Cdc37 is a molecular chaperone originally identified as being required for the Start event during the G1 phase of the cell cycle in the budding yeast *Saccharomyces cerevisiae* (25) and as a protein associating with Hsp90 and certain protein kinases in metazoan (6, 12). Cdc37 is now known to be a molecular chaperone that usually acts as a co-chaperone of Hsp90, though it also appears to have Hsp90-independent functions (see references 16 and 24 for reviews). The domain structure of mammalian Cdc37 has been elucidated, and regions involved in client binding, dimerization, and Hsp90 binding have been identified (30). The interaction between Cdc37 and Hsp90 has been characterized at the atomic level (26), and recently a structure for a complex containing Cdc37, Hsp90, and a known client, Cdk4, has been proposed (38).

A considerable body of evidence indicates that most client proteins of Cdc37 are protein kinases (reviewed in references 16 and 24; see also Cdc37 interactions at http://www.picard.ch/downloads/downloads.htm). The evidence for a protein kinase being a Cdc37 client in mammalian cells is generally biochemical; a variety of protein kinases, many with oncogenic forms, have been shown to interact physically with Cdc37, often in conjunction with Hsp90 (24). In budding yeast, evidence for Cdc37 client status is more commonly based on genetic interactions, though reduction in protein level or kinase activity in *cdc37* mutants has been demonstrated in some cases (8, 11, 29). In addition, a few direct interactions between Cdc37 and client kinases have been reported (1, 4, 19). We and others previously described the identification of the *cdc37* gene of the fission yeast *Schizosaccharomyces pombe* (34, 39) and characterization of its protein product (36). *S. pombe* Cdc37 binds Hsp90, though, as observed in *S. cerevisiae*, far more weakly than the metazoan protein. Interestingly, the region of Cdc37 believed to interact with Hsp90 does not appear to be essential for function and cell viability in either yeast (15, 36). Two likely clients of Cdc37—Cdc2 (see below) and a signaling kinase, Spc1 (also called Sty1) of the mitogen-activated protein kinase family—have been shown to interact directly with Cdc37 (34, 37).

*S. pombe* is an excellent model organism for study of the cell cycle, as the mechanisms by which it regulates the key events of DNA replication, mitosis, septation, and cell division, are well understood. Relevant to this work are the controls that govern the G2-mitosis transition (21) and septum formation (14). Entry into G2 is regulated by the activity of the cyclin-dependent kinase Cdc2 in a complex with an activator, the B-type cyclin Cdc13, which is required for kinase activity. This complex is normally held inactive during G2 by phosphorylation of a crucial tyrosine residue of Cdc2, Tyr15 (21). Removal of the phosphate by Cdc25 activates the complex and promotes mitosis. Pathways that block entry into mitosis generally do so by maintaining or increasing the level of phosphorylation of Tyr15 (21, 23). At restrictive temperature, *S. pombe* temperature-sensitive *cdc37* (*cdc37ts*) mutants show greatly reduced Cdc2 kinase activity and consequent cell cycle arrest, predominantly in G2. Phosphorylation of Cdc2 Tyr15 is not detectably affected in *cdc37ts* mutants, but the association of Cdc2 with Cdc13 is greatly reduced, accounting for the reduction in kinase activity (37).

Septum formation in *S. pombe* is controlled by a set of pathways that make up the septum initiation network (reviewed in reference 14). In outline, the system is centered on a scaffold consisting of Cdc11 and Sid4, which is tethered to the spindle pole body (spb) throughout the cell cycle. On this
scaffold, the various regulators of septum initiation assemble. Relevant to this study are the Ras-like Spg1 GTPase and the protein kinase Cdc7. Spg1 binds constitutively to the Cdc11 scaffold, and its nucleotide binding is regulated by Byr4 and Cdc16, which act as a GTPase-activating protein. During interphase, Cdc7 is delocalized within the cell, but in early mitosis it appears at the recently divided spbs, and this seems to be essential for correct septation (18). Localization of Cdc7 to the spbs apparently occurs through binding specifically to the GTP-bound form of Spg1. Later in mitosis, Spg1 is converted to the GDP form at one of the daughter spbs, and Cdc7 binding is lost at that spb (14). At the end of mitosis, Cdc7 delocalizes again. The kinase activity of Cdc7 is required for septation, but not for localization of Cdc7 at the spb, which is mediated by a different domain or domains of the protein (18).

Synthetic lethality resulting from combining two nonlethal mutations in different genes can be a useful indicator that the two genes are involved in the same biological process. On this basis, synthetic-lethal screens (10) have frequently been used to identify new genetic elements that interact with a known component of a pathway or process. Several Cdc37 candidate clients were identified in S. cerivisiae as showing synthetic lethality with a cdc37ts mutant (19). We previously observed that S. pombe cdc37 temperature-sensitive mutations interact lethally with temperature-sensitive mutations in spo11 (Hsp90) (36), cdc2 (37), and orb5 (I. Martin, unpublished observation), which encodes casein kinase II (31). In the present study, our aim was to identify new client proteins and/or cochaperones of Cdc37 by carrying out a genetic screen for mutations showing synthetic lethality with a cdc37ts mutation at a temperature permissive for this mutation alone. We report here the identification of Cdc7, a protein kinase required for septum formation and subsequent cell division, as a candidate Cdc37 client.

### MATERIALS AND METHODS

**S. pombe strains and procedures.** The strains used in this work are shown in Table 1. Strains carrying cdc37 temperature-sensitive mutations were described previously (34, 36). Strains carrying tagged cdc7 alleles were gifts from Veihurst Simans (28, 32). The tagged pcp1 allele pcp1-mcherry was a kind gift from Kevin Hardwick (Institute of Cell Biology, Edinburgh, United Kingdom). Crosses were carried out by standard procedures and analyzed by tetrad dissection or random spore analysis. Transformation of S. pombe strains was carried out by electroporation.

### RESULTS

**Synthetic-lethal screen.** Synthetic lethality between two mutations is an indicator of genetic interaction between the two genes concerned and may be due to a physical interaction between the respective gene products. Screens for mutations showing synthetic lethality with a known mutation have been used in yeasts and other organisms to identify interacting genes (and gene products) (10).

| Strain no. | Genotype | Source |
|------------|-----------|--------|
| ED0862     | leu1-32 ura4-D18 h+ | Laboratory stock |
| ED1538     | cdc37-681 leu1-32 ura4-D18 h+ | CA1388 from K. Shiozaki (34) |
| ED1586     | cdc37-681 leu1-32 ura4-D18 ade6-210 h+ | Laboratory stock |
| ED1587     | cdc7-24 leu1-32 h+ | Laboratory stock |
| ED1591     | cdc7-GFP(ura4+) pcp1-mcherry leu1-32 ura4-D18 h+ | This study; cdc7-GFP allele from V. Simans (32) |
| ED1592     | cdc7-184 cdc7-GFP(ura4+) pcp1-mcherry leu1-32 ura4-D18 h+ | This study; cdc7-GFP allele from V. Simans (32) |
| ED1594     | cdc7-HA(ura4+) leu1-32 ura4-D18 h+ | This study; cdc7-HA allele from V. Simans (28) |
| ED1595     | cdc7-HA(ura4+) leu1-32 ura4-D18 h+ | This study; cdc7-HA allele from V. Simans (28) |
| ED1596     | cdc7-HA(ura4+) cdc7-184 leu1-32 ura4-D18 h+ | This study; cdc7-HA allele from V. Simans (28) |
| ED1598     | cdc7-HA(ura4+) cdc7-184 leu1-32 ura4-D18 h- | This study; cdc7-HA allele from V. Simans (28) |
| ED1599 (J322) | cdc37-681 leu1-32 ura4-D18 syn-322 [pREP82-cdc37] h- | This study |
| ED1600     | cdc2-L7 cdc7-HA(ura4+) leu1-32 ura4-D18 h+ | This study |
| ED1605     | syn322 (= cdc7-322) leu1-32 ura4-D18 h- | This study |
| ED1606     | cdc37-184 pcp1-mcherry leu1-32 ura4-D18 h+ | This study |

**Table 1. S. pombe strains used**

**Gene libraries and plasmids.** The plasmid pREP82-cdc37+ carries the ura4+ selectable marker and expresses cdc37+ in a thiamine-repressible manner; the use of this strain in the plasmid shuffle assay has been described previously (36). Genomic plasmid libraries in the vector pLSK+ (33) were a kind gift from Stuart MacNeil; the vector carries S. cerevisiae LEU2, which complements S. pombe leu1-32.

**Protein extraction, Western blotting, immunoprecipitation, and Cdc7 kinase assays.** Protein extraction, Western blotting, immunoprecipitation, and Cdc7 kinase assays were carried out essentially as described previously (37). The buffer for preparing native protein extracts and carrying out immunoprecipitation experiments was 50 mM Tris-HCl (pH 7.2), 1% Complete Inhibitor (Roche), 1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium orthovanadate, 0.1 mM EDTA, 1 mM dithiothreitol, 60 mM β-glycerophosphate, and 1% (vol/vol) NP-40. Mono- clonal antibody 12AC5 (Roche) was used for immunoprecipitation and Western blot analysis of hemagglutinin (HA)-tagged Cdc7.

Cdc7 kinase assays were based on the procedure described by Fankhauser and Simans (7) using myelin basic protein (Sigma) as a substrate. In outline, 4 × 10⁸ cells were harvested, washed, and broken in 1 ml native buffer with glass beads on a Ribo lysar. One hundred microliters of extract was added to 20 μl Protein A-Sepharose beads prebound with 10 μl 12AC5 antibody (400 μg/ml) and incubated for 2 h at 4°C on a rotating wheel. After being washed three times, the beads were resuspended in 20 μl kinase buffer (25 mM MOPS [morpholinopropansulfonic acid], pH 7.2, 1 mM dithiothreitol, 1% Complete Inhibitor [Roche], 0.1 mM sodium orthovanadate, 15 mM EDTA, 15 mM MgCl2, 60 mM β-glycerophosphate). Ten microliters of beads was incubated at 37°C for 5 min, and 10 μl kinase reaction buffer (500 μg/ml myelin basic protein, 20 μM ATP, 2 μM [γ-32P]ATP [10 μCi/ml; Amersham; 3,000 Ci/mmol]) was added. After 15 min at 37°C, the reaction was stopped by the addition of 2× sodium dodecyl sulfate (SDS) gel loading buffer and heated at 100°C for 3 min; 10 μl was ran on an SDS-polyacrylamide gel electrophoresis (PAGE) gel, which was dried and autoradiographed.

**Cytological methods.** For visualization of the nuclear DNA, cells were washed and fixed in methanol at −20°C or in 3.7% (wt/vol) formaldehyde, mounted in antifade solution containing DAPI (4,6-diamidino-2-phenylindole) (10 μg/ml), and examined under UV excitation on a Zeiss fluorescence microscope (2). Cells walls and septa were visualized by inclusion of Calcofluor (Sigma) (2). Cdc7-green fluorescent protein (GFP) and Pcp1-mcherry were visualized using blue and green excitation filter sets, respectively, on an Intelligent Imaging Innovations (3) Marianas system, which incorporates a Zeiss Axiosvert microscope, CoolSnap charge-coupled device, and Slidebook (3i) software.
We adopted the following strategy. Strain ED1538 (obtained from K. Shiozaki as CA1388) (34) has the genotype cdc37-681 leu1-32 ura4-D18. It was transformed with pREP82-cdc37, in which the coding region of cdc37 is expressed from a weak version of the thiamine-repressible nmt1 promoter (9), to generate ED1595. This strain requires leucine but not uracil, as the plasmid carries ura4+. At 25 to 28°C, the plasmid is not essential for viability of ED1595, which can therefore grow in the presence of 5-fluoroorotic acid (5-FOA), which selects against ura4+ cells. ED1595 was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine for 60 min, which resulted in 68% lethality. The mutagenized cells were stored at −75°C in 20% glycerol. For screening, aliquots were plated on EMMG plus Leu, incubated at 28°C for 3 days, and then replicated to EMMG plus Leu plus Ura plus 5-FOA. After 2 days of growth, colonies growing on the plates without FOA, but not on the plates with FOA, were picked and retested. Strains with this growth pattern are unable to lose the plasmid and are therefore 5-FOA sensitive; some of these strains were expected to carry a mutation conferring synthetic lethality with cdc37-681. As a cross-check, these colonies were also tested for inability to grow on medium containing thiamine: synthetic-lethal mutants should not be able to tolerate repression of cdc37 expression from the pREP82 plasmid (39).

From about 40,000 colonies screened, 12 candidate mutants were obtained, 2 of which were rejected, as the plasmid had apparently integrated into the chromosome. The next stage in analysis was to introduce S. pombe genomic libraries into each of the 10 remaining strains and to select for plasmids able to rescue the synthetic lethality. Colonies carrying rescuing plasmids were able to grow in the presence of 5-FOA because they were no longer dependent on the pREP82-cdc37 plasmid, which carries the ura4+ marker. Of the 10 strains screened, plasmids were isolated that could rescue 5. One of these strains, J322, was of particular interest, and its analysis forms the basis for the work described below.

Characterization of strain J322. As explained above, J322 appears to harbor a mutation synthetically lethal with cdc37-681. We noted that J322 showed a slight elongation phenotype at 36°C in comparison with the parent strain, ED1595 (Fig. 1B), and this suggested that a cell cycle defect was conferred by the synthetic-lethal mutation present in the strain.

J322 is unable to grow in the presence of 5-FOA because it requires the pREP82-cdc37 plasmid for viability (Fig. 1A, middle). However, eight transformants of J322 carrying library plasmids were 5-FOA resistant and therefore not dependent on pREP82-cdc37 (Fig. 1A, right, J322/lib). The library plasmids from these strains were rescued into E. coli and analyzed. Only one plasmid, pJ322-H7, from the HindIII library, was able to rescue J322 on retest.

The truncated cdc7 gene in plasmid pJ322-H7 is responsible for rescue of J322. The sequences of the ends of the pJ322-H7 insert were determined and compared with the S. pombe genomic database at the Sanger Centre. Both sequences were present on chromosome 2, with the coordinates of the HindIII sites at the ends of the insert being 3223842 and 3230393.

This region of the genome contains a 3′ truncation of the cdc7 gene (whose coding region extends from coordinates 3226430 to 3222871, beyond the end of the insert), the entire pppk24 gene, and the spn7 gene truncated at the 5′ end of the coding region. It seemed unlikely that spn7 was responsible for the rescue activity because of the 5′ truncation and because the gene is expressed only during meiosis and sporulation (17). Little is known about pppk24, although it is an inessential gene predicted to encode a protein kinase (5). The best candidate for the rescuing activity appeared to be cdc7, because it encodes an essential protein kinase required for septum formation and cell division (7), consistent with the observed cell cycle defect of J322. Cdc7 is a protein of 1,062 amino acids, but the truncated gene present on pJ322-H7 is predicted to encode only the first 709 amino acid residues. This might, however, retain function, as it includes the amino-terminal kinase domain and because a similar truncation encoding the first 640 amino acids has been reported to be functional (7). Also present in both truncations is the region between residues 250 and 535, which is required for binding to Spg1, although part of a larger region (residues 360 to 870) reported to be required for localization to the spb is absent (18). To further our investigation, we obtained a plasmid expressing the full-length cdc7 gene, pREP41-cdc7 (28), and introduced it into J322. The resulting transformant was able to grow on 5-FOA (Fig. 1A, right, J322/cdc7+), demonstrating that the synthetic lethality of J322 was suppressible by cdc7. Indeed, pREP41-cdc7 showed stronger suppression than the library plasmid pJ322-H7.

FIG. 1. Growth and cell morphology of synthetic-lethal strain J322. (A) Effect of 5-FOA on growth of ED1595, mutant strain J322 (ED1599), and derivatives of J322. Left, ED1595 and J322 on medium lacking 5-FOA; middle, same strains on medium containing uracil and 5-FOA; right, J322 transformed with rescuing library plasmid pJ322-H7 (lib) and with pREP41-cdc7+ (cdc7) on medium with uracil and 5-FOA. The strains were streaked and incubated at 28°C for 3 days without 5-FOA and 4 days with 5-FOA. (B) Cell morphologies of ED1595 and J322 after growth at 36°C on EMMG plus leucine.
asked whether the defect in J322 was in the cdc7 gene. (A) (Left) Tetrads from a cross between ED1587 (cdc7-24) and ED1586 (cdc37-681) show predominantly three viable spores at 25°C, suggesting a synthetic-lethal interaction. (Right) Tetrads from J322 × ED0862 (cdc37-77) show a similar pattern. (B) Morphologies (at 36°C) of three viable spore colonies from a single tetrad. One shows normal wild-type (w-t) morphology, one shows morphology typical of cdc37-681 (modest elongation and curved cells), and the other shows great elongation and cell lysis.

The synthetic-lethal mutation in J322 lies in cdc7. We next asked whether the defect in J322 was in the cdc7 gene itself or in another gene whose defect could be suppressed by increased cdc7 expression. J322 was outcrossed to a cdc7+ strain, and the progeny were analyzed by tetrad analysis. Most of the tetrads had only three viable spores (Fig. 2A). These consisted of one wild-type spore, one spore with characteristic cdc37-681 phenotype (moderate elongation and curved cells), and one spore with very elongated cells, some of which showed lysis (Fig. 2B). The last phenotype is similar to that of cdc7 and other septum initiation mutants (7, 22). The segregation pattern observed is that of tetratype tetrads, with the double mutant carrying cdc7-322 and the other spore colonies from a single tetrad. One shows normal wild-type (w-t) morphology, one shows morphology typical of cdc37-681 (modest elongation and curved cells), and the other shows great elongation and cell lysis.

We were interested to know whether a known cdc7 mutant, cdc7-24 (22), would be synthetically lethal with cdc37-681, and we crossed two strains carrying these mutations with one another. The segregation pattern in tetrads (Fig. 2A) showed predominantly tetratype segregation with the presumptive double-mutant progeny absent (i.e., three viable spores). The phenotypes of viable spores were consistent with this, and backcrosses of the viable spores from two tetratype tetrads showed that none of the viable spores carried both cdc37 and cdc7 mutations. Nonparental ditype tetrads were also found, consisting of only two viable spores with the wild-type phenotype. These results show that cdc7-24 is synthetically lethal with cdc37-681. A further demonstration of the interaction between cdc7-24 and cdc37 is the observation that overexpression of cdc37 from pREP1 suppresses the growth defect and elongation phenotype of cdc7-24 (not shown).

Two cdc7-like spore colonies from separate tetrads from the cross J322 × cdc7+ were crossed with a strain carrying cdc7-24. Spores from these crosses were allowed to grow up at 25°C and were then tested at 36°C. In each cross, all of more than 100 progeny showed an elongated-cell phenotype and lysis, indicating that the synthetic-lethal mutation in J322 was very closely linked to cdc7. Taken together with the other observations presented here (and below), this result demonstrates that the synthetic-lethal mutation is indeed an allele of cdc7, which we have designated cdc7-322.

Mutant phenotype of cdc7-322. Defects in genes of the septum initiation network, such as cdc7, result in defects in the regulation of septum formation (14). The cdc7-24 mutation causes an inability to form septa at restrictive temperature of 36°C while allowing continuing rounds of DNA replication and mitosis, resulting in the formation of cells with two, four, and more nuclei. Cell lysis is also caused by the cdc7-24 mutation (22). As cdc7-322 shows temperature-sensitive cell elongation and lysis, we asked whether multinucleate cells formed. Figure 3 shows that, indeed, the cdc7-322 mutant leads to the formation of cells with two and four nuclei in a manner similar to that of cdc7-24.

Defects of Cdc7 in cdc37ts mutants. The product of cdc7 is a protein kinase required for the initiation of septation (7). cdc7 mRNA, protein, and protein kinase levels do not change during the cell cycle (7, 27), but the location of at least a proportion of the Cdc7 protein is cell cycle regulated (7, 32). During early mitosis, Cdc7 localizes to the spb and is distributed to both poles of the spindle when the spb divides. Cdc7 is rapidly lost from one of the spbs, however, so that only one dot is visible in mid- to late mitosis (32).

Kinase activity. We set out to ask in what ways a Cdc37 defect might affect Cdc7 function: by altering its stability, kinase activity, or localization. First, we examined the level of Cdc7-HA protein in strains expressing Cdc7-HA as their only source of Cdc7 (7). The levels of Cdc7 protein in two different cdc37 mutants (cdc37-681 and cdc37-184) were unaltered after 3 h at restrictive temperature (Fig. 4A). We tested the kinase activity of immunoprecipitated Cdc7-HA using myelin basic protein as a substrate. Cdc7 kinase activity present in immunoprecipitates of the two cdc37ts mutants was seen to decrease...
cycle was affected by compromised Cdc37 function. As a first step in this investigation, we constructed strains in which Cdc7 was tagged with GFP (32) and the spb component Pcp1 was tagged with mCherry (a modified red fluorescent protein) and which carried either cdc37ts or one of two ts alleles of cdc37. Strain ED1591 carrying wild-type cdc37ts, and GFP-tagged Cdc7 was morphologically normal at 25 and 36°C, as previously reported for similar strains lacking the tag on Pcp1 (32). To our surprise, strain ED1592, which carries cdc37-184 and cdc7-GFP, showed some elongation at 25°C and greatly elongated cells at 36°C, in which signs of lysis were evident (Fig. 5A). In contrast, strain ED1606, which carries the same cdc37 mutation but untagged Cdc7, showed typical cdc37ts morphology: moderately elongated curved cells at 36°C and essentially normal at 25°C (Fig. 5A). Thus, it appears that the modified but functional Cdc7-GFP shows an interaction with compromised Cdc37. Even over a short time course, the proportion of binucleate cells in the double mutant increased greatly compared with the single cdc37-184 mutant (Fig. 5B), strongly suggesting a delay in septation. Presumably, the GFP tag on Cdc7 affects its function in a way that has no observable phenotypic effect in a cdc37ts background but has a much more drastic effect when Cdc37 is also compromised. A further effect of the interaction, to increase the proportion of septated cells, is considered in the Discussion below. A double-mutant strain combining cdc37-681 and cdc7-GFP showed a phenotype similar to but weaker than cdc37-184 cdc7-GFP (not shown).

**Cdc7 localization in cdc37ts strains.** Cdc7 has no specific localization during interphase (32). However, early in mitosis, it localizes to the spb, and this appears to be necessary for proper septum formation (18). Given the genetic interactions between cdc37 and cdc7 and the loss of Cdc7 kinase activity in cdc37 mutants, we wondered whether Cdc7 localization to the spb also required Cdc37 activity. We examined strains ED1591 and ED1592, both of which carry tagged alleles of Cdc7 and Pcp1 (a spb component), after growth at 28°C followed by 2 h of incubation at 36°C; in each case, two cells are shown in the figure. The cells were fixed and examined by fluorescence microscopy. Mitotic ED1591 (cdc37ts) cells consistently showed two red dots at the periphery of the nucleus, corresponding to Pcp1-mCherry at the spb (Fig. 6 and not shown). Early in mitosis (Fig. 6a), two green dots indicating the presence of Cdc7-GFP were visible, coincident with the Pcp1 dots. Later in mitosis (Fig. 6b), only one Cdc7 dot was seen, consistent with previous reports (32). In ED1592, which carries cdc37-184, a single green dot was visible in late-mitotic cells (Fig. 6c and d). This indicates that in this cdc37ts cdc7-GFP double-mutant strain, which is defective in septation at 36°C, Cdc7 still localizes to the spb during mitosis. It thus seems unlikely that Cdc37 plays a role in the localization of Cdc7.

**DISCUSSION**

In this paper, we describe the isolation of mutations in *S. pombe* that are synthetically lethal with a temperature-sensitive mutation in the gene encoding the molecular chaperone Cdc37. One of these mutant strains, J322 (strain ED1599), carries a mutant allele of cdc7, as ascertained by several criteria. A plasmid isolated from a genomic *S. pombe* library that rescues the synthetic lethality carries a truncated form of cdc7;
full-length cdc7 rescues more effectively. A known cdc7 mutant, cdc7-24, also shows synthetic lethality with cdc37-681. The synthetic-lethal mutation in a cdc37+/H11001 genetic background shows a temperature-sensitive phenotype similar to that of cdc7-24, generating multinucleate cells at 36°C because of a defect in septation (Fig. 3). Furthermore, the synthetic-lethal mutation in J322 is tightly linked to cdc7. This and other evidence lead us to conclude that the mutation lies within cdc7: we have named it cdc7-322.

The genetic interactions between cdc37 and cdc7 suggested that Cdc7 might be a client protein of Cdc37, and we investigated this biochemically. In cdc37ts mutant cells, Cdc7 kinase activity was reduced progressively with time after transfer to the restrictive temperature, while the Cdc7 protein level remained constant (Fig. 4). The reduction in kinase activity appears to be due to a direct effect of impaired Cdc37 function on Cdc7 activity. An alternative explanation, that the reduction in Cdc7 kinase activity is an indirect effect of cdc37ts-mediated cell cycle arrest in G2, can be ruled out for the following reasons. G2 arrest in cdc37ts mutants is due to compromised Cdc2 activity (37). Cdc7 kinase activity is unaffected in arrested cdc2 mutants (Fig. 4) and in G2-arrested cdc25 cells in which...
S. cerevisiae client protein of Cdc37. It is noteworthy that the Cdc2 is inactive (32). On this basis, we propose that Cdc7 is a critical client of Cdc37 under these conditions. In contrast, in cdc37ts strains in which Cdc7 is tagged with GFP, the arrest phenotype is mixed, especially in cdc37-184 cdc7-GFP strains. In addition to G2 cells, a substantial proportion of cells resemble arrest cdc7ts mutant cells in showing elongation, an increased proportion of binucleate cells, and some cell lysis (Fig. 5). This suggests strongly that Cdc7 function is compromised by the presence of the GFP tag and that the activity of the tagged protein is highly dependent on Cdc7 function. Cdc7 thus appears to be a critical client of Cdc37 under these conditions, lending further support to the proposal that Cdc7 is a client of Cdc37. The mixed arrest phenotype of cdc37-184 cdc7-GFP cells is presumably due to both Cdc2 and Cdc7 activities being compromised to some extent, explaining the presence of both binucleate and unseptate binucleate cells. This could in principle be a dynamic situation in which cells transiently arrest at each of the cell cycle arrest points and escape at a low rate, an idea consistent with observations discussed below. It is also possible that the kinase activities of Cdc2 and Cdc7 affect one another, as has been demonstrated for their S. cerevisiae orthologues, Cdc28 and Cdc15 (13), and this might influence the nature and proportions of cellular phenotypes.

It seems likely that similar considerations apply to the lethal phenotype of cdc37ts cdc7ts strains at 25 or 28°C, which is permissive for the single mutants (Fig. 1A and 2A), although it is not possible to determine the cellular morphologies of these double-mutant strains because their viability depends on the presence of a rescuing plasmid.

The question arises as to whether Cdc37 is required for cell cycle processes other than the G2-M transition and septum initiation. It is interesting that about 35% of cdc7-GFP cdc37-184 cells are binucleate and septated after 4 h of incubation at 36°C (Fig. 5B). One possible explanation is that, in addition to septum formation, efficient septum cleavage requires Cdc7 function. This model would additionally require some escape of cells at a low rate from the blocks to cell cycle progress at G2-M and septum initiation. Cells escaping from G2-M would undergo mitosis and then arrest transiently as binucleate unseptate cells before escaping from that block and forming a septum. Failure to cleave septa in these cells would lead to accumulation of septated cells, as observed. Similarly, the presence of 15 to 20% binucleate septated cells when cdc37ts cdc7ts cells are incubated at 36°C for 4 to 6 h (37) (Fig. 5B) could be explained in a similar manner. Two explanations can be proposed for the defect in septum cleavage. First, Cdc37 might be directly required for activity of a protein needed for septum cleavage, most likely a (currently unknown) protein kinase. Alternatively, a proportion of the septa formed when Cdc37 function is impaired may be aberrant and unable to be cleaved. Uncleavable septa have been observed when regulation of septum initiation is defective, for instance, when spg1 is overexpressed or in other situations discussed in elsewhere (28).
ACKNOWLEDGMENTS

Initial stages of this work were supported by grant C14479 from the BBSRC. We thank the Darwin Trust of Edinburgh for a studentship for J. Liang.

We thank Ina Martin, Emma Turnbull, and Stuart MacNeill for helpful discussions and support. We are also grateful to Emma Turnbull for comments on the manuscript. We are indebted to Kevin Hardwick and members of his laboratory, particularly Josefin Fernius, for help with and advice about protein kinase assays and Karen May (the Hardwick laboratory) and Martin White (the Leach laboratory) for access to equipment and help with fluorescence microscopy.

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