The *Saccharomyces cerevisiae* Spindle Pole Body Duplication Gene MPS1 Is Part of a Mitotic Checkpoint

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Abstract. M-phase checkpoints inhibit cell division when mitotic spindle function is perturbed. Here we show that the *Saccharomyces cerevisiae MPS1* gene product, an essential protein kinase required for spindle pole body (SPB) duplication (Winey et al., 1991; Lauze et al., 1995), is also required for M-phase checkpoint function. In *cdc31-2* and *mps2-1* mutants, conditional failure of SPB duplication results in cell cycle arrest with high $p34^{CDC28}$ kinase activity that depends on the presence of the wild-type *MAD1* checkpoint gene, consistent with checkpoint arrest of mitosis. In contrast, *mps1* mutant cells fail to duplicate their SPBs and do not arrest division at 37°C, exhibiting a normal cycle of $p34^{CDC28}$ kinase activity despite the presence of a monopolar spindle. Double mutant *cdc31-2, mps1-1* cells also fail to arrest mitosis at 37°C, despite having SPB structures similar to *cdc31-2* single mutants as determined by EM analysis. Arrest of mitosis upon microtubule depolymerization by nocodazole is also conditionally absent in *mps1* strains. This is observed in *mps1* cells synchronized in S phase with hydroxyurea before exposure to nocodazole, indicating that failure of checkpoint function in *mps1* cells is independent of SPB duplication failure. In contrast, hydroxyurea arrest and a number of other *cdc* mutant arrest phenotypes are unaffected by *mps1* alleles. We propose that the essential *MPS1* protein kinase functions both in SPB duplication and in a mitotic checkpoint monitoring spindle integrity.

THE essential processes of cell division are vulnerable to environmental perturbation and are prone to error to some degree. Eukaryotic cells have "checkpoint" mechanisms that can negatively regulate cell division when critical processes or structures are perturbed, allowing time for corrective mechanisms to respond to such forces of disorder (Hartwell and Weinert, 1989). Progress from S phase into mitosis, for example, is inhibited by a checkpoint mechanism when DNA is damaged or incompletely replicated in many cell types. In the budding yeast *Saccharomyces cerevisiae*, this response requires a number of gene products; lesions in these products eliminate cell cycle response to errors in DNA metabolism, leading to lethal division when such events occur. Some of these genes, such as *RAD9*, are not essential for growth in the absence of insult (Weinert and Hartwell, 1988; Weinert et al., 1994). Others, such as *SAD1* and DNA polymerase ϵ itself, are essential for normal growth (Allen et al., 1994; Navas et al., 1995; Weinert et al., 1994). Checkpoint regulation of cell division likely works by affecting the oscillations of cyclin/cyclin-dependent kinase complex activity that are critical for cell cycle transitions, although the actual mechanisms by which they do this are still unclear (for review see Forsburg and Nurse, 1991; Luca, 1993; King et al., 1994; Nurse, 1994).

Just as a cell's genetic material must be fully replicated and intact before segregation for division to be productive, so must the mitotic spindle be able to segregate duplicated chromosomes to the daughter cells accurately. Mitotic progress is sensitive to perturbations of the spindle apparatus, and it is becoming clear that mitotic checkpoint regulation plays an important role in linking mitotic progress to the structural and functional integrity of the spindle. Careful observation of mitosis in PtK_1 cells and large newt pneumocytes (Reider and Alexander, 1989; Reider et al., 1994) and studies using antimicrotubule drugs (Jacobs et al., 1988; Jordan et al., 1992; Andreassen and Margolis, 1994) suggest that mitotic progress is negatively regulated when spindle structure is compromised. Micromanipulation of chromosomes and spindles has shown that mechanical tension on chromosomes may be a critical parameter monitored by mitotic checkpoints (Nicklas and Ward, 1994). In budding yeast, checkpoint regulation of mitosis in response to spindle disruption depends on the products of at least three *MAD* genes and three *BUB* genes (Hoyt et al., 1991; Li and Murray, 1991). When any one of these six gene products is disrupted, yeast cells cannot arrest

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passage though M phase upon treatment with the microtubule-depolymerizing drug nocodazole. None of the genes is essential for growth under normal conditions, suggesting that their only role in the cell is in a mitotic checkpoint ensuring that progress through M phase does not occur in the absence of a functional mitotic spindle.

Centrosome duplication is among the earliest morphological signs that eukaryotic cells have begun the process of division. In animal cells, the process is marked by G1 duplication of the centrioles (Vandre and Borisy, 1989). Precise duplication of this microtubule-organizing center generates the two poles of the mitotic spindle, thereby setting up a bipolar spindle apparatus (Mazia, 1978, 1985). The cycle of centrosome duplication can proceed independently of cell cycle progress (Gard et al., 1990; Sluder et al., 1990; Balczon et al., 1995), and the regulatory pathways that link the centrosome duplication and the cell cycle remain unclear. A number of studies suggest that some factor critical in cell cycle regulation may reside at the centrosome or require centrosome duplication. For example, microsurgical removal of centrosomes from tissue culture cells prevents entry into mitosis (Maniotis and Schliwa, 1991). Removal of the structure, however, may not be equivalent to disrupting its function; deletion of the *Aspergillus nidulans* gene encoding the centrosome-associated protein γ-tubulin disrupts centrosome function and mitotic spindle formation, but does not lead to M-phase arrest despite the absence of a functional mitotic spindle (Oakley et al., 1990). To investigate how mitotic checkpoints monitor centrosome duplication, we have examined cell cycle phenotypes associated with *S. cerevisiae* conditional mutations that disrupt duplication of the spindle pole body (SPB),[1] which is the centrosome-equivalent structure in budding yeast.

The SPB is a trilaminar disk-shaped structure embedded in the nuclear envelope, which remains intact throughout cell division (Byers, 1981*a*; Winey et al., 1991). Microtubules emanate from the cytoplasmic and nucleoplasmic faces of the structure, and a thickened region of nuclear envelope called the "half bridge" is often observable adjacent to the SPB proper. SPBs are duplicated in late G1, as cells proceed from START to S phase (Byers, 1981*a*). The conditional mutations *cdc31-2*, *mps1-1*, and *mps2-1* disrupt distinct steps of SPB duplication at restrictive temperatures, leading to the formation of monopolar spindles with distinctive SPB morphologies (Byers, 1981*b*; Winey et al., 1991). The *cdc31-2* mutation disrupts an early step in the duplication process. The single SPB in *cdc31-2* strains is enlarged and has little or no half-bridge structure (Byers, 1981*b*). *CDC31* function is required before START, while both *MPS1* and *MPS2* gene functions are required at or immediately after START. The *mps1-1* lesion likely disrupts an intermediate stage of SPB duplication. In *mps1-1* strains, SPBs enlarge and develop a very large half-bridge structure but do not duplicate. The *mps2-1* mutation disrupts a late step in SPB duplication, resulting in the formation of an aberrant second SPB that is not inserted into the nuclear envelope. The *mps1-1* mutation is epistatic to *mps2-1* for SPB morphology (Winey et al., 1991). Five other distinct *mps1* alleles have subsequently been identified that share the *mps1-1* phenotype (Schutz, A., and M. Winey, personal communication): in this study we used *mps1-1* as a representative allele.

When both *cdc31-2* and *mps2-1* mutants are exposed to restrictive temperatures, they arrest division after failure of SPB duplication, resulting in cells with monopolar spindles, large buds, and G2 DNA content (Byers, 1981*b*; Schild et al., 1981; Winey et al., 1991). In marked contrast, strains carrying the *mps1-1* mutation fail to duplicate their SPBs at restrictive temperatures but do not arrest division. Rather, these cells proceed through monopolar mitosis and cytokinesis without pausing, and eventually go on to further DNA synthesis resulting in accumulation of cells with aberrantly high DNA content (Winey et al., 1991). Both *cdc31-2* and *mps2-1* strains maintain high viability at restrictive temperatures, while viability falls rapidly in *mps1-1* cultures at restrictive temperatures (Byers, 1981*b*; Schild et al., 1981; Winey et al., 1991). This phenotypic difference indicates that a monopolar spindle need not always cause mitotic arrest.

Our investigation of the *cdc31-2* and *mps2-1* mutant phenotype indicates that yeast with monopolar spindles generally arrest the cell cycle with high levels of $p34^{CDC28}$ activity, similar to the arrest phenotype observed upon treatment with nocodazole. We have found that this arrest depends upon the presence of the wild-type *MAD1* gene, indicating that a mitotic checkpoint is involved. We also found that the essential *MPS1* gene product is involved in this checkpoint. Alleles of *mps1* interfere with the *cdc31-2* arrest phenotype without affecting the characteristic *cdc31-2* mutant SPB morphology and also make cells conditionally unable to arrest in nocodazole. This latter phenotype is independent of conditional SPB duplication failure caused by *mps1* alleles and indicates that the essential protein kinase encoded by *MPS1* (Lauze et al., 1995) has roles in both SPB duplication and mitotic checkpoint function.

Materials and Methods

Strains, Cell Culture, and Genetic Techniques

The yeast strains used in this study are listed in Table I. Yeast media, genetic techniques, and molecular techniques were as described by Ausubel et al. (1994). Yeast cells were arrested in G1 with α-factor (7–10 μM) produced by custom peptide synthesis using Fmoc chemistry on a peptide synthesizer (model 488; Applied Biosystems, Inc., Foster City, CA). Hydroxyurea and nocodazole were purchased from Sigma Chemical Co. (St. Louis, MO). Hydroxyurea was used at 0.1 M. Nocodazole was used at 15 μg/ml, a concentration reported to disrupt microtubules (Jacobs et al., 1988), and added from a 1.5 mg/ml DMSO stock. The efficiency of a given arrest was monitored by determination of the budding index (proportion of budded cells in a sample of 200 cells) of briefly sonicated aliquots. G1 α-factor arrests were considered adequate when 95% of the cells were unbudded and were subsequently confirmed by flow cytometry to show that the population contained primarily cells with G1 DNA content. Early S-phase arrest was induced by releasing α-factor–arrested cultures into rich media containing 0.1 M hydroxyurea. Hydroxyurea arrests were considered adequate when >95% of the cells were budded, and 75–85% were large budded such that mother and daughter cell bodies were of equal size; arrest was later confirmed by flow cytometry to show that the population predominantly contained cells with G1/S DNA content. Cultures synchronized by treatment with α-factor or hydroxyurea were released from these blocks by rinsing with growth medium equilibrated to the appropriate temperature or drug content for the experiment. In these experiments, entry into and progression through the cell cycle were monitored

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

*Table I. Yeast Strains**

Name	Genotype	Source
WX241-2b	a, *mps1-1, ura3-52, leu2-3,112, his3Δ200*	This study
WX257-5c	a, *ura3-52, his3Δ200, leu2-3,112, trp1Δ1*	This study
WX266-2b	a/a, *ura3-52/ura3-52, leu2-3,112/leu2-3,112, his3Δ200/his3Δ200,*	This study
WX178-3c	a, *mps2-1, ura3-52, leu2-3,112, trp1Δ1*	This study
ELW65-9d	a/a, *cdc31-2/cdc31-2, ura3-52/ura3-52, leu2-3,112/leu2-3,112*	This study
ELW78-7c	a/a, *cdc31-2/cdc31-2, mps1-1/mps1-1, ura3-52/ura3-52, leu2-3,112/leu2-3,112, trp1Δ1/trp1Δ1,*	This study
ELW112-3a	a/a, *cdc31-2/cdc31-2, mad1-1/mad1-1, ura3-52/ura3-52, leu2-3,112/leu2-3,112,*	This study
ELW92	spontaneous diploid, WX241-2b	This study
DRL139-7b	α, *mad1-1, ura3-52, leu2-3,112*	Li and Murray
AS127-1a	a, *mps1-K412, ura3-52, leu2-3,112, his3Δ200, trp1Δ1*	Schutz
AS132-3a	a, *mps1-1237, ura3-52, leu2-3,112, his3Δ200,*	Schutz
AS126-5a	a, *mps1-3796, ura3-52, leu2-3,112, his3Δ200,* lys-	Schutz
AS131-2d	a, *mps1-737, ura3-52, his3Δ200, trp1Δ1*	Schutz
ELW132-32b	a, *cdc31-2, mps1-737, ura3-52, leu2-3,112, trp1Δ1*	This study
ELW134-6c	a, *cdc31-2, mps1-K412, ura3-52, leu2-3,112*	This study
ELW136-2a	a, *cdc31-2, mps1-1237, ura3-52, trp1Δ1*	This study
ELW138-14c	a, *cdc31-2, mps1-3796, ura3-52, trp1Δ1*	This study
H9C1A5	a, *cdc9-1, his7, hom3, can1^r*	Hartwell
4078-13-3	a, *cdc13-1, ura3, leu2, his7, can1^r*	Hartwell
H16C1A5	a, *cdc16-1, his7, ura1, can1^r*	Hartwell
H20C1A1	a, *cdc20-1, his7, ura1, can1^r*	Hartwell
H23C1AX	a, *cdc23-1, ade1, his7, can1^r*	Hartwell
WX258-1b	α, *cdc9-1, mps1-1, his7, can1^r*	This study
WX259-1c	a, *cdc13-1, mps1-1, can1,* ura-, his-	This study
WX265-1b	a, *cdc16-1, mps1-1, ura3-52*	This study
WX261-1a	α, *cdc20, mps1-1,* ura-, his-	This study
WX262-1b	a, *cdc23, mps1-1,* ura-, ade-, his-	This study

*Where precise identity of markers are unknown, auxotrophies are indicated.

by budding index (relative percentages of different budding morphologies) and flow cytometry.

Analysis of p34^{CDC28} Activity

H1 was prepared from calf thymus by extracting blended tissue with 0.74 N PCA, followed by precipitation with 30% TCA. The TCA pellet was then washed with cold 70% acetone, resolubilized in water, and extracted a second time with PCA. After reprecipitation with 30% TCA, the pellet was washed with cold 70% acetone, resolubilized in H_2O and buffered to pH 7–7.5 by addition of 1 M Tris base, pH 9.0, to a final concentration of ~85 mM Tris. 700 g of thymus tissue yielded ~0.8 g protein. Final stock protein concentration was adjusted to 10 mg/ml with 85 mM Tris, pH 7.5. PAGE analysis of this protein preparation showed that it is primarily histone H1.

Preparation of whole cell lysates and H1 kinase activity assays were performed essentially as described by Langan et al. (1989) with some modifications. Cells washed with cold SCE (1 M sorbitol, 100 mM sodium citrate, pH 7.5, 10 mM EDTA) were incubated at 37°C in 70 μl of 200 μg/ml zymolyase + 5 mM beta mercaptoethanol, for 12 min. The resulting spheroplasts were washed twice with cold SCE and then lysed in protein kinase lysis buffer (50 mM NaCl, 50 mM Tris, pH 7.5, 0.5 mM EDTA, 0.2 mM EGTA, 0.1% Tween-20, 0.1% Triton X-100) (Langan et al., 1989) in the presence of aprotinin, benzamidine, PMSF, leupeptin, pepstatin, soybean trypsin inhibitor, and EDTA. After clearing lysates by spinning at 14,000 *g*, extract protein concentration was adjusted to 100 μg/ml in 30-μl kinase reactions, in kinase buffer (50 mM NaCl, 50 mM Tris, pH 7.5, 15 mM $MgCl_2$, 0.4 mM EDTA, 0.2 mM EGTA, 0.8% Tween-20, 0.8% Triton X-100, 250 μM ATP) described by Langan et al. (1989) with 4 μg of histone H1 in each reaction. Reactions were immediately incubated at 30°C for 25 min, after which they were stopped by addition of 10 μl 5 × SDS/DTT sample buffer and immediately boiled for 10 min. The resulting 40-μl samples were run on a discontinuous denaturing 10% polyacrylamide gel. After running, the gel was boiled in TCA for 10 min, fixed, and Coomassie-stained to confirm uniformity of extract protein concentrations. Relative phosphorylation of H1 was quantitated using a phosphorimager (Molecular Dynamics, Inc., Sunnyvale, CA) and ImageQuant software (Molecular Dynamics, Inc.).

To examine activity of affinity-purified p34^{CDC28}, 0.3 mg of extract protein was preincubated with Sepharose (Sigma Chemical Co.) and then incubated with either p13^{suc1}-Sepharose or Sepharose for 5 h in 1 ml of protein kinase lysis buffer: (settled bead volume ~30 μl). After this incubation, the H1 kinase activity remaining in the supernatants was measured as above, adjusting protein concentrations to 100 μg/ml in 30-μl assays. The p13^{suc1}-Sepharose and Sepharose beads were washed eight times in RIPA buffer (150 mM NaCl, 1.0% NP-40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) (Harlow and Lane, 1988), twice in kinase buffer lacking ATP and H1, and finally incubated with 60 μl of assay buffer containing 0.3 mg/ml H1 for 30 min. After stopping the reactions with 20 μl of 5 × sample buffer, 40 μl of each assay was analyzed by PAGE and autoradiography.

Cytological Techniques

Yeast cells were fixed for determination of budding morphology by addition of formaldehyde to 3.7%. Immediately before scoring, samples were sonicated briefly. In scoring budding morphology, cells without obvious buds were considered unbudded, cells with buds less than approximately one-third the volume of the mother cell were considered small budded, and cells with buds of greater size were considered large budded. Cells that had multiple buds or other unusual morphologies were scored as aberrant. 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 FACScan® flow cytometer using the CELLFIT and LYSYS software packages (Becton Dickinson Immunocytometric Systems, San Jose, CA) to obtain and analyze data. Yeast cells were prepared for EM by procedures described by Byers and Goetsch (1975), and serial 70–80-nm thin sections were viewed on an electron microscope (JEOL USA, Peabody, MA).

Results

cdc31-2 and mps2-1 Strains Arrest Mitotic Progress with High p34$^{cdc2/CDC28}$ H1 Kinase Activity

Nocodazole arrest, generally considered a block to mitotic

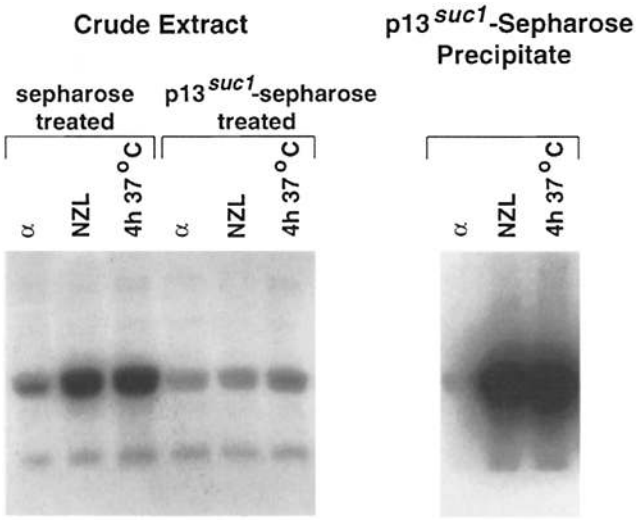


Figure 1. H1 kinase activity in extracts of variously treated *cdc31-2* cultures. The left panel shows H1 kinase activity from whole cell lysates of *cdc31-2* (ELW65-9d) cells. The major phosphorylated band is histone H1. In the left panel, extracts were treated with sepharose or p13^{suc1} sepharose. Lanes α, kinase activity in extracts of α-factor–arrested *cdc31-2* (ELW65-9d) cells; lanes *NZL*, kinase activity in extracts of such cells arrested in 15 μg/ml nocodazole; and lanes *4 h 37°C*, kinase activity in extracts of cells incubated at 37°C for 4 h. The right panel shows H1 kinase activity associated with p13^{suc1} sepharose precipitates from extracts of α-factor–arrested (*α*), nocodazole-treated (*NZL*), or 37°C-incubated (*4 h 37°C*) *cdc31-2* (ELW65-9d) cells.

progression, occurs with sustained elevation of mitotic p34^{CDC28} H1 kinase activity (Langan et al., 1989; Hadwiger et al., 1989). To determine the status of p34^{CDC28} activation at the *cdc31-2* arrest, we made crude extracts of *cdc31-2* (ELW65-9d) cultures arrested in G1 by addition of mating pheromone, in M by addition of nocodazole, or at the *cdc31-2* arrest by incubation at 37°C for 4 h, and assayed the H1 kinase activity in these extracts. Extracts of restrictive temperature–arrested *cdc31-2* cells had H1 kinase activity comparable to that observed in extracts of nocodazole-arrested cells, and significantly higher than observed in α-factor–arrested cells (Fig. 1, *left*). Most of the H1 kinase activity present in extracts of nocodazole and 37°C-arrested *cdc31-2* cells could be depleted with p13^{suc1} Sepharose, a reagent that specifically precipitates p34^{CDC28} kinase complex (Hadwiger et al., 1989). As in crude extracts, material precipitated from extracts of nocodazole and 37°C-arrested *cdc31-2* cells with p13^{suc1} Sepharose had higher H1 kinase than the p13^{suc1} precipitate from extracts of α-factor–arrested cells (Fig. 1, *right*).

This experiment shows that progress through mitosis is blocked at the *cdc31-2* arrest, with elevated p34^{CDC28} kinase activity. To determine if other parts of the cell cycle are affected in *cdc31-2* strains, we compared synchronous *cdc31-2* cultures with similarly treated wild-type cells. After synchronizing wild-type (WX266-2b) and *cdc31-2* (ELW65-9d) strains in early S phase with hydroxyurea (see *Materials and Methods*), we released the cultures into drug-free medium at 37°C, sampling at regular intervals to measure H1 kinase activity, percentage of large-budded

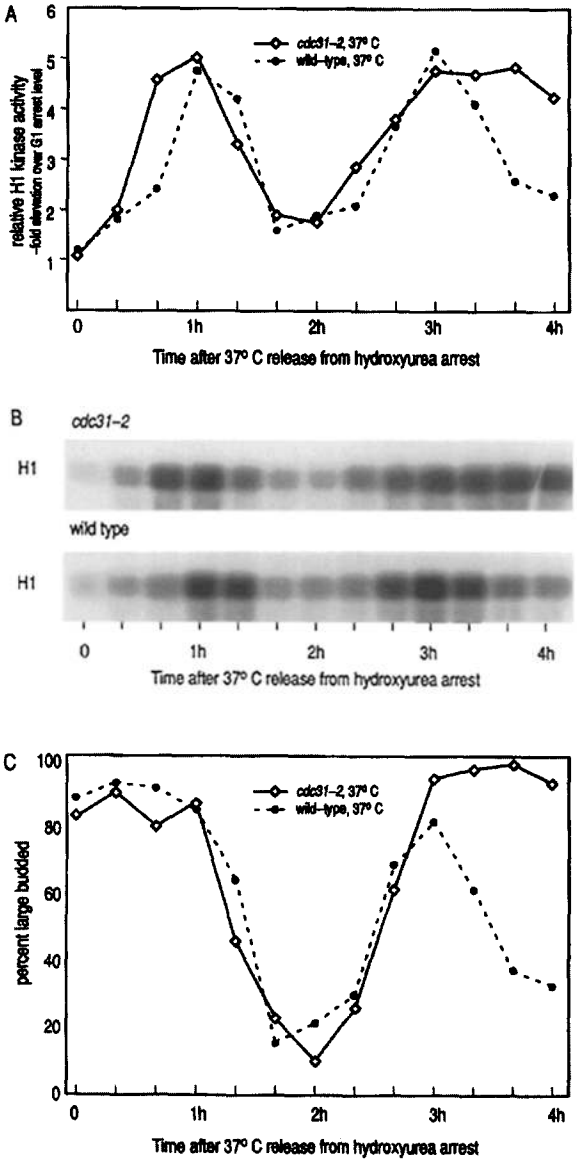


Figure 2. Cell cycle events in synchronized *cdc31-2* and wild-type cultures at 37°C. (*A*) H1 kinase activity in whole cell extracts of *cdc31-2* (ELW65-9d) and wild-type (WX266-2b) cultures synchronized in early S phase and then released into 37°C medium. (*B*) Raw data for *A*. (*C*) The percentages of large budded cells in these cultures at successive times.

cells, and DNA content. Because budding yeast cells synchronized in early S phase have duplicated their SPBs (Byers and Goetsch, 1975), the *cdc31-2* cells in this experiment should divide normally once and fail to duplicate their SPBs in G1 of the subsequent cell cycle. After release at 37°C, both *cdc31-2* and wild-type cells went through one division. After this first cycle, *cdc31-2* cells arrested with large buds while wild-type cells completed a second division (Fig. 2 *C*). H1 kinase activity rose and fell during the first division in both cultures, as expected during normal mitosis. In the second cycle, this activity rose at about the same time in the wild-type and *cdc31-2* cultures. After this, H1 kinase activity dropped in wild-type cells but remained high in *cdc31-2* cells, coincident with large-budded arrest

(Fig. 2, *A* and *B*). Flow-cytometric analysis showed that cells with G2 DNA content appeared in both wild-type and *cdc31-2* cultures at similar times, indicating that DNA synthesis was not slowed in *cdc31-2* cells as they came to arrest (not shown). Therefore, progress through S phase into mitosis, with coincident activation of $p34^{CDC28}$, is apparently not affected by failure of SPB duplication due to the *cdc31-2* allele.

To determine if the features of *cdc31-2* monopolar arrest are restricted to the *cdc31-2* lesion, we performed a similar set of experiments on an *mps2-1* mutant strain. The *mps2-1* mutation disrupts insertion of the nascent SPB into the nuclear envelope, a late step in SPB duplication. As with *cdc31-2*, the *mps2-1* lesion leads to accumulation of cells with large buds and G2 DNA content at restrictive temperatures (Winey et al., 1991). Synchronized *mps2-1* (WX178-3c) cells at restrictive temperatures behaved as *cdc31-2* cells did, arresting with large buds and elevated H1 kinase activity (not shown).

cdc31-2, mad1-1 Cells Fail to Arrest at 37°C

Failure of SPB duplication gives rise to an aberrant monopolar spindle. In this case, mitotic arrest may depend on the function of a mitotic checkpoint that monitors spindle structure. To test this idea, we examined the cell cycle phenotype of a *cdc31-2, mad1-1* double mutant. The *mad1-1* mutation renders cells unable to arrest mitosis upon nocodazole treatment, and is thought to disrupt the checkpoint monitoring mitotic spindle function. (Li and Murray, 1991). Asynchronous *cdc31-2* (ELW65-9d) and double mutant *cdc31-2, mad1-1* (ELW112-3a) cells were shifted to 37°C for 4 h. Double mutant *cdc31-2, mad1-1* cells exhibited no evidence of cell cycle arrest. After this shift, budding morphology was as follows: in the *cdc31-2* culture, 94% of the cells were large budded, and 6% were unbudded; in the *cdc31-2, mad1-1* culture, 47% were unbudded, 29% were large budded, 5% were small budded, and 19% were multiply budded. In contrast to the flow-cytometric profile of *cdc31-2* cells exposed to 37°C for 4 h, flow cytometry of the 37°C-treated *cdc31-2, mad1-1* culture revealed that this culture contained cells with higher than normal DNA content as well as cells with apparently lower than normal DNA content (Fig 3). The latter cells were most likely aploid or hypoploid daughter cells resulting from monopolar mitosis followed by cytokinesis; Winey et al. (1991) and Baum et al. (1988) showed that such cells are produced when cytokinesis occurs in cells with malformed spindles. Taken together with the lack of morphologically defined arrest, these data suggest that cytokinesis occurred in many of the *cdc31-2, mad1-1* cells without proper chromosome segregation and that such divisions were followed by subsequent DNA replication in the cell bodies that received chromosomal DNA. The presence of multiply budded cells suggests that cytokinesis was not completely efficient, however. The rise and fall of H1 kinase activity in synchronous *cdc31-2, mad1-1* (ELW112-3a) cells at 37°C resembled that of wild-type cells (not shown), in contrast to the sustained elevated activity seen in *cdc31-2* single mutant cells. Therefore, the *MAD1* checkpoint function is required for *cdc31-2* arrest as it is for nocodazole arrest, although *mad1-1* strains do not undergo cytokinesis efficiently in nocodazole (see Fig. 8).

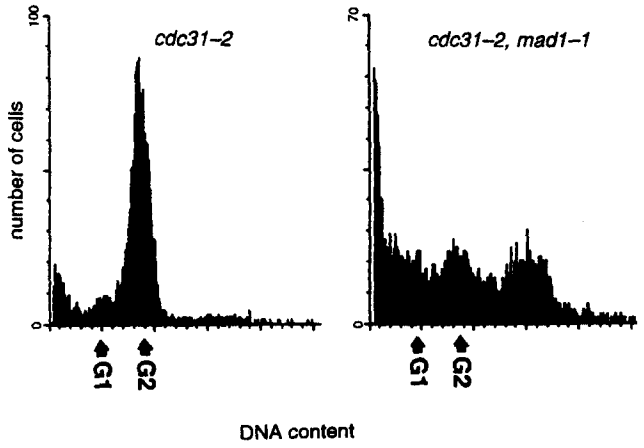


Figure 3. Flow-cytometric analysis of DNA content in *cdc31-2* and *cdc31-2, mad1-1* cells at restrictive temperature. Cultures of *cdc31-2* cells (ELW65-9d) arrest with G2 DNA content after 4 h at 37°C, while cultures of *cdc31-2, mad1-1* cells (ELW112-3a) do not exhibit such an arrest. Note presence of cells with higher and lower DNA content than normal. The x-axis is relative DNA content, and the y-axis indicates cell number. The approximate positions of the centers of G1 and G2 DNA content peaks in these cultures before shift to 37°C (*arrows*) are labeled G1 and G2.

mps1-1 Cells Fail to Duplicate Their SPBs and Do Not Arrest the H1 Kinase Activity Cycle

The cell cycle phenotype of *mps1-1* cells is strikingly different from the *cdc31-2* and *mps2-1* phenotypes. Winey et al. (1991) showed that *mps1-1* cells conditionally failed in SPB duplication without apparent cell cycle arrest. If this phenotype reflects a failure of checkpoint control, *mps1-1* cells should go through S phase, M phase, and cytokinesis with wild-type timing, and $p34^{CDC28}$ activity should rise and fall as in wild-type cells. Wild-type (WX257-5c) and *mps1-1* (WX241-2b) cultures synchronized in G1 with α-factor were released into 30°C medium (restrictive for *mps1-1*) and sampled at intervals for measurement of bulk H1 kinase activity, flow-cytometric measurement of DNA content, and scoring of budding morphology. The rise and fall of H1 kinase activity in a synchronized *mps1-1* culture was similar to wild-type in both amplitude and timing (Fig. 4, *A* and *B*). The percentages of large-budded cells present in the wild-type and *mps1-1* cultures rose until ~115 min after release from pheromone arrest. After this, the percentage of large-budded cells fell in both cultures (Fig. 4 *C*), as percentage of unbudded cells rose (Fig. 4, legend), suggesting that cytokinesis occurred with similar timing in *mps1-1* and wild-type strains. Flow cytometry showed that aploid/hypoploid cell bodies appeared in the *mps1-1* culture after 115 min and that cells with greater than normal ploidy subsequently emerged (not shown); this is consistent with previously reported analysis of the *mps1-1* phenotype (Winey et al., 1991). These results demonstrate that *mps1-1* cells do not inhibit mitotic progress or the cycle of $p34^{CDC28}$ activity when SPB duplication fails, as *cdc31-2* or *mps2-1* cells do.

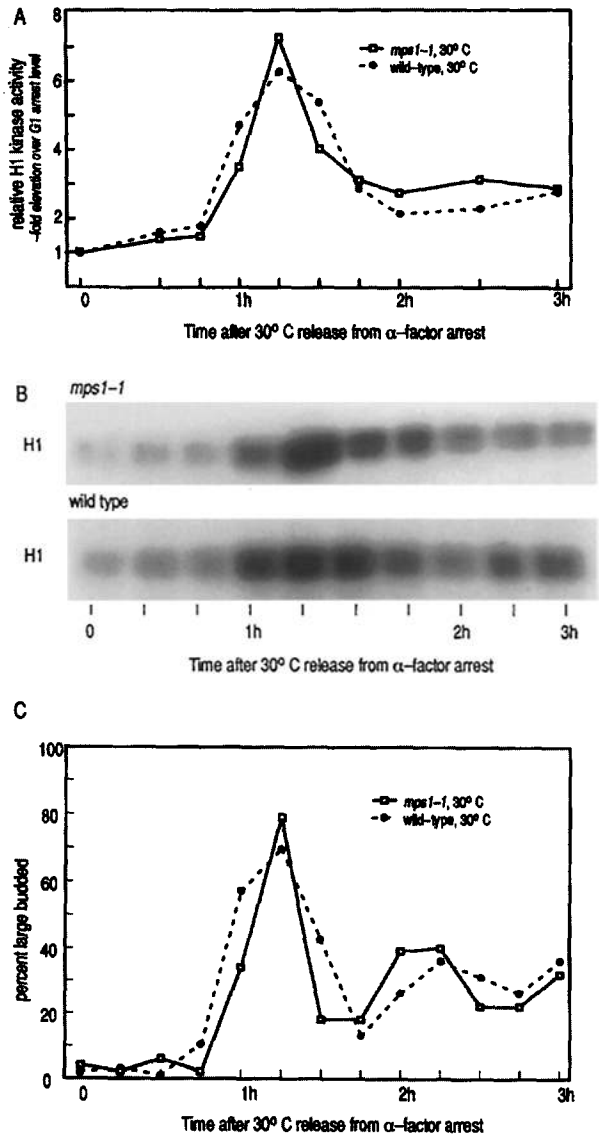


Figure 4. Cell cycle events in synchronized *mps1-1* and wild-type cultures at 30°C. (*A*) H1 kinase activity in whole cell extracts of *mps1-1* (WX241-2b) and wild-type (WX257-5c) cultures synchronized at START. (*B*) Raw data for *A*. (*C*) The percentage of cells in these cultures that had large buds at successive times. The percentage of cells in the *mps1-1* culture that were unbudded following release at 30°C rose from 11.5% at 75 min to 71% at 105 min; the percentage of cells in the wild-type culture that were unbudded rose from 18% at 75 min to 72.5% at 105 min.

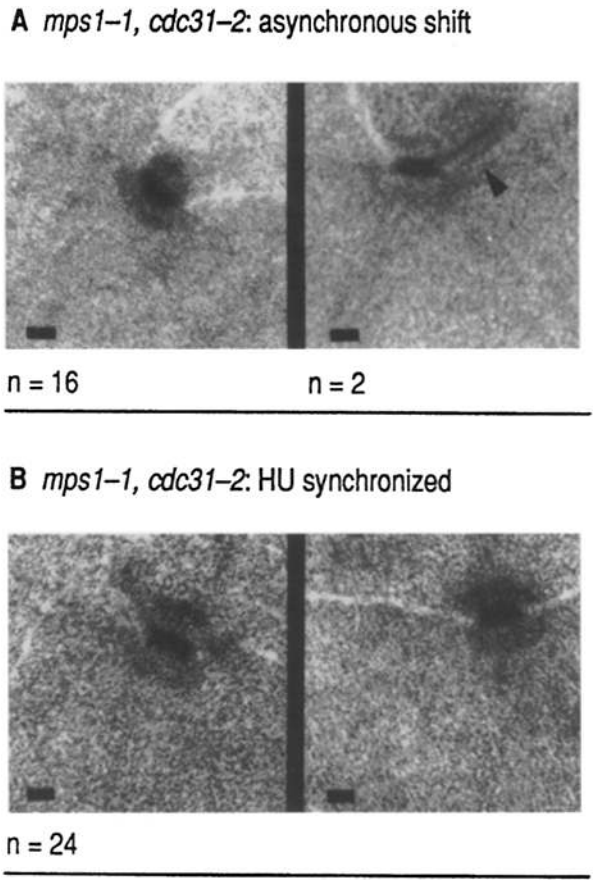


Figure 5. EM of SPB structure in a *cdc31-2*, *mps1-1* double mutant strain (ELW78-7c) at 37°C. (*A*) Electron micrographs of SPBs from an asynchronous culture shifted to 37°C. Most SPB morphologies are characteristic of *cdc31-2* single mutants, lacking pronounced half-bridge structures. More rarely, SPBs exhibit pronounced half bridges (*arrowhead*), as in *mps1-1* single mutants. (*B*) Electron micrographs of SPBs from cells synchronized by hydroxyurea treatment before transfer to 37°C. Such cells uniformly exhibit SPB structure characteristic of *cdc31-2* single mutants. In both cultures, pole morphology characteristic of *cdc31-2* single mutants predominates.

mps1-1, cdc31-2 Cells Have the cdc31-2 SPB Phenotype but Do Not Arrest Mitosis

The *mps1* phenotype shows that failure of SPB duplication does not always lead to cell cycle arrest. However, the *mps1* cell cycle phenotype could be due to structural features of SPBs in *mps1* strains at restrictive temperatures. The aberrant unduplicated SPB formed in *mps1* cells at restrictive temperatures has a distinctive, enlarged half-bridge structure relative to SPBs found in wild-type or *cdc31-2* cells when viewed in the electron microscope (Winey et al., 1991). Alternatively, the *MPS1* gene product may itself play an important role in signaling mitotic arrest after SPB duplication failure. To distinguish these possibilities, we examined SPB morphology and cell cycle phenotypes of *cdc31-2*, *mps1-1* double mutants. To ensure that phenotypes observed are not specific to the *mps1-1* allele, we examined the cell cycle phenotypes of double mutants between *cdc31-2* and four other distinct *mps1* alleles.

In these experiments, the synchronization protocol is important. The *cdc31-2* execution point precedes that of *mps1-1*, and both execution points precede S phase (Winey et al., 1991). Upon release into 37°C medium, an *mps1-1*, *cdc31-2* culture synchronized in S should be affected first by the *cdc31-2* mutation. If *cdc31-2* is epistatic to *mps1-1* with respect to SPB phenotype, all cells in this culture should have an unduplicated SPB characteristic of *cdc31-2* single mutants. In contrast, an asynchronous double mutant culture should contain some cells that have passed the *cdc31-2* execution point but not the *mps1-1* execution point. Asynchronous *mps1-1*, *cdc31-2* cells shifted to restrictive temperatures should exhibit a mixture of *cdc31-2*

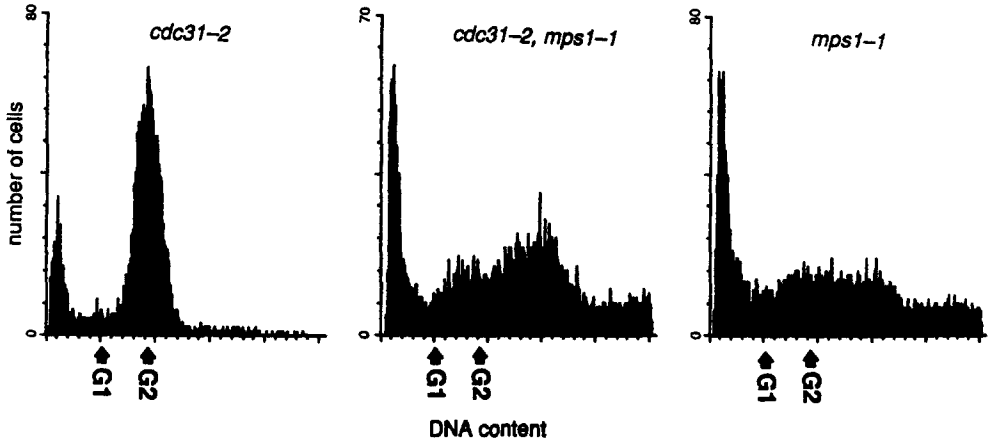
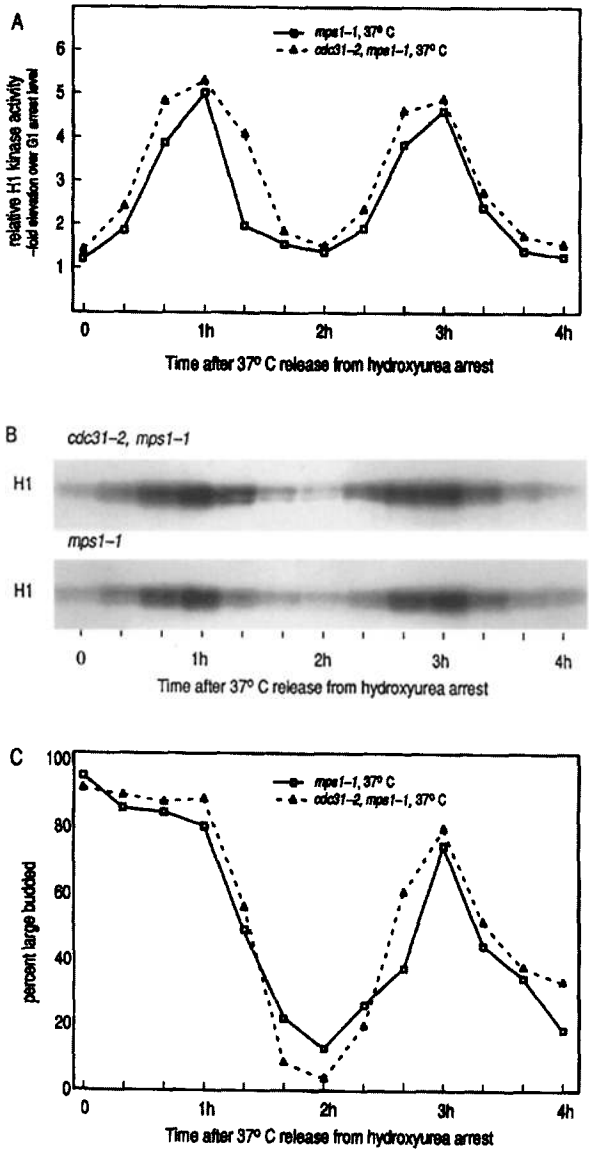


Figure 6. Flow-cytometric analysis of DNA content in double mutant *cdc31-2, mps1-1* and single mutant *cdc31-2* and *mps1-1* cells at restrictive temperature. Unlike *cdc31-2* (ELW65-9d) cells, neither *cdc31-2, mps1-1* (ELW78-7c) nor *mps1-1* (ELW92) cells exhibit cell cycle arrest. Note presence of cells with higher and lower than normal DNA content. The x-axis is relative DNA content, and the y-axis indicates cell number; each histogram represents 10,000 cells. The approximate positions of the centers of G1 and G2 DNA content peaks in the cultures before shift to 37°C (*arrows*) are labeled G1 and G2.



and *mps1-1* SPB phenotypes. We synchronized *mps1-1, cdc31-2* (ELW78-7c) cells in early S phase, released them into medium at 37°C, and fixed the cells for EM 4 h after introduction into restrictive temperature medium. We also shifted an asynchronous *mps1-1, cdc31-2* (ELW78-7c) culture to 37°C and fixed the cells for EM after 4 h. A small fraction of cells from an asynchronous *mps1-1, cdc31-2* (ELW78-7c) culture shifted to restrictive temperatures had unduplicated SPBs with a large half bridge, characteristic of *mps1-1* single mutants, while most had the *cdc31-2*–type SPB with little or no observable half bridge (Fig. 5 *A*). In the synchronized double mutant culture, no SPBs with pronounced half bridges were observed; rather, all SPBs observed were characteristic of those found in *cdc31-2* single mutants (Fig. 5 *B*). No bipolar spindles were found in either culture. This experiment demonstrates that *cdc31-2* is epistatic to *mps1-1* with respect to SPB duplication phenotype.

Although synchronized *cdc31-2, mps1-1* cells formed SPBs characteristic of *cdc31-2* single mutants at 37°C, they failed to arrest division like *cdc31-2* single mutant cells. Both *mps1-1* (WX241-2b) and *mps1-1, cdc31-2* (ELW78-7c) cultures were synchronized in early S phase with hydroxyurea, released from arrest into 37°C medium, and sampled at intervals after release. Flow-cytometric analysis showed that both cultures failed to arrest with G2 DNA content (Fig. 6). After 4 h at 37°C, both cultures contained aploid or hypoploid bodies and cells with higher than normal ploidy, similar to *mps1-1* single mutant strains (Winey et al., 1991) and *cdc31-2, mad1-1* cells at 37°C (see Fig. 3). H1 kinase activity in the synchronized *cdc31-2, mps1-1*

Figure 7. Cell cycle events in synchronized *cdc31-2, mps1-1* and *mps1-1* cultures at 37°C. (*A*) H1 kinase activity in whole cell extracts of *cdc31-2, mps1-1* (ELW78-7c) cells and *mps1-1* (ELW92) cultures synchronized in early S phase and released into 37°C medium. (*B*) Raw data for *A*. (*C*) The percentage cells of cells in these cultures that had large buds at successive times.

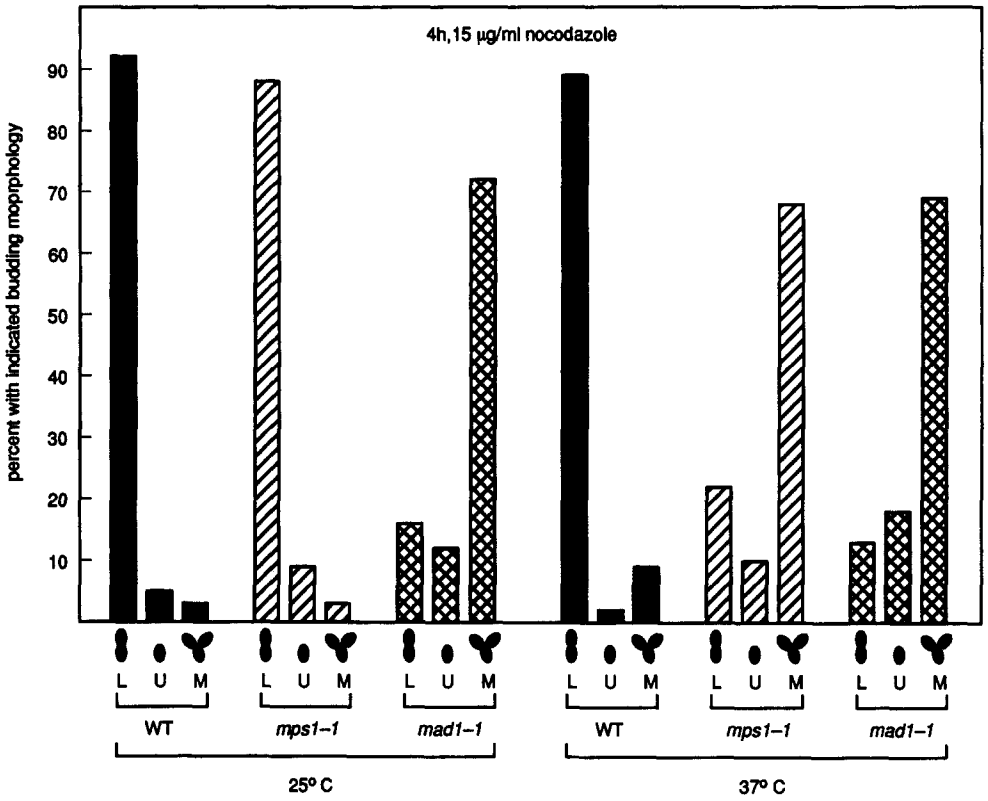


Figure 8. Failure of *mps1-1* cells to arrest in nocodazole at 37°C. Budding morphology distributions in wild-type (WX257-5c), *mps1-1* (WX241-2b), and *mad1-1* (DRL139-7b) cultures after 4-h incubation in medium containing 15 µg/ml nocodazole at either 25°C or 37°C. 200 cells were scored from each culture.

double mutant culture rose and fell in concert with budding and cytokinesis (Fig. 7), much as in *mps1-1* or wild-type strains (see Figs. 2 and 4). Hence, *mps1-1* is epistatic to *cdc31-2* with respect to cell cycle phenotype. Because the SPBs in synchronized *cdc31-2*, *mps1-1* cells after failure of SPB duplication are characteristic of a *cdc31-2* single mutant, the *mps1* cell cycle phenotype cannot be attributed to abnormal SPB morphology. The cell cycle phenotypes of double mutants carrying *cdc31-2* and one of four distinct *mps1* alleles (ELW132-32b, ELW134-6c, ELW136-2a, ELW138-14c) were also examined. They behaved similarly to *cdc31-2*, *mps1-1* cells, failing to arrest mitosis (not shown).

mps1 Cells Are Conditionally Unable to Arrest in Nocodazole

Given our observation that *mps1* cells do not arrest division when the mitotic spindle is malformed due to SPB duplication failure, we asked if *mps1* strains fail to arrest when the spindle is destroyed by the microtubule-depolymerizing drug nocodazole. While *mps1-1* cells arrested upon treatment with nocodazole at 25°C (not shown), they did not arrest in nocodazole containing medium at 37°C. Wild-type (WX257-5c), *mps1-1* (WX241-2b), and *mad1-1* (DRL139-7b) cultures synchronized at 25°C in G1 with α-factor were split four ways and released into four different media conditions: nocodazole-containing medium at 25°C and 37°C, and drug-free medium at 25°C or 37°C. The distributions of budding morphologies present after 4 h in 25°C nocodazole-containing medium are shown in the left side of Fig. 8. Wild-type and *mps1-1* cells arrested with large buds, while *mad1-1* cells became multiply budded. Flow cytometry indicated that wild-type and *mps1-1* cultures arrested with G2 DNA content, while *mad1-1* cells went on to higher ploidy, indicating that S phase had been reinitiated in the absence of chromosome segregation (not shown). In contrast, after 4 h in 37°C medium containing nocodazole, *mps1-1* strains behaved as *mad1-1* cells did, becoming mostly multiply budded (Fig. 8, *right*). Flow-cytometric analysis showed that while wild-type cells arrested with G2 DNA content at 37°C, (Fig. 9 *A*), *mps1-1* and *mad1-1* cells did not (Fig. 9, *B* and *C*). Instead, *mps1-1* and *mad1-1* cells went on to reduplicate their DNA, evidenced by the appearance of cells with higher ploidy in later time points. This experiment was repeated with strains carrying four other distinct *mps1* alleles (AS127-1a, AS132-3a, AS126-5a, AS131-2d). None of these *mps1* mutant strains exhibited cell cycle arrest in the presence of nocodazole at 37°C that could be detected by flow cytometry or examination of budding morphology (not shown).

Winey et al. (1991) and Baum et al. (1988) demonstrated that aploid and hypoploid cells are generated when cytokinesis occurs in cells with malformed mitotic spindles. It appears that cytokinesis, as evidenced by the appearance of unbudded aploid and hypoploid cell bodies, does not occur efficiently in nocodazole-treated *mad1-1* and *mps1* strains in this experiment. Although the *mps1* cells at restrictive temperature and *mad1-1* cells clearly rebudded and went on to subsequent rounds of DNA synthesis in the presence of nocodazole, neither strain produced a large number of aploid or hypoploid cell bodies. Instead, both *mad1-1* and *mps1-1* strains mostly formed multiply budded polyploid cells. This phenotype is com-

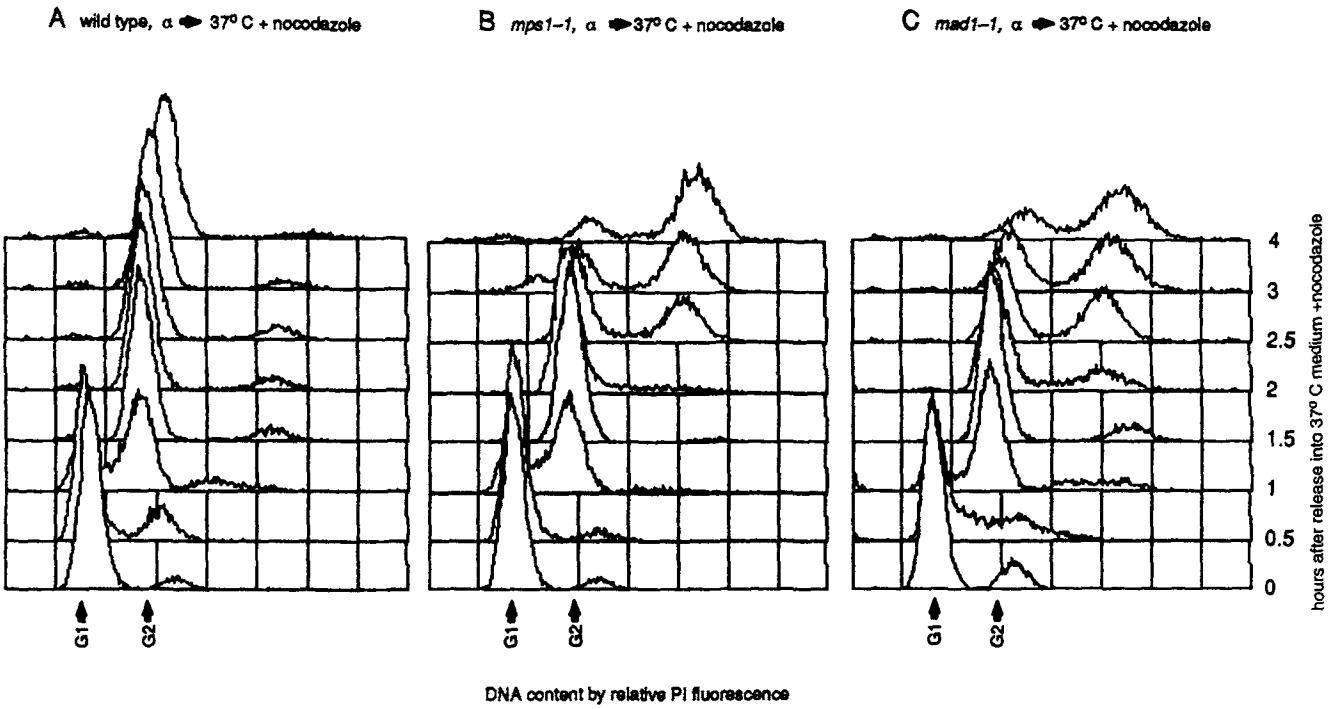


Figure 9. Failure of *mps1-1* cells to arrest in nocodazole at 37°C. Flow-cytometric profiles of (*A*) wild-type (WX257-5c) cells, (*B*) *mps1-1* (WX241-2b), and (*C*) *mad1-1* (DRL139-7b) cells sampled at intervals after release from α-factor arrest into 37°C medium containing nocodazole. Note the appearance of polyploid cells at later times (*B* and *C*). Sampling times after release from α-factor arrest are noted on the left side of *C*. The x-axis is relative DNA content, and the y-axis indicates cell number; each histogram represents 10,000 cells. The approximate positions of centers of G1 and G2 DNA content peaks from asynchronous cultures of these strains (*arrows*) are labeled G1 and G2.

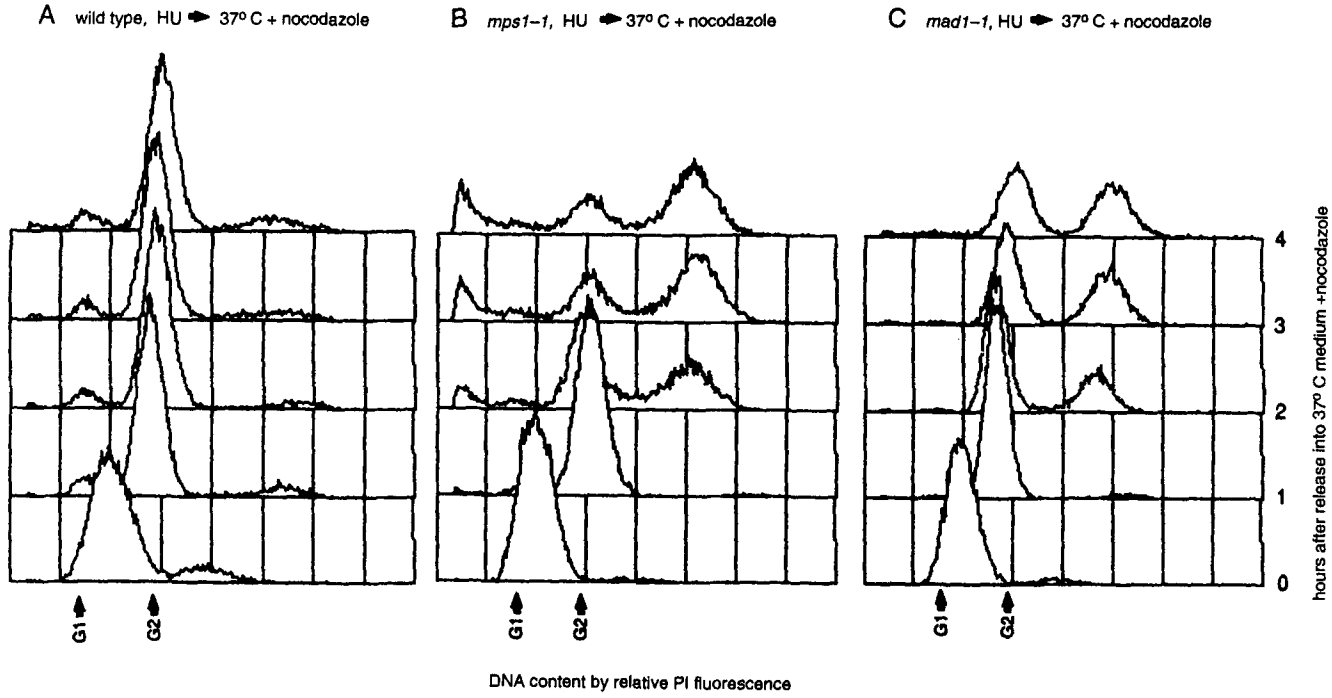


Figure 10. Failure of hydroxyurea-synchronized *mps1-1* cells to arrest in nocodazole at 37°C. Flow-cytometric profiles of (*A*) wild-type (WX257-5c), (*B*) *mps1-1* (WX241-2b), and (*C*) *mad1-1* (DRL139-7b) cells sampled at intervals after release from hydroxyurea arrest into 37°C medium containing nocodazole. As in Fig. 9, *B* and *C*, note the appearance of polyploid cells at later times (*B* and *C*). Sampling times after release from α-factor arrest are noted on the left side of *C*. Same strains released from hydroxyurea arrest into 37°C medium containing nocodazole. Notation and cell number are as in Fig. 9.

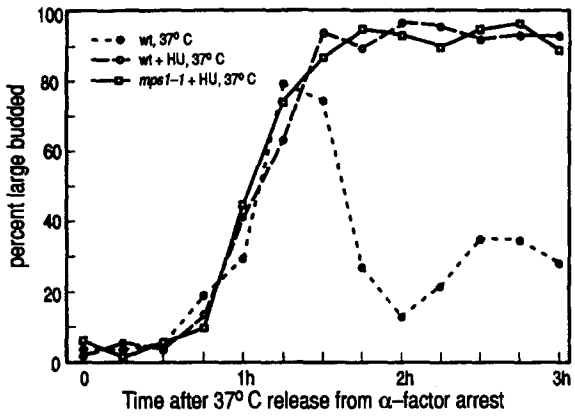


Figure 11. Response of wild-type and *mps1-1* cells to hydroxyurea at 37°C. Wild-type and *mps1-1* strains (WX257-5c, WX241-2b) were released from α-factor arrest into 37°C medium either containing 0.1 M hydroxyurea (*HU*) or lacking drug. Accumulation of large budded cells over time is shown.

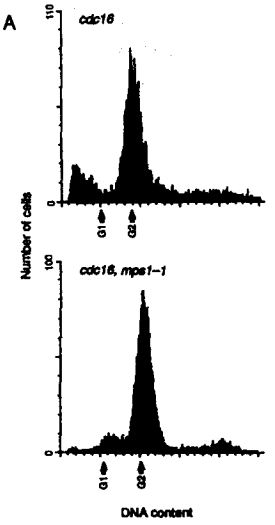
mon among *mad* and *bub* mutants in nocodazole (Hardwick, K., personal communication; Roberts, B. T., personal communication; Hoyt et al., 1991). We suspect that inefficient cytokinesis may be a secondary effect of nocodazole treatment rather than an *mps1* or *mad1* phenotype per se: both *mps1* and *cdc31-2*, *mad1-1* strains apparently execute cytokinesis after failure of SPB duplication when no nocodazole is present (Winey et al., 1991; see aploid/hypoploid cells in Figs. 3 and 6).

The *mps1* strains in the above experiment experienced two kinds of spindle defects: SPB duplication failure and microtubule depolymerization. Absence of mitotic arrest might therefore result from this compound defect. To determine if failure of nocodazole arrest in *mps1* strains requires failure of SPB duplication, we arrested the wild-type, *mps1-1*, and *mad1-1* strains mentioned above at 25°C in S phase with hydroxyurea, and then released them into 37°C medium containing nocodazole. As mentioned earlier, *S. cerevisiae* cells arrested in hydroxyurea have completed SPB duplication and formed short bipolar mitotic spindles (Byers and Goetsch, 1974). Upon release from hydroxyurea arrest into nocodazole containing medium at 37°C, wild-type strains arrested with large buds and G2 DNA content (Fig. 10 *A*). In contrast, neither *mps1* nor *mad1-1* strains arrested in the presence of nocodazole, instead rebudding and going on to subsequent DNA synthesis (Fig. 10, *B* and *C*). As above, the appearance of cells with high ploidy is evidence of this failure to arrest mitosis in the absence of a functional mitotic spindle. Four other *mps1* alleles (AS127-1a, AS132-3a, AS126-5a, AS131-2d) shared this phenotype upon release from hydroxyurea arrest into 37°C medium containing nocodazole (not shown). Hence, conditional failure of mitotic arrest in nocodazole-treated *mps1* cells is apparently not caused by failure of SPB duplication.

Wild-type MPS1 Is Not Required for Hydroxyurea Arrest or Various cdc Arrests

To determine if *mps1* alleles disrupt cells' ability to arrest division in response to inhibition of DNA synthesis, we examined how *mps1* cells at restrictive temperatures responded to hydroxyurea. Wild-type (WX257-5c) and *mps1-1* (WX241-2b) cells were synchronized in G1 with α-factor at 25°C and then released from this arrest into 25°C and 37°C medium containing or lacking hydroxyurea. Wild-type and *mps1* cultures released in the presence of hydroxyurea arrested with large buds (Fig. 11) and G1/S phase DNA content (not shown). H1 kinase activity in hydroxyurea-treated wild-type and *mps1-1* cultures rose relatively slowly, reaching a peak ~3.5 h after release from α-factor arrest into hydroxyurea-containing medium (not shown). Therefore, the wild-type *MPS1* gene product is not required for hydroxyurea arrest.

We examined the interaction of *mps1-1* with *cdc9*, *cdc13*, *cdc16*, *cdc20*, and *cdc23* mutations and found no evidence that *mps1-1* affects the cell cycle arrest phenotypes associated with these mutations. The behavior of *cdc16*, *mps1-1* double mutant cells is representative of the phenotypes observed in these double mutant strains. Asynchronous *cdc16* (H16C1A5) and *cdc16*, *mps1-1* cells (WX265-1b) were shifted from 25°C to 37°C, and the culture was



B

budding morphology after 4h at 37° C

strain	% unbudded	% large budded
WT	47	31
mps1-1	42	27
cdc9	4	89
cdc9, mps1-1	3	92
cdc13	8	92
cdc13, mps1-1	4	91
cdc16	9	88
cdc16, mps1-1	4	91
cdc20	7	93
cdc20, mps1-1	5	91
cdc23	4	92
cdc23, mps1-1	10	86

Figure 12. Cell cycle phenotypes of various *cdc* mutants in combination with *mps1-1*. (*A*) Flow-cytometric profiles of *cdc16* (H16C1A5) and *cdc16*, *mps1-1* (WX265-1b) strains shifted to 37°C for 4 h. (*B*) Percentages (rounded to the nearest integer) of cells with unbudded and large-budded morphology after a 4-h incubation at 37°C in various mutant strains. Percentages of cells with small budded or aberrant morphology are not shown.

sampled hourly for 6 h. Cells with G2 DNA content (Fig. 12 *A*) and large buds (Fig. 12 *B*) accumulated, indicating that the *cdc16* arrest phenotype is epistatic to the *mps1-1* cell cycle phenotype. Double mutant budding phenotypes are summarized in Fig. 12 *B*. It is worth noting that *cdc9* and *cdc13* arrests are dependent on the *RAD9* checkpoint (Weinert and Hartwell, 1993), indicating that the *mps1-1* lesion does not interfere with that checkpoint.

Discussion

The experiments reported here demonstrate that the wild-type *MPS1* gene product is required for an M-phase checkpoint that allows yeast cells to arrest division when the mitotic spindle is disrupted or malformed. This defines a novel role for the *MPS1* gene product, an essential protein kinase also required for SPB duplication in G1 (Lauze et al., 1995; Poch et al., 1994.; Winey et al., 1991). We have shown that failure of SPB duplication normally results in arrest of mitosis with high $p34^{CDC28}$ activity. In *mps2-1* and *cdc31-2* strains, cell cycle arrest occurs after DNA replication is complete and $p34^{CDC28}$ kinase activity rises, suggesting that events leading to the fall of $p34^{CDC28}$ kinase activity are inhibited when SPB duplication fails. In contrast, *cdc31-2, mad1-1* cells fail to arrest mitosis, indicating that the *MAD1*-dependent mitotic checkpoint (Li and Murray, 1991) is required for mitotic arrest after failure of SPB duplication. A priori, it seems reasonable that some of the same cell cycle regulators are involved in both nocodazole arrest and mitotic arrest in the presence of a monopolar spindle, because both conditions are fundamentally defects in spindle integrity. These findings suggest that the *MPS1* gene product acts in the *MAD1*-dependent mitotic checkpoint as well as in SPB duplication, the earliest structural step in bipolar spindle formation.

In *mps1* mutants, both conditional SPB duplication failure and checkpoint defects are evident. Cultures of *mps1-1* cells synchronized in G1 and released at restrictive temperatures go through budding, DNA replication, rise and fall of H1 kinase activity, and cytokinesis with essentially normal timing, ultimately committing aberrant monopolar cell division. A number of experiments performed here attempt to separate these phenotypes. Analysis of the *cdc31-2, mps1-1* double mutant phenotype is particularly informative, showing that lack of mitotic arrest in cells carrying the *mps1-1* lesion cannot be attributed to structural defects of the SPB visible in the electron microscope. Furthermore, we have found that *mps1* mutant cells are conditionally unable to arrest mitosis in the presence of nocodazole. This phenotype is shared by *mad1-1* cells, although *mad1-1* is not a conditional mutation. It is perhaps most telling that *mps1-1* cells conditionally fail to arrest in nocodazole after synchronization in S phase with hydroxyurea. Because SPB duplication and short spindle formation are complete in hydroxyurea-arrested cells, this treatment separates the *mps1-1* SPB duplication and mitotic checkpoint phenotypes. When *mps1-1* cells were released from hydroxyurea arrest into restrictive temperature medium, they appeared to undergo one normal divison, suggesting that the *mps1-1* mutation does not adversely affect spindle function after SPB duplication is complete. We propose that after SPB duplication is complete, Mps1p activity is also required for checkpoint-mediated restraint of M-phase progress upon disruption of the mitotic spindle. Because both *MAD1* and *MPS1* gene products are required for mitotic arrest in the presence of a malformed or disrupted mitotic spindle, it is possible that these two proteins are involved in the same pathway linking cell cycle progress to the status of the mitotic apparatus.

MPS1's cell cycle regulatory role appears to be specifically within the mitotic checkpoint monitoring spindle integrity. Hydroxyurea arrest and *cdc* mutant arrests that depend on *RAD9* and other S-phase checkpoint activities are unaffected by the *mps1-1* mutation. It is also interesting that the *cdc16* and *cdc23* mutations are epistatic to *mps1-1* with respect to cell cycle phenotype, considering that the *CDC16* and *CDC23* gene products are required for ubiquitin-mediated proteolysis that accompanies M-phase exit (Irniger et al., 1995). This suggests that the effects of *mps1-1* on checkpoint regulation are upstream of cyclin degradation. Although it functions in a different checkpoint, *MPS1* is comparable to the *SAD1/MEC3* gene, which is essential for viability and required for arrest of cell division specifically when DNA is damaged or unreplicated (Allen et al., 1994; Weinert et al., 1994).

MPS1 is apparently among a class of checkpoint components that serve dual roles: one in an essential process and another in regulation of the cell cycle when that process is perturbed. Li and Deshaies (1993) propose three classes of checkpoint regulators. One class of gene products might originate a signal to arrest when processes are disturbed. These proteins might also participate in critical processes or be components of structures important for cell division. Such gene products would likely be essential for normal growth and interact with a second class of "transducer" proteins that relay and amplify a signal to arrest the cell cycle. Ultimately, a signal would be relayed to a third class of effector proteins that can directly inhibit cell cycle progression. DNA polymerase ϵ itself is an example of the first class of checkpoint regulators: mutations in the COOH-terminal region of *S. cerevisiae POL2* disrupt S-phase checkpoint activity (Navas et al., 1995). The *Schizosaccharomyces pombe cut5* and *cdc18* gene products, which are critical for S-phase progression and required for arrest of the cell cycle when DNA metabolism has gone awry, also define this first class of checkpoint elements (Saka and Yanagida, 1993; Kelly et al., 1993; Saka et al., 1994). In contrast, checkpoint components that transduce information need not be essential for growth under ideal conditions because the signals they pass might never be generated in the absence of perturbation. The *RAD9* and *MAD1* gene products, for example, are both dispensable unless specific problems arise and are thought to fall into this transducer class (Li and Deshaies, 1993). The *MPS1* gene product is essential for viability, involved in SPB duplication, and required for inhibition of mitotic progress when spindle structure is disrupted. Therefore, the *MPS1* protein likely stands among the class of checkpoint components that both participate in an essential process and originate a signal to arrest division when the process fails. In this case, we propose that the *MPS1* gene product acts in both the G1 process of SPB duplication and the mitotic checkpoint monitoring spindle integrity.

The *MPS1* gene product is a protein kinase that phosphorylates itself on serine, threonine, and tyrosine and exogenous substrates on serine and threonine in vitro (Lauze et al., 1995). The *mps1-1* mutant lesion falls within the kinase domain, as do four other known alleles that exhibit the same SPB duplication and cell cycle phenotypes as *mps1-1* (Schutz, A., and M. Winey, personal communication). We predict that Mps1p's enzymatic activity is of key importance to both the protein's SPB duplication and mitotic checkpoint functions. For example, if Mps1p's kinase activity normally rises in G1 to drive SPB duplication and is inhibited once a bipolar spindle is assembled, failure or delay of SPB duplication would result in prolonged activation of the enzyme and cell cycle arrest. Alternately, there may be a burst of Mps1p kinase activity at the time of SPB duplication, with the enzyme activated a second time in mitosis if the process fails or if the spindle is destroyed by other insult. In either case, *MPS1* kinase activity might work in G2/M to inhibit mitotic progress in a *MAD1*-dependent manner, while continuing to drive processes that promote SPB duplication. Phosphorylation of *MAD1* correlates with mitotic arrests requiring that protein (Hardwick and Murray, 1995). It will be interesting to see if Mps1p phosphorylates Mad1p or other proteins believed to transduce cell cycle–arresting signals when mitotic spindle integrity is compromised.

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