

Saccharomyces cerevisiae Kinesin- and Dynein-related Proteins Required for Anaphase Chromosome Segregation

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Abstract. The *Saccharomyces cerevisiae* kinesin-related gene products Cin8p and Kiplp function to assemble the bipolar mitotic spindle. The cytoplasmic dynein heavy chain homologue Dynlp (also known as Dhclp) participates in proper cellular positioning of the spindle. In this study, the roles of these motor proteins in anaphase chromosome segregation were examined. While no single motor was essential, loss of function of all three completely halted anaphase chromatin separation. As combined motor activity was di-

minished by mutation, both the velocity and extent of chromatin movement were reduced, suggesting a direct role for all three motors in generating a chromosome-separating force. Redundancy for function between different types of microtubule-based motor proteins was also indicated by the observation that *cin8 dynl* double-deletion mutants are inviable. Our findings indicate that the bulk of anaphase chromosome segregation in *S. cerevisiae* is accomplished by the combined actions of these three motors.

MITOTIC division in eukaryotes requires the controlled segregation of chromosomes by the microtubule-based spindle. Assembly of the bipolar spindle, an early mitotic event, occurs by separation of the duplicated spindle poles and the formation of an antiparallel microtubule lattice between them. In diverse eukaryotic cell types, spindle assembly and pole separation require the actions of members of a conserved subfamily of kinesin-related motor proteins (Enos and Morris, 1990; Hagan and Yanagida, 1990; Heck et al., 1993; Hoyt et al., 1992; Roof et al., 1992; Sawin et al., 1992). A number of these spindle motors have been localized to the microtubules that lie between the poles, suggesting that they separate poles by producing sliding of the anti-parallel midzone microtubules (Hagan and Yanagida, 1992; Hoyt et al., 1992; Roof et al., 1992; Sawin et al., 1992). In the budding yeast *Saccharomyces cerevisiae*, two members of this family, Cin8p and Kiplp, act redundantly in mitosis. Although neither is essential for viability, the function of at least one is required during spindle assembly to separate the spindle poles (Hoyt et al., 1992; Roof et al., 1992) and, until the onset of anaphase, to prevent the inward collapse of separated poles (Saunders and Hoyt, 1992).

Before anaphase, the *S. cerevisiae* spindle and associated nucleus is properly positioned in the neck between the

mother and bud cell bodies such that the subsequent mitotic division equally segregates the nuclear contents to the two progeny cells. This motility event, often referred to as nuclear migration, is accomplished in part by the cytoplasmic dynein heavy chain product of the *DYN1* gene (also known as *DHCl*). Although not essential for this process (or viability), *dyn1* mutant cells exhibit frequent nuclear migration failures, a rare occurrence in wild-type cells (Eshel et al., 1993; Li et al., 1993). It has been demonstrated that nuclear migration and proper spindle positioning are accomplished by the microtubules that extend out from the spindle poles into the cytoplasm (cytoplasmic or astral microtubules; Palmer et al., 1992; Sullivan and Huffaker, 1992). The *DYN1* gene product (Dynlp) has not been localized, but its role in nuclear migration suggests it may pull on the poles through an interaction with the cytoplasmic microtubules (Eshel et al., 1993; Li et al., 1993).

In anaphase, the assembled and positioned spindle physically segregates replicated chromosomes by two mechanisms: sister kinetochore movement toward opposite poles (anaphase A) and spindle elongation, resulting in further separation of the poles and the attached chromosomes (anaphase B). During anaphase B in *S. cerevisiae*, poles separate from six to eight times the preanaphase distance (1–1.5 μm before anaphase to 8–10 μm after; Byers and Goetsch, 1974; Peterson and Ris, 1976). Since the chromatin is always closely associated with one of the separating poles, it is clear that anaphase B is the major mechanism by which chromosomes are physically segregated in this cell type. A contribution from anaphase A movement is possible, but it must be

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small. (In *S. cerevisiae*, movement of individual chromosomes relative to the pole is difficult to observe because of incomplete chromatin condensation [Peterson and Ris, 1976].)

We initiated the following study because the identities of the motors that accomplish anaphase chromosome segregation were not yet known. A role for kinesin-related motors was suggested by the finding that antibodies to the kinesin motor domain blocked elongation of diatom spindles in vitro, although the lack of astral microtubules in these preparations obscures their possible contribution to elongation (Hogan et al., 1993). The previously described roles for *S. cerevisiae* Cin8p, Kiplp, and Dynlp made these gene products good candidates for the anaphase chromosome segregating motors. In the study reported here, we found that these motors function in an overlapping manner during anaphase, and that spindle elongation and chromosome segregation could be completely arrested only when the activities of all three were eliminated.

Materials and Methods

Yeast Strains and Media

The yeast strains used in these experiments are derivatives of S288C, and they are listed in Table I. All are closely related, having been derived by extensive backcrossing or by transformation. The temperature-sensitive *cin8* allele (*cin8-3*), the *cin8* deletion allele (*cin8::LEU2*), and the *kipl* deletion allele (*kipl::HIS3*) were described in Hoyt et al. (1992). For *cin8::HIS3*, the same region of *CIN8* was deleted as was for *cin8::LEU2*, but a DNA fragment carrying *HIS3* was inserted instead. The *dyn1* deletion allele (*dyn1::URA3* "R2" allele) was described in Eshel et al. (1993). *rho*⁻ strains were created from *rho*⁺ strains by overnight growth in 10 μg/ml ethidium bromide. All physiological characterizations were performed using rich growth media (YPD; Sherman et al., 1983).

"Fixed-cell" Anaphase Assay

Cells were treated with 0.1 M hydroxyurea (Sigma Immunochemicals, St. Louis, MO) at 26°C for 4–5 h in YPD media and released into fresh media at 26°C. After 60 min, the cultures were split and placed at 33°C and 26°C. Aliquots were fixed briefly with 70% ethanol, washed and stained with 4',6'-diamidino-2-phenylindole (DAPI)¹ (see Hoyt et al., 1992). Antitubulin immunofluorescence microscopy was performed as described in Hoyt et al. (1992).

"Real-time" Anaphase Assay

rho⁻ cells were treated with 0.1 M hydroxyurea and released into fresh media plus 0.5 μg/ml DAPI for 45 min at 26°C. The cells were then centrifuged, resuspended in a small volume of the same supernatant, and placed on ice. 2.5-μl aliquots were placed between a glass slide and coverslip, the edges were sealed, incubated at 23–26°C, and examined every 2.5 min using low level ultraviolet illumination and computer-enhanced video microscopy (Palmer et al., 1989). When anaphase initiated (as evidenced by the beginning of chromatin separation), the temperature was changed by pumping 50% ethanol at 33°C through a jacketed stage and a coiled tube surrounding the objective. The temperature change took ~2 min, and the actual temperature under the microscope objective varied between 32 and 34°C, depending on the room temperature.

Demonstration of *cin8-Δ dyn1-Δ* Lethality

To test whether the *cin8-Δ dyn1-Δ* genotype is mitotically viable, the following strains were constructed: MAY3184 = *cin8::LEU2 dyn1::URA3 lys2-80l* carrying a plasmid with *CIN8* and *LYS2* and MAY3190 = *cin8::HIS3 dyn1::URA3 cyh2^R* carrying a plasmid with *CIN8* and *CYH2*. During mi-

Table I. Yeast Strains

Strain	Genotype
MAY591	MAT α <i>lys2-80l his3-Δ200 leu2-3, 112 ura3-52</i>
MAY2169	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 cin8-3 kipl::HIS3</i>
MAY2249	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 cin8-3 kipl::HIS3 rho⁻</i>
MAY2250	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 rho⁻</i>
MAY2252	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 cin8-3 rho⁻</i>
MAY3170	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 cin8-3 kipl::HIS3 dyn1::URA3</i>
MAY3179	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 cin8-3 dyn1::URA3</i>
LMAY3184	MAT α <i>lys2-80l his3-Δ200 leu2-3, 112 ura3-52 cin8::LEU2 dyn1::URA3</i> (pMA1260 = <i>CIN8 LYS2 CEN</i>)
MAY3190	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 trp1-Δ1 cyh2R cin8::HIS3 dyn1::URA3</i> (pMA1208 = <i>CIN8 CYH2 LEU2 CEN</i>)
MAY3192	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 cin8-3 kipl::HIS3 dyn1::URA3 rho⁻</i>
MAY3193	MAT α <i>lys2-80l his3-Δ200 leu2-3, 112 ura3-52 cin8-3 kipl::HIS3 rho⁻</i>
MAY3303	MAT α <i>ade2-101 his3-Δ200 leu2-3, 112 ura3-52 kipl::HIS3 dyn1::URA3 rho⁻</i>

otic growth, MAY3184 did not give rise to cells able to grow on α -amino adipate, indicating that the plasmid carrying the dominant *LYS2* allele could not be lost (Sikorski and Boeke, 1991). Similarly, MAY3190 did not give rise to cells able to grow on cycloheximide, indicating that the plasmid carrying the dominant *CYH2* allele could not be lost. These findings demonstrate that survival of the *cin8-Δ dyn1-Δ* genotype was not possible without either of the *CIN8*-containing plasmids.

Results

Anaphase Proficiency Assays

Various genotypic combinations consisting of a temperature-sensitive allele of *CIN8* (*cin8-3*) and deletion alleles of *KIPI* (*kipl-Δ*) and *DYNI* (*dyn1-Δ*) were created (Hoyt et al., 1992; Eshel et al., 1993). Two assays were then used to examine the anaphase proficiency of the different genotypes. In the first assay, cell cultures were synchronized with hydroxyurea, a DNA synthesis inhibitor that blocks the *S. cerevisiae* cell cycle at a stage that follows spindle assembly but that precedes anaphase (Pringle and Hartwell, 1981). The cells were then released from the block and incubated at a permissive temperature (26°C) until the onset of anaphase. At this point, half of each culture was shifted to 33°C to inactivate Cin8-3p and the other half was maintained at 26°C. Aliquots were taken at various time points, fixed, stained for DNA, and examined for chromatin separation (Fig. 1). This assay examines the separation of one chromatin mass into two, a relatively early anaphase event.

The inactivation of Cin8p function or the absence of Kiplp and Dynlp, individually or together, did not inhibit the separation of chromatin into two masses (results not shown). *cin8-3 kipl-Δ* and *cin8-3 dyn1-Δ* cells are inviable at 33°C (Hoyt et al., 1992, and see below), yet under these experimental conditions, they were also able to achieve

1. Abbreviation used in this paper: DAPI, 4',6'-diamidino-2-phenylindole.

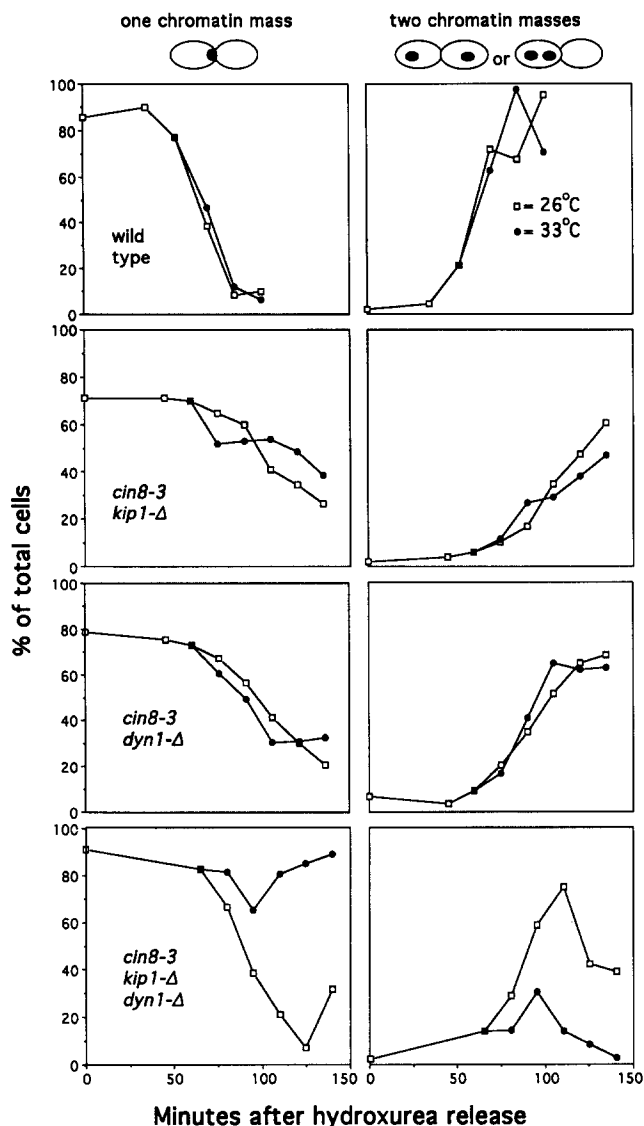


Figure 1. Chromosome segregation in wild-type and *cin8 kipl dyn1* mutant cells. The ability of cells to accomplish mitotic chromatin separation was examined by determining the percentage of large-budded cells with one chromatin mass (preanaphase) or two chromatin masses (anaphase or postanaphase) after release from a hydroxyurea-induced S-phase arrest. After 60 min of release at 26°C, the cultures were split and placed at 26°C (open squares) and 33°C to inactivate Cin8p (closed circles). In the absence of Dynlp, some cells separated their chromatin entirely within the mother cell (Eshel et al., 1993; Li et al., 1993). A reduction in the number of large budded cells with two chromatin masses (rightmost column) is a result of cytokinesis and eventually occurs in all cultures. The experiments were performed for each genotype three or more times with similar results. Yeast strains MAY591 (wild type), MAY2169 (*cin8-3 kipl-Δ*), MAY3179 (*cin8-3 dyn1-Δ*), and MAY3170 (*cin8-3 kipl-Δ dyn1-Δ*) are shown.

anaphase chromatin separation (Fig. 1). In contrast, loss of all three gene functions rendered cells incapable of chromatin separation. These results indicate that the activity of at least one of these three motors is required to separate chromatin, and they suggest that these motors overlap for anaphase function.

When preanaphase *cin8-3 kipl-Δ* cells are temperature shifted, bipolar spindles rapidly collapse with separated poles coming together. When these cells enter anaphase, however, the spindles become resistant to collapse (Saunders and Hoyt, 1992). To rule out the possibility that a major spindle structural disruption, such as collapse, was responsible for the inability of the *cin8-3 kipl-Δ dyn1-Δ* triple mutant to complete anaphase at 33°C, spindles were observed by immunofluorescence microscopy. Characteristic of spindles that have entered anaphase, the triple mutant spindles remained bipolar, but were unable to elongate as did the similarly treated wild-type spindles (Fig. 2). Inhibition of elongation was not caused by structural collapse; however, a more subtle change in spindle structure, not detected in these experiments, cannot be ruled out. In a separate experiment, the rates of bud formation and bud enlargement were the same in *cin8-3 kipl-Δ dyn1-Δ* and wild-type cells at a nonpermissive temperature, indicating that the mutant cells do not have a nonspecific block for growth (results not shown).

A second anaphase assay allowed us to determine if these gene products are specifically required for chromosome segregation rather than an essential event required immediately before segregation. Live cells were stained with DAPI (a fluorescent label for DNA), and they were observed individually in real time with computer-enhanced video microscopy (Palmer et al., 1989). When the cells entered anaphase, as determined by the onset of chromatin separation, the microscope stage was shifted from room temperature (23–26°C) to 33°C. The temperature change took ~2 min. Relative to the above fixed-cell assay, this real-time assay examined chromatin movement in a later interval of anaphase, including the period after the chromatin had separated into two distinct masses.

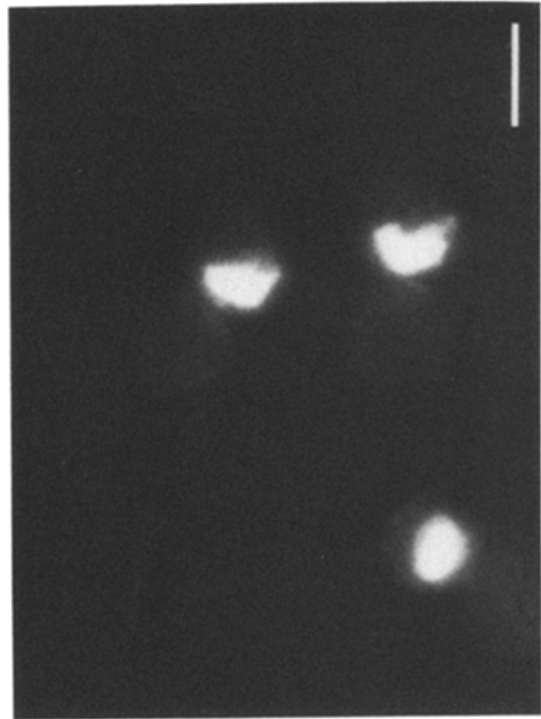
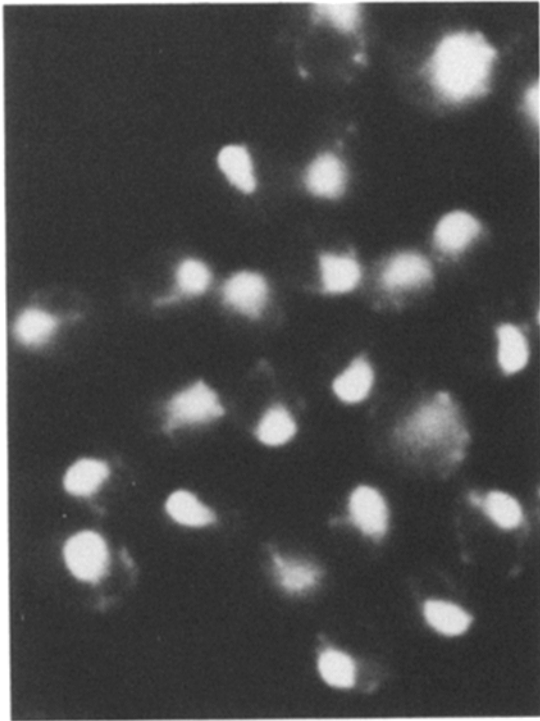
Wild-type cells switched to 33°C (and mutant cells left at room temperature) continued to separate their chromatin at a normal rate (Figs. 3 and 4). In contrast, chromatin movement, especially at the leading edge, stopped prematurely in all *cin8-3 kipl-Δ dyn1-Δ* mutant cells tested. In most cases (10 out of 16), this occurred within 3–5 min of the start of the temperature shift. Most of the residual movements in the triple mutant cells occurred during the 2 min in which the stage temperature was changing (Fig. 4 E). It is possible, however, that some of the residual movements observed represent a contribution from other unidentified motors.

These real-time experiments, like the fixed-cell assay of Fig. 1, suggest that the function of Cin8p, Kiplp, or Dynlp is essential for chromatin movement and indicate that their activity is required to sustain as well as initiate movement. In addition, the real-time assay demonstrated a general stepwise reduction in separation proficiency with compounded loss of gene product activity (Fig. 4). The relative contribution to chromatin movement appears to be Cin8p, then Kiplp, and then Dynlp. As their combined activity was diminished, both the velocity and extent of chromatin movement was reduced, with the triple mutant being most severely affected. This suggests a direct role for these gene products in generating motile force.

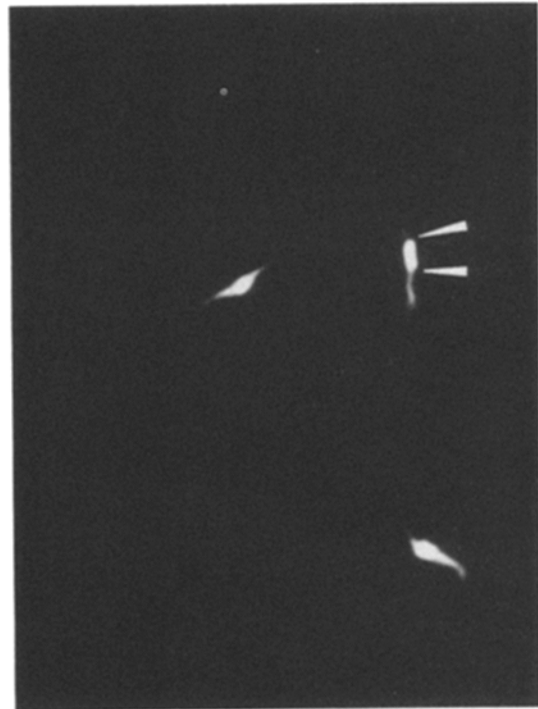
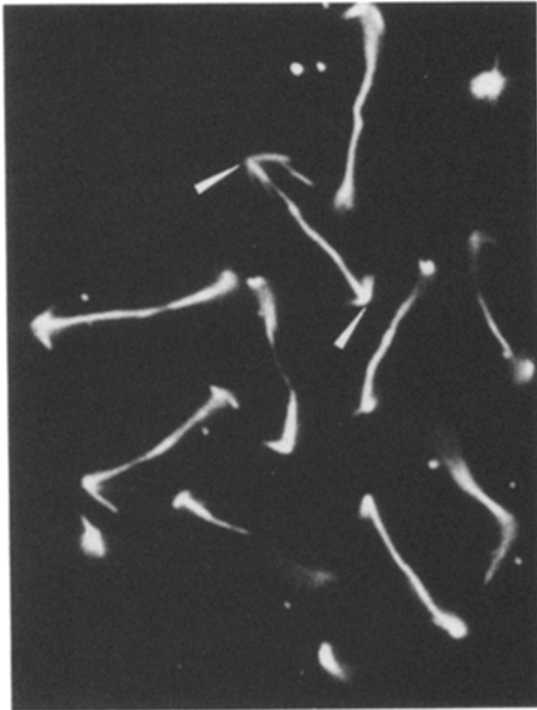
Either CIN8 or DYN1 Is Required for Viability

Further support for an overlap in function between the *CIN8*, *KIPI*, and *DYN1* gene products comes from the observation

DAPI



anti-tubulin



wild
type

cin8-3
kip1-Δ
dyn1-Δ

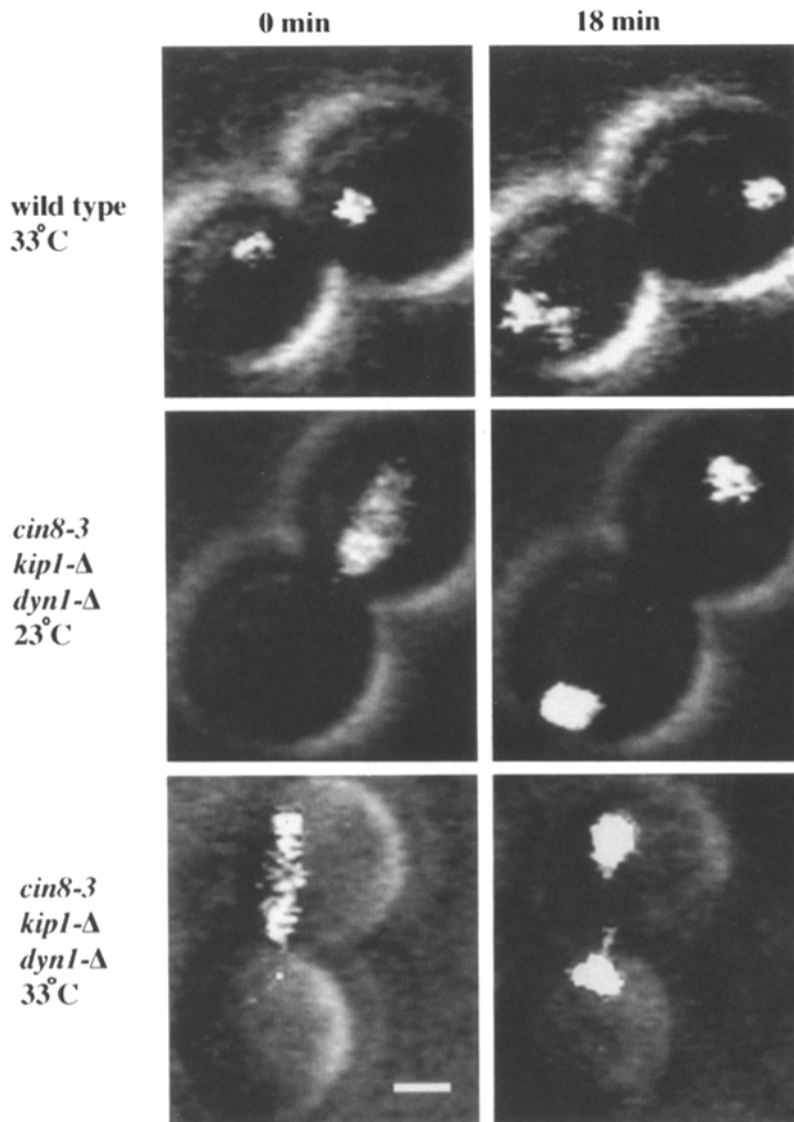


Figure 3. Chromatin movement stops with loss of Cin8p, Kiplp, and Dynlp function. Cells of various genotypes were arrested with hydroxyurea and released. Chromosome segregation was examined in real time using DAPI staining and computer-enhanced video microscopy (Palmer et al., 1989). In the two rows labeled 33°C, a temperature shift to 33°C was initiated at 0 min (the shift took ~2 min). The row labeled 23°C was not shifted to the higher temperature. The leading edges of chromatin in the triple mutants usually stopped quickly after a temperature shift to 33°C, although the lagging chromatin between the edges continued to advance until it caught up with the leading edges to form two tight masses. The triple mutant cell was observed for another 42 min at 33°C, revealing no further separation. *rho*⁻ yeast strains MAY2250 (wild type) and MAY3192 (*cin8-3 kipl-Δ dyn1-Δ*) are shown. Bar, 1.5 μm.

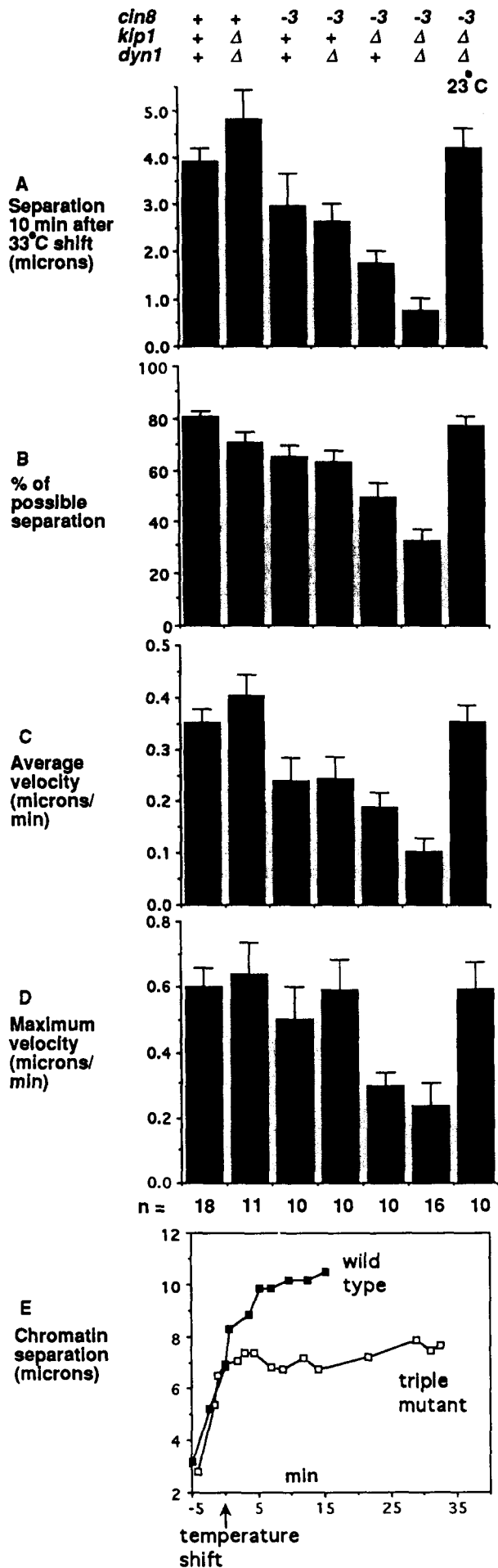
that two of the three possible double-deletion mutant genotypes are inviable. The inviability of the *cin8-Δ kipl-Δ* genotype was reported previously (Hoyt et al., 1992; Roof et al., 1992). We report here that *cin8-Δ dyn1-Δ* double mutants are also inviable; *cin8-Δ dyn1-Δ* mutants carrying *CIN8* on a plasmid were unable to survive plasmid loss at 26°C (see Materials and Methods). Neither of the single mutants were significantly affected for growth rate at this temperature. In addition, similar to deletion of *KIP1* (Hoyt et al., 1992), deletion of *DYN1* greatly enhanced the temperature sensitivity of *cin8-3* cells. *cin8-3* single mutants were only inviable when incubated at 37°C. *cin8-3 dyn1-Δ* and *cin8-3 kipl-Δ* double mutants were inviable at 33°C and above. Such “syn-

thetic” interactions were not observed in double mutants constructed with *dyn1-Δ* and deletions of two other genes encoding kinesin-related motors, *KAR3* (Meluh and Rose, 1990) and *KIP2* (Roof et al., 1992).

Discussion

Two assays were used to examine the anaphase proficiency of cells deficient for various combinations of motor proteins. In the fixed-cell assay, cells were examined for their ability to separate chromatin into two distinct masses, a relatively early anaphase event. In the real-time assay, chromatin movement in individual cells was examined in the anaphase

Figure 2. Spindle structure in wild-type and mutant cells. Cells were treated with hydroxyurea at 26°C for 4 h, released into fresh media at 26°C for 1 h, transferred to 33°C for 1 h, and then fixed. Spheroplasted cells were stained with DAPI and antibodies directed against tubulin. Wild-type cells were able to form long spindles under these conditions with well-separated chromatin, indicating that anaphase had proceeded normally (average spindle length = 7.2 μm [*n* = 26]). In contrast, most (~65%) *cin8-3 kipl-Δ dyn1-Δ* mutant cells arrested with short but intact bipolar spindles (average spindle length = 1.7 μm [*n* = 130]). The arrows point to the two poles of bipolar spindles. Spindles in *cin8-3 kipl-Δ dyn1-Δ* mutant cells collapsed when shifted to 33°C immediately after hydroxyurea synchronization (not shown). Yeast strains MAY591 (wild type) and MAY3170 (*cin8-3 kipl-Δ dyn1-Δ*) are shown. Bar, 5 μm.



period extending from the beginning of separation until movement stopped. In both assays, cells deficient for the function of all three motors, Cin8, Kipl and Dyn1, were most severely compromised for their ability to segregate chromosomes. In the fixed-cell assay, only the triple mutant was unable to separate chromatin. Although it is possible that other motors may also contribute to this process, these findings indicate that the bulk of anaphase chromosome segregation in *S. cerevisiae* is accomplished by the combined actions of Cin8p, Kiplp and Dynlp. This is the first demonstration of a set of defined motor proteins required to sustain chromosome segregation.

It is likely that the effects on chromatin movement we observed reflect the ability of the spindle to elongate (anaphase B). In *S. cerevisiae*, the extent of chromatin separation compared to the relatively short kinetochore-to-pole distance indicates that most chromosome segregation is due to spindle elongation and not kinetochore movement to the poles (anaphase A). In addition, our real-time observations demonstrated effects of motor loss during the period in which the chromatin had already separated further than the length of a preanaphase spindle. Recent electron microscopic studies of serially-sectioned *S. cerevisiae* spindles (Winey, M., C. L. Mamay, E. T. O'Toole, D. N. Mastronarde, T. H. Giddings, Jr., K. L. McDonald, and J. R. McIntosh, manuscript submitted for publication) and analysis of the nuclear positions of centromeric DNA sequences (Guacci, V., E. Hogan, and D. Koshland, unpublished observations) indicate that anaphase A movement must be virtually complete at this stage. Therefore, the simplest hypothesis to explain our findings would have all three of these motors directly producing the force that elongates the spindle. However, our studies cannot rule out less direct effects on spindle elongation caused by loss of motor function.

Our findings indicate that kinesin-related and dynein-related motors can cooperatively accomplish a single motil-

Figure 4. Quantitative analysis of chromatin movement. Video images of cells treated as in Fig. 3 were examined and the amount of chromatin separation determined by measuring the position of the leading edges of the chromatin masses before and ~ every 2.5 min after the temperature shift was initiated. Note that it took ~2 min for the stage to reach 33°C after the shift was initiated. (A) The amount of chromatin separation in the first 10 min after the initiation of the temperature shift, (B) the percentage of the remaining distance to the edge of the cell (as determined by the corresponding phase microscopy image) that the leading edge of the chromatin traveled after initiation of the temperature shift, (C) the average relative velocity of chromatin separation from the temperature shift to maximum separation, and (D) the maximum velocity of chromatin separation for any three consecutive time points are shown. Only cells that had unambiguously begun chromosome segregation before the temperature shift and in which the chromosomes had segregated to both sides of the neck by the end of the experiment were counted. Segregation to both cell bodies ensured that chromatin movement was not blocked by inaccurate positioning of the spindle in the *dyn1* mutants. The rightmost set of columns refer to *cin8-3 kip1-Δ dyn1-Δ* cells left at 23°C. n = sample size; bracket, standard error of the mean of the data. *rho*⁻ strains MAY250 (wild type), MAY3303 (*kip1-Δ dyn1-Δ*), MAY2252 (*cin8-3*), MAY3193 (*cin8-3 dyn1-Δ*), MAY2249 (*cin8-3 kip1-Δ*), and MAY3192 (*cin8-3 kip1-Δ dyn1-Δ*) are shown. (E) Chromatin leading edge separation as a function of time for a typical wild-type cell (closed symbols) and a typical *cin8-3 kip1-Δ dyn1-Δ* triple mutant cell (open symbols).

ity event. Overlap in function between Cin8p and Dynlp was indicated by the additive effects of mutations on anaphase proficiency and also the inviability of double deletion mutants. Although cells compromised for both Cin8p and Dynlp functions were able to perform anaphase chromatin separation under our experimental conditions, they did so with reduced proficiency (Fig. 4). Perhaps inefficient chromatin separation contributes to the inviability of the double deletion mutants. Alternatively, Cin8p or Dynlp may be required for some other essential process that Kiplp is unable to accomplish by itself. *cin8-3 dynl-Δ* mutants shifted to elevated temperatures arrested growth with heterogeneous cell morphologies making it difficult to determine the primary block to cell cycle progression (Saunders, W. S., and M. A. Hoyt, unpublished observations).

We obtained the first functional evidence for a role for cytoplasmic dynein in anaphase chromosome segregation. Previous studies have suggested mitotic roles for cytoplasmic dynein-related motors. Antibody inhibition experiments performed with mammalian cells indicated that cytoplasmic dynein may be required to assemble the spindle (Vaisberg et al., 1993). This finding is consistent with a pole-separating role for cytoplasmic dynein. In mammalian cells, some of the cytoplasmic dynein is located at the kinetochores during mitosis, suggesting an additional role in chromosome-to-pole movement (Pfarr et al., 1990; Steuer et al., 1990; Wordeman et al., 1991). Our findings suggest a role for cytoplasmic dynein in spindle elongation in *S. cerevisiae*, but an additional role in chromosome-to-pole movement is also possible.

Cin8p and Kiplp colocalize with the microtubules between the spindle poles (Hoyt et al., 1992; Roof et al., 1992) and probably function to push the poles apart (Saunders and Hoyt, 1992). The mechanism by which Dynlp functions is unknown, but its role in spindle positioning suggests it may pull on the poles through an interaction with cytoplasmic microtubules (Eshel et al., 1993; Li et al., 1993; Palmer et al., 1992; Sullivan and Huffaker, 1992). Evidence from a variety of sources suggests that forces acting on microtubules between the spindle poles (Cande and McDonald, 1985; Masuda et al., 1990; Sullivan and Huffaker, 1992) or outside the poles (Aist et al., 1991; Mastronarde et al., 1993; Waters et al., 1993), or both (Aist and Bayles, 1991), contribute to spindle elongation (reviewed in Ault and Rieder, 1994; Saunders, 1993). We propose that anaphase spindle elongation involves a combination of outward pulling on the spindle poles by cytoplasmic dynein and pushing on the poles from within by the same kinesin-related proteins responsible for spindle assembly. Furthermore, as Cin8p and Kiplp (and probably Dynlp) are active prior to anaphase, spindle elongation may not require additional anaphase-specific motors, but rather be a consequence of a secondary event (such as sister kinetochore disjunction) allowing already active motors to elongate the spindle.

Note. After the experiments reported here were completed, we discovered that the *cin8-3 kipl-Δ dynl-Δ* genotype (i.e., MAY3170) caused instability of karyotype, even at the permissive growth temperature. When MAY3170 was crossed to a wild-type strain, 61% of the spore products of meiosis were inviable, and a number of the viable spores were non-maters. These are characteristics of aneuploid strains. Three

other independently derived strains with the same genotype also displayed these characteristics, but the less severely compromised motor mutant strains (i.e., double-motor mutants) did not. While aneuploidy is an expected phenotype for mitotic motor mutants, this finding raised the possibility that the chromatin separation phenotype observed for the triple mutants may be in some way affected by the aneuploid state. To explore this possibility, MAY3170 was transformed with plasmids carrying *CIN8* or *KIPL*. As expected, the *CIN8* plasmid relieved the temperature sensitivity of this strain, while the *KIPL* plasmid only had a marginal effect (*KIPL* cannot rescue the colony growth defect caused by loss of *CIN8* and *DYNI*). Fixed-cell anaphase chromatin separation assays were performed on MAY3170 and the two transformants. Unlike untransformed MAY3170, both transformants were able to accomplish the separation of chromatin when shifted to 33°C at the onset of anaphase (data not shown). Therefore, the reduced proficiency of chromatin separation exhibited by *cin8-3 kipl-Δ dynl-Δ* strains at 33°C reflected loss of function of the three motors, and it was not caused by their aneuploid state.

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