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A checkpoint that monitors cytokinesis in *Schizosaccharomyces pombe*

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SUMMARY

Cell division in *Schizosaccharomyces pombe* is achieved through the use of a medially positioned actomyosin ring. A division septum is formed centripetally, concomitant with actomyosin ring constriction. Genetic screens have identified mutations in a number of genes that affect actomyosin ring or septum assembly. These cytokinesis-defective mutants, however, undergo multiple S and M phases and die as elongated cells with multiple nuclei. Recently, we have shown that a mutant allele of the *S. pombe drc1*$^+$/*cps1*$^+$ gene, which encodes a 1,3-β-glucan synthase subunit, is defective in cytokinesis but displays a novel phenotype. *drc1*-191/*cps1*-191 cells are capable of assembling actomyosin rings and completing mitosis, but are incapable of assembling the division septum, causing them to arrest as binucleate cells with a stable actomyosin ring. Each nucleus in arrested *cps1*-191 cells is able to undergo S phase but these G_2 nuclei are significantly delayed for entry into the M phase. In this study we have investigated the mechanism that causes *cps1*-191 to block with two G_2 nuclei. We show that the inability of *cps1*-191 mutants to proceed through multiple mitotic cycles is not related to a defect in cell growth. Rather, the failure to complete some aspect of cytokinesis may prevent the G_2/M transition of the two interphase-G_2 nuclei. The G_2/M transition defect of *cps1*-191 mutants is suppressed by a mutation in the *wee1* gene and also by the dominant *cdc2* allele *cdc2*-1w, but not the *cdc2*-3w allele. Transient depolymerization of all F-actin structures also allowed a significant proportion of the *cps1*-191 cells to undergo a second round of mitosis. We conclude that an F-actin and Wee1p dependent checkpoint blocks G_2/M transition until previous cytokinesis is completed.

Key words: Cytokinesis, Checkpoint, *Schizosaccharomyces pombe*, Cps1p

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe* has emerged as an attractive model organism in the recent years for the study of cytokinesis (Balasubramanian et al., 1997; Gould and Simanis, 1997; Le Goff et al., 1999a). Cell division in *S. pombe* is achieved through a medially positioned actomyosin ring structure, which is assembled at the onset of anaphase (Bahler et al., 1998). At the end of anaphase and following disassembly of the mitotic spindle, the actomyosin ring constricts. The division septum is assembled in a centripetal manner concomitant with actomyosin ring constriction. Genetic studies in *S. pombe* have identified genes important for various steps in cytokinesis (Nurse et al., 1976; Chang et al., 1996; Balasubramanian et al., 1998; Bahler and Pringle, 1998; Bahler et al., 1998; Liu et al., 1999).

The genes *mid1*, *plo1* and *pom1* are required to position the actomyosin ring and the division septum, but are not required for assembly of either the actomyosin ring or the division septum. It has been proposed that Mid1p and Plo1p act in a signaling pathway that integrates nuclear positioning with the position of the actomyosin ring (Sohrmann et al., 1996; Bahler et al., 1998). The genes *cdc3*, *cdc4*, *cdc8*, *cdc12*, *rng2*, *rng3*, *rng4*, *rng5*/*myo2* and *act1* (collectively referred to as the *rng* genes) are required for the assembly of the actomyosin ring (Gould and Simanis, 1997; Eng et al., 1998; Kitayama et al., 1997; May et al., 1997; Bezanilla et al., 1997; Motegi et al., 1997; Naqvi et al., 1999). The identity of the products of the *rng* genes as actin cytoskeletal elements is consistent with the idea that they interact to effect actomyosin ring assembly. Following actomyosin ring assembly, the function of the ring component Cdc15p, a SH3 domain containing protein, is required to assemble F-actin patches adjacent to the actomyosin ring (Fankhauser et al., 1995; Balasubramanian et al., 1998). A third group of genes (collectively referred to as the *sid* genes, including *cdc7*, *cdc11*, *cdc14*, *sid1*, *sid2*, *spg1*/*sid3* and *sid4*), which regulates division septum assembly following actomyosin ring assembly, has also been identified from genetic studies (Nurse et al., 1976; Schmidt et al., 1997; Balasubramanian et al., 1998). The *sid* gene products encode signaling molecules that are localized to the spindle pole body (Sohrmann et al., 1998; Sparks et al., 1999). Furthermore, the product of the *sid2* gene has also been shown to localize to the division site late in mitosis (Sparks et al., 1999). The products of the *sid* genes are thought to initiate actomyosin ring constriction and division septum assembly in response to signals originating at the spindle pole body (Sohrmann et al., 1998; Sparks et al., 1999).

Recently, we have reported the characterization of a novel cytokinesis mutant, *drc1*-191/*cps1*-191 (Ishiguro et al., 1997; Liu et al., 1999; Le Goff et al., 1999b). In the remainder of this paper this gene will be referred to as *cps1*-191. Cps1p is a 1,3-β-glucan synthase subunit, which presumably acts in the assembly of the septum-polymer 1,3-β-glucan. Unlike other cytokinesis mutants, which accumulate multiple nuclei, the *cps1*-191 mutant arrests the cell cycle with a stable actomyosin ring and two G_2 nuclei, which are significantly delayed for entry into a subsequent M phase. In this study, we have investigated the mechanism causing the arrest of the *cps1*-191 mutant with two G_2 nuclei following failed cytokinesis. Based on a variety of genetic and physiological studies we conclude that the failure of cytokinesis in these cells is perceived by a 'cytokinesis-monitoring system' that delays entry into the next mitotic cycle. We show that Wee1p and an intact F-actin cytoskeleton are required for the G_2 arrest following failed cytokinesis in the *cps1*-191 mutant.

MATERIALS AND METHODS

S. pombe strains, media and reagents

The *S. pombe* strains used in this study are: *leu1*-32 *ura4*-D18 h- (wild type), *cps1*-191 (Liu et al., 1999), *cps1*-UV1 (this study), *cdc7*-24 (Nurse et al., 1976), *wee1*-50 (Nurse, 1975), *cdc12*-112 (Nurse et al., 1976), *cdc2*-3w (Fantes, 1981), *cdc2*-1w (Nurse and Thuriaux, 1980), *cps1*-191 *wee1*-50, *cps1*-191 *cdc2*-1w, *cps1*-191 *cdc2*-3w, *rad1*::*ura4* (Sunnerhagen et al., 1990) and *cps1*-191 *rad1*::*ura4*. YES medium was used for cell culture (Moreno et al., 1991). Genetic crosses were performed by mixing appropriate strains of opposing mating types on YPD plates, and recombinant strains were selected by tetrad dissection. Double mutants were typically isolated from NPD tetrads. The genotypes of double mutants were further confirmed by outcrosses to wild-type cells. Hydroxyurea (HU) and rhodamine-conjugated phalloidin were purchased from Sigma chemicals (St Louis, MO, USA) and Latrunculin A (LatA) was purchased from Molecular Probes (Eugene, OR, USA). HU was used at 12 mM final concentration while LatA was used at 100 μM final concentration. Cell stainings with DAPI and rhodamine-conjugated phalloidin were performed as described (Balasubramanian et al., 1997).

Isolation of the *cps1*-UV1 allele

To isolate cytokinesis-defective mutants, the *S. pombe* wild-type strain of the genotype *ura4*-D18 *leu1*-32 was mutagenized with UV light. Briefly, an overnight culture of this strain was plated on 35 large Petri dishes such that approximately 11,000 cells were seeded on each plate. The plated cells were exposed to short wavelength UV light for 1 minute, which was previously determined to result in 90% killing (K. Wong and M. Balasubramanian, unpublished observations), and allowed to recover and form colonies at 26°C. Colonies that formed at 26°C were replica-plated to 36°C on plates containing phloxin B to identify temperature-sensitive lethal mutants. One mutant isolated in this screen displayed a phenotype similar to that of *cps1*-191 (Liu et al., 1999) and was confirmed to be an allele of *cps1* (referred to as *cps1*-UV1).

RESULTS

The *cps1*-191 mutant arrests with two interphase G_2 nuclei and a stable actomyosin ring (Liu et al., 1999). The previously characterized cytokinesis mutants fall into two broad categories, the actomyosin ring assembly/function mutants (*rng* mutants) and the septum initiation defective mutants (*sid* mutants). To better characterize the *cps1*-191 nuclear division defect, we first quantified the rate of cell and nuclear division in wild-type cells, the *cps1*-191 mutant, a representative *rng* mutant (*cdc12*-112) and a representative *sid* mutant (*cdc7*-24) after the shift to the restrictive temperature (36°C) for these temperature-sensitive mutants. Wild-type cells grew and divided normally upon shift to 36°C, as seen by the increase in cell number and the number of nuclei/ml of the culture (Fig. 1A). By contrast, division of *cps1*-191, *cdc12*-112 and *cdc7*-24 cells ceased approximately 2 hours after the shift to 36°C (Fig. 1A). As expected, the number of nuclei continued to increase in *cdc12*-112 as well as in *cdc7*-24 cells until 6 hours after the shift to 36°C, whereas the number of nuclei increased only marginally in the case of the *cps1*-191 mutant (Fig. 1A). The *cps1*-191 cells did not become highly elongated and >90% of the cells had arrested with two interphase nuclei (Fig. 1B). However, *cdc12*-112 and *cdc7*-24 cells elongated substantially and accumulated between 4-8 nuclei (Fig. 1B). In agreement with previous results, the *rng* mutants accumulated nuclei more slowly compared to the *sid* mutants (Nurse et al., 1976; Gould and Simanis, 1997). Nevertheless, all *rng* mutants tested accumulated at least four nuclei upon shift to 36°C for 6 hours (Nurse et al., 1976; data not shown). By contrast, only 10% of *cps1*-191 cells contained four nuclei even 8 hours after shift to 36°C, while the rest of the cells were binucleate (Liu et al., 1999; data not shown). These studies showed that *cps1*-191 is a unique cytokinesis mutant that arrests with two interphase nuclei.

Since all our studies and conclusions on *cps1* were based on a single temperature-sensitive mutant allele of *cps1*, *cps1*-191, we sought to isolate additional mutant alleles. In a screen aimed at isolating cytokinesis defective mutants, we isolated four more alleles of *cps1* (see Materials and Methods; K. Wong, H. Wang and M. Balasubramanian, unpublished observations), which displayed effects similar to that observed in *cps1*-191. The characterization of one such mutant, *cps1*-UV1, which carries a mutation different from that in *cps1*-191 (J. Liu and M. Balasubramanian, unpublished observations), is shown in Fig. 2. Upon shift to 36°C, the *cps1*-UV1 mutant fails to assemble a division septum and arrests with a stable actomyosin ring that persists into interphase (Fig. 2B). A time course analysis of cell division profile showed that cell number increase ceased in a manner similar to that in *cps1*-191, *cdc7*-24 and *cdc12*-112 mutants. However, unlike in case of *cdc7*-24 and *cdc12*-112 mutants, and similar to the *cps1*-191 mutant, progression through additional rounds of mitosis was blocked in the *cps1*-UV1 mutant (Fig. 2A). In the remainder of this study we consider the mechanism(s) that cause arrest of the *cps1* mutants with two interphase nuclei. Given that *cps1*-191, *cps1*-UV1 and four other alleles of *cps1* behave similarly, we have used the *cps1*-191 mutant in further analyses.

Entry into mitosis requires the attainment of a critical cell size in *S. pombe* (Nurse, 1975). It was therefore possible that the *cps1*-191 mutant, which is defective for cell elongation (Liu et al., 1999), does not accumulate cell mass sufficient to allow the two G_2 nuclei to proceed into the M phase. If lack of cell size and cell mass increase were to block G_2/M transition in *cps1*-191 cells, highly elongated *cps1*-191 cells produced by artificial means should accumulate more than two nuclei upon shift to 36°C. Alternatively, the failure to complete cell division might activate a cytokinesis-monitoring system that prevents a

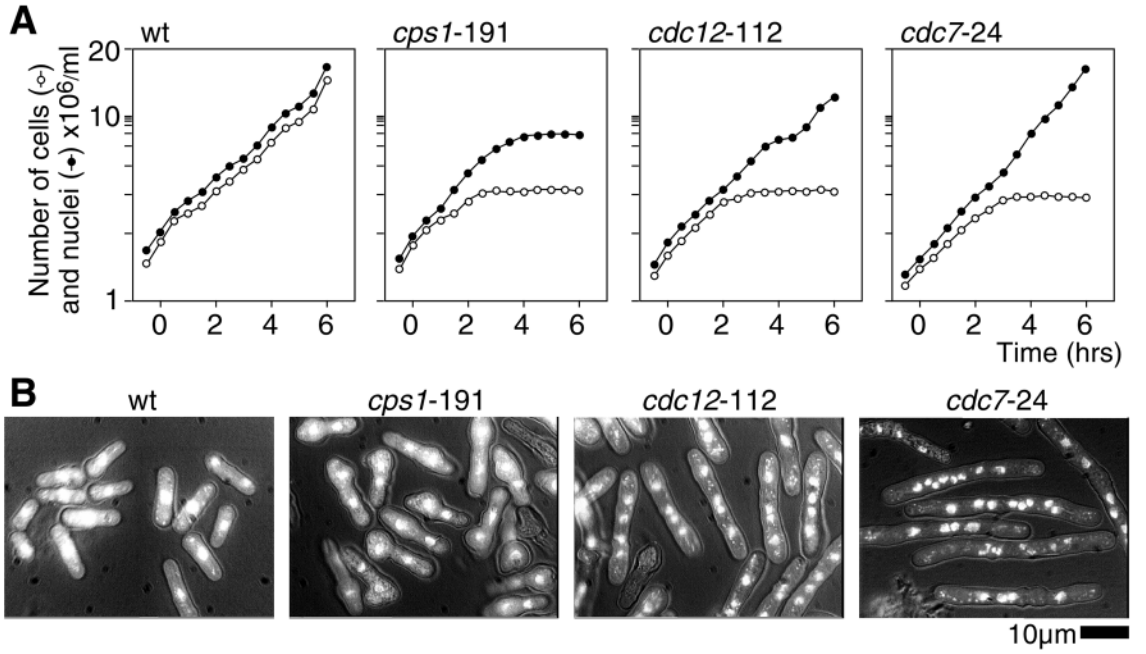

Fig. 1. (A) Cell division and nuclear division profiles of wild-type, *cps1*-191, *cdc12*-112 and *cdc7*-24 mutants. (B) Nuclear morphology of the indicated strains following a 6-hour shift to 36°C. Cells of the indicated genotypes were cultured to exponential growth phase at 24°C and shifted to 36°C. Samples were taken at 30-minute intervals, fixed and stained with DAPI. Cell number and number of nuclei per ml of culture was quantified and cells at the 6-hour time point in B were viewed using a fluorescence microscope and a phase contrast/UV filter combination.

subsequent mitotic cycle in heat-arrested *cps1*-191 cells. If such a cytokinesis monitoring system were to block G_2/M transition in *cps1*-191, highly elongated *cps1*-191 cells produced by artificial means would still arrest with two nuclei following a temperature shift to the restrictive temperature. To address these possibilities, *cps1*-191 cells were treated at the permissive temperature with hydroxyurea (HU), a DNA synthesis inhibitor. Treatment with HU served two purposes. First, it allowed synchronization of cells in the S phase. Second, it allowed cells to grow during the S phase block, resulting in a population of elongated cells. As a control, *cdc7*-24 mutant cells were also arrested with HU at the permissive conditions. Following HU arrest >90% of both *cps1*-191 and *cdc7*-24 cells arrested with a single nucleus (Fig. 3A,B). HU-arrested *cps1*-191 and *cdc7*-24 cells were reinoculated into fresh medium and shifted to the restrictive temperature (36°C) for 5 hours, fixed and stained with DAPI to visualize the nuclei. Interestingly, while the majority of *cdc7*-24 cells accumulated >4 nuclei (60% with four nuclei and 15% with eight nuclei; Fig. 3A,B), *cps1*-191 still arrested with two nuclei (70% with two nuclei and only 3% with four nuclei). Cells with eight nuclei were not observed. Comparison of the length of HU-treated *cps1*-191 cells with that of untreated cells showed that those treated were at least twice the size of the untreated cells (Fig. 3, w/o HU and HU release). These observations suggest that the arrest of *cps1*-191 at the G_2/M boundary is not due to a defect in cell growth. Instead it is likely that some aspect of completion of cytokinesis is checked by a 'cytokinesis monitoring system', which allows entry into the subsequent M phase only upon successful completion of cytokinesis.

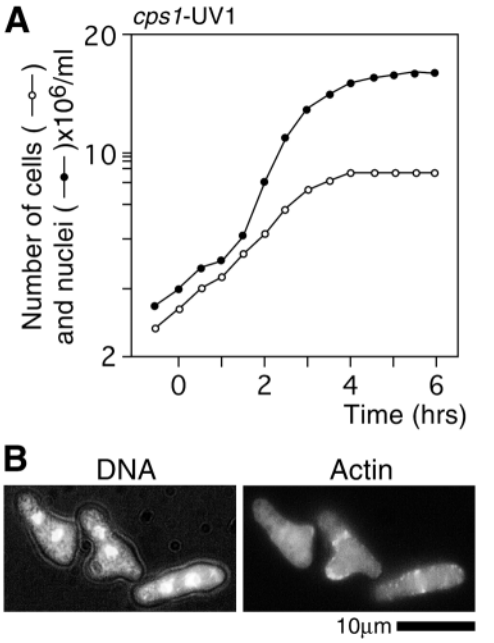

Fig. 2. The phenotype of the *cps1*-UV1 mutant. The cps1-UV1 mutant was cultured at 24°C to exponential phase and shifted to 36°C. Samples were taken at 30-minute intervals, fixed and stained with DAPI and rhodamine-conjugated phalloidin to visualize chromosomal DNA and F-actin, respectively. Cell numbers and number of nuclei per ml of culture were quantified and cells at the 6-hour time point in B were viewed using a fluorescence microscope.

A morphogenesis checkpoint that delays onset of mitosis until bud assembly is completed has been described in the budding yeast *S. cerevisiae* (Barral et al., 1999; McMillan et al., 1998; Lew and Reed, 1995). It has been shown that the function of Swe1p (homolog of the *S. pombe* Wee1p) is necessary for the G_2 delay observed when bud assembly is

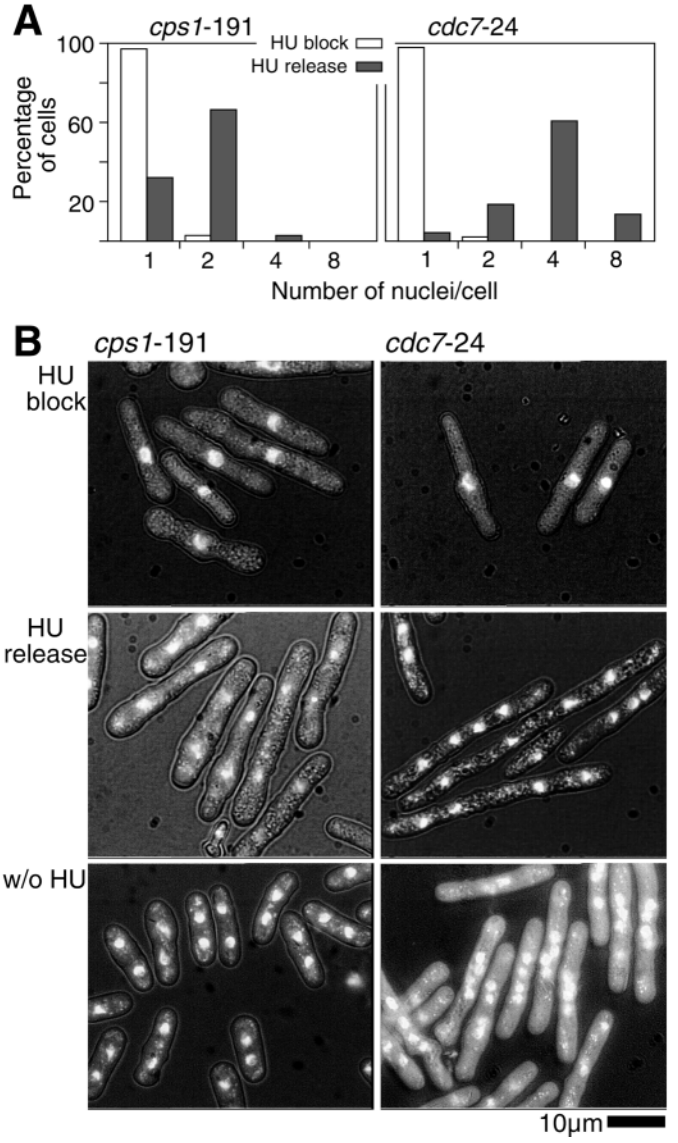

Fig. 3. Arrest of *cps1*-191 mutants with two nuclei is not due to insufficient cell mass increase. Cells of the genotypes *cps1*-191 and *cdc7*-24 were grown to exponential phase at 24°C and treated with 12 mM hydroxyurea (HU) for 6 hours and shifted to 36°C for 1 hour. HU-treated cells were washed with prewarmed YES medium and inoculated into fresh YES medium and incubated at 36°C for a further 4 hours, fixed and stained with DAPI. HU-treated cells prior to release from HU are labeled HU block. Cells released from HU block are labeled HU release. HU-untreated cells were also shifted to 36°C at the time when cells treated with HU were released into fresh growth medium (w/o HU). (A). Percentage of cells with one, two, four and eight nuclei after HU and heat block of *cps1*-191 and *cdc7*-24. (B) Photomicrographs of DAPI-stained *cps1*-191 and *cdc7*-24 cells.

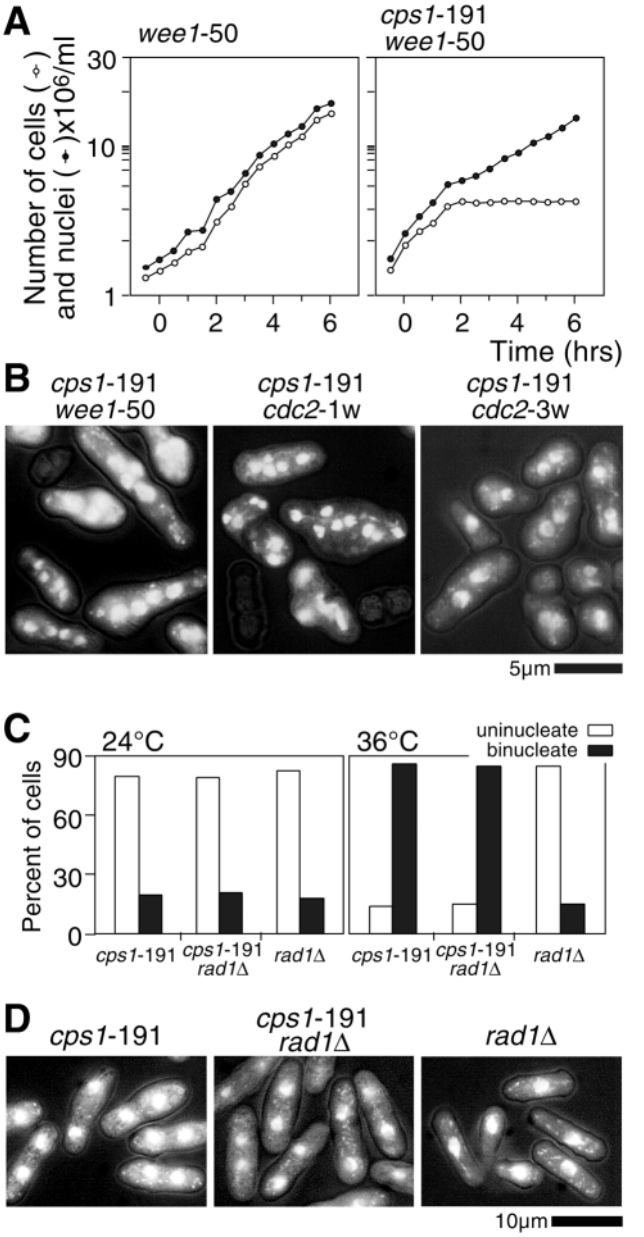

Fig. 4. Suppression of G_2 delay of *cps1*-191 by *wee1*-50 and *cdc2*-1w, but not by *cdc2*-3w or *rad1::ura4*. (A) Cells of the genotypes *wee1*-50 and *wee1*-50 *cps1*-191 were cultured to exponential growth phase at 24°C and shifted to 36°C. Samples were taken at 30-minute intervals, fixed and stained with DAPI. Cell numbers and number of nuclei per ml of culture were quantified. (B) Cells of the indicated genotypes were cultured to exponential growth phase at 24°C and shifted to 36°C. Samples were taken 6 hours after the shift, fixed and stained with DAPI to detect nuclear morphology. (C) Cells of the genotypes *cps1*-191, *cps1*-191 *rad1::ura4* and *rad1::ura4* were cultured to exponential growth phase at 24°C and shifted to 36°C. Samples were taken 4 hours after the shift, fixed and stained with DAPI and the percentage of cells with one and two nuclei was quantified. (D) Nuclear morphology of 4-hour heat-arrested *cps1*-191, *cps1*-191 *rad1::ura4* and *rad1::ura4* cells.

slowed down. We therefore addressed whether the G_2/M transition defect in heat-arrested *cps1*-191 cells is alleviated in the presence of a mutation in the *wee1* gene. We constructed a double mutant strain harboring the *wee1*-50 and the *cps1*-191 mutations. The *wee1*-50 mutant is temperature-sensitive, growing as well as dividing at the same size as wild-type cells at 24°C and dividing at a reduced size at 36°C. *wee1*-50, *cps1*-191 and *cps1*-191 *wee1*-50 cells were grown at 24°C and shifted to 36°C. Samples were taken at 30 minute intervals for 6 hours and the increase in cell and nuclear number was

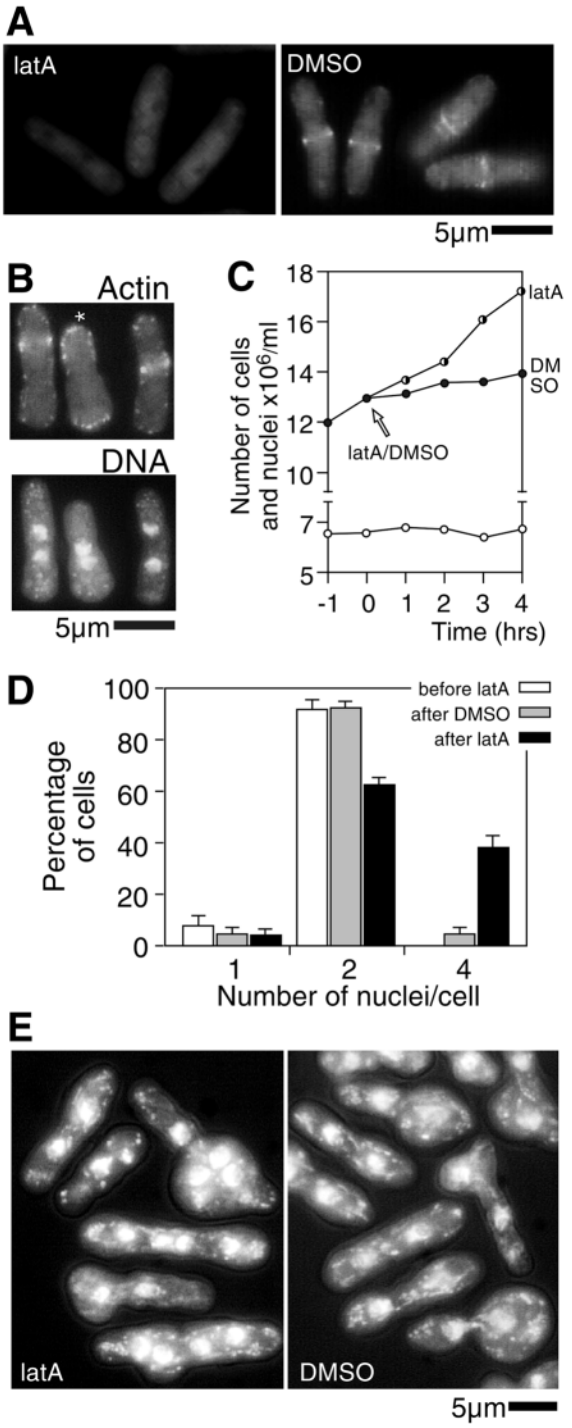

Fig. 5. Transient depolymerization of all F-actin structures partially relieves the G_2/M transition defect of *cps1*-191. (A) *cps1*-191 cells were cultured to exponential growth phase at 24°C and shifted to 36°C for 4 hours, then treated with either Latrunculin A (LatA) or DMSO for 30 minutes, fixed and stained with rhodamine-conjugated phalloidin and DAPI to visualize F-actin and nuclei. Cells remaining in culture were then washed three times with prewarmed YES medium and released into fresh YES medium at 36°C. Samples were taken at 30 minutes and at hourly intervals, fixed and stained with rhodamine-conjugated phalloidin and DAPI. (B) 30 minutes after release, F-actin containing rings appeared in approximately 50% of the cells, while the rest had F-actin at the cell tips (*). (C) Cell number remained constant upon treatment with LatA or DMSO, but number of nuclei/ ml of culture increased significantly in cells treated with LatA but not in cells treated with DMS O. Open circles, cell number; closed circles, number of nuclei/ml of culture after DMSO treatment; half-filled circles, number of nuclei/ml of culture after Lat A treatment. (D) Quantification of % cells with one, two and four nuclei (4 hours after treatment) following treatment with DMSO or LatA. (E) Nuclear morphology of cells treated with DMSO or LatA 4 hours after treatment with DMSO or LatA.

quantified (see Fig. 1A for *cps1*-191 and Fig. 4A for *wee1*-50 and *cps1*-191 *wee1*-50 cells). As expected, *wee1*-50 cells continued to grow and divide at 36°C. Cell number increase stopped approximately 2 hours after shift of both *cps1*-191 cells as well as *cps1*-191 *wee1*-50 cells (see Fig. 1A for *cps1*-191 and Fig. 4A for *cps1*-191 *wee1*-50 cells). Interestingly, even though the number of nuclei stopped increasing in the *cps1*-191 mutant, the number of nuclei continued to increase in the *cps1*-191 *wee1*-50 double mutant. Microscopic examination showed that the *cps1*-191 *wee1*-50 cells had accumulated between 4-8 nuclei (Fig. 4B), unlike the *cps1*-191 single mutant (Fig. 1B). These observations suggested that a pathway involving Wee1p might be important in delaying G_2/M transition in heat-arrested *cps1*-191 cells.

We then addressed whether the effect of Wee1p on G_2/M transition in the *cps1*-191 mutant was mediated through the Cdc2p-kinase, which governs G_2/M transition and is negatively regulated through phosphorylation by Wee1p (Gould and Nurse, 1989; Nurse, 1990). To this end, we constructed double mutants *cdc2*-1w *cps1*-191 and *cdc2*-3w *cps1*-191. The *cdc2*-1w and *cdc2*-3w are dominant mutant alleles of *cdc2* that cause cells to divide at a reduced size, resulting in a 'wee' morphology. The *cdc2*-1w mutant mimics the *wee1* mutant in that the *cdc2*-1w cells are no longer sensitive to over-production of Wee1p and do not show additive effects when combined with the *wee1* mutants (MacNeill et al., 1989). By contrast, *cdc2*-3w mutants show strong negative interactions leading to 'mitotic catastrophe' when combined with *wee1* mutants. Thus, *cdc2*-3w appears to function independently of Cdc25p (MacNeill et al., 1989). The *cps1*-191 *cdc2*-1w and *cps1*-191 *cdc2*-3w mutants were cultured at 24°C and shifted to 36°C for 6 hours, fixed and stained with DAPI to visualize the nuclei (Fig. 4B). Interestingly, we found that whereas *cdc2*-3w only weakly suppressed the G_2/M defect of *cps1*-191 (less than 10% cells with four nuclei) the *cdc2*-1w was more effective in suppressing the G_2/M defect of *cps1*-191 (60% cells with four nuclei and 10% cells with eight nuclei). This analysis suggested that a Wee1p mediated function of Cdc2p was important for the G_2 delay observed in *cps1*-191 mutants.

S. pombe cells prevent entry into mitosis if the DNA is damaged or if DNA replication is incomplete (Rowley, 1992). G_2 delay observed in these situations depends on the functions of *rad1*, *rad3*, *rad9* and *rad17* genes (Rowley, 1992). To address whether these genes played a role in the G_2 delay observed in the *cps1*-191 mutant we combined *cps1*-191 with a representative mutant *rad1::ura4* (Sunnerhagen, 1990), which is defective in G_2 arrest in response to DNA damage as well as in response to unreplicated DNA (Rowley, 1992). Cells of the genotypes *cps1*-191, *rad1::ura4* and *cps1*-191 *rad1::ura4* were grown at 24°C and shifted to 36°C. At the permissive temperature *cps1*-191, *cps1*-191 *rad1::ura4* and

rad1::ura4 mutants resembled wild-type cells in that approximately 80% cells were uninucleate and the rest were binucleate (Fig. 4C). Upon shift to 36°C for 4 hours, approximately 85% of *cps1*-191 *rad1::ura4* cells, similar to the *cps1*-191 mutant (86% binucleate cells), contained two nuclei (Fig. 4C,D). The *rad1::ura4* single mutant resembled wild-type cells at the higher temperature as well in that 85% of the cells were uninucleate, while the rest were binucleate (Fig. 4C,D). Thus, elements that control the DNA damage and unreplicated DNA checkpoint pathways do not regulate the G_2 delay in *cps1*-191 cells.

The experiments discussed earlier suggest the existence of a 'cytokinesis monitoring system', which blocks G_2/M progression until previous cytokinesis is completed. One major difference between *cps1*-191 and other cytokinesis mutants is that the actomyosin rings remain stable in the absence of septum deposition in heat-arrested *cps1*-191 mutants (Liu et al., 1999). On the other hand, *rng* mutants fail to assemble proper actomyosin rings and the *sid* mutants assemble actomyosin rings that disassemble at the end of anaphase (Gould and Simanis, 1997). We have previously shown that the *sid* mutant phenotype is epistatic to the *cps1*-191 phenotype (Liu et al., 1999). In addition, some *rng* mutants were also found to be partially epistatic to the *cps1*-191 phenotype (Liu et al., 1999). It therefore seemed possible that the continued presence of the actomyosin ring in interphase-arrested *cps1*-191 cells prevented entry into a subsequent M phase. Consistent with this, *cps1::ura4*$^+$ cells that are capable of actomyosin ring assembly and disassembly accumulate multiple nuclei (Liu et al., 1999).

To directly test whether the presence of the actomyosin ring or other stable F-actin structures prevented G_2/M transition, *cps1*-191 cells were arrested at 36°C for 4 hours and treated either with Latrunculin A (LatA), a drug that prevents actin polymerization, or with DMSO (solvent) as a control. Following a 30-minute treatment with LatA or DMSO at 36°C, cells were washed with growth medium and reinoculated into fresh medium at 36°C. Samples were taken at hourly intervals and fixed and stained with DAPI and rhodamine-conjugated phalloidin to visualize nuclei and F-actin, respectively. As expected, F-actin structures were abolished in cells treated with LatA whereas stable actomyosin rings were detected in all binucleate cells in the culture treated with DMSO (Fig. 5A). Interestingly, we found that upon washing out the LatA, approximately 50% cells were capable of reassembling actomyosin rings within 30 minutes while the rest of the binucleate cells were incapable of assembling F-actin containing rings (Fig. 5B, asterisk). The reason for the reappearance of F-actin containing rings in only half of the LatA treated *cps1*-191 cells is presently unclear. Comparison of the number of nuclei in cells treated with LatA and those treated with DMSO showed that the number of nuclei increased more rapidly in cells treated with LatA (Fig. 5C). 4 hours after treatment with LatA approximately 40% cells contained four nuclei, whereas only 5% cells treated with DMSO contained four nuclei (Fig. 5D,E). The vast majority of cells treated with DMSO arrested with two nuclei. Cells not treated with either DMSO or LatA behaved in a manner similar to those treated with DMSO (data not shown). It should be noted however, that cells that accumulated four nuclei upon LatA treatment were also more elongated than untreated and DMSO treated cells (Fig. 5E). These results suggest that the persistence of an F-actin dependent structure in interphase cells prevents G_2/M transition in heat-arrested *cps1*-191 cells. Presently it is unclear whether the actomyosin ring itself or some other structure that is normally dependent on F-actin for its integrity is important in preventing entry into the M phase in heat-arrested *cps1*-191 cells.

DISCUSSION

In this paper, we have presented evidence for a surveillance mechanism that halts entry into subsequent mitosis until cytokinesis is properly executed. We conclude that the presence of some F-actin dependent structure(s) is perceived by a 'cytokinesis-monitoring system' in heat-arrested *cps1*-191 cells. This conclusion is supported by the observation that treatment of heat-arrested *cps1*-191 cells with an actin polymerization inhibitor allows a further round of mitosis. We hypothesize that this cytokinesis-monitoring system in turn responds by preventing G_2/M transition of the two interphase G_2 nuclei found in these cells (Fig. 6). The 'cytokinesis checkpoint' might also prevent cell elongation in arrested *cps1*-191 cells. However, we have shown that the defect in G_2/M transition in these cells is not due to insufficient cell mass increase, since elongated *cps1*-191 cells (produced by HU treatment) shifted to the restrictive temperature still arrest with only two nuclei. The inability of *cdc2*-3w, which allows G_2/M transition at a reduced size, to suppress the G_2/M transition defect of arrested *cps1*-191 cells also argues against the possibility that insufficient cell mass increase alone prevents entry of the two G_2 nuclei into M phase. Given that *wee1*-50 and *cdc2*-1w mutants suppress the G_2/M defect in these cells we conclude that the functions of Wee1p and Cdc2p are involved in preventing G_2/M transition in arrested *cps1*-191 cells (Fig. 6).

Recently, in a parallel study, Le Goff et al. (1999b) have arrived at similar conclusions on the existence of a septation checkpoint in *S. pombe* through the analysis of a different allele of *cps1*, *cps1*-N12. Our study is consistent with the previous study (Le Goff et al., 1999b) and furthers their findings, by ruling out the lack of attainment of critical size as a possible reason for *cps1* mutants being defective in mitotic entry following failed cytokinesis. In addition, we have also shown that the checkpoint is activated in response to the persistence of some F-actin dependent structure that is normally disassembled upon completion of cytokinesis. Finally, we have shown that elements that control the G_2 delay in response to damaged DNA and unreplicated DNA are not important for the G_2 delay observed in cps1-191 mutants.

If a checkpoint-like 'cytokinesis monitoring system' was to operate in assessing completion of cytokinesis, a key question relates to the nature of the signal that activates this cytokinesis monitoring system. The nature of the signal is unclear. However, based on data presented in this paper and in Liu et al. (1999), we consider two possibilities. Given that the *cps1*-191 mutant arrests with a stable actomyosin ring and two G_2 nuclei, one possibility is that the persistence of an actomyosin ring or other F-actin dependent structures might prevent entry into a subsequent round of mitosis. Consistent with this, we have found that brief treatment of arrested *cps1*-191 cells with the

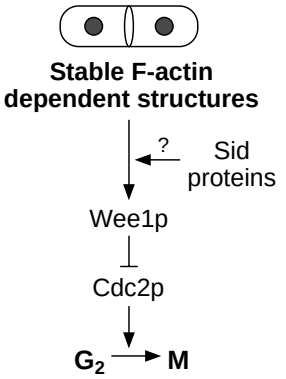

Fig. 6. Schematic representation of the G_2 delay in heat-arrested *cps1*-191 mutant cells. The persistence in interphase of an actomyosin ring or other F-actin dependent structure(s) that are normally present only during cytokinesis in *cps1*-191 mutant cells is detected by the 'cytokinesis-monitoring system'. This 'cytokinesis-monitoring system' prevents G_2/M transition. The block in nuclear division in the absence of cytokinesis requires the function of Wee1p and Cdc2p. The Sid proteins might play a role in the signal transduction pathway that causes the G_2 delay.

actin polymerization inhibitor Lat A allows at least 40% of cells to undergo a second round of mitosis. A further test of this hypothesis would be the ability of F-actin stabilizing molecules such as phalloidin or jasplakinolide to activate the cytokinesis checkpoint in wild-type cells. However, since cells are impermeable to phalloidin and jasplakinolide does not appear to stabilize F-actin structures in *S. pombe* (J. Liu and M. Balasubramanian, unpublished observations), we have been unable to further explore this possibility. Presently it is unclear if the presence of the actomyosin ring itself or some other structure that normally depends on F-actin for its integrity serves as the source of this signal that blocks G_2/M transition. Alternatively, Cps1p itself might activate the cytokinesis monitoring system, since *cps1*-null mutants proceed through multiple mitotic cycles and accumulate up to 32 nuclei, whereas the *cps1*-191 mutant arrests with two nuclei (Liu et al., 1999). In this model, the product of the *cps1*-191 allele is presumed only to be defective in the enzymatic synthesis of 1,3-β-glucan, but is capable of transducing the signal resulting from lack of septum assembly. By contrast, in the absence of Cps1p both enzymatic synthesis and perception of failed cytokinesis are affected. This model is similar to that proposed from studies of mutations in the DNA polymerase gene *pol1* (D'Urso et al., 1995). These studies have shown that temperature-sensitive mutations in the *pol1* gene prevent DNA synthesis but activate the unreplicated DNA checkpoint, whereas *pol1*-null mutants are unable to activate the unreplicated DNA checkpoint and undergo mitosis in the absence of DNA replication.

If a cytokinesis-checkpoint were to halt a subsequent mitotic cycle in *cps1*-191 mutants, it is curious to note that other cytokinesis mutants undergo multiple mitotic cycles. The *rng* mutants fail to assemble organized actomyosin rings and assemble improper septa, whereas the *sid* mutants assemble actomyosin rings that disassemble at the completion of anaphase. It is possible that this cytokinesis monitoring system itself is triggered only after assembly of the actomyosin ring and after signaling by the Sid group of proteins. We have found that the mitotic defect of the *cps1*-191 mutant is only partially suppressed in the *rng cps1*-191 double mutants (J. Liu, H. Wang and M. K. Balasubramanian, unpublished observations). Temperature-sensitive alleles of all known *rng* mutants are known to deposit 'spotty septa', suggesting that they assemble improper rings (Nurse et al., 1976; Chang et al., 1996; Balasubramanian et al., 1998). It is also interesting to note that the rate of accumulation of nuclei is slower in the *rng* mutants compared to the *sid* mutants (Nurse et al., 1976). Again, the rather slow accumulation of the improper septa (or disassembly of the improperly formed rings) might slow mitotic entry in these mutants. By contrast, *sid* mutants and *cps1*-191 *sid* double mutants accumulate nuclei more rapidly than the *cps1*-191 single mutant (Liu et al., 1999; J. Liu, H. Wang and M. K. Balasubramanian, unpublished observations). An attractive possibility is that the Sid proteins are required for septum assembly as well as to prevent a subsequent round of mitosis in the absence of cytokinesis. Interestingly, previous studies have shown that overproduction of a Sid-protein Cdc14p causes cells to arrest at G_2/M in a *wee1* dependent manner, suggesting that some of the Sid proteins are capable of blocking mitotic entry (Fankhauser and Simanis, 1993). Isolation of additional alleles of the *sid* genes with properties similar to the *cps1*-191 allele will establish if the *sid* gene products indeed are also required to monitor completion of some aspect of cytokinesis.

A further question relating to checkpoint-regulatory events concerns the advantage of such a surveillance mechanism for the survival of cells. The cytokinesis checkpoint might be useful in preventing formation of multinucleate cells, which are prone to increases in ploidy by fusion of interphase nuclei. Compounds such as aculeacin A, papulacandin B and other echinocandins, which are known to inhibit 1,3-β-glucan synthases, are made by members of the fungal kingdom (Denning, 1997). It is possible that in the wild, *S. pombe* cells exposed to such compounds might be able to prevent an increase in ploidy through the use of a cytokinesis checkpoint that restrains entry into mitosis until the previous septation event is completed.

In summary, through the analysis of *cps1*-191, we have provided evidence for a checkpoint mechanism that halts entry into a subsequent round of mitosis until successful completion of cytokinesis. The ability of *wee1* and *cdc2*-1w to override the G_2 delay seen in the two interphase nuclei of arrested *cps1*-191 cells suggests that the known elements of mitotic control are required for this G_2 delay. Finally, the source of the signal assessed by the 'cytokinesis monitoring system' is dependent on F-actin. Future studies should assess whether the actomyosin ring itself or other F-actin dependent structures serve to signal failure of completion of cytokinesis, and also identify other elements that might function in this checkpoint-like mechanism.

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