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The *Schizosaccharomyces pombe* Cdc7 Protein Kinase Required for Septum Formation Is a Client Protein of Cdc37[▽]

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Cdc37 is an essential molecular chaperone found in fungi and metazoa whose main specificity is for certain protein kinases. Cdc37 can act as an Hsp90 cochaperone or alone; in yeasts, the interaction with Hsp90 is weak and appears not to be essential for Cdc37 function. Numerous genetic interactions between Cdc37 and likely client proteins have been observed in yeasts, but biochemical confirmation has been reported in only a few cases. We and others have generated and characterized temperature-sensitive *cdc37* alleles in *S. pombe* and have used them to investigate the cellular roles of Cdc37: previous work has shown that mitotic Cdc2 is a major client. In this paper, we describe a screen for mutations synthetically lethal with a *cdc37ts* mutant with the aim of identifying genes encoding further client proteins of Cdc37. Ten such strains were isolated, and genomic libraries were screened for rescuing plasmids. In one case, a truncated *cdc7* gene was identified. Further experiments showed that the mutation in this strain was indeed in *cdc7*. Cdc7 is a protein kinase required for septum initiation, and we show that its kinase activity is greatly reduced when Cdc37 function is impaired. Cdc7 normally locates to the spindle pole body during mitosis, and this appears to be unaffected in the *cdc37ts* mutant. Other evidence suggests that, in addition to mitosis and septum initiation, Cdc37 may also be required for septum cleavage.

Cdc37 is a molecular chaperone originally identified as being required for the Start event during the G_1 phase of the cell cycle in the budding yeast *Saccharomyces cerevisiae* (25) and as a protein associating with Hsp90 and certain protein kinases in metazoa (6, 12). Cdc37 is now known to be a molecular chaperone that usually acts as a cochaperone 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 G_2-mitosis transition (21) and septum formation (14). Entry into G_2 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 G_2 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 G_2. 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

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TABLE 1. *S. pombe* strains used

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. Simanis (32)
ED1592	*cdc37-184 cdc7*-GFP(*ura4*$^+$) *pcp1*-mcherry *leu1-32 ura4-D18 h*$^-$	This study; *cdc7-GFP* allele from V. Simanis (32)
ED1594	*cdc7-HA*(*ura4*$^+$) *leu1-32 ura4-D18 h*$^+$	This study; *cdc7-HA* allele from V. Simanis (28)
ED1595	*cdc37-681 leu1-32 ura4- D18* [pREP82-cdc37] *h*$^-$	This study
ED1596	*cdc7-HA*(*ura4*$^+$) *cdc37-681 leu1-32 ura4-D18 h*$^-$	This study; *cdc7-HA* allele from V. Simanis (28)
ED1598	*cdc7-HA*(*ura4*$^+$) *cdc37-184 leu1-32 ura4-D18 h*$^-$	This study; *cdc7-HA* allele from V. Simanis (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; *cdc7-HA* allele from V. Simanis (28)
ED1605	*syn322* (= *cdc7-322*) *leu1-32 ura4-D18 h*$^-$	This study
ED1606	*cdc37-184 pcp1*-mcherry *leu1-32 ura4-D18 h*$^-$	This study

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. cerevisiae* 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 *swo1* (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 Viesturs Simanis (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.

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 pALSK+ (33) were a kind gift from Stuart MacNeill; 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. Monoclonal 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 Simanis (7) using myelin basic protein (Sigma) as a substrate. In outline, 4×10^8 cells were harvested, washed, and broken in 1 ml native buffer with glass beads on a Ribolyser. 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 [morpholinepropanesulfonic acid], pH 7.2, 1 mM dithiothreitol, 1× Complete Inhibitor [Roche], 0.1 mM sodium orthovanadate, 15 mM EGTA, 15 mM $MgCl_2$, 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 μCi [γ-^{32}P]ATP [10 mCi/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 run 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). Cell 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 (3i) Marianas system, which incorporates a Zeiss Axiovert microscope, CoolSnap charge-coupled device, and Slidebook (3i) software.

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).

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 *ppk24* 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 *ppk24*, 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-*cdc37* (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-*cdc37* showed stronger suppression than the library plasmid pJ322-H7.

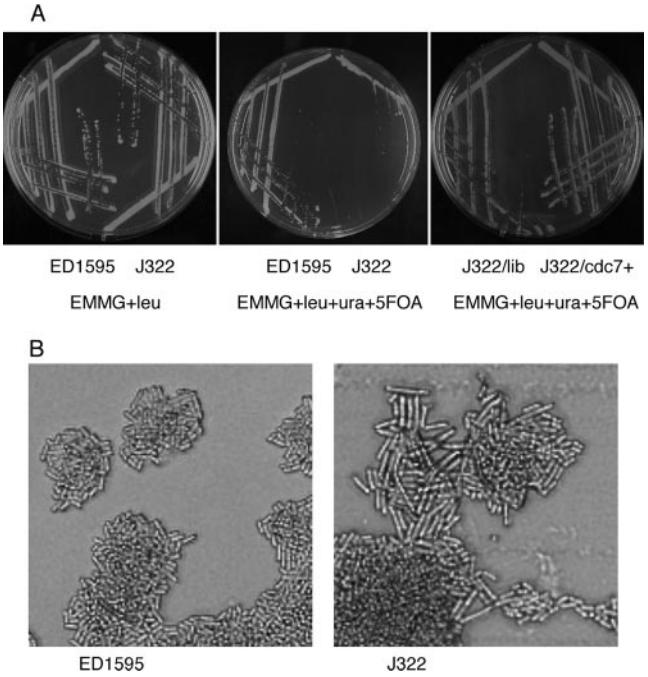

FIG. 1. Growth and cell morphology of synthetic-lethal strain J322. (A) Effect of 5-FOA on growth of ED1595, mutant strain J322 (ED1599), and derivatives of J322. Left, ED1595 and J322 on medium lacking 5-FOA; middle, same strains on medium containing uracil and 5-FOA; right, J322 transformed with rescuing 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.

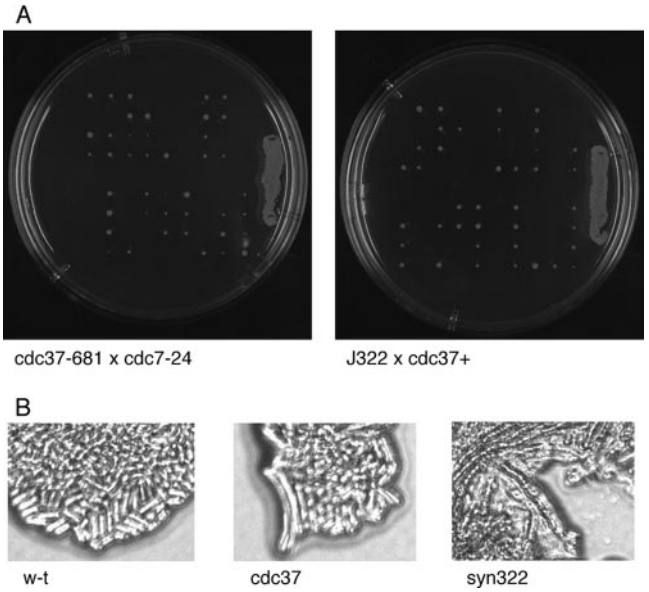

FIG. 2. The *syn-322* mutation in J322 is similar to *cdc7-24*. (A) (Left) Tetrads from a cross between ED1587 (*cdc7-24*) and ED1586 (*cdc37-681*) show predominantly three viable spores at 25°C, suggesting a synthetic-lethal interaction. (Right) Tetrads from J322 × ED0862 ($cdc37^+$) show a similar 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 $cdc37^+$ 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 *cdc37-681* and the *cdc7*-like mutation being nonviable. In one tetrad with four viable spores, one spore had apparently retained the pREP82-*cdc37* plasmid, as it was Ura^+.

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 pREP41 suppresses the growth defect and elongation phenotype of *cdc7-24* (not shown).

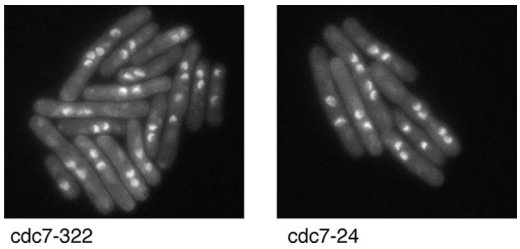

FIG. 3. *cdc7-322* blocks septum formation at 36°C and allows the formation of multinucleate cells. Cells of ED1605 (*cdc7-322*) and ED1587 (*cdc7-24*) were grown in liquid yeast extract at 25°C and shifted to 36°C for 3 h. The cells were fixed in methanol, stained with DAPI and calcofluor, and examined on a fluorescence microscope. In both strains, elongated cells with two and four nuclei were present, indicating a block to septation while the mitotic cycle continued.

Two *cdc7*-like spore colonies from separate tetrads from the cross J322 × $cdc37^+$ 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

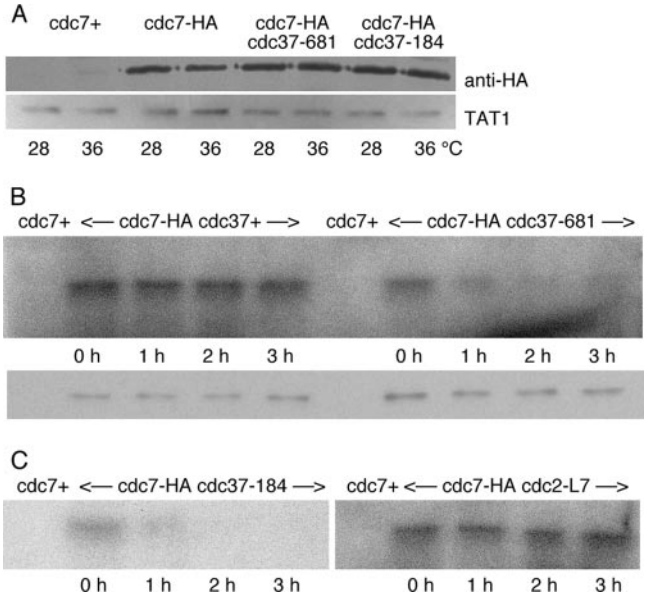

FIG. 4. Cdc7 kinase activity requires Cdc37 function. (A) The Cdc7 protein level is unaffected in *cdc37* temperature-sensitive mutants at restrictive temperature. Cells of strains ED0862 (*cdc7*$^+$), ED1594 (*cdc7-HA*), ED1596 (*cdc7-HA cdc37-681*), and ED1598 (*cdc7-HA cdc37-184*) were grown in liquid yeast extract (YE) at 28°C and shifted to 36°C. Samples were taken immediately before the shift and 3 h later, and total protein extracts were prepared. Aliquots of the extracts were separated by SDS-PAGE and probed for the HA epitope (top) and for α-tubulin using TAT1 antibody (bottom). (B) Cells of ED0862 (*cdc7*$^+$ *cdc37*$^+$), ED1594 (*cdc7-HA cdc37*$^+$), and ED1596 (*cdc7-HA cdc37-681*) were grown in liquid YE at 28°C and shifted to 36°C. Immediately before the shift (0 h) and at hourly intervals after the shift, native protein extracts were prepared and Cdc7-HA was immunoprecipitated; part of the washed immunoprecipitate was assayed for Cdc7 protein kinase activity. Kinase assay products were separated by SDS-PAGE and autoradiographed (top). A separate part of each extract was separated by PAGE and probed for Cdc7-HA (bottom). (C) Cells of ED1598 (*cdc7-HA cdc37-184*) and ED1600 (*cdc7-HA cdc2-L7*) were treated as described for panel B; kinase reactions are shown.

rapidly after a shift to restrictive temperature (Fig. 4B and C). As these mutants differ in the Cdc37 amino acid substitutions they contain (36), loss of Cdc7 kinase activity is very likely to be due to general loss of Cdc37 function rather than to any allele-specific effects. In contrast, the Cdc7 kinase activity of a *cdc37*$^+$ strain remained constant for 3 h at restrictive temperature. One consequence of shifting *cdc37ts* strains to restrictive temperature is that they arrest in G_2 because of compromised Cdc2 function (37). It was conceivable that the observed fall in Cdc7 kinase activity might be a consequence of reduced Cdc2 kinase activity and/or G_2 arrest, rather than a direct effect of the loss of Cdc37 function. We tested the kinase activities of Cdc7 in two *cdc2ts* strains, *cdc2-L7* and *cdc2-33*. In neither case was Cdc7 kinase activity affected (Fig. 4C and data not shown). This is consistent with the observation that *cdc25-22* arrested cells showed no major reduction in Cdc7 kinase activity (32) and demonstrates that the loss of Cdc7 kinase activity in *cdc37* mutants is likely to be a direct effect of compromised Cdc37. Thus, Cdc7 needs Cdc37 activity for normal function.

Genetic interaction between *cdc37-681* and *cdc7*-GFP. We wished to test whether the localization of Cdc7 during the cell 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 *cdc37*$^+$ or one of two *ts* alleles of *cdc37*. Strain ED1591 carrying wild-type *cdc37*$^+$, 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 *cdc37*$^+$ 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 (*cdc37*$^+$) 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*;

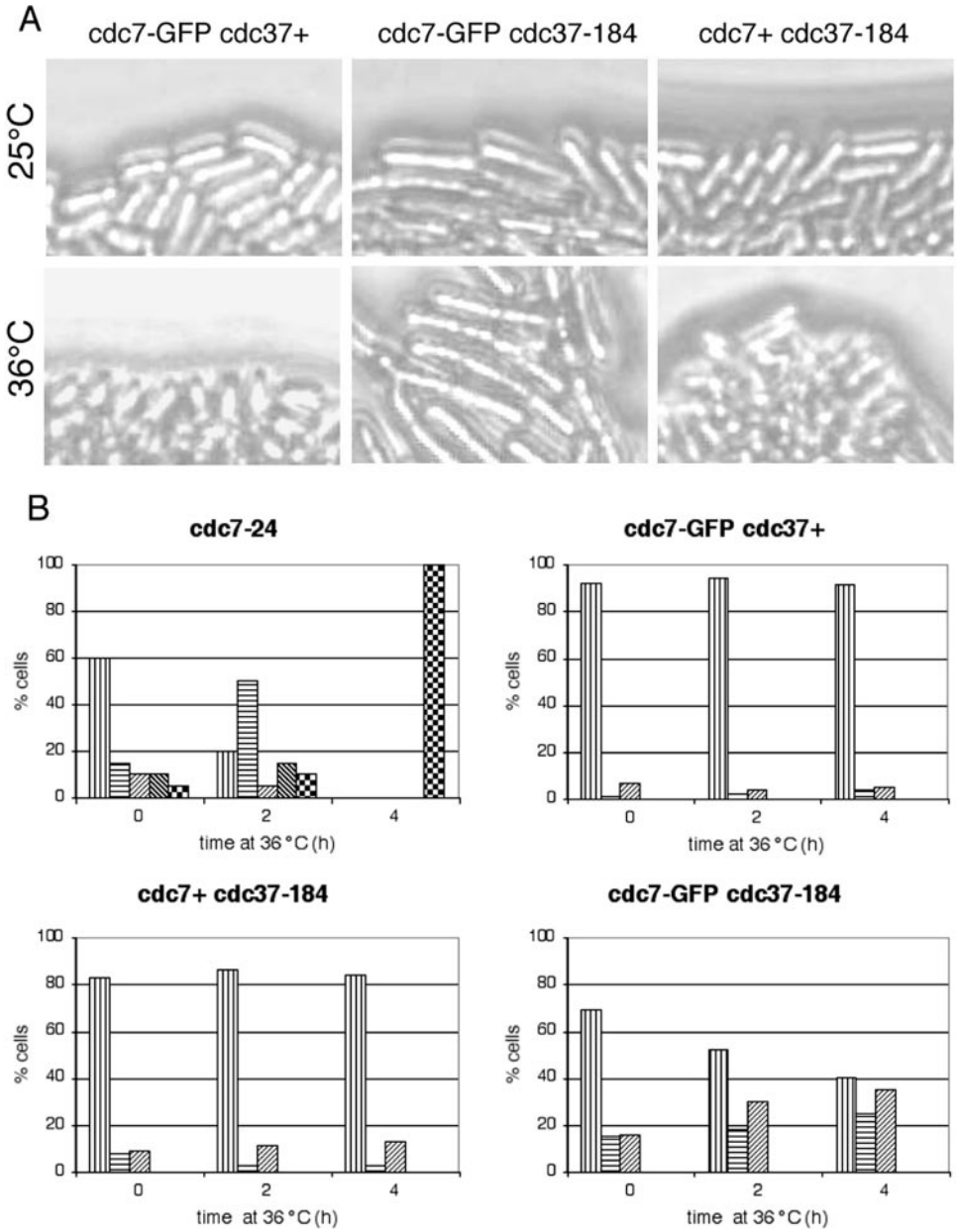

FIG. 5. A GFP tag on Cdc7 affects the arrest phenotype of *cdc37-184*. (A) Cells of ED1591 (*cdc7*-GFP *cdc37*$^+$), ED1592 (*cdc7*-GFP *cdc37-184*), and ED1606 (*cdc7*$^+$ *cdc37-184*) (all also *pcp1*-mcherry) were grown on solid medium at 25 and 36°C. Note the great elongation of ED1592 at 36°C. (B) Proportions of cells with various phenotypes after growth in liquid medium at 25°C and shift to 36°C for 0, 2, and 4 h. Vertical stripes, uninucleate cells; horizontal stripes, binucleate cells with no septum; upward hatch, binucleate septated cells; downward hatch, cells with three nuclei; cross hatch, cells with 4 or more nuclei.

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*$^+$ 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 G_2, can be ruled out for the following reasons. G_2 arrest in *cdc37ts* mutants is due to compromised Cdc2 activity (37). Cdc7 kinase activity is unaffected in arrested *cdc2* mutants (Fig. 4) and in G_2-arrested *cdc25* cells in which

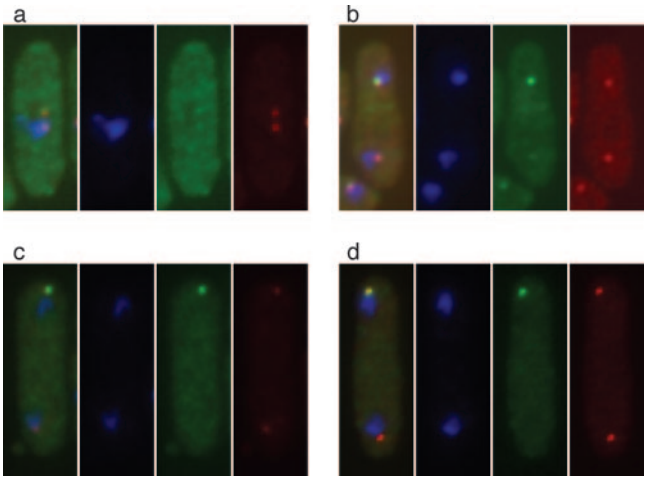

FIG. 6. Cdc37 is not required for localization of Cdc7 to the spb. Cells of strains ED1591 (*cdc7*-GFP *pcp1*-mcherry) and ED1592 (*cdc37-184 cdc7*-GFP *pcp1*-mcherry) were grown in liquid medium at 25°C and shifted to 36°C for 2 h, at which time samples were taken and processed for fluorescence microscopy. (a and b) ED1591 cells. (c and d) ED1592 cells. For each cell, a merged image is shown in the left-hand column, followed by DAPI (blue), Cdc7-GFP (green), and Pcp1-mcherry (red).

Cdc2 is inactive (32). On this basis, we propose that Cdc7 is a client protein of Cdc37. It is noteworthy that the *S. cerevisiae* orthologue of *S. pombe* Cdc7, Cdc15, behaves genetically as a Cdc37 client (19), although no biochemical confirmation of this has been reported. However, Ste11, whose kinase domain is very similar to that of Cdc7, is a genuine Cdc37 client (1).

The localization of Cdc7 to the spb during mitosis, believed to be essential for correct septation (18, 32), appears to be unaffected by *cdc37ts* mutations (Fig. 6). Our observations are consistent with the finding that Cdc7 kinase activity is not required for correct localization of the protein to the spb. Cdc7 has been shown to dimerize in vivo, and this is required for association with Spg1 and presumably also for septum initiation (18). It is possible that Cdc37 may be required to promote Cdc7 dimerization, though this possibility requires further investigation.

In some situations, particularly in metazoa, Cdc37 works together with Hsp90, and it has been described as a cochaperone of Hsp90 (16, 24). In yeasts, the interaction between the two proteins appears to be weak, and the presumptive Hsp90-binding regions of *S. pombe* Cdc37 (35) and *S. cerevisiae* Cdc37 (15) are not required for function and cell viability. We looked for genetic interactions between Cdc7 and Hsp90 by crossing *cdc7-24* with a temperature-sensitive Hsp90 mutant, *swo1-26* (3), but no synthetic lethality was observed. This contrasts with the behavior of *cdc2* mutants, where at least one mutant allele (*cdc2-33*) shows synthetic lethality with *cdc37ts* mutants (37), and with *swo1-w1*, a mutant allele similar to *swo1-26* (20). However, Cdc2 kinase activity is reduced in *cdc37ts* mutant cells (37) while it is unaffected in *swo1-w1* (20). The relationship between Cdc37 and Hsp90 and their roles in maintaining the activities of protein kinases may differ for different kinases (and perhaps different organisms), and further studies will be needed to unravel this complexity.

In otherwise wild-type cells, *cdc37ts* mutants show reduced Cdc2 kinase activity and predominantly arrest in G_2 as uninucleate cells with replicated DNA content and interphase microtubule arrays (37). This indicates that Cdc2 is the most 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 G_2 cells, a substantial proportion of cells resemble arrested *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 Cdc37 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 uninucleate and unseptated 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 G_2-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 Cdc37 function. This model would additionally require some escape of cells at a low rate from the blocks to cell cycle progress at G_2-M and septum initiation. Cells escaping from G_2-M would undergo mitosis and then arrest transiently as binucleate unseptated 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 cdc7*$^+$ 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).

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REFERENCES

1. **Abbas-Terki, T., O. Donze, and D. Picard.** 2000. The molecular chaperone Cdc37 is required for Ste11 function and pheromone-induced cell cycle arrest. FEBS Lett. **467:**111–116.
2. **Alfa, C., P. Fantes, J. Hyams, M. McLeod, and E. Warbrick.** 1993. Experiments with fission yeast: a laboratory course manual. Cold Spring Harbor Press, Cold Spring Harbor, NY.
3. **Aligue, R., H. Akhavanniak, and P. Russell.** 1994. A role for Hsp90 in cell-cycle control: Wee1 tyrosine kinase activity requires interaction with Hsp90. EMBO J. **13:**6099–6106.
4. **Bandhakavi, S., R. O. McCann, D. E. Hanna, and C. V. C. Glover.** 2003. A positive feedback loop between protein kinase CKII and Cdc37 promotes the activity of multiple protein kinases. J. Biol. Chem. **278:**2829–2836.
5. **Bimbo, A., Y. H. Jia, S. L. Poh, R. K. M. Karuturi, N. den Elzen, X. Peng, L. L. Zheng, M. O'Connell, E. T. Liu, M. K. Balasubramanian, and J. H. Liu.** 2005. Systematic deletion analysis of fission yeast protein kinases. Eukaryot. Cell **4:**799–813.
6. **Cutforth, T., and G. M. Rubin.** 1994. Mutations in *hsp83* and *cdc37* impair signaling by the sevenless receptor tyrosine kinase in *Drosophila*. Cell **77:** 1027–1036.
7. **Fankhauser, C., and V. Simanis.** 1994. The cdc7 protein-kinase is a dosage-dependent regulator of septum formation in fission yeast. EMBO J. **13:**3011–3019.
8. **Farrell, A., and D. O. Morgan.** 2000. Cdc37 promotes the stability of protein kinases Cdc28 and Cak1. Mol. Cell. Biol. **20:**749–754.
9. **Forsburg, S. L.** 1993. Comparison of *Schizosaccharomyces pombe* expression systems. Nucleic Acids Res. **21:**2955–2956.
10. **Forsburg, S. L.** 2001. The art and design of genetic screens: yeast. Nat Rev. Genet. **2:**659–668.
11. **Gerber, M. R., A. Farrell, R. J. Deshaies, I. Herskowitz, and D. O. Morgan.** 1995. Cdc37 is required for association of the protein kinase Cdc28 with G_1 and mitotic cyclins. Proc. Natl. Acad. Sci. USA **92:**4651–4655.
12. **Hunter, T., and B. W. Sefton.** 1980. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc. Natl. Acad. Sci. USA **77:**1311–1315.
13. **Jaspersen, S. L., and D. O. Morgan.** 2000. Cdc14 activates Cdc15 to promote mitotic exit in budding yeast. Curr. Biol. **10:**615–618.
14. **Krapp, A., M. P. Gulli, and V. Simanis.** 2004. SIN and the art of splitting the fission yeast cell. Curr. Biol. **14:**R722–R730.
15. **Lee, P., J. Rao, A. Fliss, E. Yang, S. Garrett, and A. J. Caplan.** 2002. The Cdc37 protein kinase-binding domain is sufficient for protein kinase activity and cell viability. J. Cell Biol. **159:**1051–1059.
16. **MacLean, M., and D. Picard.** 2003. Cdc37 goes beyond Hsp90 and kinases. Cell Stress Chaperones **8:**114–119.
17. **Mata, J., R. Lyne, G. Burns, and J. Bahler.** 2002. The transcriptional program of meiosis and sporulation in fission yeast. Nat. Genet. **32:**143–147.
18. **Mehta, S., and K. L. Gould.** 2006. Identification of functional domains within the septation initiation network kinase, Cdc7. J. Biol. Chem. **281:**9935–9941.
19. **Mort-Bontemps-Soret, M., C. Facca, and G. Faye.** 2002. Physical interaction of Cdc28 with Cdc37 in *Saccharomyces cerevisiae*. Mol. Genet. Genomics **267:**447–458.
20. **Munoz, M. J., and J. Jimenez.** 1999. Genetic interactions between Hsp90 and the Cdc2 mitotic machinery in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **261:**242–250.
21. **Nurse, P.** 1990. Universal control mechanism regulating onset of M-phase. Nature **344:**503–508.
22. **Nurse, P., P. Thuriaux, and K. Nasmyth.** 1976. Genetic control of cell division cycle in fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **146:**167–178.
23. **O'Connell, M. J., N. C. Walworth, and A. M. Carr.** 2000. The G_2-phase DNA-damage checkpoint. Trends Cell Biol. **10:**296–303.
24. **Pearl, L. H.** 2005. Hsp90 and Cdc37—a chaperone cancer conspiracy. Curr. Opin. Genet. Dev. **15:**55–61.
25. **Reed, S. I.** 1980. The selection of *S. cerevisiae* mutants defective in the start event of cell division. Genetics **95:**561–577.
26. **Roe, S. M., M. M. U. Ali, P. Meyer, C. K. Vaughan, B. Panaretou, P. W. Piper, C. Prodromou, and L. H. Pearl.** 2004. The mechanism of Hsp90 regulation by the protein kinase-specific cochaperone p50 (cdc37). Cell **116:** 87–98.
27. **Rustici, G., J. Mata, K. Kivinen, P. Lio, C. J. Penkett, G. Burns, J. Hayles, A. Brazma, P. Nurse, and J. Bahler.** 2004. Periodic gene expression program of the fission yeast cell cycle. Nat. Genet. **36:**809–817.
28. **Schmidt, S., M. Sohrmann, K. Hofmann, A. Woollard, and V. Simanis.** 1997. The Spg1p GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. Genes Dev. **11:**1519–1534.
29. **Schutz, A. R., T. H. Giddings, E. Steiner, and M. Winey.** 1997. The yeast CDC37 gene interacts with MPS1 and is required for proper execution of spindle pole body duplication. J. Cell Biol. **136:**969–982.
30. **Shao, J. Y., A. Irwin, S. D. Hartson, and R. L. Malts.** 2003. Functional dissection of Cdc37: characterization of domain structure and amino acid residues critical for protein kinase binding. Biochemistry **42:**12577–12588.
31. **Snell, V., and P. Nurse.** 1994. Genetic analysis of cell morphogenesis in fission yeast—a role for casein kinase II in the establishment of polarized growth. EMBO J. **13:**2066–2074.
32. **Sohrmann, M., S. Schmidt, I. Hagan, and V. Simanis.** 1998. Asymmetric segregation on spindle poles of the *Schizosaccharomyces pombe* septum-inducing protein kinase Cdc7p. Genes Dev. **12:**84–94.
33. **Tanaka, K., and H. Okayama.** 2000. A Pcl-like cyclin activates the Res2p-Cdc10p cell cycle "start" transcriptional factor complex in fission yeast. Mol. Biol. Cell **11:**2845–2862.
34. **Tatebe, H., and K. Shiozaki.** 2003. Identification of Cdc37 as a novel regulator of the stress-responsive mitogen-activated protein kinase. Mol. Cell. Biol. **23:**5132–5142.
35. **Turnbull, E. L., I. V. Martin, and P. A. Fantes.** 2004. Functional analysis of the *Schizosaccharomyces pombe* molecular chaperone Cdc37. Mol. Biol. Cell **15:**368A–368A.
36. **Turnbull, E. L., I. V. Martin, and P. A. Fantes.** 2005. Cdc37 maintains cellular viability in *Schizosaccharomyces pombe* independently of interactions with heat-shock protein 90. FEBS J. **272:**4129–4140.
37. **Turnbull, E. L., I. V. Martin, and P. A. Fantes.** 2006. Activity of Cdc2 and its interaction with the cyclin Cdc13 depend on the molecular chaperone Cdc37 in *Schizosaccharomyces pombe*. J. Cell Sci. **119:**292–302.
38. **Vaughan, C. K., U. Gohlke, F. Sobott, V. M. Good, M. M. U. Ali, C. Prodromou, C. V. Robinson, H. R. Saibil, and L. H. Pearl.** 2006. Structure of an Hsp90-Cdc37-Cdk4 complex. Mol. Cell **23:**697–707.
39. **Westwood, P. K., I. V. Martin, and P. A. Fantes.** 2004. Fission yeast Cdc37 is required for multiple cell cycle functions. Mol. Genet. Genomics **271:**82–90.