Time-Lapse Microscopy Reveals Unique Roles for Kinesins during Anaphase in Budding Yeast

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Abstract. The mitotic spindle is a complex and dynamic structure. Genetic analysis in budding yeast has identified two sets of kinesin-like motors, Cin8p and Kip1p, and Kar3p and Kip3p, that have overlapping functions in mitosis. We have studied the role of three of these motors by video microscopy of motor mutants whose microtubules and centromeres were marked with green fluorescent protein. Despite their functional overlap, each motor mutant has a specific defect in mitosis: cin8Δ mutants lack the rapid phase of anaphase B, kip1Δ mutants show defects in the slow phase of anaphase B, and kip3Δ mutants prolong the duration of anaphase to the point at which the spindle becomes longer than the cell. The kip3Δ and kip1Δ mutants affect the duration of anaphase, but cin8Δ does not.

Key words: mitosis • kinesin • microtubule • anaphase • yeast

The mitotic spindle segregates the chromosomes into the daughter cells during cell division. The dynamic behavior of the spindle is controlled by motor proteins that move along microtubules and by the polymerization and depolymerization of microtubules. Motor proteins are required to assemble and maintain a bipolar spindle, to regulate microtubule dynamics, and to orient the spindle in the cell. After the spindle has formed, motor proteins mediate spindle elongation and chromosome separation during anaphase (reviewed in McIntosh and Pfarr, 1991; Inoue and Salmon, 1995; Vernos and Karsenti, 1996).

In vertebrate cells, the roles of motor proteins have been dissected by inactivating motors in living cells and egg extracts (Rodionov et al., 1993; Vaisberg et al., 1993; Lombillo et al., 1995; Walczak et al., 1996; Heald et al., 1997). These approaches are powerful, but can produce complex results because many motors have overlapping functions and antibody inactivation or immunodepletion experiments can also affect proteins that interact with the target motor protein. Budding yeast is an attractive alternative for studying the roles of specific motors in mitosis: the genome sequence (Goffeau et al., 1996) provides a complete inventory of microtubule motors, yeast genetics allows inactivation of individual motors, and marking centromeres and microtubules with green fluorescent protein (GFP) makes it possible to study spindle and chromosome dynamics in living cells (Straight et al., 1997). Yeast contain six kinesin related proteins (Cin8p, Kar3p, Kip1p, Kip2p, Kip3p, and Smy1p) and a single dynein (Meluh and Rose, 1990; Hoyt et al., 1992; Lillie and Brown, 1992; Roof et al., 1992; Eshel et al., 1993; Cottingham and Hoyt, 1997; DeZwaan et al., 1997). This paper analyzes the mitotic roles of three of the six kinesin motors: Cin8p, Kip1p and Kip3p. We did not study Smy1p since it doesn’t appear to be involved in mitosis (Lillie and Brown, 1992, 1994) or Kip2p because it primarily affects cytoplasmic microtubules rather than the intranuclear microtubules of the mitotic spindle (Huyett et al., 1998). We were unable to study kar3Δ mutants, because most cells arrest in mitosis under the conditions required for microscopy.

Cin8p and Kip1p kinesins belong to the bimC/Cut7 class of microtubule motors that have roles in spindle formation and spindle elongation in other organisms (reviewed in Kashina et al., 1997). Kip3p, is a novel kinesin that does not easily fit into the known kinesin subfamilies but has been shown to be important for spindle positioning (Cottingham and Hoyt, 1997; DeZwaan et al., 1997). The minus end–directed motor Kar3p antagonizes the activity of Cin8p/Kip1p to ensure proper spindle assembly and elongation (Saunders and Hoyt, 1992; Saunders et al., 1997) and is also thought to participate in the positioning of the spindle (Cottingham and Hoyt, 1997; DeZwaan et al., 1997). In addition to the kinesin family of proteins, dynein also has roles in spindle positioning, assembly and elonga-
The Cin8p and Kip1p proteins are thought to be plus end directed motors that have overlapping roles in pushing the spindle pole bodies apart. cin8 was isolated as a mutant that exhibited elevated chromosome loss (Hoyt et al., 1990). At 37°C cin8Δ mutants arrest in mitosis with duplicated spindle poles but fail to form a bipolar spindle. The defect in cin8Δ mutants can be overcome by mild overexpression of Kip1p, suggesting that the two motors have redundant functions during mitosis (Hoyt et al., 1992). Furthermore, cin8Δ kip1Δ double mutants are inviable and fail to form a bipolar spindle (Hoyt et al., 1992; Roof et al., 1992). Cin8 and Kip1 are required for both assembly and maintenance of the bipolar spindle: bipolar spindles in kip1Δ cells that carry a temperature sensitive allele of cin8 collapse when cells are shifted to the nonpermissive temperature. This collapse is partially rescued in cells lacking Kar3p, suggesting that the length of the spindle is controlled by the balance between forces generated by Cin8p and Kip1p that push the spindle pole bodies apart and those generated by Kar3p that pull them together (Saunders and Hoyt, 1992).

Kip3p plays a role in the migration of the nucleus to the neck between the mother and daughter cells and the proper alignment of the mitotic spindle before anaphase (Cottingham and Hoyt, 1997; DeZwaan et al., 1997). One explanation for these roles is that Kip3p, like certain other motors (Endow et al., 1994; Waleczak et al., 1996), can destabilize microtubules.

Since the spindle is a dynamic structure, we investigated its behavior by time lapse microscopy of wild-type and mutant cells whose centromeres and microtubules were marked with GFP. Kip1p and Cin8p have distinct roles during anaphase chromosome separation and spindle elongation. Although both motors are required for normal elongation of the spindle, Cin8p is most important early in anaphase when rapid separation of the chromosomes occurs, and Kip1p is required late in anaphase for robust elongation of the spindle. Analysis of Kip3p mutants shows that Kip3p does not play a role in the absolute rates of anaphase spindle elongation but is involved in the proper timing of spindle disassembly. Thus, despite the functional overlap between them, each of the three kinesin motors contributes to a particular event during anaphase.

**Materials and Methods**

**Strains and Media**

Yeast were grown either in YPD (10 g/liter yeast extract, 20 g/liter Bacto-Peptone, 20 g/liter Dextrose) supplemented with 50 mg/ml adenine-HCl, and 50 mg/ml l-tryptophan or in complete synthetic medium lacking histidine (CSM-HIS; Sherman et al., 1974) supplemented with 50 mg/ml adenine-HCl, 50 mg/ml l-tryptophan, and 6.5 g/liter NaCitrate. Yeast strains are listed in Table I and are all isogenic to W303 (AFS34). Yeast transformations were performed using the lithium acetate method (Ito et al., 1983). Plasmids were propagated in _Escherichia coli_ strain TG1 (Sambrook et al., 1989) in medium containing 100 µg/ml ampicillin except for Lac operator repeat plasmids which were propagated in _E. coli_ STB2 (GIBCO BRL, Gaithersburg, MD). GFP-Lac repressor and GFP-Tubulin fusions were induced as described (Straight et al., 1996, 1997).

**Plasmids and DNA Manipulation**

**Construction of Motor Protein Deletions.** The cin8Δ::LEU2 deletion plasmid was provided by Andrew Hoyt (Hoyt et al., 1992). cin8 was deleted in strain AFS34, then the LEU2 marked cin8 deletion was changed to TRP1 by integrating the marker switching plasmid pL7 from F. Cross (Cross, 1997).

Kip1p was amplified from yeast genomic DNA by PCR using the following oligonucleotide pair: 5′-GGCATGGATGAA-

**Table I. Yeast Strains**

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Relevant genotype</th>
<th>Plasmid</th>
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<tbody>
<tr>
<td>AFS34 (W303-1a)</td>
<td>MA Ta ade2-1 can1-100 ara3-1 leu2-3,112 his3-11,15 trp1-1</td>
<td>pAFS78</td>
</tr>
<tr>
<td>AFS403</td>
<td>MA Ta his3-11,15::GFP-LacI-HIS3 leu2-3,112::lacO-LEU2 ara3-1::GFP-TUB1-URA3</td>
<td>pAFS59</td>
</tr>
<tr>
<td>AFS426</td>
<td>MA Ta cin8Δ::TRP1 his3-11,15::GFP-LacI-HIS3 leu2-3,112::lacO-LEU2 ara3-1::GFP-TUB1-URA3</td>
<td>pAFS91</td>
</tr>
<tr>
<td>AFS404</td>
<td>MA Ta kip1Δ::HIS3 his3-11,15::GFP-LacI-HIS3 leu2-3,112::lacO-LEU2 ara3-1::GFP-TUB1-URA3</td>
<td>pAFS91</td>
</tr>
<tr>
<td>AFS417</td>
<td>MA Ta kip1Δ::HIS3 his3-11,15::GFP-LacI-HIS3 leu2-3,112::lacO-LEU2 ara3-1::GFP-TUB1-URA3</td>
<td>pAFS91</td>
</tr>
<tr>
<td>AFS501</td>
<td>MA Ta his3-11,15::GFP-LacI-HIS3 leu2-3,112::lacO-LEU2 ara3-1::GFP-TUB1-URA3</td>
<td>pAFS144</td>
</tr>
</tbody>
</table>

All strains are isogenic to AFS34 (W303-1a from the laboratory of R. Rothstein). Only the genotype that differs from AFS34 is shown.

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The brightest GFP (GFP12) was mutated at codon 163 from GTT to GCT changing Val163 to Ala163. This change resulted in the same amino acid change described by Siemering et al. (1996) for the GFP-B mutant but used a different codon. Based on the work of Siemering et al. we then combined our GFP12 mutant with the Ser175→Gly mutation to give GFP13 (S6ST, V163A, and S175G).

Certain mutations in the lac repressor cause increased affinity between the repressor and operator DNA (Miller and Reznikoff, 1980). We mutated the GFP-lac repressor fusion in pAFS78 from Pro3→Tyr5 to give the lacI-12 mutation (Schmitz et al., 1978). This Lac repressor mutant was then fused to the GFP12 and GFP13 sequences exactly as described for pAFS78 to give pAFS135 and pAFS144, respectively. Plasmids pAFS135 and pAFS144 were linearized with NheI and transformed into yeast strain AFS34 for expression of GFP-Lac fusions in yeast. Lac operators were introduced at the centromere of chromosome III as previously described using pAFS59 (Straight et al., 1996). GFP-Tubulin was expressed by integration of pAFS91 as previously described (Straight et al., 1997).

**Time-Lapse Microscopy and Image Analysis**

Images were acquired as described (Straight et al., 1997) except that the actual length of spindles in kip3Δ cells was calculated by tracing fluorescence intensities in three-dimensional image stacks using the program 3D Model that had been customized for length measurement (Chen et al., 1996). The data for wild-type spindle elongation is previously published (Straight et al., 1997) and used only for comparison to the motor mutant data.

**Results**

Positioning, assembly and elongation of the yeast spindle during mitosis requires microtubule motor proteins. Anaphase has two components: anaphase A, the movement of the chromosomes towards the spindle poles and anaphase B, the separation of the spindle poles. In budding yeast, anaphase B has two phases, a rapid initial elongation of the spindle that is followed by a period of slower elongation (Kahana et al., 1995; Yeh et al., 1995; Straight et al., 1997). To study the contributions of individual motor proteins to anaphase, we performed time lapse video microscopy on yeast cells individually deleted in three kinesin genes to anaphase, we performed time lapse video microscopy (Kahana et al., 1995; Yeh et al., 1995; Straight et al., 1997). We measured the separation between the spindle and between the sister centromeres as cells went from metaphase to anaphase. These distances allow us to quantify chromosome to pole movement during anaphase A and the increase in the separation between the poles during anaphase B.

**Cin8p Is Required for the Rapid Phase of Mitotic Spindle Elongation**

Fig. 1 shows records of mitosis in cin8Δ mutants. During metaphase, cin8Δ cells had shorter spindles (1–1.5 μm) than wild-type cells (1.5–2 μm; Figs. 1 A and 4). The suggestion that Cin8p is required for the full separation of the spindle pole bodies during metaphase is consistent with the role for Cin8p in spindle assembly described by Saunders and Hoyt (Saunders and Hoyt, 1992).

During anaphase, cin8Δ cells exhibited a defect in the rapid phase of spindle elongation. In wild-type cells the initial rapid separation of the spindle pole bodies (0.54 μm/min) was followed by a slower phase (0.21 μm/min; Table II; Straight et al., 1997). cin8Δ cells showed a uniformly slow spindle elongation whose rate (0.19 μm/min) was statistically indistinguishable from the slow phase of anaphase B in wild-type cells (Table II; Fig. 1 A). This defect suggests that Cin8p has a specific role in the initial rapid separation of the spindle poles but that other activities drive the slower phase of mitotic spindle elongation. The rapid initial separation of the centromeres in wild-type cells was not affected in the cin8Δ mutant (Fig. 1 B) suggesting that other factors are responsible for the initial separation. These could include the activity of other motors or the release of tension when the linkage between the sister chromatids dissolves.

![Figure 1](https://example.com/figure1.png)

**Table II. Spindle Dynamics in Wild-type and Kinesin Mutants**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fast phase</th>
<th>Slow phase</th>
<th>Anaphase B</th>
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<tbody>
<tr>
<td></td>
<td>μm/min</td>
<td>μm/min</td>
<td>μm/min</td>
</tr>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cin8Δ</td>
<td>0.28 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>6.95 ± 0.68</td>
</tr>
<tr>
<td>kip1Δ</td>
<td>0.51 ± 0.03</td>
<td>0.12 ± 0.03</td>
<td>9.79 ± 0.43</td>
</tr>
<tr>
<td>kip3Δ</td>
<td>0.56 ± 0.04</td>
<td>0.17 ± 0.02</td>
<td>12.31 ± 0.25</td>
</tr>
</tbody>
</table>

Rates of spindle elongation were calculated using linear regression analysis in the same time intervals used to calculate the wild-type rates for the fast and slow phases of anaphase. Measurements shown in bold are significantly different from wild-type rates and all rates are displayed as the mean ± SE. The average breakdown length and the average duration of anaphase were calculated using the final timepoint before spindle disassembly. The total number of records analyzed for measurements of WT, cin8Δ, kip1Δ, and kip3Δ are n = 6, n = 5, n = 5, and n = 7, respectively.
Although cin8Δ cells are defective for the rapid phase of anaphase B, the longer slow phase of anaphase B allows them to complete mitosis, cin8Δ spindles disassemble at the same time after anaphase initiation as do wild-type cells (Table II; Fig. 2) but with a shorter length (7.0 ± 0.7 μm) than that of wild-type cells (9.5 ± 0.5 μm). Much of this length difference can be accounted for by the slower elongation of cin8Δ spindles during the 5 min when wild-type cells are elongating their spindle rapidly ([0.55 μm/min [WT] − 0.2 μm/min [cin8Δ]] × 5 min = 1.8 μm).

**Kip1 Affects the Slow Phase of Mitotic Spindle Elongation**

Cin8p and Kip1p have overlapping roles during spindle assembly and elongation (Roof et al., 1992; Saunders et al., 1997; Saunders and Hoyt, 1992). We examined kip1Δ cells during mitosis to determine whether differences exist between the two motors. Consistent with prior measurements in cells arrested in S phase (Saunders et al., 1997), and like cin8Δ cells (1.2 μm), kip1Δ cells (1.2 μm) had shorter metaphase spindles than wild-type cells (1.8 μm; see Fig. 4). This result supports the idea that Cin8p and Kip1p work together to maintain the mitotic spindle at its proper metaphase length (Saunders and Hoyt, 1992). The distances we have measured are slightly larger than the distances measured in hydroxyurea arrested cells for wild-type, cin8Δ and kip1Δ (Saunders et al., 1997). Our measurements of metaphase spindle length were made using video records of the 20–30 min preceding sister chromatid separation. The differences between hydroxyurea arrested and G2/M cells may reflect a difference between cells arrested by the DNA replication checkpoint and mitotic cells, a difference between cells with unreplicated centromeres and replicated centromeres, or differences between measurements made on live and fixed samples.

During anaphase, kip1Δ cells exhibited a normal rapid elongation phase (0.51 μm/min; Fig. 3 A; Table II) in contrast to cin8Δ cells, whose spindles elongate slowly. However, during the slow phase of spindle elongation in anaphase B, the spindles of kip1Δ cells (0.12 μm/min) elongated more slowly than wild-type spindles (0.2 μm/min; Fig. 3 A; Table II). This suggests that Kip1p and Cin8p have distinct roles in anaphase B: Cin8p is required for rapid elongation at the beginning of mitosis and Kip1p is more important during the slower phase of spindle elongation.

The period from the onset of anaphase to spindle breakdown was ~15 min longer in kip1Δ cells than it was in wild-type or cin8Δ cells. The longer duration of anaphase compensated for the slower rate of spindle elongation with the result that kip1Δ cells elongated their spindles to the same final length as wild-type cells (9.5–10 μm; Figs. 3 A and 6; Table II). We could not tell whether the loss of Cin8p or Kip1p affected chromosome to pole movement (anaphase A) because the shorter metaphase spindle in cin8Δ and kip1Δ cells made it impossible to measure this movement.

**Kip3 Affects Microtubule Length and Spindle Breakdown in Mitosis**

Deletion of KIP3 increases the metaphase spindle length compared with wild-type cells. Metaphase spindles in kip3Δ cells were on average 0.4 μm longer than those in wild-type cells (Figs. 4 and 5 A). This increase is similar to that seen in the comparison between fixed kip3Δ and wild-type cells arrested in S phase (Cottingham and Hoyt, 1992).
Unlike Cin8p and Kip1p, Kip3p opposes extension of the metaphase spindle, either by exerting an inward directed force or by reducing the length of spindle microtubules. Because kip3Δ cells have longer astral microtubules and their spindles are resistant to depolymerization with the drug benomyl (Cottingham and Hoyt, 1997; DeZwaan et al., 1997), destabilization of the central spindle microtubules by Kip3p is the more likely mechanism for regulating spindle length.

Examining kip3Δ cells during anaphase revealed a specific role for Kip3 in regulating spindle breakdown. Initial anaphase spindle elongation occurred normally in kip3Δ cells, consistent with measurements of spindle pole separation in fixed samples (DeZwaan et al., 1997). The rates of the rapid (0.56 μm/min) and slow phases (0.17 μm/min) of spindle elongation in kip3Δ cells were statistically indistinguishable from those of wild-type cells (Fig. 5A; Table II). Although the rates of spindle elongation in wild-type and kip3Δ are similar, kip3Δ cells broke down their spindles 12 min later than wild-type cells (Fig. 2; Table II). This observation contrasts with earlier measurements on populations of fixed kip3Δ cells, that showed that the distance between fluorescently labeled spindle pole bodies stopped increasing at the same time in wild-type and kip3Δ cells (DeZwaan et al., 1997). However, in all but one of our sequences (n = 7), spindles persisted in kip3Δ cells after the time at which the spindle in wild-type cells broke down. This difference is probably explained because previous work assumed that spindle breakdown occurred at the time when the distance between the spindle poles stopped increasing.

Cells lacking Kip3p elongate their spindles beyond the length of wild-type cells. Although the pole to pole distance in kip3Δ stops increasing at ~10 μm (Fig. 5A), the spindles keep elongating, making them bend before they break down (Fig. 5D). To confirm this interpretation, we measured the length of the bent kip3Δ spindles in three-dimensional space. Although the spindle pole bodies were only separated by 10 μm, the actual spindle length increased to more than 12 μm (Figs. 5C and 6; Table II). Kip3p may therefore be involved in destabilizing microtubules at the end of mitosis so that the spindle can disassemble at the proper time and at the proper length. Bowed spindles have also been observed in wild-type fission yeast, although in this organism the spindle poles do not have to reach the ends of the cell for bending to occur (Hagan and Hyams, 1996).

Motor Mutants Delay the Onset of Anaphase

We investigated the effect of motor mutations on the interval between spindle assembly and the onset of anaphase. Wild-type, cin8Δ, kip1Δ, and kip3Δ mutants that contained GFP-marked tubulin and CENIII were arrested in G1 by treatment with the yeast mating pheromone α-factor. The cells were released from this arrest and population samples were removed at intervals and scored for spindle elongation and sister chromatid separation. Cells deleted for Kip1p or Kip3p initiated anaphase at the same time as wild-type cells and progressed through anaphase with similar kinetics (Fig. 7). Cells lacking Cin8p showed a dramatic delay in the initiation of anaphase. 90 min after release from α-factor, >30% of wild-type, kip1Δ, and kip3Δ cells had initiated anaphase compared with only 4%
of cin8Δ cells. When >80% of wild-type, kip1Δ and kip3Δ cells had initiated anaphase (t = 120), <25% of cin8Δ cells had separated their sister chromatids (Fig. 7). We have also observed delays in the onset of anaphase in cells lacking Kar3p (data not shown). The prolonged metaphase of motor mutants is probably due to the action of the spindle checkpoint (reviewed in Nicklas, 1997; Rudner and Murray, 1996), which delays anaphase until all chromosomes are correctly aligned on a bipolar spindle. Inactivation of the checkpoint allows cells with spindle defects to exit from mitosis and kills kar3Δ and cin8Δ mutants, which have the most profound delays in mitosis (Roof et al., 1992; Saunders and Hoyt, 1992; Saunders et al., 1995). These observations did not reveal that Cin8p and Kip1p are important for different phases of anaphase. We have shown that Cin8p functions early in the separation of the spindle pole bodies and is required for the rapid phase of anaphase spindle elongation. Unlike Cin8p, Kip1p plays no role in controlling the rapid phase of spindle elongation. Cells deleted for Kip1p perform the initial phase of anaphase normally but are then compromised in the slower phase of spindle elongation.

The difference between the rates of the two phases of anaphase B in wild-type cells could be explained in many ways. One explanation is that the rate of the rapid phase is set by the rate at which motors slide the microtubules from opposite poles of the spindle past each other and that the rate of the slow phase is set by the rate of the microtubule growth required to maintain an overlap zone as the spindle extends. In the rapid phase, Cin8p would move microtubules past each other more rapidly than Kip1p. During the slow phase, the deletion of KIP1 would affect the rate of microtubule growth, but the deletion of CIN8 would not. Cut7p, a fission yeast homologue of Cin8p and Kip1p, localizes to the midzone of the spindle during anaphase B (Hagan and Yanagida, 1992). Experiments in isolated diatom spindles suggest that the rate of spindle elongation is strongly influenced by factors that control the speed at which microtubules grow (Masuda and Cande, 1987). Another possibility is that both rates of anaphase B are set by the speed of microtubule sliding and that Cin8p is a faster motor that is only active during early anaphase B, whereas Kip1p is a slower motor active throughout anaphase B.

Cells lacking Kip3p delay spindle breakdown and their final spindles are so long that they become physically deformed when they run into the ends of the cell. Previous studies suggested that Kip3p may regulate the stability of microtubules (Cottingham and Hoyt, 1997). In our experiments, metaphase kip3Δ spindles were longer than those of wild-type cells in agreement with spindle length measurements in hydroxyurea arrested cells (Cottingham and Hoyt, 1997). Once anaphase began, kip3Δ cells behaved exactly like wild-type cells until the time of spindle breakdown: when wild-type spindles broke down, kip3Δ spindles remained intact and continued to elongate. The ability of the spindle to elongate beyond the cell length has implications for the mechanism of spindle elongation. The buckling of the spindle in kip3Δ indicates that some or all of the force elongating the spindle must be generated within the spindle, pushing the poles apart. If spindle elongation was driven by a pulling force generated by interactions of astral microtubules with the cortex at the ends of the cell, the length of the spindle could not exceed that of the cell. In the phytopathogenic fungus Nectria haematococca and in rat kangaroo kidney epithelial cells (PtK2 cells), severing the central spindle during anaphase increased the rate of spindle pole separation suggesting that interactions in the central spindle were limiting the rate of spindle elongation and that astral pulling forces can drive spindle elongation (Aist et al., 1991, 1993). We do not know if the apparent discrepancy between these observations and our own is due to different factors regulating the extent versus the speed of anaphase B, or differences between the mechanism of spindle elongation in different organisms.

**Figure 6.** Motors regulate the final length of the anaphase spindle. Spindle breakdown length in wild-type and motor mutants. The final breakdown length of the anaphase spindle was calculated using the timepoint before spindle breakdown in strains AF503 (WT), AF526 (cin8Δ), AF504 (kip1Δ), and AF517 (kip3Δ).

**Figure 7.** Motor mutants delay anaphase onset. cin8Δ causes a delay in the entry into anaphase. Strains AF501 (WT), AF526 (cin8Δ), AF504 (kip1Δ), and AF517 (kip3Δ) were synchronized in G1 by treatment with 10 μg/ml α-factor. Cells were released from the block and were assayed for sister chromatid separation at 15 minute intervals as previously described (Straight et al., 1996). The percentage of cells with separated sister chromatids versus time is shown.
Regulation of Spindle Breakdown

In most organisms, the position of the metaphase spindle dictates the plane of cell division (Rappaport, 1996), ensuring that each daughter cell receives one set of chromosomes. In budding yeast, cell division occurs at the neck that separates mother and bud and this site is defined before spindle formation. As a result, cells must ensure that the anaphase spindle passes through the neck so that the mother receives one set of chromosomes and the bud the other. Studies of dynein mutants reveal a mechanism to achieve this: the anaphase spindle does not break down until one of its poles and the associated set of chromosomes has entered the bud (Yeh et al., 1995).

Our analysis of kinesin mutants suggests that the regulation of spindle breakdown is complex. Compared with wild-type cells, the spindles of cin8Δ break down at the same time after the onset of anaphase and at shorter final lengths, those of kip1Δ break down later but at the same final length and those of kip3Δ cells break down later and at a greater final length.

One explanation of these differences is that compression of the spindle as it contacts the ends of the cell at the end of anaphase promotes spindle disassembly by promoting the action of microtubule-stabilizing factors. This idea is supported by the observation that microtubules assembled from pure tubulin can be extensively bent in vitro without causing them to break. In contrast, bent microtubules of lower radius of curvature are observed to break more frequently than straight microtubules in the cytoplasm is thought to break in the cytoplasm. In this hypothesis, changes in the cell cycle timing of spindle elongation, absolute spindle length, spindle microtubule dynamics, and motor protein activity. Determining how these properties are built into the chemistry of motor proteins and how they are regulated by the cell should be informative in understanding the regulation of anaphase.

Genetic Redundancy and Functional Overlap

The distinct phenotypes of cin8Δ and kip1Δ cells have implications for the concepts of genetic redundancy and functional overlap between related genes. Groups of two or more genes are said to be redundant if deletion of a single member of the family produces little or no phenotype, but deletion of all members of the group is lethal. KIP1 and CIN8 are partially redundant since kip1Δ mutants have very mild phenotypes and mild overexpression of Kip1p suppresses the phenotype of cin8Δ (Hoyt et al., 1992; Roel et al., 1992). One interpretation of these observations is that the two motors perform essentially identical functions and differ only in their level of expression. If this were so, the phenotypes of kip1Δ and cin8Δ might differ quantitatively but not qualitatively. Our observation that two mutants have distinct effects on different aspects of anaphase argues that the motors have distinct functions, but that these functions have overlapping roles in producing spindle elongation. We believe that careful study of genes that appear to be redundant will reveal that in most cases their functions are qualitatively as well as quantitatively different.

The phenotypes of motor mutants highlight the distinction between minimal and full systems for performing complex cellular tasks like chromosome segregation. A minimal system suffices to perform a task at a level that allows cells to reproduce indefinitely under optimal conditions. Cells with two functional motors can function at this level (Hoyt, A., personal communication). A full system performs a task with high fidelity in the face of environmental, physiological, and genetic perturbations. For mitosis one criterion of the full system is a very low rate of errors in chromosome segregation. Lack of Cin8p disrupts the full system, since cin8Δ mutants were first found because they lead to errors in chromosome segregation (Hoyt et al., 1990). A more general criterion for including a function in the full system is that it makes cells more susceptible to genetic perturbations. Mutations in any one of the mitotic motors (Cin8p, Kip1p, Kip3p, Kar3p, and dynein) make at least one of the remaining mitotic motors essential for cell proliferation (Hoyt et al., 1992; Roel et al., 1992; Cottingham and Hoyt, 1997; DeZwaan et al., 1997). Different organisms may use different additional functions.
to convert a minimal system into one full. Thus fission yeast appear to possess a single motor, Cut7, of the BimC class, whereas budding yeast possess two, Cin8 and Kip1. This difference may reflect the historical accidents of evolution, or the more critical role of spindle elongation in budding yeast, where one of the daughter nuclei must be transported through the bud neck to produce two viable and genetically identical progeny. Detailed analysis of the requirements for mitosis in budding yeast and other organisms should illuminate how minimal systems for chromosome segregation arose and were then evolved into the highly efficient and regulated machines found today.

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References


