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Second-head on-rate correction</td>
<td>0 – 8</td>
<td>Free parameter, depends on number of MTs in 40 nm attachment radius</td>
</tr>
<tr>
<td>$r_M$</td>
<td>Radius of 2nd Motor Head attach point relative to 1st Motor Head attach point</td>
<td>40 nm</td>
<td>(Kashina et al., 1996) (53 nm)</td>
</tr>
<tr>
<td>$F_c$</td>
<td>Critical Force for force-dependent off-rates</td>
<td>6 pN</td>
<td>(Valentine et al., 2006), 5-7 pN (Korneev et al., 2007), 2 pN</td>
</tr>
</tbody>
</table>
Table S6: Model Variable Summary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Typical Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( v_i )</td>
<td>Velocity of ( i^{th} ) motor</td>
<td>0-50 nm/sec</td>
</tr>
<tr>
<td>( N_{m,att} )</td>
<td>Number of attached motors</td>
<td>~50</td>
</tr>
<tr>
<td>( F_{L,i} )</td>
<td>Load force on ( i^{th} ) motor</td>
<td>0 – 6 pN</td>
</tr>
<tr>
<td>( \Delta x_{m,i} )</td>
<td>Stretch of ( i^{th} ) motor in the x direction</td>
<td>0 - 30 nm</td>
</tr>
<tr>
<td>( k_{off,i} )</td>
<td>Force corrected off-rate constant for ( i^{th} ) motor</td>
<td>0.3 – 1.0 sec(^{-1})</td>
</tr>
<tr>
<td>( k_{on,1} )</td>
<td>Pseudo first-order motor on-rate constant, 1(^{st}) head</td>
<td>30-35 sec(^{-1})</td>
</tr>
<tr>
<td>( k_{on,2} )</td>
<td>Pseudo first-order motor on-rate constant, 2(^{nd}) head</td>
<td>0-500 sec(^{-1})</td>
</tr>
<tr>
<td>([Tub])</td>
<td>Concentration of tubulin polymer in nucleus [=(1600 \text{ units}/\mu m)\times(\text{total MT polymer length in } \mu \text{m})], assuming a 1.5 \mu m diameter nucleus</td>
<td>30-35 \mu M</td>
</tr>
</tbody>
</table>

*Simulation Methods: “Self-Organized” Model*

The effect of kinesin-5 motors on catastrophe frequency at kMT plus-ends is modeled according to the following:

\[
k_c = k_{c,0} + \beta_m M_T
\]  

(5)

where \( k_c \) is the catastrophe frequency for a given kMT plus-end, \( k_{c,0} \) is the basal rescue frequency in the absence of kinesin-5 motors, \( M_T \) is the total number of kinesin-5 motors within 16 nm (1\(^{st}\) or 2\(^{nd}\) tubulin subunit) of the plus-end, and \( \beta_m \) is the sensitivity factor for the effect of motors on basal catastrophe frequency. Model parameter values and typical variable values for the results shown in Fig. S13 and Fig. 7 are shown in Table S7. Cin8p motor parameters and variables are as listed in Tables S3-S6. In addition to the model assumptions outlined in Table S4, kMT plus-end catastrophe frequency is increased upon sister kinetochore crossing, which is unlikely to occur in vivo due to steric hindrance of kinetochores and chromatin (Gardner et al., 2008).
Table S7: “Self-Organized” Model for MT Dynamics: Simulation Parameters

<table>
<thead>
<tr>
<th>Category</th>
<th>Symbol</th>
<th>Description</th>
<th>Wild-Type</th>
<th>cin8A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>$k_{c,0}$</td>
<td>Basal Catastrophe Frequency (no Motor) (min$^{-1}$)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Parameter</td>
<td>$\beta_m$</td>
<td>Catastrophe Sensitivity Factor per Motor at Kinetochore (min$^{-1}$)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Parameter</td>
<td>$V_g$</td>
<td>Growth Rate (µm/min)</td>
<td>1.2$^a$</td>
<td>1.9$^a$</td>
</tr>
<tr>
<td>Parameter</td>
<td>$V_s$</td>
<td>Shortening Rate (µm/min)</td>
<td>1.2$^a$</td>
<td>1.9$^a$</td>
</tr>
<tr>
<td>Parameter</td>
<td>$N_{kMT}$</td>
<td>Number of kMTs</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Variable</td>
<td>$k_c$</td>
<td>Catastrophe Frequency (min$^{-1}$)</td>
<td>0.25-30$^b$</td>
<td>0.25-10$^b$</td>
</tr>
<tr>
<td>Variable</td>
<td>$M_T$</td>
<td>Total Number of Motors at Kinetochore</td>
<td>0.5±0.9$^c$ (out of 90 motors)</td>
<td>0.1±0.4$^c$ (out of 20 motors)$^e$</td>
</tr>
<tr>
<td>Variable</td>
<td>$k_r$</td>
<td>Mean Rescue Frequency (min$^{-1}$)</td>
<td>9-24$^d$</td>
<td>9-13$^{f,d}$</td>
</tr>
</tbody>
</table>

$^a$In all simulations, growth and shortening rates are assumed to be equal.
$^b$Catastrophe frequency depends on the number of motors near the kinetochore, as described in equation (5)
$^c$mean±sd per kinetochore (typical in simulation)
$^d$Rescue frequency is dependent on distance between sister kinetochores, such that high tension at the kinetochore promotes rescue, as in Gardner et al., 2005.
$^e$In the cin8Δ simulation, Kip1p motors are still present, and so a smaller number of kinesin-5 motors are simulated.
$^f$In the cin8Δ simulation, tension-dependent rescue frequency decreases near to basal (no tension) values as a natural consequence of reduced sister kinetochore separation distances.
### Table S8: Relevant Plasmids and Strains

<table>
<thead>
<tr>
<th>Plasmid Name</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMA1214</td>
<td>GAL1-CIN8 plasmid</td>
<td>M.A. Hoyt</td>
</tr>
<tr>
<td>pMA1186</td>
<td>cin8A plasmid</td>
<td>M.A. Hoyt</td>
</tr>
<tr>
<td>pB1585</td>
<td>3xGFP plasmid</td>
<td>D. Pellman</td>
</tr>
<tr>
<td>pRS406</td>
<td>mCherry-TUB1 plasmid</td>
<td>S. Reed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEF473A</td>
<td>MATa trp1-63 leu2-1 ura3-52 his3-200 lys2-801</td>
<td>J. Pringle</td>
</tr>
<tr>
<td>KBY2012</td>
<td>As YEF473A except cse4::HYG SPC29-CFP-KAN pKK1</td>
<td>C. Pearson</td>
</tr>
<tr>
<td>KBY2270</td>
<td>As YEF473A except cin8::LEU2 cse4::HYG SPC29-CFP-KAN pKK1</td>
<td>This Study</td>
</tr>
<tr>
<td>KBY2129</td>
<td>As YEF473A except GFP-TUB1::leu2_1, SPC29RFPKAN</td>
<td>C. Pearson</td>
</tr>
<tr>
<td>KBY2275</td>
<td>As YEF473A except cin8::LEU2 GFP-TUB1::leu2_1, SPC29RFPKAN</td>
<td>This study</td>
</tr>
<tr>
<td>KBY7001</td>
<td>As YEF473A except kip1::NAT cse4::HYG SPC29-CFP-KAN pKK1</td>
<td>This Study</td>
</tr>
<tr>
<td>KBY8030A</td>
<td>As YEF473A except mCherry-TUB1:URA3</td>
<td>This Study</td>
</tr>
<tr>
<td>KBY8023</td>
<td>As YEF473A except Cin8-GFP:His3MX6, NDC80-Cherry:KAN</td>
<td>This Study</td>
</tr>
<tr>
<td>KBY8052</td>
<td>As YEF473A except CIN8-GFP:His3, SPC29RFP:HYG</td>
<td>This Study</td>
</tr>
<tr>
<td>DCB 411.1</td>
<td>As YEF 473A except CIN8-3GFP-HIS3 mCherry-TUB1:URA3</td>
<td>This Study</td>
</tr>
<tr>
<td>DCB 421.1</td>
<td>As YEF473A except CIN8-NLSdelta-3GFP-HIS3 mCherry-TUB1:URA3</td>
<td>This Study</td>
</tr>
<tr>
<td>DCB 430</td>
<td>As YEF473A except pGAL-CIN8-HIS3 cse4::HYG SPC29-CFP-KAN pKK1</td>
<td>This Study</td>
</tr>
<tr>
<td>KBY8033A</td>
<td>As YEF473A except pGAL cin8:HIS GFP-TUB1::leu2_1, SPC29RFPKAN</td>
<td>This Study</td>
</tr>
<tr>
<td>DCB 204.1</td>
<td>As YEF473A except HHT1::TRP1 KAN-GAL1p-HHT2 ura3::NUF2-GFP-URA3 SPC29-RFP HYG</td>
<td>D. Bouck</td>
</tr>
<tr>
<td>DCB 208.1</td>
<td>As YEF473A except HHT1::TRP1 KAN-GAL1p-HHT2 ura3::NUF2-GFP-URA3 SPC29-CFP-HIS3 cin8::LEU2</td>
<td>D. Bouck</td>
</tr>
</tbody>
</table>
Supplemental References


of dam1 couples kinetochores to microtubule plus ends at metaphase. Curr Biol 16, 1489-1501.


