

Mitotic role for the Cdc28 protein kinase of *Saccharomyces cerevisiae*

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ABSTRACT The Cdc28 protein kinase functions in the G₁ to S phase transition of the cell cycle of the budding yeast *Saccharomyces cerevisiae*. This is in contrast with observations of the homologous protein kinase from a variety of metazoans, where activity and function are associated with the G₂ to M phase transition. We present evidence that the Cdc28 protein kinase is also required for mitosis and that this function is executed in the G₂ interval of the cell cycle. We show, in addition, that the protein kinase is highly active during this phase of the cell cycle. The dual role of the Cdc28 protein kinase in the *S. cerevisiae* cell cycle thus parallels that demonstrated for the cdc2 protein kinase of the fission yeast *Schizosaccharomyces pombe*.

The characterization of a highly conserved protein kinase in a wide variety of eukaryotic organisms has led to the convergence of a number of active areas of biological investigation. Members of the Cdc28/cdc2 protein kinase family, defined initially by mutational analysis of the cell cycles of budding and fission yeasts, respectively, are associated with cell cycle transitions in mitosis and meiosis (1–4). Parallel to the genetic analyses, most biochemical and physiological work has implicated this kinase in the induction of the mitotic state or in the progression from meiotic prophase to metaphase. In somatic cells and cleavage embryos, kinase activity has been shown to peak at the G₂/M phase boundary (5–7). The same active protein kinase has been shown to induce the maturation of oocytes in marine invertebrates and frogs, where it is a component of maturation-promoting factor (8–12). In all of these cases, the active enzyme is a heteromultimeric protein of imprecisely determined stoichiometry composed of a 34-kDa catalytic subunit and 45- to 60-kDa regulatory subunit known as cyclin and, under some circumstances, other polypeptides (6, 7, 13–18). The mitotic and meiotic activities of the kinase, usually assayed by its ability to phosphorylate histone H1 *in vitro*, correlate with the assembly of these protein complexes as well as with covalent modifications of the catalytic subunit, specifically tyrosine and threonine dephosphorylations (5, 11, 19–22). The activity of the kinase decays rapidly as cells complete mitosis and there is some evidence that this decay is essential to reestablish interphase (5–7, 14, 23). The catastrophic proteolysis of cyclin is thought to cause or facilitate this inactivation of the kinase (6, 24).

In contrast to the assignment of a mitotic role through biochemical studies, a role in the G₁ to S phase transition has been established through genetic analysis in budding and fission yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively (1–3). In fact, in budding yeast, most analysis has concentrated on the G₁ function of the Cdc28 protein kinase since the standard mutant alleles utilized do not confer a significant defect in mitotic functions (1, 2). In budding yeast, an active Cdc28 protein kinase complex has been characterized, the presence of which correlates with

a cell's ability to undergo the transition from G₁ to S phase (15, 25). External signals that cause cells to arrest in G₁ phase lead to dissociation of the active complexes (15). Although the precise composition and stoichiometry of G₁ complexes remain to be determined, there is some evidence that cyclin-like proteins perform an analogous function to that of cyclins in the G₂ to M phase transition (26–29). The products of the genes *CLN1*, *CLN2*, and *CLN3* are polypeptides with limited primary structure similarity to mitotic cyclins (26, 27). These have been shown to associate with the *CDC28* gene product in G₁ (45) and genetic evidence suggests that they are rate-limiting for the G₁ to S phase transition and required for active G₁ Cdc28 protein kinase complexes (26–29). Presumably, a similar regulatory system exists in fission yeast where the cdc2 protein kinase is also required for the G₁ to S phase transition (3), although no G₁ activity has been detected (17, 18). It is not known whether the homologous protein kinase from animal cells also has a G₁ role. However, Cdc2Hs, the human homolog, can perform the G₁ function in budding and fission yeast (30, 31).

A mitotic role for the Cdc28 protein kinase has been reported (32). Piggott *et al.* (32) described a mutant allele, *cdc28-1N*, which they claimed arrested with a homogeneous G₂ cell division cycle phenotype. They also reported that other recessive *cdc28* mutations conferred a G₂ phase cell cycle defect that was normally not observed because the execution point for this function was in late G₁, not well separated from the earlier execution point associated with G₁ arrest (32). Most cells in an asynchronous culture would be expected to exhibit arrest at the first of two closely spaced *CDC28*-dependent events. Both of the genetic results reported by Piggott *et al.* (32) have been difficult to reproduce (see *Discussion*). On the other hand, the Cdc28 protein kinase is active when cells are arrested in G₂ or M phase using mutants and inhibitors (15, 25, 33). Furthermore, the Cdc28 protein kinase can perform mitotic functions when expressed in fission yeast in place of the Cdc2 protein kinase (34). These observations and the high degree of conservation of mitotic control through the eukaryotic phylogeny led us to reinvestigate the possibility of a mitotic role for the Cdc28 protein kinase in *Sa. cerevisiae*. We report here that based on genetic and biochemical criteria, the Cdc28 protein kinase plays a role in mitotic induction analogous to that of the cdc2 protein kinase of fission yeast and of its homologs in animal cells.

MATERIALS AND METHODS

Strains and Growth Media. All yeast strains were congenic with BF264-15D, which has been described (35). Additional markers were introduced from plasmids by transformation (36, 37). Standard culture media for *Sa. cerevisiae* were used in all experiments except as specified (38).

Conditional Shift Experiments. Wild-type, *cdc28-4*, and *cdc28-13* cells were grown in rich medium (YEFD) to a concentration of 2–4 × 10⁶ cells per ml at 23°C at which time hydroxyurea (39) was added to 0.2 M. Incubation at 23°C continued for 4 hr and then the culture was shifted to 38°C. After 1 hr at 38°C, cells were collected on 2.5-cm membranes (0.45-μm pore size) using a Millipore filtration apparatus and

quickly resuspended in fresh medium without hydroxyurea at 38°C. Incubation was continued under these conditions for the remainder of the experiment. As specified, samples were removed and fixed for microscopic analysis and for flow cytometric analysis.

To arrest *CDC28* and *cdc28* mutant cells in G₂ phase, isogenic derivatives were prepared in which the *MIH1* (40) gene was deleted and a copy of the *Sc. pombe weel* mitotic inhibitor gene under control of the regulatable *Sa. cerevisiae GAL1* promoter was integrated (40). Cells were grown in rich medium containing the neutral carbon source raffinose (YEPrf) at 23°C. Galactose was added to 2% (wt/vol) and cells were grown at 23°C for 3.5 hr at which time most cells had a distinctive morphology associated with G₂ arrest in the context of an *mih1/weel* block (40). Cells were shifted to 38°C for 0.5 hr and then filtered and resuspended in prewarmed YEPD (repressing) medium. Incubation for the remainder of the experiment was at 38°C or at 36°C.

Microscopy and Flow Cytometry. For determination of cell number and budding index, cells were fixed in 3.7% (vol/vol) formaldehyde. After fixation overnight, cell aggregates were disrupted with a 20-sec pulse using a Braun sonicator and a needle probe. A Leitz SM-Lux phase-contrast microscope fitted with a ×40 objective and a hemacytometer were used for counting and determination of budding index (percentage of budded cells).

For fluorescence microscopy and flow cytometry, cells were stained with propidium iodide as described (41). Flow cytometric analysis using a FACS IV analyzer (Becton Dickinson) has been described (41). For analyses where cell volume increased dramatically during the course of the experiment after an extended cell cycle arrest, an upward drift of peaks was unavoidable due to increasing cytoplasmic autofluorescence. Fluorescence microscopy of propidium iodide-stained cells was performed using a Zeiss Axiophot photomicroscope fitted with a ×100 objective. A combination of fluorescence and phase-contrast optics was used to accentuate cellular contours simultaneously with nuclear staining. Tri-X film (Kodak) was used.

Determination of Cdc28 Protein Kinase Activity. Cells were synchronized either by arrest and subsequent release using mating pheromone or by zonal elutriation. Synchronization by the mating pheromone has been described (25). For synchronization by centrifugal elutriation, 1 liter of cells growing in minimal sucrose medium (YNBSuc) at 5×10^7 cells per ml was loaded on a Beckman JE10X rotor at 4200 rpm and a flow rate of 35 ml/min. Small unbudded presumably newly released daughter cells were obtained by increasing the flow rate to 40 ml/min. A mixed population containing larger and budded cells was obtained by stepping the flow rate to 55 ml/min. Cdc28 protein kinase assays were performed essentially as described (15, 25).

RESULTS

Cdc28 Protein Kinase Is Required After an S-Phase Block to Induce Mitosis. When temperature-sensitive *cdc28* mutants were arrested in S phase using the inhibitor hydroxyurea and then shifted to the restrictive temperature at the time of removal of the inhibitor, most cells progressed from S phase through mitosis and were arrested in G₁ (unpublished results). These observations suggest that the Cdc28 protein kinase performs no essential role late in the cell cycle. This interpretation, however, is difficult to reconcile with a large body of experimental observations in other systems. A possible explanation for the failure to detect a mitotic role for Cdc28 is that the mitotic demand for kinase activity may be lower than that in G₁ coupled with a significant time requirement for decay of mutant Cdc28 protein. Alternatively, the G₁ forms of the temperature-sensitive mutant kinase may be

more thermolabile than the mitotic forms. Either of these might cause the G₁ function to become limiting first in a population of asynchronous or synchronous mutant cells shifted to the restrictive temperature. We therefore modified the experiment described above by using a more extreme restrictive temperature than is normally employed to facilitate the decay of the thermolabile mutant protein. In addition, the mutant cells were preincubated at the restrictive temperature for 1 hr after synchronization but prior to removal of the inhibitor to allow time for the temperature-induced decay to be complete. *cdc28-4*, *cdc28-13*, and congenic wild-type cells were arrested in S phase by incubation with hydroxyurea at 23°C. After 4 hr, most of the mutant and wild-type cells had become synchronized, as determined by an increase in budded cells to almost 100% and by flow cytometric analysis and morphological observation (Fig. 1). At this time cells were shifted to 38°C and incubated in the continued presence of hydroxyurea for 1 hr. Hydroxyurea was then removed by filtration with prewarmed medium and incubation was continued at 38°C in the absence of hydroxyurea. After removal of hydroxyurea, wild-type cells recovered and resumed cycling as shown by the appearance of a steady-state fraction of unbudded cells and a dramatic increase in cell number (Fig. 1 A and B). *cdc28-13* mutant cells, after a lag, completed mitosis and became arrested in G₁, as shown by an approximate doubling of cell number and the accumulation of unbudded cells with a 1n complement of nuclear DNA (Fig. 1 A–C). This result is consistent with previous observations as well as the proposal that the Cdc28 protein kinase does not have an essential mitotic function. *cdc28-4* mutant cells, on the other hand, remained budded during the same time course, showed no cell number increase, and accumulated with a 2n complement of nuclear DNA (Fig. 1 A–C). Examination of the arrested *cdc28-4* mutant cells stained with propidium iodide by fluorescence microscopy revealed that most have a nonextended nucleus in or near the bud aperture. This morphological phenotype is indicative of arrest in G₂ (Fig. 1E). Viability of these arrested cells was confirmed by shifting the temperature to 23°C at the end of the time course shown. Greater than 80% of the cells were then able to complete division (data not shown). Parallel observation of the arrested *cdc28-13* mutant cells stained with propidium iodide revealed morphological and nuclear phenotypes associated with arrest in G₁ (Fig. 1D). Specifically, the cells were unbudded and had asymmetric cytoplasmic projections, and the nucleus was centrally located. Thus of the two *cdc28* mutations tested, only one revealed an essential function for the encoded protein kinase between the end of S phase and mitosis. The other, although conferring a lag in completion of the cell cycle relative to wild type, was not sufficiently defective in G₂/M-phase functions to cause arrest in G₂. These results indicate a G₂ function for the Cdc28 protein kinase executed at some point during or after S phase of the cell cycle.

Execution Point for the G₂ Phase Cdc28 Function Is in G₂. To localize the execution point of the Cdc28 protein kinase mitotic function, *cdc28* mutant and wild-type cells were first arrested in G₂ mutationally. Cells containing a deletion of the gene *MIH1*, which encodes a mitotic inducer, and a copy of the *Sc. pombe weel* gene, which encodes a mitotic inhibitor, expressed under control of the regulable *GAL1* promoter, arrest in G₂ when grown in galactose (40). When significant fractions of mutant and wild-type populations were arrested in G₂ in this manner, as determined by morphological observation (data not shown), and then shifted to restrictive temperature (38°C) followed by release from the G₂ block by filtration into glucose-containing medium, the wild-type cells were able to complete mitosis and initiated additional cell cycles (Fig. 2B). The *cdc28-13* mutant, in this case, was unable to complete mitosis at 38°C, as determined by cell

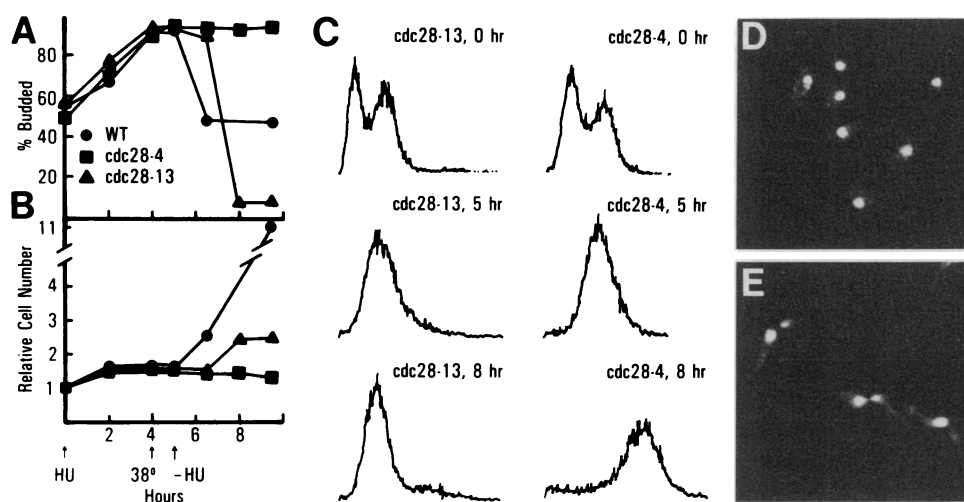


FIG. 1. Cdc28 function relative to an S-phase block. Cells were arrested in S phase with hydroxyurea, shifted to 38°C for 1 hr, and then released from the S-phase block. (A) Percentage of budded cells in the population as a function of time. (B) Cell number as a function of time. Points of addition of hydroxyurea (HU), shift to 38°C, and removal of hydroxyurea (-HU) are indicated. ●, Wild type (WT); ■, *cdc28-4*; ▲, *cdc28-13*. (C) Flow cytometric analysis of *cdc28-13* (profiles on left) and *cdc28-4* (profiles on right) mutant populations as a function of time. Times are 0 hr (top profiles); 5 hr (middle profiles), and 8 hr (bottom profiles). The ordinate for each plot represents frequency while the abscissa represents relative fluorescence. At the beginning time point (0 hr), the left peak represents cells with a 1n content of DNA (G₁ cells) and the right peak represents cells with a 2n content of DNA (G₂ + M phase cells). Note that there is a rightward drift (increased fluorescence) during each time course as a result of cell enlargement and cytoplasmic autofluorescence. (D) Fluorescent/phase-contrast micrograph of propidium iodide-stained *cdc28-13* mutant at 8 hr. (E) Fluorescent/phase-contrast micrograph of propidium iodide-stained *cdc28-4* mutant at 8 hr. (×350.)

number, flow cytometry, and cellular morphology (Fig. 2 A–C and E). These cells were viable, however, after release from the *wee1/MIH1* block at 38°C, as demonstrated by their ability to complete mitosis and arrest in G₁ after a shift down to 36°C (Fig. 2 A–D). Presumably, the *cdc28-13* mutation can establish a G₂ block at 38°C in this experiment because mutational perturbations in the *wee1/MIH1* regulatory network attenuate Cdc28 function in G₂. The inability of these *cdc28* mutant cells to recover at restrictive temperature from a previously established G₂ block localizes the execution point for the Cdc28 mitotic function to G₂.

H1 Kinase Activity Through the Yeast Cell Cycle. In many cell types, the noncore histone H1 becomes phosphorylated during mitosis. This reversible phosphorylation has been associated with chromosome condensation during the early stages of mitosis. The protein kinase implicated in mitotic phosphorylation of histone H1 is the maturation-promoting factor complex containing cyclins and p34, the product of the *CDC28/cdc2* homologs in these species (5–14). Similarly, the Cdc28 protein kinase in *Sa. cerevisiae* has been shown to be capable of efficiently phosphorylating histone H1 from animal sources (15), although the presence of a histone H1

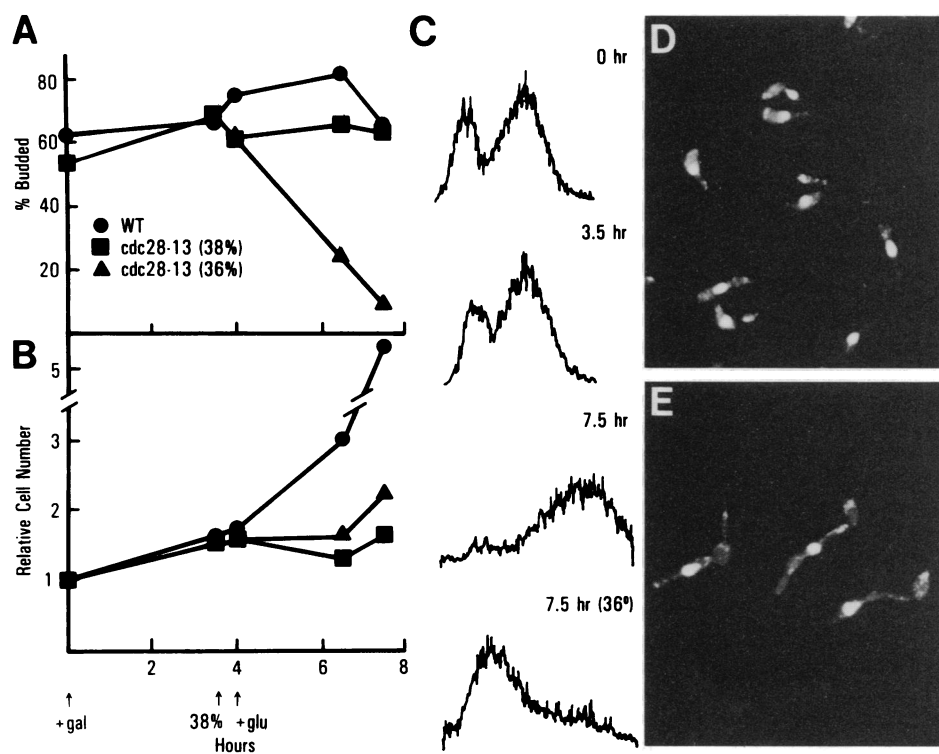


FIG. 2. Cdc28 function relative to a G₂ block. Cells were arrested mutationally in the G₂ phase of the cell cycle. Arrest was initiated at 0 hr by addition of galactose followed by elevation to 38°C after 3.5 hr and filtration into preheated glucose-based medium at 4.0 hr. Subsequent incubation was at 38°C or 36°C. (A) Percent budded cells as a function of time. (B) Cell number as a function of time. Times of galactose addition (0 hr), shift to 38°C (3.5 hr), and shift to glucose (4.0 hr) are indicated. ●, Wild type (WT); ■, *cdc28-13* at 38°C; ▲, *cdc28-13* at 36°C. (C) Flow cytometric analysis of *cdc28-13* mutant. Times are 0 hr (top profile), 3.5 hr (second profile), 7.5 hr with final incubation at 38°C (third profile), and 7.5 hr with final incubation at 36°C (bottom profile). Flow cytometric data are as in Fig. 1. (D) Fluorescent/phase-contrast micrograph of propidium iodide stained *cdc28-13* mutant at 7.5 hr (36°C). (E) Fluorescent/phase-contrast micrograph of propidium iodide-stained *cdc28-13* mutant at 7.5 hr (38°C). (×480.)

homolog in yeast remains to be conclusively demonstrated. Nevertheless, we considered the ability to phosphorylate histone H1 a reasonable measure of G₂/M activity of the Cdc28 protein kinase. Yeast cells of mating type a were presynchronized by incubation with the mating pheromone α factor. After removal of mating pheromone by filtration, cells progress through at least one cell cycle with a high degree of synchrony. At regular intervals subsequent to release from the mating pheromone block, cells were harvested, lysates were prepared, and Cdc28 activity was assayed using bovine histone H1 as a substrate. As can be seen in Fig. 3A, Cdc28-dependent histone H1 kinase activity was periodic and accumulated in the latter part of the cell cycle. Based on analysis of cell and nuclear morphologies (Fig. 3A), the peak of protein kinase activity corresponded best to the fractions enriched for mitotic cells. Because the synchrony had decayed somewhat by the end of the first cell cycle, this experiment did not have sufficient resolution to determine precisely when histone H1 kinase activity was inactivated.

To investigate the timing of histone H1 kinase activation, a population of growing cells was fractionated by centrifugal elutriation to obtain newly released G₁ cells. Selection for small size gave a relatively pure population of daughter cells in G₁. When assayed for histone H1 kinase activity, extracts from these cells showed relatively little activity (Fig. 3B). Thus by the time cells have completed mitosis and entered G₁, the Cdc28 protein kinase has been inactivated. Extracts from elutriated fractions containing a heterogeneous population of cells that have progressed further through the cell cycle, as determined by size and degree of budding, showed high levels of histone kinase activity (Fig. 3B).

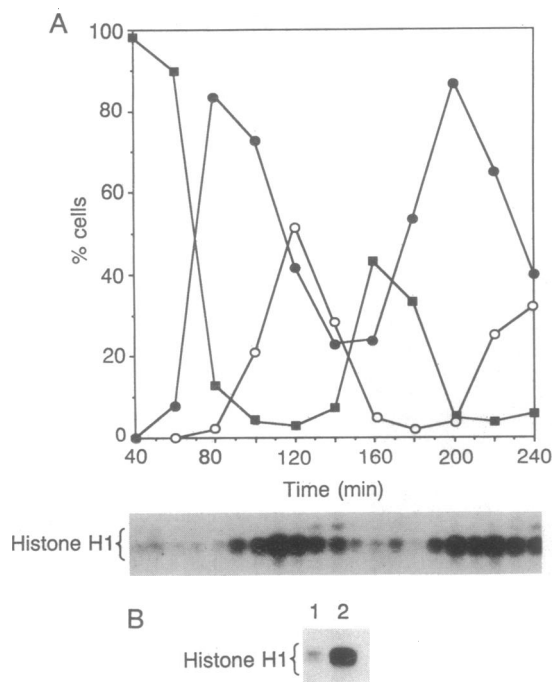


FIG. 3. Histone H1 kinase activity through the cell cycle. (A) Cells synchronized by mating pheromone block and release were assayed for Cdc28 protein kinase activity using histone H1 as a substrate as a function of progression through the cell cycle. The percentages of cells with small buds (●), mitotic nuclei (○), and no buds (■) are indicated as parameters of synchrony and cell cycle progression. Labeled histone H1 is indicated after SDS/PAGE of reaction products and autoradiography. (B) Recently abscised daughter cells and a heterogeneous population of cells prepared by centrifugal elutriation were assayed for Cdc28 protein kinase activity, as described in A. Lanes: 1, newly released daughter-enriched population; 2, mixed population.

The substrate activity of an endogenous protein, p40, that coimmunoprecipitates with Cdc28 protein kinase complexes from asynchronous cellular lysates has been reported (25). By using synchronous cycling cultures, complexes capable of phosphorylating p40 *in vitro* were enriched in early G₁ fractions. However, we now believe that result to be the consequence of *in vitro* mixing of free p40, which accumulates in G₁, and mitotic Cdc28 complexes that we show here to be extremely active and that will inevitably contaminate G₁ fractions because of the short interval between mitosis and G₁. Thus, as synchrony improves, the window of p40 phosphorylation becomes increasingly restricted. The cell cycle profile of p40 phosphorylation based on a highly synchronous culture can be seen in Fig. 3A, where p40 is the unmarked species of slightly lower mobility than that of histone H1.

DISCUSSION

Mitotic Role for Cdc28. It has long been perplexing that the Cdc28 protein kinase, clearly a structural homolog of the mitotic inducers for a broad spectrum of eukaryotic organisms, did not have a clearly demonstrable mitotic role (1, 2). It was furthermore shown that the *CDC28* gene could rescue the G₁ and mitotic defects conferred by a mutation in the homologous gene, *cdc2*⁺, of *Sc. pombe* (34). This suggested that the Cdc28 protein kinase could assume a mitotic role when expressed in other organisms. One report (32) claimed that the Cdc28 protein kinase was required to proceed through mitosis in *Sa. cerevisiae*. This conclusion was based on an allele, *cdc28-1N*, which was reported to confer cell cycle arrest only in G₂ and the demonstration that another allele, *cdc28-6* (2), which normally confers arrest in G₁, could arrest cells late in the cell cycle if they had been synchronized in G₁. However, the execution point for this cell cycle block was determined to be in G₁ (32). Our attempts to reproduce these experiments have been unsuccessful. We found that *cdc28-1N* mutant cells grow poorly at all temperatures but eventually arrest in G₁ at the restrictive temperature, although not necessarily on the first cell cycle (M. D. Mendenhall and S.I.R., unpublished data). Our attempts to produce G₂ arrest in other *cdc28* mutants after release from a mating pheromone block were similarly unsuccessful. Flow cytometric analysis indicated that the arrest was heterogeneous relative to cell cycle position and transient in duration. Furthermore, a similar transient arrest was observed with wild-type cells, probably a result of heat shock during a sensitive window of the cell cycle (M. D. Mendenhall and S.I.R., unpublished data).

The results reported here clearly establish a mitotic role for the Cdc28 protein kinase. *cdc28* mutant cells, if prearrested in S or G₂ phase, can be homogeneously synchronized in G₂ by a shift to a high restrictive temperature (38°C). However, an asynchronous culture shifted to the same restrictive temperature arrests with >80% of the population in G₁ (data not shown). There are a number of trivial explanations for this apparent paradox, most of which are probably related to properties intrinsic to temperature-sensitive mutant proteins. (i) Decay of the activity of a thermolabile protein most likely takes time. Hence we began the restrictive temperature incubation 1 hr prior to removal of the S-phase arresting agent. (ii) The G₁ to S phase transition controlled by the Cdc28 protein kinase may require a higher level of activity than the G₂ to M phase transition, explaining the bias toward arrest in G₁ as the thermolabile function decays. We therefore used the maximum restrictive temperature tolerated in our genetic background without disturbance of growth and division, 38°C. It is noteworthy that most previous work with the alleles *cdc28-4* and *cdc28-13* has been at the minimum restrictive temperature, 34–36°C. At these temperatures, arrest in G₂ is not observed for either allele using our protocol,

suggesting that enough residual activity remains for the mitotic function even though the cells cannot proceed through G₁ to S phase. This is undoubtedly the reason that the *cdc28-13* allele arrested in G₁ after completing mitosis even at 38°C (Fig. 1). The completion of mitosis, however, did lag relative to the wild-type controls in that experiment (Fig. 1) and was blocked completely in the subsequent experiment (Fig. 2), indicating that reduced Cdc28 protein kinase activity conferred by the *cdc28-13* allele has an impact on mitosis.

In the fission yeast, *Sc. pombe*, the homologous gene *cdc2*⁺ has execution points in G₁ and G₂ (3). Temperature-sensitive alleles do not appear to show an arrest-point bias at any restrictive temperature, although distinguishing G₁ arrest from G₂ arrest in fission yeast is technically more difficult than in budding yeast. It is likely that these differences reflect quantitative aspects of protein kinase function rather than qualitative differences. Thus, it appears that, for all their obvious dissimilarity, these highly diverged yeasts share a common central organization to their cell cycles based on a single protein kinase. The *mih1/wee1* G₂ arrest used in our experiments to localize the mitotic Cdc28 execution point is analogous to a similar mutational arrest in *Sc. pombe* mediated by the *cdc25* and *wee1* mutations (40, 42, 43). In addition, tyrosine phosphorylation of the *cdc2* protein kinase associated with this G₂ arrest (22) also occurs under parallel circumstances with the Cdc28 protein kinase (unpublished observations).

Our findings provide a landmark for the cell cycle of budding yeast. Prior investigations have proposed that mitosis in *Sa. cerevisiae* is controlled and begins prior to S phase (32). This view has been based on the previous difficulty in defining a G₂ function for *CDC28* in budding yeast and on cytological grounds—that a spindle-like structure is observed during most of the cell cycle (44). Our demonstration of a role for *CDC28* after S phase indicates that mitosis in budding yeast is controlled late in the cell cycle, as is the case with most other eukaryotes. Given the particular structural features of the budding yeast mitotic apparatus, it is possible that mitosis is regulated at two points, with aspects related to spindle assembly controlled early and control of mitotic segregation of chromosomes occurring late.

Timing of the Cdc28 Protein Kinase Function. Based on the experiments presented here, it can be said that *CDC28* must function in G₂ for cells to enter into mitosis. A G₁ function for the kinase has also been defined genetically (1, 2). When assayed in synchronous populations of cells using histone H1 as a substrate, Cdc28 protein kinase activity accumulates to high levels during the G₂/M phase interval, corresponding to the genetically determined mitotic function. Only low levels of activity appear in G₁. This is consistent with our observation that complexes containing the Cdc28 protein kinase and G₁ cyclins, which presumably constitute the active G₁ kinase, have low but detectable histone H1 kinase activity (45). Thus most of the histone kinase activity observed corresponds to the mitotic forms that presumably contain mitotic cyclins. The genes encoding such cyclins have recently been identified in *Sa. cerevisiae* (H. Richardson and S.I.R., unpublished data). Furthermore, the observation that histone H1 kinase activity is low in newly released postmitotic cells is consistent with the demonstration in other systems that maturation-promoting factor function is not required after metaphase (6, 8). In these respects the requirements for and observations of Cdc28 kinase activity parallel those defined for homologs in other organisms.

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