Supplemental materials

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Supplemental note 1

Kar3 localization at the microtubule ends and at kinetochores.

Kar3-4GFP signals were detected at the plus ends of growing microtubules (Fig. 1 B). However, these signals were almost abolished during the shrinkage of microtubules. A similar result was also obtained for the Kar3 orthologue in *Arabidopsis* (Ambrose et al., 2005). Thus, Kar3 behaves like other microtubule plus end–tracking proteins (+TIPs; Carvalho et al., 2003; Akhmanova and Hoogenraad, 2005).

Although intense Kar3-4GFP signals were found at spindle poles, the signal was weak between poles. The total amount of such signals (derived from pole to pole microtubules and 16 pairs of bioriented sister kinetochores) was only one to five times greater than the intensity of Kar3-4GFP signals at an uncaptured *CEN3* (unpublished data). This suggests that the amount of Kar3 at kinetochores decreases upon sister kinetochore biorientation, which is in agreement with a recent study (Tytell and Sorger, 2006).

Our result is consistent with a previous report that Klp2, a kinesin-14 family member in fission yeast, localizes at kinetochores during mitosis (Troxell et al., 2001).

Supplemental note 2

Scoring kinetochore transport modes in cells with a marker at the plus ends of shrinking microtubules.

Microtubules sometimes overlap when they extend from spindle poles, and, for example, two microtubules with different lengths could overlap with each other. In this situation, if the shorter microtubule undergoes the end-on pulling of *CEN3*, it might be wrongly interpreted as *CEN3* sliding along the longer microtubule. However, such a situation can usually be discerned by the observation of different intensities of tubulin signals from the spindle pole to *CEN3* and from *CEN3* to the microtubule plus end (K. Tanaka et al., 2005). Nonetheless, we wanted to confirm that our assessment of sliding does not include end-on pulling by overlapping shorter microtubules. If *CEN3* was transported with end-on pulling by an overlapping shorter microtubule, we would expect the Dam1 complex, which should localize at the plus end of the shorter microtubule (Fig. 6 B), to colocalize continuously with *CEN3* during its transport (Fig. 7 A). Therefore, we made *KAR3*$^{+}$ and *kar3-64* strains harboring Ask1-4GFP (a component of the Dam1 complex) and recounted sliding and end-on pulling, etc. We observed sliding and end-on pulling, etc. (Fig. S1 A) with a similar frequency to that shown in Figs. 2 A and 8 A, thus excluding the possibility that we confused sliding and end-on pulling in a substantial number of cells. In particular, in Figs. 2 A and 8 A, we observed *CEN3* sliding along microtubules in ~14–24% of *kar3*Δ and *kar3-64* cells (including cells that showed conversion from sliding to end-on pulling). Consistent with these results, in 4/34 *kar3-64* cells, although *CEN3* (labeled with CFP) moved toward a spindle pole, Ask1-4GFP did not continuously colocalize with *CEN3* at least for some time (unpublished data). This suggests that even when Kar3 is missing or defective, *CEN3* is able to move along the microtubule lateral surface in a small number of cells.

Supplemental note 3

How does *CEN3* reach the spindle by sliding in a small number of *kar3*Δ cells?

CEN3 still reached the spindle by sliding along microtubules in 11% of *kar3*Δ cells. This may be the result of one-dimensional diffusion as suggested below (Fig. 3). However, given that diffusion along microtubules should not show preferential direction, how can *CEN3* reach a spindle pole by sliding in this small percentage of *kar3*Δ cells? This may be explained as follows: if overall *CEN3* motion by diffusion was, by chance, toward the microtubule distal end, it is likely that *CEN3* becomes eventually tethered at the microtubule end and transported by microtubule end-on pulling. In fact, the microtubule end-on pulling is established more frequently in *kar3*Δ cells, as shown below (Fig. 4). Conversely, if *CEN3* moves particularly fast toward a spindle pole by diffusion along the microtubule lateral surface, it may escape conversion to the end-on pulling and may eventually reach a spindle pole.

Supplemental note 4

A lack of microtubule rescue during end-on pulling cannot be solely caused by the loss of Stu2 at *CEN3*.

After nuclear microtubules shrank, they showed regrowth (i.e., rescue) only when kinetochores were associated with their lateral sides (before sister kinetochore biorientation) but not in the absence of associated kinetochores (K. Tanaka et al., 2005). This microtubule rescue occurred (1) before their shrinking plus ends reached kinetochores or (2) when their plus ends arrived at kinetochores. Our previous data showed that Stu2 was loaded on kinetochores before their capture by microtubules and suggested that Stu2 had a central role in kinetochore-dependent microtubule rescue (K. Tanaka et al., 2005). Given this, a lack of microtubule rescue during the microtubule end-on pulling of *CEN3* might be caused by the absence of Stu2 at this centromere. Therefore, we visualized Stu2 with GFP and *CEN3*/microtubules with CFP. We found that Stu2 still localized at *CEN3* during end-on pulling (Fig. S1 B). Thus, it is unlikely that a lack of microtubule rescue during the end-on pulling of kinetochores is caused solely by the loss of Stu2 at kinetochores.

Supplemental note 5

CEN3 detachment from microtubules during microtubule-dependent transport.

We quantified how often *CEN3* detached from microtubules during its transport in *KAR3*$^+$ wild-type cells. *CEN3* occasionally (13/263; 4.9%) detached from microtubules during sliding, whereas such detachment was never (0/88; 0%) observed during end-on pulling. Therefore, *CEN3* detachment is more frequent during sliding than during end-on pulling ($P = 0.044$; Fisher's exact test). After detachment, in the majority of cases, *CEN3* was recaptured quickly by the same microtubule or by another microtubule.

Supplemental note 6

How we avoided potential bias in evaluating a role of Kar3 in *CEN3* sliding.

To evaluate the contribution of Kar3 to *CEN3* sliding along microtubules, we focused on the kinetics of *CEN3* motion in a time interval between initial *CEN3* capture by the microtubule lateral surface (the start of continuous overlap between the two objects) and the shrinking microtubule plus end reaching *CEN3* (Fig. 3). For this analysis, we also excluded samples in which microtubule rescue happened before the microtubule plus end reached *CEN3*. By choosing this time interval, we could avoid potential bias in evaluating Kar3 contribution to *CEN3* sliding because during this time frame, there was no option for conversion to end-on pulling (which is suppressed by Kar3; Fig. 4). Simply comparing the distance that *CEN3* travelled by sliding (as categorized in Fig. 2 A) in *KAR3*$^+$ and *kar3*Δ cells would have mainly analyzed *CEN3* sliding that had escaped conversion to end-on pulling. It is likely that this category would have shown a faster than typical poleward motion of *CEN3* (note that *CEN3* sliding velocity has a relatively large variation, probably because pausing occurs frequently and stochastically during sliding) by which it adventitiously delays the microtubule distal end reaching *CEN3* after microtubule shrinkage, therefore making conversion to end-on pulling less likely (supplemental note 3). Moreover, the rate of this conversion would have been different between *KAR3*$^+$ and *kar3*Δ cells (Fig. 4).

Consistent with the aforementioned consideration, the mean velocity of *CEN3* transport in *KAR3*$^+$ during sliding (0.71 μm/min; Fig. 2 D), in which *CEN3* was transported for 1 μm or longer without conversion to end-on pulling, was higher than the mean displacement of *CEN3* obtained in Fig. 3 A (bottom left; 0.39 μm/min). This difference could be also explained by the fact that in a few *KAR3*$^+$ cells, *CEN3* showed little motion along microtubules (see supplemental note 7); *CEN3* behavior in such cells was taken into account in Fig. 3 A but not in Fig. 2 D.

Supplemental note 7

CEN3 motion and the amount of Kar3 at *CEN3*.

In a few *KAR3*$^+$ cells, *CEN3* did not move preferentially toward a spindle pole but rather moved for a short length in both directions from its original position when initially captured by a microtubule. In such cells, we assume that Kar3 was not significantly involved in *CEN3* motion along microtubules. We tried to correlate the amount of Kar3 at *CEN3* with the mode of *CEN3* motion along microtubules, but we did not find substantial correlation (unpublished data). We presume that some stochastic elements are also involved in determining whether Kar3 at *CEN3* drives its poleward motion.

Supplemental note 8

No motor proteins other than Kar3 are involved in *CEN3* transport along microtubules.

Because *CEN3* motility in the absence of Kar3 is consistent with one-dimensional diffusion along the microtubule lateral surface, it is unlikely that motor proteins other than Kar3 affect *CEN3* motion along microtubules. Nonetheless, we wanted to obtain evidence supporting this notion. We previously studied *CEN3* transport in all other single deletion mutants of the microtubule-dependent motor proteins Cin8, Kip1, Kip2, Kip3, and Dyn1. In these mutants, *CEN3* was transported as in wild-type cells (K. Tanaka et al., 2005).

Next, we visualized these motors by fusing them with four tandem GFPs (Fig. S2 A, green) and, in the same strain, also visualized microtubules and *CEN3*, both marked with CFP (Fig. S2 A, red). Using this, we could distinguish nuclear and cytoplasmic microtubules by noting whether microtubules of interest captured *CEN3* and whether they extended within or outside of the nucleus (whose shape was made visible by enhancing the CFP signal from the small fraction of TetR-3CFP remaining unbound to *tet* operators; unpublished data).

Cin8 and Kip1 signals were only detected along the spindle (between two spindle poles; Hildebrandt and Hoyt, 2000) but usually not along nuclear microtubules extending off the spindle axis or along cytoplasmic microtubules (exceptionally, they were weakly detected along a region where two or more nuclear microtubules overlapped; note that the microtubules should have overlapped with the same orientation in this situation; unpublished data). Kip2 and Dyn1 signals were only detected at the plus ends of cytoplasmic microtubules (Hildebrandt and Hoyt, 2000) and also along cytoplasmic microtubules, albeit weakly, but not on the spindle or on nuclear microtubules. Kip3 localized on the spindle and at the end of growing microtubules (Gupta et al., 2006; Varga et al., 2006). Although microtubules shrank, Kip3 signals at the microtubule ends became weaker. Kip3 signals were also weakly localized along microtubules and predominantly moved toward their plus ends (Gupta et al., 2006; Varga et al., 2006).

Although Cin8, Kip1, and Kip3 localize at kinetochores in metaphase (i.e., after sister kinetochore biorientation; Tytell and Sorger, 2006), these proteins were not detected at *CEN3* before its capture by microtubules or during its poleward transport by microtubules. Among all six microtubule-dependent motor proteins, only Kar3 was detected at *CEN3* before its capture by microtubules and during its poleward transport by microtubules, and only Kar3 and Kip3 were detected on nuclear microtubules in our system. However, it is still possible that other motor proteins were under the detection level and, thus, missed (see supplemental note 20).

Because Kip3 localized along microtubules, we next addressed a potential overlap in function between Kip3 and Kar3 in kinetochore transport by investigating *CEN3* transport in a *kar3-64 kip3*Δ double mutant. However, this double mutant

showed only a minor defect in *CEN3* transport (Fig. S2 B) similar to *kar3-64* and *kar3*Δ single mutants (Figs. 2 A and 8 A). In addition, because Dyn1 is the only microtubule minus end–directed motor other than Kar3 (Hildebrandt and Hoyt, 2000), we also addressed a potential functional overlap between Dyn1 and Kar3 in kinetochore transport by investigating *CEN3* transport in the *kar3-64 dyn1*Δ double mutant. However, this double mutant also showed only a minor defect in kinetochore transport (Fig. S2 B), similar to *kar3-64* and *kar3*Δ single mutants. Thus far, we have not found any evidence that motor proteins other than Kar3 are involved in kinetochore transport along microtubules.

Supplemental note 9
The amount of Kar3 at *CEN3* and establishment of microtubule end-on pulling.

Given that Kar3 partially suppresses the establishment of the microtubule end-on pulling of *CEN3*, the amount of Kar3 at *CEN3* may influence the choice between microtubule rescue and the establishment of end-on attachment when the microtubule plus ends reach *CEN3* after microtubule shrinkage. However, thus far, we have not found a considerable correlation between the choice and Kar3 amount at *CEN3* (unpublished data). For instance, in some cells, the Kar3-4GFP signal was clearly detected at *CEN3* during microtubule end-on pulling (Fig. S2 C). We assume that some stochastic elements might be also involved in the choice between the two options and, therefore, could obscure a possible correlation between the choice and the Kar3 amount at *CEN3*. Nonetheless, the amount of Kar3 at kinetochores eventually decreases upon sister kinetochore biorientation (supplemental note 1); by that time, all kinetochores probably attach to the plus ends of microtubules (see supplemental note 26).

Supplemental note 10
CEN3 transport by microtubules in *kar3-1* cells.

We explored the possibility that the inhibition of end-on pulling establishment in *kar3-1* (compared with *kar3*Δ; Fig. 4 B) was the result of *kar3-1* molecules, which might bind all along microtubules in large amounts, rather than the result of *kar3-1* molecules loaded at kinetochores. If this was the case, this mutant might also change the dynamics of non-*CEN*–associated microtubules in the nucleus. However, as far as we could detect, the rate of depolymerization and polymerization of non-*CEN*–associated microtubules in *kar3-1* was similar to that in *KAR3*$^+$ and *kar3*Δ (not depicted; Fig. S3 A). Non-*CEN*–associated nuclear microtubules did not show rescue in *kar3-1*, similar to those in *KAR3*$^+$ and *kar3*Δ. Given these results, we do not think our data in Fig. 4 B can be readily explained as the effect of *kar3-1* molecules locating along microtubules; rather, we propose that *kar3-1* molecules loaded at kinetochores are involved in this inhibition.

The *kar3-1* mutant suppressed the establishment of the microtubule end-on pulling of *CEN3* more frequently than in *KAR3*$^+$ cells (although it was not statistically significant with the number of samples in this study; Fig. 4 B). If this is generally the case, this may be because wild-type Kar3 molecules associate and dissociate from microtubules in every ATPase cycle (Endow, 2003), whereas *kar3-1* rigor mutant molecules associate with microtubules but do not often dissociate (Meluh and Rose, 1990; Maddox et al., 2003).

To explore further defects in microtubule-dependent *CEN3* transport in *kar3-1*, we needed to observe how *CEN3* interacted with individual microtubules extending from a spindle pole. Such an observation was possible for a short time after *CEN3* was initially captured by a microtubule, which allowed us to collect data in Fig. 4 B. However, it was difficult to observe *CEN3* interaction with individual microtubules at later time points in the *kar3-1* mutant because additional microtubules grew from spindle poles and overlapped with the first microtubule, whereas *CEN3* on microtubules often failed to be transported quickly toward a spindle pole.

Supplemental note 11
Kar3 directly and indirectly suppresses the establishment of end-on pulling.

Altogether, these data suggest that Kar3 partially suppresses the establishment of end-on pulling by both direct and indirect mechanisms. First, Kar3 drives poleward kinetochore sliding along microtubules and, thereby, delays the microtubule distal end reaching kinetochores by microtubule shrinkage. Second, when the microtubule distal end reaches *CEN3* after shrinkage, Kar3 reduces the frequency of the establishment of end-on pulling, probably by anchoring *CEN3* to the microtubule lateral surface in a way that is not consistent with end-on pulling.

Supplemental note 12
The microtubule shrinkage rate is similar during *CEN3* sliding and in the absence of associated *CEN3*.

We previously showed that the microtubule shrinkage rate during *CEN3* sliding along the microtubule lateral side was not significantly different from that in the absence of *CEN3* associated with microtubules (Fig. 3 in K. Tanaka et al., 2005).

Supplemental note 13
dam1 and *kar3* mutants show a synergistic effect on chromosome loss.

We analyzed the frequency of chromosome loss in wild-type, *dam1-1*, and *kar3-64* single mutants and in a *dam1-1 kar3-64* double mutant. We monitored the loss of a centromere-containing nonessential liner marker chromosome as a red sector in a colony (Spencer et al., 1990). Red sectors were visible when the *ade2-101* mutant was no longer suppressed by the *SUP11* gene that was harbored by the marker chromosome on the culture plates with low adenine concentration. After colonies were grown at 25°C for 4 d and subsequently left at 4°C for 1 d, >300 colonies were observed for the presence and absence of red sectors (colonies that were entirely red were excluded from observation). In wild-type, *dam1-1*, *kar3-64*, and *dam1-1 kar3-64* cells, 0, 8.2, 0.3, and 80.1% of colonies showed one or more red sectors, respectively. Therefore, *dam1* and *kar3* mutants show a synergistic effect on chromosome loss.

Supplemental note 14
Nature of *kar3-64* mutation.

kar3-64 is a recessive temperature-sensitive allele that was isolated by Cottingham et al. (1999). As in *kar3*Δ cells, *kar3-64* cells showed mislocalized nuclei, a shorter metaphase spindle, and longer cytoplasmic and nuclear microtubules at restrictive temperature compared with *KAR3*$^+$ wild-type cells (Cottingham et al., 1999; unpublished data). We also compared the frequency of microtubule rescue and establishment of end-on pulling between *kar3-64* and *KAR3*$^+$ cells, when microtubule plus ends caught up with *CEN3*, as performed in Fig. 4 but at 35°C. Like *kar3*Δ cells (Fig. 4), *kar3-64* cells showed a significantly higher frequency of end-on pulling establishment than *KAR3*$^+$ cells (not depicted).

Supplemental note 15

Microtubule dynamics during end-on attached standstill.

During the end-on attached standstill of *CEN3* in *kar3-64 dam1-1* cells, its poleward transport is defective, and microtubule dynamics is also altered (e.g., microtubule depolymerization does not happen). It is interesting to discover whether the primary reason for this standstill is a lack of kinetochore transport or altered microtubule dynamics. To address this, we compared the polymerization and depolymerization rate of nuclear microtubules that were not associated with *CEN3* in *KAR3*$^+$ *DAM1*$^+$ wild-type, *kar3-64*, and *dam1-1* single mutants and in the *kar3-64 dam1-1* double mutant. We did not find a substantial difference between these strains (unpublished data), which is consistent with end-on attached standstill being primarily caused by a lack of kinetochore transport. We also found that non-*CEN*–associated nuclear microtubules showed no rescue in these strains. Our data suggest that the *dam1-1* mutant is defective in converting microtubule depolymerization into the puling force of *CEN3*. On the other hand, the Dam1 complex is still assembled (at least partially) in the *dam1-1* mutant (see supplemental note 16), and we assume that the complex binds *CEN3* because of a residual function in this mutant. The combination of these two situations could somehow cause the Dam1 complex to be stuck at the microtubule end, leading to the end-on attached standstill phenotype. For microtubules involved in end-on attached standstill, polymerization occasionally occurred but more slowly than non-*CEN*–associated microtubules (unpublished data).

Supplemental note 16

Residual function of the *dam1-1* mutant allows the maintenance of *CEN3*–microtubule attachment.

If residual function of the *dam1-1* mutant protein is sufficient to maintain *CEN3*–microtubule attachment, at least some part of the Dam1 complex must localize at *CEN3* during the microtubule end-on attachment of *CEN3* in *dam1-1* cells. Indeed, we found that another Dam1 complex component, Dad1, localized at *CEN3* in this situation (unpublished data). Note that in fission yeast, Dad1 localizes at kinetochores with different timing from other Dam1 complex components (i.e., Dad1 binds kinetochores throughout the cell cycle; Sanchez-Perez et al., 2005). This is not the case in budding yeast (in which, similarly to Ask1 and Dam1 [Fig. 7], Dad1 did not localize at *CEN3*), whereas it was uncaptured by microtubules or transported along the lateral surface of microtubules (not depicted).

If residual function of the *dam1-1* mutant protein is sufficient to maintain *CEN3*–microtubule attachment, we should observe more frequent the detachment of centromeres from microtubules by modifying *dam1-1* into a more defective mutant. This was indeed the case as we describe here. To make a more defective mutant allele than *dam1-1*, we added a heat-labile degron tag (Dohmen et al., 1994) at the N terminus of the *dam1-1* protein (*dam1-1-td*). Both *dam1-1* and *dam1-1-td* cells showed disruption of the spindle (the spindle poles moved away from each other) during metaphase arrest by Cdc20 depletion, which can be at least partly caused by a defect in maintaining sister kinetochore biorientation (Janke et al., 2002). This spindle disruption occurred more quickly in *dam1-1-td* cells after a shift to restrictive temperature, suggesting that the *dam1-1-td* mutant is more defective than *dam1-1*. We subsequently tried to study how *CEN3* capture and transport by microtubules were impaired in the *dam1-1-td* mutant using our *CEN3* reactivation system. However, because of the rapid spindle disruption in the *dam1-1-td* mutant, spindle poles drifted close to *CEN3* and captured it with very short microtubules; thus, high resolution observations were not possible. Consequently, instead, we investigated whether a GFP-labeled centromere stayed attached to a spindle pole or microtubules during metaphase without regulating the centromere activity (Fig. S4 A). 1.2% of *dam1-1* cells showed centromere detachment from a spindle pole and microtubules, and, as we predicted, *dam1-1-td* cells showed more frequent centromere detachment (18.3%). Such a percentage of detachment is probably an underestimation because we were not able to discern detachment if the centromere was recaptured by microtubules rapidly after detachment.

Our data suggest that *dam1-1* is defective, particularly in converting microtubule depolymerization into the kinetochore pulling force, whereas *spc34-3* is partly defective in tethering kinetochores at the microtubule plus ends (Fig. S3 B). *dam1-1-td* also showed partial defects in tethering kinetochores at microtubules (Fig. S4 A). In retrospect, because *dam1-1* has such a defect but yet can be combined with *kar3-64* at a permissive temperature (*spc34-3* and *dam1-1-td* showed synthetic lethality in our attempts to combine them with *kar3-64*), we could identify a role of the Dam1 complex in converting microtubule depolymerization into the kinetochore pulling force.

Supplemental note 17

More information on transient *CEN* detachment from microtubules in normal S phase.

In early S phase, both *CEN5* and *CEN15* showed transient detachment from microtubules with similar frequency when cells started budding (bud index of 5–30% after α-factor arrest and release) and typically moved up to 1.4 ± 0.6 μm (mean ± SD) from a spindle pole (our unpublished data; note that in early S phase, spindle pole bodies are undergoing duplication and have not yet separated from each other; Lim et al., 1996). However, the peak in detachment frequency for *CEN5* was ~5 min earlier than for *CEN15* (our unpublished data). In fact, DNA replication happens 4 min earlier at *CEN5* than at *CEN15* (Raghuraman et al., 2001). The time difference between *CEN5* and *CEN15* detachment, which was also found in *dam1-1* and *kar3-*

64 single mutants and in the *dam1-1 kar3-64* double mutant (unpublished data), is therefore consistent with the notion that *CEN*s detach because centromere DNA replication causes transient kinetochore disassembly. Although the bud index increased from 5 to 30% after α-factor arrest and release, the majority of *CEN*s (>75%) showed evidence of detachment (our unpublished data). Nonetheless, the same *CEN* seldom, if ever, repeatedly detached from microtubules during a single S phase.

Supplemental note 18
Rationale in analyzing discernible microtubule end-on pulling and end-on attached standstill.

In normal S phase, it was generally difficult to distinguish *CEN* transport by sliding along microtubules and by microtubule end-on pulling. This was because microtubules were short and frequently overlapped in this condition, which is in contrast to the centromere reactivation system in metaphase-arrested cells. For instance, even if *CEN*s appeared to move along the lateral sides of microtubules, they may have been caught by the ends of shorter overlapping microtubules. Nonetheless, when *CEN*s localized at the ends of microtubules and there were no other microtubules extending beyond *CEN*s, we could reasonably conclude that *CEN*s actually localized at the microtubule ends. For instance, if *CEN*s were pulled poleward in such a configuration, we could conclude it was end-on pulling (discernible microtubule end-on pulling in Fig. 9 A). On the other hand, if *CEN*s took such a configuration but were not transported, we judged it as end-on attached standstill (discernible end-on attached standstill in Fig. 9 A).

Supplemental note 19
***CEN* transport velocity after *CEN* recapture by microtubules in S phase.**

We measured the transport velocity of *CEN*s after they had detached from and were subsequently recaptured by microtubules during S phase. First, in wild-type cells, we analyzed *CEN* transport velocity during discernible end-on pulling and during any *CEN*–microtubule interaction; *CEN* transport velocity was significantly higher in the former than in the latter (Fig. S4 B). *CEN* transport velocity during discernible end-on pulling in the normal cell cycle was similar to that during end-on pulling in the *CEN* reactivation system at 35°C (Fig. S3 A). We also found that wild-type and *kar3-64* cells showed a similar velocity of *CEN* transport during discernible end-on pulling in S phase (Fig. S4 B). Next, we compared *CEN* transport velocity in wild-type, *kar3-64*, and *dam1-1* cells in which *CEN* reached the vicinity of a spindle pole (Fig. S4 B). Compared with wild-type cells, *CEN* transport velocity was significantly higher in *kar3-64* cells (in which *CEN*s could be transported mainly by end-on pulling; $P = 0.025$) and was lower in *dam1-1* cells (in which *CEN*s could be transported mainly by sliding; $P = 0.065$). These results are consistent with the notion, which was originally obtained by the *CEN* reactivation system, that kinetochores are transported with either sliding or end-on pulling in the normal cell cycle and that sliding (during which *CEN*s are transported more slowly; Fig. 2 D) is dependent on Kar3, whereas end-on pulling (during which *CEN*s are transported more rapidly; Fig. 2 D) is dependent on the Dam1 complex.

Supplemental note 20
Localization of Kar3 at authentic centromeres in S phase.

We studied the localization of Kar3 at authentic centromeres (i.e., without regulation by an adjacent *GAL1-10* promoter) and in normal cell cycles (i.e., without cell cycle arrest). To this end, we visualized kinetochore components (both Mtw1 and Ndc80) with CFP (three tandem copies) and Kar3 with GFP (four tandem copies). In early S phase, kinetochores occasionally showed transient detachment from a spindle pole but were subsequently captured by microtubules and reached the spindle pole (K. Tanaka et al., 2005; our unpublished data). We detected Kar3 signals on ~30% of kinetochores that were transported toward a spindle pole (Fig. S4 C) but not on the remaining 70%. This relatively high percentage of kinetochores without detectable Kar3 signals may be caused by the presence of only a low number of Kar3 molecules at kinetochores, which could not be detected by our method.

To evaluate how many GFP molecules we can detect at kinetochores, we observed cells containing all Mtw1-GFP (single GFP), Ndc80-3CFP, and Ctf19-3CFP. Mtw1-GFP signals were very rarely detected at CFP-labeled individual kinetochores (presumably, an unduplicated kinetochore or a single sister kinetochore pair) but were often visible at more intense CFP signals, where, presumably, multiple sister kinetochore pairs were closely grouped (unpublished data). On the other hand, Ctf19-4GFP was visible at the majority of single pairs of sister kinetochores (unpublished data). Because it is estimated that six to seven Mtw1 and three Ctf19 molecules are present at each sister kinetochore (Joglekar et al., 2006), we assume that the detection limit of GFP with our system is ~20 (15–24) molecules gathered closely together. Given this assumption, we would not be able to detect four or fewer Kar3-4GFP molecules if they were present at a single pair of sister kinetochores.

Supplemental note 21
Both Kar3 and the Dam1 complex are involved in microtubule-dependent kinetochore transport in normal S phase.

Both Kar3 and the Dam1 complex are clearly involved in microtubule-dependent kinetochore transport in the normal cell cycle because the *kar3-64 dam1-1* double mutant shows much more severe defects than each single mutant in the poleward kinetochore transport in normal S phase (Fig. 9 A). Our data also imply that the role of Kar3 in kinetochore transport is not merely a backup (i.e., when end-on pulling is defective) but that it promotes sliding in at least a substantial portion of S-phase cells because (1) kinetochore transport velocity was faster in the *kar3-64* mutant than in wild-type (Fig. S4 B; this suggests that more cells show end-on pulling, which transports kinetochores more rapidly than sliding, in the *kar3-64* mutant), (2) frequency of discernible end-on pulling was higher in the *kar3-64* mutant than in wild type (Fig. 9 A), and (3) Kar3 localized at kinetochores during their poleward transport at least in some part of S-phase cells (Fig. S4 C).

Supplemental note 22
Which kinetochore component provides direct contact with microtubules?

Neither Kar3 nor the Dam1 complex is required for kinetochore interaction with the microtubule lateral surface (K. Tanaka et al., 2005). On the other hand, we previously showed that this interaction requires multiple kinetochore complexes such as the Mtw1, Ctf19, and Ndc80 complexes (K. Tanaka et al., 2005). So which kinetochore complex provides direct contact with a microtubule? Recent data suggest that the Ndc80 complex directly binds a microtubule in vitro (Cheeseman et al., 2006; Wei et al., 2007). The Ndc80 complex binds all along microtubules, not preferentially at the microtubule end, and, therefore, we presume it provides direct contact with the microtubule lateral surface. For kinetochore association with the plus end of a microtubule, we propose that the Dam1 complex provides a direct contact with a microtubule (Fig. 10). However, we cannot rule out that in addition to the Dam1 complex, the Ndc80 complex may also mediate the end-on attachment by directly contacting a microtubule.

Supplemental note 23

Dynamics of the Dam1 complex at the microtubule plus end is very different from that of +TIPs.

The increase in the amount of the Dam1 complexes at the plus ends of shrinking microtubules is a unique behavior and is in sharp contrast to several other molecules, such as Bim1, Bik1, Stu2, Kip2, Dyn1, Kar9 (Carvalho et al., 2003; Akhmanova and Hoogenraad, 2005), Kip3 (Gupta et al., 2006; Varga et al., 2006), and Kar3 (supplemental note 1), which are collectively called microtubule plus end–tracking proteins (+TIPs). All of these +TIPs also localize at the plus ends of microtubules, but their amount at the microtubule ends increases only during microtubule growth and rapidly decreases during microtubule shrinkage. The unique behavior of the Dam1 complex in vivo is consistent with the ring structure formed by this complex in vitro, which would allow collection by splaying protofilaments at the plus ends of shrinking microtubules.

Supplemental note 24

Two *CEN*s captured by the same microtubule.

We analyzed the behavior of two centromeres that were simultaneously activated in metaphase arrest. For this analysis, we made a strain with two homologous sets of *CEN3*s, both of which were regulated under the *GAL1-10* promoter. These two homologous sets of *CEN3*s were sometimes captured by the lateral side of a single microtubule and simultaneously transported poleward by sliding along the microtubule (unpublished data).

Supplemental note 25

Another possible advantage of sliding over end-on pulling.

To ensure sister kinetochore biorientation, Ipl1 (aurora B orthologue) phosphorylation of the Dam1 complex facilitates kinetochore–microtubule reorientation (Biggins et al., 1999; Cheeseman et al., 2002; Tanaka et al., 2002, 2005; Zhang et al., 2005). However, this may function only when kinetochores are associated with the Dam1 complex in a microtubule end-on configuration. During kinetochore sliding, in which the Dam1 complex is not yet loaded onto kinetochores, reorientation may not require the Ipl1 kinase and, therefore, may more readily happen.

Supplemental note 26

Do kinetochores attach to the ends of microtubules after biorientation (during metaphase)?

Stable maintenance of biorientation crucially requires Dam1 complex function (Janke et al., 2002). This suggests that by metaphase, most, if not all, kinetochores are attached to microtubule plus ends in budding yeast as in vertebrate cells provided that the Dam1 complex tethers kinetochores at microtubule plus ends. Two other pieces of indirect evidence also suggest that kinetochores attach to microtubule plus ends during metaphase in budding yeast. First, electron microscopy studies suggest that the number of nuclear microtubules (excluding pole to pole microtubules) that extend from one spindle pole in metaphase cells is approximately identical to the number of yeast chromosomes (Winey et al., 1995; O'Toole et al., 1999). The simplest explanation for this is that each kinetochore attaches to the plus end of a single microtubule in budding yeast. If kinetochores stay associated laterally with microtubules, two or more kinetochores on different chromosomes could still associate with a single microtubule in metaphase (we have indeed found that this occurs; supplemental note 24). Assuming that nuclear microtubules always work either as pole to pole microtubules or as kinetochore-associated microtubules, this would allow the number of kinetochore-associated microtubules to be somewhat smaller than the number of sister chromatids on the metaphase spindle; only the end-on model is consistent with identical numbers of kinetochores and kinetochore-associated microtubules. Second, evidence for end-on attachment could also be provided by studying the localization of bona fide kinetochore proteins relative to that of microtubule plus ends in the metaphase spindle. Such data are available for Ndc10 (Muller-Reichert et al., 2003), and, although some Ndc10 shows dispersed localization along spindle microtubules in metaphase, it clearly shows preferential localization at the microtubule plus ends. Such preferential Ndc10 accumulation at the microtubule plus ends probably corresponds to the location of kinetochores and, therefore, is consistent with end-on attachment.

Supplemental note 27

Function of the Dam1 complex during anaphase and G1 phase.

Metaphase is followed by anaphase A, in which the kinetochore–spindle pole distance is shortened, and anaphase B, in which the distance between two spindle poles is enlarged. In budding yeast, although chromosome segregation mainly depends on anaphase B, cells also undergo anaphase A for a short period (Tanaka et al., 2000; Pearson et al., 2001; Winey and O'Toole, 2001). Anaphase A probably depends on microtubule depolymerization at microtubule plus ends (i.e., at the kinetochore), as microtubules are dynamic only at their plus ends in this organism (Maddox et al., 2000; K. Tanaka et al., 2005). We envisage that the Dam1 complex also plays the same role in anaphase A as we found in prometaphase (i.e., it is required to tether kinetochores at the microtubule plus ends and to convert microtubule depolymerization into the kinetochore pulling force). In addition, the Dam1 complex is presumably crucial in tethering kinetochores at the microtubule plus ends during ana-

phase B, telophase, and G1 phase. Consistent with this notion, we found that the Dam1 complex colocalizes with kinetochores during anaphase A and B using time-lapse microscopy (unpublished data); previous data also suggested that the Dam1 complex colocalizes with kinetochores from metaphase to telophase as well as during G1 phase (Cheeseman et al., 2001; He et al., 2001; Janke et al., 2002; Li and Elledge, 2003). Such functions of the Dam1 complex might explain why kinetochores show abnormal movement (Jaqaman et al., 2006) and often localize more distantly from a spindle pole (albeit still attached to microtubules) in G1 phase (see the relevant Results section in the main text) when this complex is defective.

We attempted to observe how anaphase A proceeds in mutants of the Dam1 complex components. However, such a study was technically difficult because in Dam1 complex component mutants, sister kinetochore biorientation could not be maintained, and kinetochores were already pulled toward spindle poles before the onset of anaphase A at a restrictive temperature.

Supplemental note 28
Kinetochore sliding along microtubules in metazoan cells.

In metazoan cells, the nuclear envelope breaks down in mitosis (open mitosis), whereas in budding yeast, the nuclear envelope remains intact throughout the cell cycle (closed mitosis). If dynein is involved in kinetochore sliding along microtubules in metazoan cells, eukaryotic cells must have acquired an ability to use cytoplasmic dynein for kinetochore transport after they developed open mitosis. Nonetheless, it is still possible that kinesin-14 family members also regulate kinetochore sliding along microtubules in metazoan cells, as in budding yeast.

Kinetochore sliding along microtubules could be more complex in vertebrate cells because after kinetochores arrive at a spindle pole, CENP-E (a kinesin-7 family member) could also drive this process, but toward the microtubule plus ends, to facilitate chromosome congression to the metaphase plate (Kapoor et al., 2006). Note that there is no obvious orthologue of kinesin-7 found in the budding yeast genome (Hildebrandt and Hoyt, 2000).

Supplemental note 29
More information on yeast strain construction.

KAR3, *DAM1*, *ASK1*, *DAD1*, *CIN8*, *KIP1*, and *DYN1* were tagged with four tandem copies of GFP at their C termini at their original gene loci by a one-step PCR method (Maekawa et al., 2003). *MTW1*, *NDC80*, and *CTF19* were tagged with three copies of CFP at their C termini at their original gene loci by a one-step PCR method (unpublished data). *KIP2-4GFP*, *KIP3-4GFP*, and *STU2-4GFP* were previously reported (K. Tanaka et al., 2005; Varga et al., 2006). The tagged *KAR3*, *DAM1*, *ASK1*, *DAD1*, *STU2*, *MTW1*, and *NDC80* were functional because these cells grew normally (note that *DAM1*, *ASK1*, *DAD1*, *STU2*, *MTW1*, and *NDC80* are essential genes and that *kar3*Δ cells show slow growth). The tagged *KIP2* should be functional as previously reported (Carvalho et al., 2004). The tagged *KIP3* was functional as previously described (Varga et al., 2006). The tagged *CIN8* was functional because its combination with *kip1*Δ did not lead to synthetic lethality, which is in contrast to *cin8*Δ (Hildebrandt and Hoyt, 2000). The tagged *KIP1* was functional because its combinations with *cin8*Δ did not lead to synthetic lethality, which is in contrast to *kip1*Δ (Hildebrandt and Hoyt, 2000). The tagged *DYN1* was functional because its combination with *kar3*Δ did not lead to synthetic lethality, which is in contrast to *dyn1*Δ (Cottingham et al., 1999).

Constructs of *PGAL-CEN3-tetOs* (K. Tanaka et al., 2005), *TetR-GFP* (Michaelis et al., 1997), *TetR-3CFP* (Bressan et al., 2004), *CFP-TUB1* (Janke et al., 2002), *PMET3-CDC20* (Uhlmann et al., 2000), *CEN5-tetOs* (Tanaka et al., 2000), *CEN15-lacOs* (Goshima and Yanagida, 2000), and *GFP-lacI* (Straight et al., 1996) were previously described. *YFP-TUB1* plasmid (pDH20) was obtained from the Yeast Resource Centre. *dam1-1-td* was made as described in supplemental note 16. Strains with gene deletions were obtained from EUROSCARF or made by us using a one-step PCR method (Amberg et al., 2005).

Supplemental note 30
More methods in analyzing the dynamics of kinetochores and microtubules.

To score sliding and end-on pulling, we only analyzed transport events in which *CEN* reached the vicinity of a spindle pole or ended up showing standstill. For instance, if *CEN* had detached from microtubules during transport, the transport mode observed before this detachment was not taken into account. In any case, such *CEN* detachment was a rare event (supplemental note 5).

Laterally attached standstill was scored when *CEN3* was at the lateral side of microtubules and when the change in the *CEN3*–spindle pole distance was $<$300 nm in $>$90% of time points and $<$500 nm continuously for 6 min or longer. Once *CEN3* showed such behavior, it usually continued to do so until the end of observation. End-on attached standstill was scored when *CEN3* was at the plus ends of microtubules and the *CEN3*–spindle pole distance was not shortened by $>$500 nm for a period of 6 min or longer. Again, once *CEN3* showed such behavior, it continued to do so until the end of observation in most cases. Note that during end-on attached standstill, the *CEN*–spindle pole distance occasionally increased relatively briefly ($<$6 min) and slowly ($<$0.5 μm/min).

The following precautions were taken to minimize potential misinterpretation of the mode of kinetochore transport as a result of overlapping microtubules (supplemental note 2): (1) if additional microtubules grew (judged by tubulin signal intensity) overlapping with the microtubule that captured *CEN3* within 2 min after *CEN3* capture, such cells were not included in scoring; (2) if additional microtubules grew (judged by tubulin signal intensity) overlapping with and extending beyond the microtubule, which showed end-on attached standstill of *CEN3*, for 20% or more time points during the period of this standstill, such cells were not included in scoring (the number of such cases was $<$5% of the scored number of end-on attached standstill).

When *CEN3* was captured by microtubules in the *CEN3* reactivation system, *CEN3* approached the side of microtubules that had already extended from spindle poles in some cells. In

others, *CEN3* was located in the path of microtubule extension. In the latter case, the plus ends of microtubules seemingly passed through *CEN3* without causing the immediate motion of *CEN3*. After a short interval, *CEN3* started moving along the microtubule. In such cases, it was difficult to determine whether *CEN3* was actually captured by microtubules until *CEN3* started moving along them. In figures, the time point at which *CEN3* had started continuous colocalization with a microtubule was defined as the point of *CEN* capture by the microtubule.

Supplemental note 31
More information for the legend of Fig. 6 C.
We analyzed microtubule shrinkage that occurred for 1 μm or longer, and relevant microtubules (or regions along a microtubule) had none (pale blue) or at least one (pink) discrete GFP signal whose intensity was 200 U or higher when measured with the default setting of SoftWorx (Applied Precision) software. Before shrinkage of the microtubule, Dam1 complex signals were not present along a microtubule (middle depiction); alternatively, they were distributed either along a microtubule (top depiction) or close to the microtubule plus end but not close to a pole (bottom depiction; we did not find any microtubules with the reversed pattern of Dam1 complex distribution).

For the microtubules of the top and middle depictions, we measured GFP intensity at the microtubule plus end when it first exceeded 200 U after microtubule catastrophe (after the microtubule had started shrinkage; pre-measurement) and 10 s (one time point) before the microtubule plus end reached a spindle pole (postmeasurement for the top and middle depictions; post2 measurement for the bottom depiction). For the microtubules of the bottom schematic depiction, we also measured the GFP intensity when all Dam1 complex signals along the microtubule had been collected by the microtubule end (postmeasurement).

Occasionally, the intensity of the Dam1 complex signal at the microtubule plus end suddenly decreased by >30% relative to the signal at time points one before and after it. Such a sudden and transient signal decrease could happen when the microtubule plus ends drift away from focal planes, and such time points were removed from further analyses.

Fold increase was calculated by dividing the increase of the signal intensity (subtraction of pre-signal intensity from post-signal intensity) by pre-signal intensity. For the second measurement of the fold increase (bottom depiction), we also divided the increase of the signal intensity (subtraction of post-signal intensity from post2 signal intensity) by pre-signal intensity rather than by post-signal intensity to determine whether there was a further increase in GFP accumulation during the second stage of microtubule shrinkage. During the relevant observation, GFP intensity decreased by <20% as a result of photobleaching, as judged from changes of GFP signal intensity on the spindle.

We statistically analyzed differences in the fold increase of Dam1 complex signals at the shrinking microtubule ends. In the presence (top depiction; $n = 10$) and absence (middle depiction; $n = 7$) of Dam1 complex signals along the extent of microtubule shrinkage, the fold increase was significantly higher in its presence than in its absence ($P = 0.0002$; unpaired *t* test), and the fold increase divided by the length of microtubule shrinkage also showed similar tendency ($P = 0.023$; unpaired *t* test). When the fold increase was compared on the same microtubules (bottom depiction; $n = 5$) during collection of the Dam1 signals along the microtubule and after it, the fold increase was significantly higher during than after it ($P = 0.0033$; paired *t* test), and the fold increase divided by the length of microtubule shrinkage showed similar tendency ($P = 0.0076$; paired *t* test).

Supplemental note 32
More information for the legends of Figs. 9 and S4 B.
Time-lapse images were collected every 7.5 s for 8 min. We analyzed *CEN* capture and transport by microtubules only after the detachment of either *CEN* from microtubules was observed at two or more time points. Such *CEN* detachment happened almost exclusively during early S phase (bud index of 5–30% after α-factor arrest and release). *CEN*s were scored as reaching the vicinity of a spindle pole when they were 0.6 μm or less from the center of the spindle pole signal within 2 min after recapture by microtubules (*CEN*s moved poleward for 0.7–1.5 μm with microtubules associated). If *CEN*s did not reach the proximity of the spindle pole within 2 min, they usually remained attached to microtubules somewhat distant (>1.0 μm) from a spindle pole until the end of observation. Discernible end-on pulling was scored when *CEN* was discernibly at the distal ends of microtubules at two thirds (or more) of time points after being recaptured by microtubules and before reaching the vicinity of a spindle pole (<0.6 μm from the center of the pole signal). Discernible end-on attached standstill was scored when *CEN* was discernibly at the distal ends of microtubules and *CEN*–spindle pole distance was not shortened for 350 nm or more during a continuous 3 min or longer. In Fig. 9 B, note that the other *CEN* (i.e., whose detachment was not shown in these images) was sometimes out of focus and, therefore, is not indicated and that the *dam1-1 kar3-64* cell shown here has a shmoo but not a bud.

To determine the *CEN* transport velocity shown in Fig. S4 B (pink bars), *CEN*–spindle pole distance was measured when *CEN* was initially captured by microtubules after detachment and when *CEN* reached the vicinity of a spindle pole (<0.6 μm from the center of the pole signal). To determine the *CEN* transport velocity shown in Fig. S4 B (shaded red bars), *CEN*–spindle pole distance was measured when end-on pulling first became discernible after recapture by microtubules and when *CEN* reached the vicinity of a spindle pole (or when end-on pulling became indiscernible). In both cases, to calculate the *CEN* transport velocity, the difference between the two distances was divided by the time taken.

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