

MPS1 and *MPS2*: Novel Yeast Genes Defining Distinct Steps of Spindle Pole Body Duplication

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Abstract. It is crucial to the eucaryotic cell cycle that the centrosome undergo precise duplication to generate the two poles of the mitotic spindle. In the budding yeast *Saccharomyces cerevisiae*, centrosomal functions are provided by the spindle pole body (SPB), which is duplicated at the time of bud emergence in G1 of the cell cycle. Genetic control of this process has previously been revealed by the characterization of mutants in *CDC31* and *KARI*, which prevent SPB duplication and lead to formation of a monopolar spindle. Newly isolated mutations described here (*mps1* and *mps2*, for monopolar spindle) similarly

cause monopolar mitosis but their underlying effects on SPB duplication are unique. The *MPS1* gene is found by electron microscopy to be essential for proper formation of the site at which the new SPB normally arises adjacent to the existing one. By contrast, a mutation in *MPS2* permits duplication to proceed, but the newly formed SPB is structurally defective and unable to serve as a functional spindle pole. Distinct temporal requirements for the *CDC31*, *MPS1*, and *MPS2* gene functions during the SPB duplication cycle further demonstrate the individual roles of these genes in the morphogenetic pathway.

FAITHFUL duplication of the centrosome is crucial to the eucaryotic cell cycle. This organelle, which serves as the focus of microtubule organization in an interphase cell, must be precisely duplicated to create either pole of the mitotic spindle as the cell enters mitosis (for review, see Brinkley, 1985; McIntosh, 1983). Duplication of the centrosome in many eucaryotes is coupled to duplication of the centrioles situated within it (see review by Sluder, 1989). The centrioles themselves have been shown by studies of labeled tubulin incorporation to undergo duplication in a conservative manner, such that each parental centriole remains intact while a new centriole is assembled from new material at an adjacent site (Kochanski and Borisy, 1990). The centrioles are then segregated in a semiconservative pattern, each centrosome retaining a former centriole while also gaining a new one (Kochanski and Borisy, 1990). It remains unknown whether other centrosomal components, which are poorly delineated by any available cytological methods, undergo similar patterns of duplication and segregation. Nor do we understand much about the functions that control centrosomal duplication. Inhibition of protein synthesis either in fertilized sea urchin eggs (Sluder et al., 1990) or in *Xenopus* blastulae (Gard et al., 1990) blocks the cell cycle, but centrosomal duplication continues to occur. In these experiments, the demonstrable depletion of maturation-promoting factor upon inhibition of protein synthesis is thought to ex-

plain the failure of cell cycle progression (Gard et al., 1990; Sluder et al., 1990). If this is the case, then the agent that promotes centrosome duplication must differ and therefore remains to be identified.

A similar uncoupling of cell cycle control from spindle pole formation has been achieved by mutation in yeast cells (Baum et al., 1988; Uzawa et al., 1990), where centrosomal function is provided by the discrete spindle pole body (SPB),¹ which is situated within the nuclear envelope. Electron microscopic analysis of the SPB in *Saccharomyces cerevisiae* has shown its duplication and separation to be precisely controlled in wild-type cells (Byers and Goetsch, 1974; for review, see Byers, 1981a), as well as in the *cdc* (cell division cycle) mutants (Byers and Goetsch, 1974). When most *cdc* mutants are arrested by transfer to the nonpermissive temperature, the SPB cycle uniformly becomes arrested at a stage appropriate to other indices of cell cycle progression, including DNA synthesis and bud formation (Byers and Goetsch, 1975). A defect specific to SPB duplication has, however, been recognized in the formation of monopolar spindles by *cdc31* strains subjected to the nonpermissive temperature (Byers, 1981b). Here, bud formation and DNA synthesis proceed in the absence of SPB duplication and the cells then undergo monopolar mitosis (Schild et al., 1981), all of the chromosomes segregating to the single functional pole, thereby causing a doubling of ploidy in the single surviving daughter cell. The *CDC31* gene has been found to encode an essential Ca²⁺-binding protein similar to calmodu-

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1. *Abbreviation used in this paper:* SPB, spindle pole body.

lin (Baum et al., 1986), but the manner in which calcium may regulate SPB duplication remains obscure.

Effects on SPB duplication similar to those shown for *cdc31* have also been reported for conditional loss-of-function and over-expression alleles of the *KARI* gene (Rose and Fink, 1987). The original mutation in *KARI* was recognized by its defect in karyogamy, or nuclear fusion (Conde and Fink, 1976), which is known from cytological analysis to be initiated by fusion of the SPBs (Byers and Goetsch, 1975). Coincidence of defects in karyogamy and SPB duplication, in conjunction with the finding that a hybrid product, when overexpressed, can be seen to be localized in the vicinity of the SPB (Vallen, L., and M. Rose, personal communication), strongly suggests a functional role for the *KARI* gene product within the SPB.

We report here the isolation of temperature-sensitive mutations in two new loci (*MPS1* and *MPS2*, monopolar spindles); mutants in both of these genes are specifically defective in duplication of the SPB at the nonpermissive temperature. Electron microscopy has revealed that the new mutants display forms of the SPB that are distinct from wild type, as well as from each other or from that observed for *cdc31* or *kar1*. The basis for these differences has been investigated by determining the stage at which each gene function is required during SPB duplication. These order-of-function studies demonstrate that strains mutant in *MPS1*, *MPS2*, and *CDC31* are defective in temporally distinct stages of the SPB duplication pathway.

Materials and Methods

Yeast Strains, Cell Culture, and Genetic Techniques

Yeast media and genetic techniques were as described by Hartwell (1967) and Sherman et al. (1971). The yeast strains used in this study are listed in Table I. The collection of temperature-sensitive yeast strains (Hartwell, 1967) used here was obtained from Michael Culbertson. The original isolates of *mps1-1* and *mps2-1* were successively outcrossed to strain S288C and its derivatives: R14, M10, and M11 (Table I). Diploid strains 3264N-5c and 4024N-16d, homozygous at the mating type locus, were constructed by selecting rare tetraploids between H243-13-2 and D326-1 or D402-1, and the diploid meiotic products from sporulation of these tetraploids were screened for strains homozygous for mating type and the *mps* loci. Diploid strains Wx209-8a and Wx217-10b, also homozygous for mating type, were isolated by transient exposure of an appropriate haploid strain to the nonpermissive temperature and screening for clones of diploid cells.

The *mps1-1* mutation maps to chromosome IV, 5 cM distal to *cdc7*. This location was determined by tetrad analysis of a cross of a *mps1-1*, *trp1* strain (Wx161-3a, Table I) to a *cdc7* strain (H91-3-4, Table I), and by examining segregation of flanking markers in recombinant tetrads. The data resulting from this cross are presented in Table II. The gene order is *trp1* - *CENIV* - *cdc7* - *mps1*.

The *mps2-1* mutation maps to chromosome VII, 21 cM proximal to *cyh2*. The *mps2-1* mutation complements *cri3*, which maps to the same region of chromosome VII (McCusker and Haber, 1988). A five-point mapping cross was carried out, in which the pertinent markers are *mps2*, *cyh2* (Wx193-7b, Table I) X *leu1*, *trp5*, *met13* (REE963, Table I). The data from this cross are presented in Table III. The gene order on this arm of chromosome VII is supported by examination of the segregation of flanking markers in recombinant tetrads. The gene order is *CENVII* - *leu1* - *trp5* - *mps2* - *cyh2* - *met13*.

Cell Synchronization and Release from Arrest

Cells were arrested in G1 with α -factor (7–10 μ M) obtained from Sigma, Star Biochemical (Torrance, CA), or as a custom synthesis using F-MOC chemistry on a peptide synthesizer (model 488; Applied Biosystems Inc., Foster City, CA). The efficiency of a given arrest was monitored by determining the budding index (the proportion of budded cells in a sample of 200

Table I. Strain List

| Strain | Genotype | Source |
|-----------|--|------------------|
| S288C | α <i>gal2</i> | R. K. Mortimer* |
| R14 | <i>a leu2-3</i> | R. F. Gaber† |
| M10 | <i>a leu2-3,112 trp1-7 ura3-52</i> | W. R. Boorstein§ |
| M11 | α <i>leu2-3,112 trp1-7 ura3-52</i> | W. R. Boorstein§ |
| H243-13-2 | α/α <i>trp1/trp1 ura1/ura1</i> | D. Hawthorne |
| Wx209-8a | <i>a/a cdc31-2/cdc31-2, ade2/ade2, trp1/trp1</i> | This study |
| D326-1 | <i>a/\alpha mps1-1/mps1-1 trp1/trp1 leu2-3,112/+ ura3-52/ura3-52</i> | This study |
| D402-1 | <i>a/\alpha mps2-1/mps2-1 trp1/trp1 leu2-3,112/+ ura3-52/ura3-52</i> | This study |
| 3264N-5c | <i>a/a mps1-1/mps1-1 trp1/trp1 Ura⁻</i> | This study |
| 4024N-16d | <i>a/a mps2-1/mps2-1 trp1/trp1 Ura⁺</i> | This study |
| Wx217-10b | <i>a/a mps1-1/mps1-1 trp1/trp1 mps2-1/mps2-1 cyh2/cyh2 ura3-52/ura3-52 His⁻</i> | This study |
| Wx161-3a | <i>a mps1-1 his7 trp1 ura3-52</i> | This study |
| H91-3-4 | α <i>cdc7 ade2 ura3 leu2 lys2</i> | L. H. Hartwell |
| Wx193-7b | <i>a mps2-1 cyh2 can1 ura3-52 leu2-3,112</i> | This study |
| REE963 | α <i>spo11::URA3 ura3 lys2 tyr1 leu1 trp5 met13 his6 lys1 ade2 aro7</i> | R. E. Esposito† |

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Table II. Tetrad Data for Mapping *mps1-1*

| Gene pair | PD | T | NPD | Total | cM |
|-------------------|----|---|-----|-------|----|
| <i>mps1, cdc7</i> | 18 | 2 | 0 | 20 | 5 |
| <i>mps1, trp1</i> | 16 | 4 | 0 | 20 | 10 |
| <i>cdc7, trp1</i> | 18 | 2 | 0 | 20 | 5 |

PD is parental ditype, T is tetratype, NPD is nonparental ditype, and the mapping function is $[(T \times 6 \text{ NPD}) / \text{total asci}] \times 50 = \text{cM}$.

Table III. Tetrad Data for Mapping *mps2-1*

| Gene pair | PD | T | NPD | Total | cM |
|--------------------|----|----|-----|-------|----|
| <i>mps2, cyh2</i> | 32 | 23 | 0 | 55 | 21 |
| <i>mps2, trp5</i> | 18 | 21 | 2 | 41 | 41 |
| <i>mps2, met13</i> | 16 | 31 | 0 | 47 | 33 |
| <i>mps2, leu1</i> | 20 | 33 | 3 | 56 | 46 |
| <i>cyh2, met13</i> | 32 | 15 | 0 | 47 | 16 |
| <i>cyh2, trp5</i> | 11 | 26 | 4 | 41 | 61 |
| <i>trp5, leu1</i> | 26 | 15 | 0 | 41 | 18 |

Definitions and the mapping function are given in Table III.

cells) of briefly sonicated aliquots. Arrests were deemed adequate when 95% of the cells in the culture were unbudded, as later confirmed by flow cytometry. Cells were released from arrest by two rinses in growth media equilibrated to the temperature being used for the release. Subsequent reentry into the cell cycle and cell cycle arrest were monitored by budding index and flow cytometry.

Cytological Techniques

All cytological experiments were carried out with diploid strains, which have larger SPBs and spindles that are more readily visualized by both

fluorescence and electron microscopy. Preparation of cultures for immunofluorescent staining entailed fixation with formaldehyde, spheroplasting, and incubation with antibodies as described by Kilmartin and Adams (1984) and modified by Jacobs et al. (1988), using the rat monoclonal antibody YOL1/34 (anti- α -tubulin) and FITC-conjugated goat anti-rat antibodies (Accurate Chemical & Scientific Corp., Westbury, NY). DNA was stained with DAPI (1.0 μ g/ml; Sigma Chemical Co., St. Louis, MO). Stained cells were viewed with a Nikon Microphot FX fluorescent microscope and photographed with Kodak Kodachrome 200 Professional film.

Yeast cells were prepared for flow cytometry by the method of Hutter and Eipel (1979) using the DNA stain, propidium iodide (Sigma Chemical Co.). Stained cells were analyzed on a Becton Dickinson FACSCAN flow cytometer using the CELLFIT and LYSYS software packages to obtain and analyze data.

Yeast cells were prepared for thin-sectioning by procedures described by Byers and Goetsch (1974, 1975). Serial sections were viewed on a Philips EM300 electron microscope.

Results

Isolation of the *mps* Mutations

Previous studies of *cdc31* (Byers, 1981b; Schild et al., 1981) and *kar1* (Rose and Fink, 1987) have demonstrated that these mutants share several common phenotypes, including formation of a monopolar spindle at the point of temperature-sensitive arrest. The accompanying disorganization of the mitotic spindle provides an easily recognized defect that could serve as the basis for identifying other mutants defective in SPB duplication. We therefore screened a collection of temperature-sensitive-for-growth yeast strains (Hartwell, 1967) for members exhibiting this cytological phenotype. Mutant cells which had been incubated at 36°C for 4 h were subjected to immunofluorescent staining of their microtubules and the stained cells were scored in a double-blind assay for the presence of aberrant microtubule arrays (Materials and Methods). During the budded phase of the cell cycle, wild-type strains contain mitotic spindles that appear as straight bundles of microtubules (Fig. 1A), whereas mutants of interest lacked such arrays when incubated at the nonpermissive temperature (Fig. 1, B and C). In all, twelve recessive temperature-sensitive mutations, representative of ten complementation groups, were found to be unable to

form normal mitotic spindles at 36°C; each segregated as a single nuclear locus in crosses to wild-type strains (data not shown).

Further analysis of the mutants included flow cytometric assays to characterize the overall pattern of chromosome replication and segregation, and electron microscopy to specify the state of the SPBs. By these criteria, mutations defining two of the ten complementation groups displayed defects specific to SPB duplication (described below). These SPB-defective mutations were outcrossed six times to wild-type strains to verify that the temperature-sensitive mutation cosegregated with the aberrant microtubule organization phenotype and to generate the strains used for genetic mapping, and for the following phenotypic characterization (see Table I, Materials and Methods). These two complementation groups, *mps1* and *mps2* (monopolar spindle), identify new loci on chromosomes IV and VII, respectively (Tables II and III, Materials and Methods).

The Single SPB in *mps1-1* Strains Has an Enlarged Half-Bridge

The *mps1-1* mutation maps to a new centromere-linked locus on chromosome IV, 5 cM distal to *cdc7* (see Table II, Materials and Methods). Fig. 1B shows the microtubule organization observed in large-budded cells of an *mps1-1* strain 4 h after transfer to 36°C. These cells display disorganized arrays of microtubules and a single region of DAPI-stained chromosomal DNA, suggesting that the cells have not entered mitosis. The apparent disruption of spindle organization is consistent with gross defects in chromosome segregation. Flow cytometry (Fig. 2A) reveals that *mps1-1* cells, when incubated at 36°C, accumulate substantially more DNA per cell than that in similarly treated control cells. The *mps1-1* mutation is lethal; cells transferred to 36°C for 4 h continue budding but yield <10% viable cells when restored to the permissive temperature (25°C). When survivors were plated and the resulting colonies tested for their ploidy by flow cytometry, 80% of the cell clones had DNA contents greater than the initial strain (data not shown). The generation of such polyploids by transient exposure to the nonper-

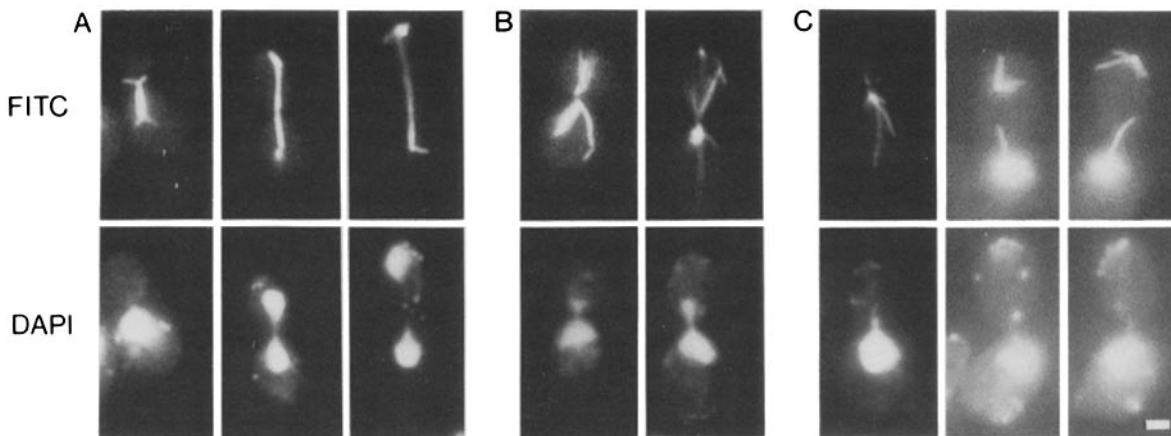


Figure 1. Immunofluorescent staining of wild-type and *mps* mutant yeast strains. Yeast cells were stained to display microtubules (FITC) and DNA (DAPI) as described in Materials and Methods. The yeast strains, (A) wild-type (a diploid obtained by mating M10 and M11, Table I), (B) *mps1-1* (D326-1), and (C) *mps2-1* (D402-1), were grown at 25°C and transferred to 36°C for 4 h. The wild-type strain (A) exhibits normal mitotic spindles, whereas these structures are absent in large-budded cells of *mps* strains incubated at the nonpermissive temperature. Bar, 1.0 μ m.

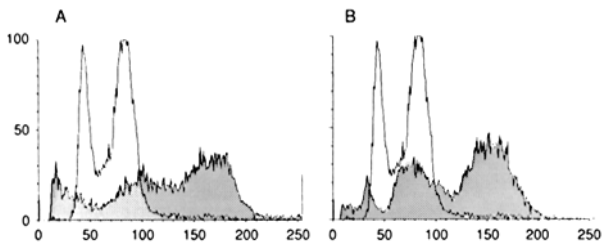


Figure 2. Flow cytometric analysis of *mps* mutant strains. The histogram of wild-type strain (M10/M11, open curve) serves as a standard for the histograms of the mutant (stippled curve) strains (A) *mps1* (D326-1) and (B) *mps2* (D402-1), after 4 (D326-1) or 6 h (D402-1) at 36°C. In the histogram of the wild-type strain, the left peak is G1 cells and the right peak is G2 and M cells. The *mps* mutants display cells of increased ploidy (peaks to the right of the wild-type G2/M peak). Similar results were obtained with rho^o derivatives of D402-1 and D326-1 (data not shown), indicating that mitochondrial DNA is not the source of the unusual DNA content observed in these mutants. In these histograms the x-axis is relative DNA content as expressed in propidium iodide fluorescence, and the y-axis is the relative number of cells normalized to the maximum peak. Each sample represents 10,000 cells.

missive temperature is consistent with the observed defects in chromosome segregation.

The defects in microtubule organization and chromosome segregation seen in a *mps1-1* cell were found to result from the formation of a monopolar spindle. Only a single SPB per nucleus was found when budded cells of a *mps1-1* mutant incubated at 36°C were analyzed by electron microscopy of serial sections (Fig. 3, B and C), whereas budded cells of the same strain grown at 25°C contained two SPBs per nucleus (Fig. 3 A), each acting as a pole of the mitotic spindle as in wild type. The monopolar spindles observed in *mps1-1* strains are similar to those seen in *cdc31* and *kar1* strains, except that the SPB itself is morphologically unique. Whereas the half-bridge associated with the SPB in a cell arrested by *cdc31* or *kar1* is small and indistinct (Byers, 1981b; Rose and Fink, 1987), the half-bridge in an *mps1-1* cell transferred to 36°C is strikingly enlarged (Fig. 3, B and C), extending 180 nM ($s = 31$ nM, $n = 28$) outward from the edge of the SPB, while that observed for the same strain at permissive temperature extended half as far (90 nM, $s = 8$ nM, $n = 5$; Fig. 3 A). A similar enlargement of the half-bridge (to a length of 150 nM) has previously been found for the satellite-bearing SPB borne by cells arrested in G1 by α -factor or by temperature-sensitivity for *CDC28* (Byers and Goetsch, 1974), but the *mps1* defect shown here uniquely leads to formation of an enlarged half-bridge in the absence of satellite formation.

Strains Containing *mps2-1* Produce a Monopolar Spindle Plus a Defective SPB

The aberrant microtubule organization observed in large-budded cells of an *mps2-1* strain 4 h after transfer from 23 to 37°C is shown in Fig. 1 C. About half the cells treated in this manner were found to acquire two foci of microtubule staining. Unlike the wild-type controls (Fig. 1 A), however, these foci are not connected to one another by a mitotic spindle. When incubated at 37°C, strains containing the *mps2-1*

mutation become arrested in the cell cycle, yielding large-budded cells with a G2 content of DNA. Unlike *mps1-1*, most *mps2-1*-arrested cells remain viable (>90%, data not shown, also see Fig. 4 C). Nevertheless, upon continued incubation at this temperature, missegregation of chromosomes results in the accumulation of cells with DNA contents greater than the control wild-type strain (Fig. 2 B). The *mps2-1* mutation identifies a new locus on the left arm of chromosome VII, 21cM proximal to *cyh2* (see Table III, Materials and Methods). Many of the phenotypic features displayed by *mps2* are shared by a previously reported mutation, *ndc1-1*, which maps to a distinct locus (Thomas and Botstein, 1986).

Strains carrying the *mps2-1* mutation give rise to a monopolar spindle that differs from that observed in *mps1*, *cdc31*, or *kar1* strains. Electron microscopy has revealed that *mps2-1* is unique among these mutations in that it causes the formation of two structurally distinct SPBs (Fig. 3, D and E). One SPB lies at the focus of both cytoplasmic and nuclear microtubules, forming a monopolar spindle similar to that seen in *cdc31* or *kar1* strains. This monopolar spindle is presumed to be dysfunctional in a manner similar to that observed for *cdc31* or *kar1*, as it gives rise to monopolar chromosome segregation and subsequent polyploidization. The second SPB is incapable of acting as a functional mitotic pole, as it lacks any attached nuclear microtubules. Nevertheless, there are cytoplasmic microtubules associated with this structure, thereby explaining why it is recognizable as a focal point for microtubule organization in cells subjected to immunofluorescent staining of tubulin. This interpretation is supported by the asymmetric distribution of DAPI staining in *mps2-1* strains at the arrest: chromosomal DNA appears to be associated exclusively with one focus of microtubule staining (presumably the SPB that bears nuclear microtubules), but not the other (the upper ones in Fig. 1 C).

Besides lacking nuclear microtubules, the defective SPB in *mps2-1* strains has some other interesting cytological features. First, it does not appear to be inserted into the nuclear envelope like a functional SPB, but seems instead to reside on the cytoplasmic surface of the envelope (Fig. 3 E). It has, in some instances, been found to be associated with a half-bridge (Fig. 3 E), but this feature could not be demonstrated in all cases. Consistently, the defective SPB appears to lack the "inner plaque," the diffuse layer of material on the nuclear face of the functional SPB in which the nuclear microtubules terminate (Rout and Kilmartin, 1990). Finally, the defective SPB is often found to have migrated into the distal region of the bud, where it resides on the tip of a thin extension of the nuclear envelope (Fig. 3 F). The occurrence of this extensive movement in the absence of any spindle microtubules suggests that some other agent is responsible for its movement.

Functions of the MPS Genes, but Not CDC31, Are Required After α -Factor Arrest

Structural differences between the SPBs in cells subject to mutational inactivation of *CDC31*, *MPS1*, and *MPS2* indicate that these genes may be responsible for distinct functions in the duplication pathway. We undertook to identify the stage of SPB duplication during which each genetic function was required by exploiting the reversible arrest of SPB duplication brought about by treatment of the cells with α -factor. This treatment blocks the cell-cycle in G1 at an intermediate

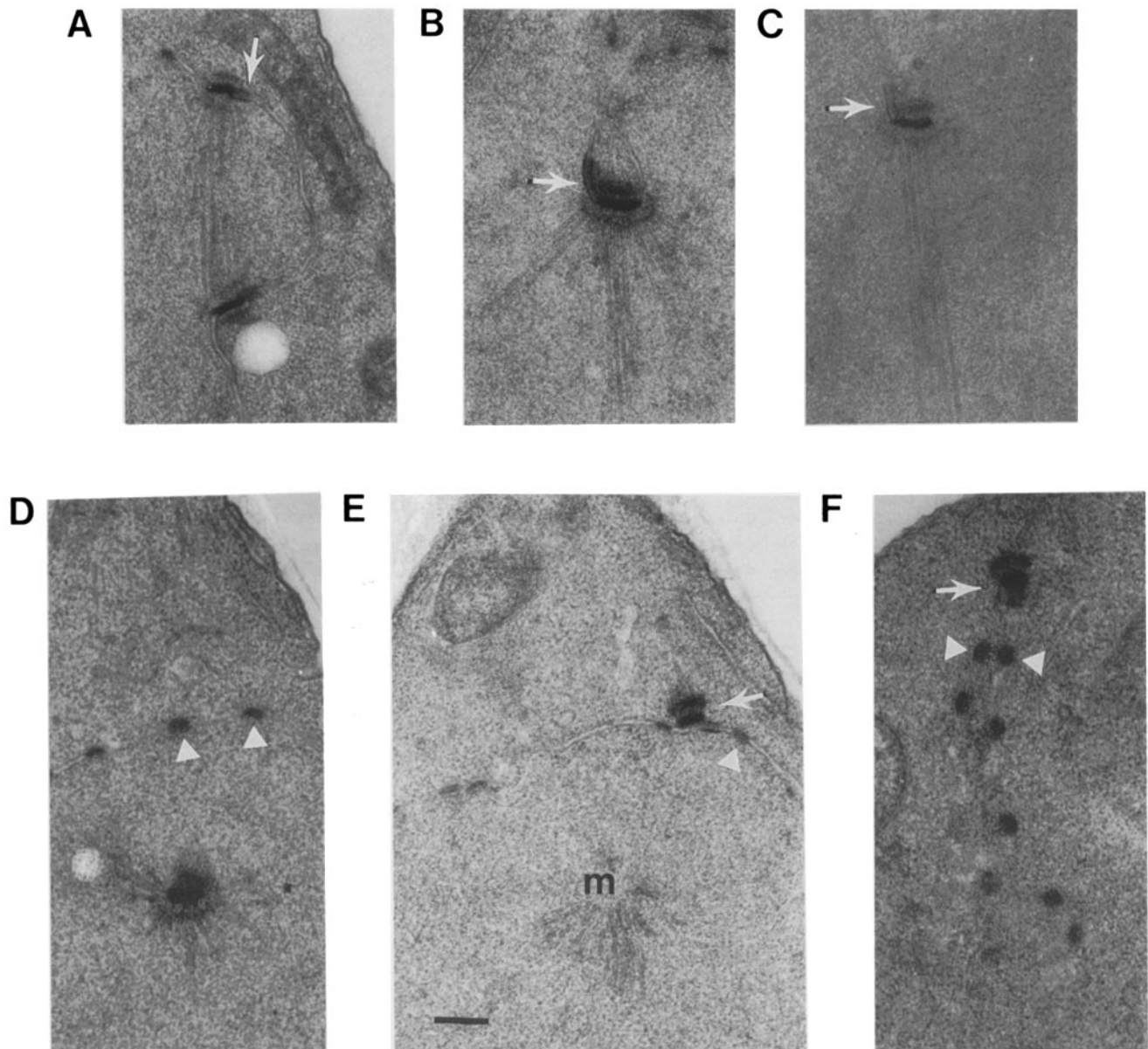


Figure 3. Electron micrographs of the *mps* mutants. A normal mitotic spindle is seen in a *mps1-1* strain (D326-1) grown at 25°C (A). In contrast, the same strain produces monopolar spindles when incubated at 36°C for 4 h (B and C). The half-bridge structures in A, B, and C are highlighted by arrows. Strains carrying the *mps2-1* mutation (D402-1) display a monopolar spindle (D) and a defective SPB (arrow, E) in serial sections. The defective SPB (E, arrow) lacks microtubules on its nuclear face and does not appear to be fully inserted into the nuclear envelope, which is marked by nuclear pores (arrowheads in D-F). The monopolar spindle (m) emanating from the functional SPB shows no apparent interaction with defective SPB (E). At later times of *mps2-1* arrest the nucleus gains a characteristic deformation (F) that bears the defective SPB (arrow) while the functional SPB (not shown) remains in the nucleus proper. Similar phenotypes were detected for 15 or more nuclei that were completely serially sectioned for each mutant strain. Bar, 0.2 μ m.

step of SPB duplication, each cell containing a single satellite-bearing SPB (Byers and Goetsch, 1975). At the next detectable step, an apparent maturation of the satellite culminates in formation of side-by-side SPBs that are interconnected by a fully formed bridge. Since satellites have not been observed in *cdc31* cells simultaneously challenged with α -factor and the nonpermissive temperature (Winey, M., and L. Goetsch, unpublished data), we are led to believe that there is a specific requirement for *CDC31* in satellite deposition. On this basis, we reasoned that if *cdc31* cells were arrested

at the satellite-bearing stage by addition of α -factor at the permissive temperature and then washed free of α -factor at the nonpermissive temperature, the preexisting satellite-bearing SPB would already have become competent to undergo duplication, and bipolar mitotic spindle formation would ensue in the absence of any active *CDC31* gene product.

This prediction was verified by releasing a *cdc31* strain from α -factor arrest at either 25 or 36°C. Progression of these cells into the cell cycle was determined by microscopic assays for bud formation and by flow cytometric assays of

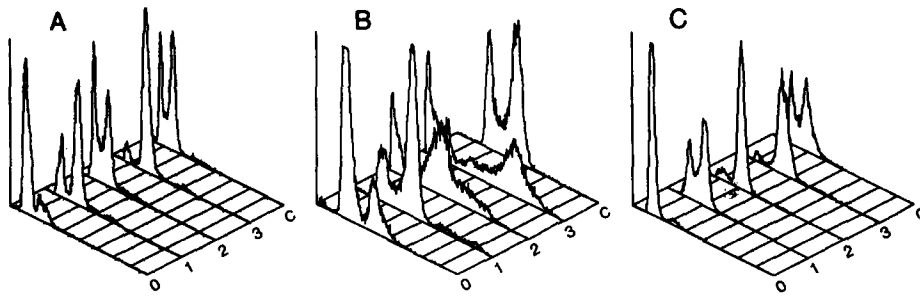


Figure 4. Flow cytometric analysis of the *cdc31* and *mps* mutants released at nonpermissive temperature from α -factor arrest. The (A) *cdc31-2*, (B) *mps1-1*, and (C) *mps2-1* strains (Wx209-8a, 3264N-5c, and 4024N-16d, respectively) were arrested with α -factor at 25°C, and then released at 36°C. Aliquots of cells for flow cytometry were taken at the arrest (0), at 1, 2, and 3 h after release from arrest (1, 2, and 3, respectively), and from

the initial culture (C) as a standard for the position of G1 and G2+M cells. The (A) *cdc31-2* strain completes an entire cell cycle (new G1 cells at 2 h) after release from arrest before arresting with G2+M DNA content (3 h). The (B) *mps1-1* strain shows no cell cycle arrest, failing in the first cycle and producing cells with aberrant DNA contents. The (C) *mps2-1* strain shows cell cycle arrest with G2+M DNA content (2 and 3 h) in the first cell cycle after release from arrest. After recovery from α -factor arrest, the generation times in this experiment are ~ 2 h. The x- and y-axes for each time-point are the same as in Fig. 2.

DNA content to monitor entry into S-phase. Bud formation and DNA synthesis were found to be completed in ~ 1.0 h (Fig. 4 A). At this time, microtubule organization was assessed by immunofluorescent staining for tubulin, and normal mitotic spindles were observed at both 25 (100%, $n = 150$) and 36°C (91%, $n = 132$). Continued incubation of the cells at 36°C showed that these cells proceeded to complete one full cell cycle and then arrest quantitatively in the next cell cycle as 91% ($n = 288$) large budded cells with G2 con-

tent of DNA (3.0-h timepoint, Fig. 4 A); 100% ($n = 180$) of the cells displayed monopolar spindles upon immunofluorescent staining. Electron microscopic analysis confirmed that the SPBs had undergone both duplication and separation to become components of a mitotic spindle (Fig. 5 A) in the first cell cycle after release from α -factor arrest and had then formed the characteristic monopolar spindle upon arrest in the second cell cycle (Fig. 5 B). Electron microscopy also served to demonstrate that the cells initially arrested by

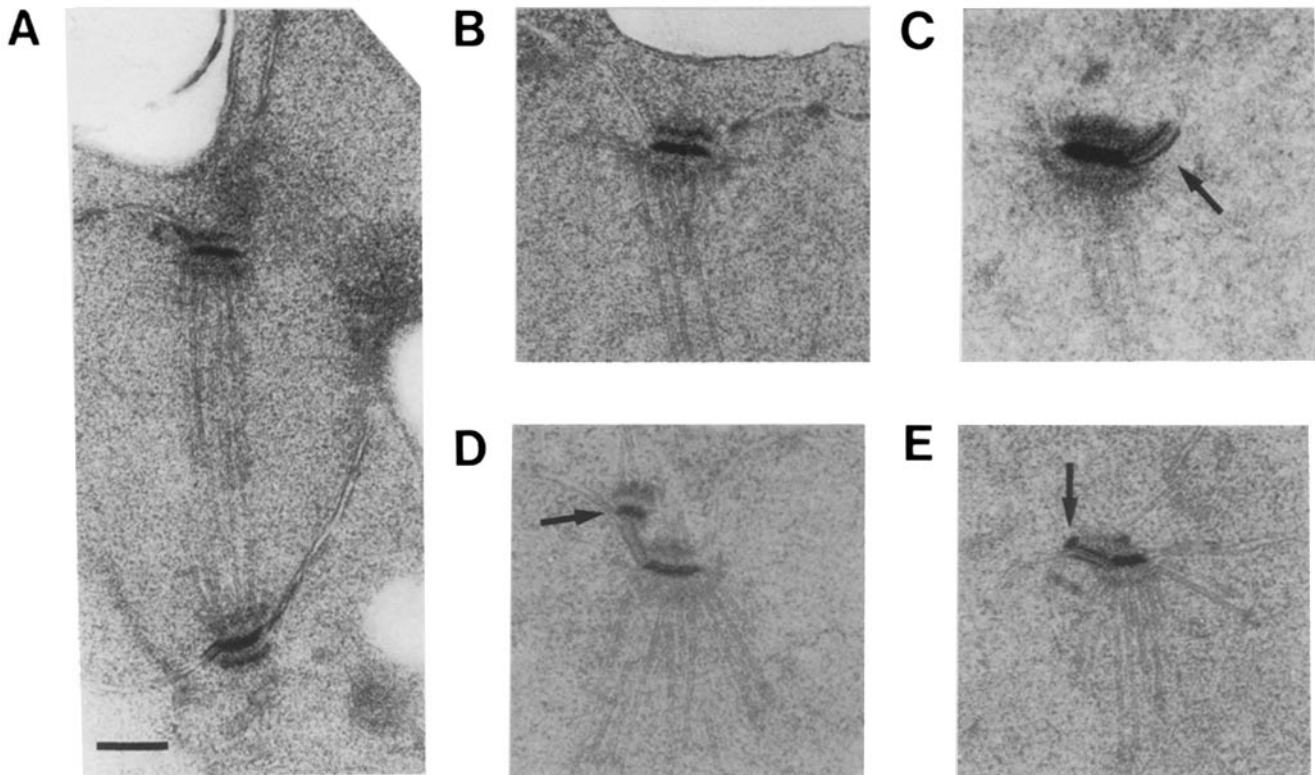


Figure 5. SPB duplication upon release from α -factor arrest without *CDC31* or *MPS* gene functions. The *cdc31-2*, *mps1-1*, and *mps2-1* strains (Wx209-8a, 3264N-5c, 4024N-16d, respectively) were arrested at 25°C with α -factor and then released from this arrest at 36°C. At various times after release the cells were prepared for electron microscopy. The *cdc31-2* strain displayed normal spindles (A) in all of 14 cells at 1 h after release, while cells allowed to continue incubation at 36°C for 3 h exhibited monopolar spindles (B) in all of 16 nuclei examined. All 16 cells of the *mps1-1* strain examined had immediately formed monopolar spindles (C) with SPBs displaying the enlarged half-bridge (arrow). The *mps2-1* strain displayed both a monopolar spindle and defective SPB (arrow, D) in each of 10 cells examined. Cells arrested by α -factor at 25°C were confirmed to contain satellite-bearing SPBs, as exhibited by the *mps2-1* strain (E, arrow marks the satellite). Bar, 0.2 μ m.

α -factor did, indeed, contain satellite-bearing SPBs (data not shown). These results demonstrate that the *CDC31* gene function had already been executed at the stage of α -factor arrest and therefore was not further required for SPB duplication subsequent to release from the arrest.

Repeating this experiment with the *mps1* and *mps2* mutants showed, on the other hand, that these gene functions have not been executed at the point of α -factor arrest, but are still required for completion of SPB duplication upon release. Flow cytometric analysis of these strains demonstrated that both of them displayed their mutant phenotypes in the first cell cycle after release from α -factor arrest at 36°C. The *mps1* mutant showed its characteristic pattern of chromosome missegregation (Fig. 4 B), and the *mps2* strain underwent G2 arrest (Fig. 4 C). In further assays of these cells, immunofluorescent staining of the microtubules showed that the cells were unable to complete SPB duplication upon release from α -factor arrest at 36°C, forming monopolar spindles in 91% ($n = 248$) of the *mps1* cells and in 98% ($n = 126$) of the *mps2* cells. Spindle formation was normal when the cells were released at 25°C (*mps1*: 94% spindles, $n = 220$; *mps2*: 98% spindles, $n = 109$). Electron microscopy of the cells that were released at 36°C revealed the same types of aberrant SPBs (Fig. 5, C and D) as those seen when these mutants were transferred directly to 36°C from logarithmic growth. Furthermore, electron microscopy of the α -factor-arrested cells confirmed that both mutants arrested with satellite-bearing SPBs (*mps1*: data not shown; *mps2*: Fig. 5 E). These results, therefore, differ from those ob-

tained for *cdc31* and suggest that *MPS1* and *MPS2* act at one or more later points in the SPB duplication pathway than does *CDC31*.

mps1-1 Is Epistatic to *mps2-1*

Having found that both *MPS1* and *MPS2* are required after the point of α -factor arrest, we sought to determine which of the two genes acts first upon release. Because the relevant mutations differ in their cytological phenotypes, it was feasible to address this issue by use of an epistasis test. A strain doubly mutant for *MPS1* and *MPS2* (Wx217-10b, Table I) was released from α -factor arrest at 36°C and was found to exhibit phenotypes similar to those of *mps1* strains. Flow cytometric analysis showed that the doubly mutant strain exhibited the rapid increase in ploidy characteristic of *mps1* strains (Fig. 6 A). Furthermore, the double mutant showed other characteristics of the *mps1* mutant including rapid death at the nonpermissive temperature (<10% viability after 4 h at 36°C) and the lack of cell-cycle arrest. Finally, electron microscopy of the *mps1*, *mps2* strain after release from α -factor arrest at 36°C revealed that the monopolar spindle found within each cell originated from a SPB that bore an enlarged half-bridge similar to that seen upon failure of the *MPS1* function alone (Fig. 6 B). The defective SPB seen in *mps2* strains was never detected in these doubly mutant cells. The phenotypic identity between *mps1* mutants and the *mps1*, *mps2* double mutant demonstrates that *mps1* failure obviates the expression of the *mps2* phenotypes, suggesting that the *MPS1* gene acts before *MPS2* in the SPB duplication pathway.

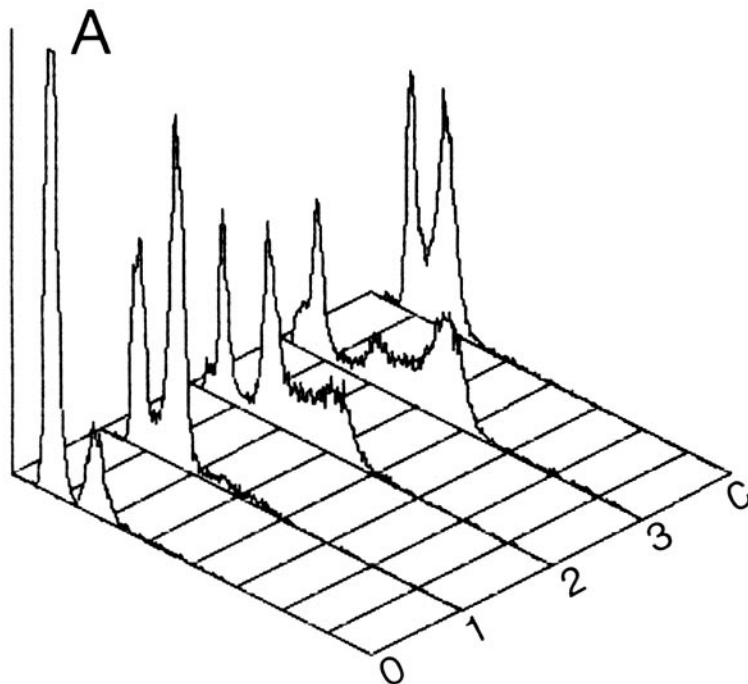


Figure 6. Epistasis test of *mps1-1* and *mps2-1*. A *mps1-1*, *mps2-1* doubly mutant strain (Wx217-10b) was arrested at 25°C with α -factor and released from this arrest at 36°C. The flow cytometric profiles (A) at the arrest and at various times after release are as in Fig. 4. The doubly mutant strain exhibits the same behavior as a *mps1* mutant strain (see Fig. 4 B). Electron microscopy of cells from this experiment revealed 13 monopolar spindles with clearly enlarged bridges (B, arrow) and five other monopolar spindles for which the bridges were not as distinct. Bar, 0.2 μ m.

Discussion

We have described the identification of two yeast genes, *MPS1* and *MPS2* (monopolar spindle), that are required for SPB duplication. Temperature-sensitive alleles of these genes share some phenotypes with the previously described monopolar spindle mutants, *cdc31* (Byers, 1981b) and *kar1* (Rose and Fink, 1987). When either of the *mps* mutants is incubated at the nonpermissive temperature, aberrant spindle organization is evident upon immunofluorescent staining of microtubules, and there are accompanying defects in chromosome segregation leading to polyploidization. Analysis by electron microscopy has revealed novel effects on SPB structure in the *mps* mutants. Strains mutant in *MPS1* were found to possess an enlarged half-bridge adjacent to the single SPB. Strains mutant in *MPS2* undergo an aberrant cycle of SPB duplication to yield one functional SPB and another that resides entirely in the cytoplasmic compartment, lacking any microtubules on its nuclear face.

Interaction of the *mps* Mutants with the Cell Cycle

The *mps* mutants differ from one another in their effects on the cell cycle. Like *cdc31* (Byers, 1981b) and *kar1* (Rose and Fink, 1987), *mps2-1* causes arrest of the cell cycle at a stage characterized by a large bud and a G2 content of DNA, whereas *mps1-1* fails to cause cell-cycle arrest upon transfer to the nonpermissive temperature. Hartwell and Weinert (1989) have argued that arrest of the cell cycle might result either from an intrinsic defect in a cellular component which normally provides an appropriate substrate for the next step of the cycle, or from an extrinsic controlling function that assesses completion of prerequisite events before allowing cell cycle progression. Indeed, *RAD9* has been identified as an extrinsic control function, or checkpoint, that is required for cell-cycle arrest in response to DNA damage (Weinert and Hartwell, 1988). One might postulate that the arrests observed in the *cdc31*, *kar1*, and *mps2* mutants are examples of intrinsic blocks to cell-cycle progression because the formation of a monopolar spindle does not provide the appropriate structure for mitosis. However, the failure of *mps1* strains to undergo cell cycle arrest reveals that formation of a monopolar spindle per se does not necessarily cause arrest, thereby implicating some sort of extrinsic control in the cell cycle arrests observed for the *cdc31*, *kar1*, and *mps2* mutants.

The Spindle Pole Body Duplication Pathway

We have shown that the monopolar spindle mutants provide a basis for genetic dissection of the SPB duplication pathway, which previously was based largely on cytological criteria (Byers and Goetsch, 1975). SPB duplication, in brief, begins late in G1 when the satellite, seen in the electron microscope as a small tuft of darkly staining material, appears on the cytoplasmic surface of the half-bridge at a site distal to the SPB proper (Fig. 7; Byers and Goetsch, 1975). The satellite persists until a bona fide daughter SPB, fully embedded in the nuclear envelope, arises at the same site. Duplication therefore appears to be a conservative process, the existing SPB remaining intact while the satellite serves as the precursor to the new SPB. Upon completion of this duplication event, the side-by-side SPBs are connected by a complete bridge, which is later severed as the SPBs undergo separation to form a mitotic spindle, leaving a half-bridge on each SPB. We reasoned that failures in distinct steps of the SPB duplication pathway might be responsible for the novel SPB morphologies observed among mutants that undergo monopolar spindle formation. Our experimental results support this conclusion and can be integrated with previous cytological findings (Byers, 1981a) to suggest the pathway for SPB duplication shown in Fig. 7.

Of the three genes investigated, *CDC31* appears to be required first in SPB duplication, possibly playing a role in satellite formation. Although satellite formation itself is difficult to monitor in a quantitative manner, it has been possible to analyze more rigorously whether *CDC31* is required before or after the stage in which cells first display a satellite. We have found, in fact, that an entire cell cycle is completed by *cdc31* strains released from α -factor arrest (when the satellite is present) at the nonpermissive temperature, indicating the absence of any essential role for *CDC31* after satellite formation is completed. It is possible, on the basis of these findings, that execution of the *CDC31* function late in one cycle of ongoing growth serves to permit both satellite formation and SPB duplication in the subsequent cycle. This notion is supported by the recent finding that when *cdc31* cells are brought to arrest in an earlier phase of G2 by treatment with nocodazole and then released from the drug at the nonpermissive temperature, they complete mitosis and then become arrested with a monopolar spindle in the subsequent cell cycle (Winey, M., unpublished observation).

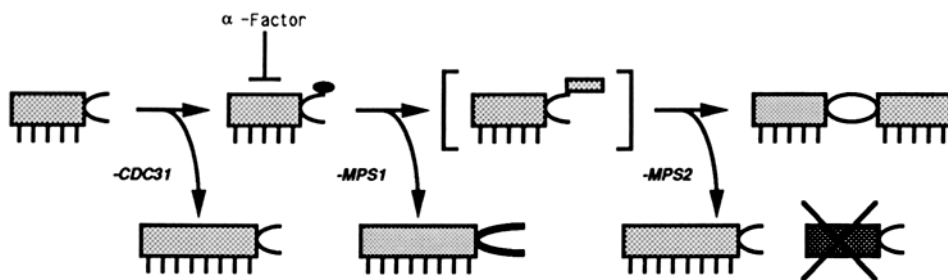


Figure 7. Proposed pathway of SPB duplication in *Saccharomyces cerevisiae*. This schematic representation of the SPB duplication pathway is based on previous cytological analysis (Byers, 1981a), and the results reported here. Microtubules are displayed only on the nuclear side of the SPB to indicate its orientation. The steps dependent on the *MPS1*, *MPS2*, and *CDC31* gene activities

are indicated, as are the types of SPBs that result from failure in the given step. As diagrammed, the SPBs associated with monopolar spindles are seen to increase in size, as characterized by Byers (1981b). The bracketed structure is a possible intermediate between satellite-bearing and duplicated-but-unseparated SPBs suggested by the *mps2* phenotype (see Fig. 5 D).

The next known gene function to act in SPB duplication is *MPS1*. The satellite-bearing SPB of a *mps1-1* cell arrested with α -factor at the permissive temperature is unable, upon removal of α -factor, to progress through further stages of SPB duplication at the nonpermissive temperature. Because *MPS1* is required after α -factor arrest, whereas the *CDC31* function has already been executed at this stage, we believe that *MPS1* may also act after *CDC31* in an uninterrupted cell cycle. Further information on the order of gene functions is gained from the finding that *mps1* is epistatic to *mps2*, the doubly mutant strain being phenotypically identical to a strain mutant in *mps1* alone. Although we believe this to indicate that *MPS1* acts before *MPS2*, we must entertain the alternative interpretation that *MPS2* is required before *MPS1* but that development of the *mps2* mutant phenotype requires *MPS1* gene activity. We discount the latter interpretation because the appearance in *mps2* strains of the defective SPB, which is not seen in *cdc31* or *mps1* mutants, provides further evidence that *mps2* strains have progressed farther through the SPB duplication pathway than the other mutants.

For the reasons indicated above, we conclude that *MPS2* acts later in the pathway than either *CDC31* or *MPS1*. In the absence of *MPS2* function, a satellite-bearing SPB proceeds through a defective duplication cycle to yield only one functional SPB, which we presume to be the same one that originally was present, while the satellite gives rise to the defective SPB. The defective SPBs observed in *MPS2* mutants appear mature on their cytoplasmic faces, from which cytoplasmic microtubules emanate, but seem not to have become inserted into the nuclear envelope. Failure of insertion into the envelope would prevent access of the nuclear face to the nucleoplasm and thereby preclude the assembly of spindle microtubules. Surprisingly, the defective SPB is able to undergo separation from the functional SPB without being attached to any spindle microtubules. The mechanism of this separation remains obscure, but may possibly involve activities of the cytoplasmic microtubules associated with the defective SPB.

The phenotypes of *mps1* and *mps2* establish certain aspects of the SPB duplication pathway that previously were supported only by circumstantial evidence. As noted above, it has been known for some time that satellite formation on the half-bridge anticipates appearance at a similar position of the second SPB proper, but there was no direct evidence that the satellite actually gave rise to the nascent SPB. No intermediate steps in the enlargement of the satellite or its insertion into the nuclear envelope had been observed (Byers and Goetsch, 1975), so it was conceivable that SPB duplication entailed a simple redistribution of the material that previously comprised the existing SPB. However, the simultaneous presence of both a normal SPB and an aberrant one after failure of the *MPS2* function now provides compelling evidence that the nascent SPB is newly formed at the site previously occupied by the satellite while the existing SPB remains intact. Furthermore, this phenotype uniquely demonstrates that the possible enlargement of the satellite to form the nascent SPB can occur independently of its insertion into the nuclear envelope. These findings, in conjunction with the evidence that failure of SPB duplication in *mps1* cells results primarily from a defect in the site at which satellite formation occurs (on the half-bridge), substantiate the aforementioned model that SPB duplication proceeds by a conserva-

tive mechanism. That is to say, the role of the existing SPB in duplication may be simply to ensure the availability of an adjacent site for assembly of the nascent SPB.

Finally, it should be noted that the screening method utilized here to identify *MPS1* and *MPS2*, though time-consuming, might profitably be pursued to greater extent because only a single allele of each gene has yet been isolated. Further screening could yield additional alleles that might differ in phenotype and thereby offer further insight into the functions mediated by these genes. In addition, other genes specifically required for SPB duplication could be identified. Regardless, genetic characterization of the two genes identified here already provides a basis for analyzing these SPB-related functions at the molecular level. Recent advances in the isolation of yeast SPBs and their immunochemical characterization provides improved methods for analyzing SPB assembly and in establishing whether these gene products are themselves components of the SPB (Rout and Kilmartin, 1990). These approaches, coupled with recent advances in the mass isolation of mammalian centrosomes (Komesli et al., 1989) and new assays for their activity (Klotz et al., 1990), should also permit us to search for conserved biochemical functions that may be involved in the duplication and regulation of centrosome-like organelles in a broader range of organisms.

We would like to thank Mike Culbertson for supplying the collection of temperature-sensitive yeast strains, and Don Hawthorne, Lee Hartwell, and John McCusker for providing other yeast strains. We appreciate the communication of data prior to publication by Liz Vallens and Mark Rose. We are indebted to Jeff McGrew and Todd Seeley for critically reading the manuscript, to Dean Toy and Andrea Burger for technical assistance, and to Bryan Jensen for computer graphics. Peptide synthesis was carried out by Dr. Patrick Chou at the Chemical Synthesis Facility, Howard Hughes Medical Institute, University of Washington, and flow cytometry was carried out at the University of Washington Center for AIDS Research with the assistance of Mary Touma supported by National Institutes of Health (NIH) grant IP30-27757.

This work was supported by NIH grant GM18541 to B. Byers and NIH Postdoctoral Fellowship GM12701 to M. Winey.

Received for publication 7 March 1991 and in revised form 8 May 1991.

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