We report here the identification of CDC37, which encodes a putative Hsp90 co-chaperone, as a multicopy suppressor of a temperature-sensitive allele (cka2-13\(^{ts}\)) of the CKA2 gene encoding the α\(^{\prime}\) catalytic subunit of protein kinase CKII. Unlike wild-type cells, cka2-13 cells were sensitive to the Hsp90-specific inhibitor geldanamycin, and this sensitivity was suppressed by overexpression of either Hsp90 or Cdc37. However, only Cdc37 was capable of suppressing the temperature sensitivity of a cka2-13 strain, implying that Cdc37 is the limiting component. Immunoprecipitation of metabolically labeled Cdc37 from wild-type versus cka2-13 strains revealed that Cdc37 is a physiological substrate of CKII, and Ser-14 and/or Ser-17 were identified as the most likely sites of CKII phosphorylation in vivo. A cdc37-S14,17A strain lacking these phosphorylation sites exhibited severe growth and morphological defects that were partially reversed in a cdc37-S14,17E strain. Reduced CKII activity was observed in both cdc37-S14A and cdc37-S17A mutants at 37 °C, and cdc37-S14A or cdc37-S14,17A overexpression was incapable of protecting cka2-13 mutants on media containing geldanamycin. Additionally, CKII activity was elevated in cells arrested at the G1 and G2/M phases of the cell cycle, the same phases during which Cdc37 function is essential. Collectively, these data define a positive feedback loop between CKII and Cdc37. Additional genetic assays demonstrate that this CKII/Cdc37 interaction positively regulates the activity of multiple protein kinases in addition to CKII.

Protein kinase CKII is an essential, ubiquitous serine/threonine/tyrosine protein kinase of unknown function. From most sources, the enzyme is composed of two polypeptide subunits, α (35–44 kDa) and β (24–28 kDa), which combine to form an α\(_2\)β\(_2\) tetramer (for review, see Refs. 1–4). In Saccharomyces cerevisiae, the enzyme consists of two distinct catalytic subunits, α and α\(^{\prime}\) (encoded by the genes CKA1 and CKA2, respectively), and two regulatory subunits, β and β\(^{\prime}\) (encoded by the genes CKB1 and CKB2, respectively). CKII recognizes Ser/Thr (or in exceptional cases Tyr) (5) in an acidic environment (2). CKII phosphorylates a broad spectrum of endogenous substrates involved in transcription, translation, signal transduction, and other functions.

Cdc37 was initially isolated in a genetic screen for mutants defective in progression through Start (6). Cdc37 function is required for the proper association of Cdc28 (yeast CDK1) with both G1 and mitotic cyclins, thus demonstrating a role for Cdc37 in G1/M progression as well (7). Additional cdc37 mutants generated by Dey et al. (8) arrest in both G1 and G2/M when shifted to restrictive temperature. Cdc37 interacts genetically with several different protein kinases that are involved in diverse cellular roles. The list of genetic interactors of CDC37 include CDC28 (7), KIN28 (8), mammalian v-Src when expressed in yeast (8), MPS1 (10), STE11 (11), and CAK1 (12).

Consistent with the genetic results in yeast, mammalian CDC27 was found to encode the p50 subunit of an Hsp90 molecular chaperone complex that exhibits specificity for protein kinases (13). Although Hsp90 recognizes a diverse collection of client proteins, including steroid receptors, protein kinases, and others, Cdc37/Hsp90 complexes appear to interact exclusively with the catalytic subunit of protein kinases, with one notable exception (14, 15). Binding sites for the protein kinase client and for Hsp90 have been mapped to the N- and C-terminal regions of Cdc37, respectively (13, 15, 16), providing further support for the notion that Cdc37 functions as a kinase-targeting co-chaperone of Hsp90. Cdc37 has also been demonstrated to possess chaperone activity on its own, independent of Hsp90 (17).

We show here that the CKII/Cdc37 connection is unique among the known interactions between Cdc37 and protein kinases because the highly conserved N terminus of Cdc37 contains an evolutionarily conserved CKII consensus site that is phosphorylated by CKII in vivo. Further, CKII and Cdc37 constitute a positive feedback loop. Although Hsp90 inhibition reduces CKII function in vivo, Cdc37 and not Hsp90 is the limiting factor in maintaining CKII function. We also present genetic evidence that CKII-mediated phosphorylation of Cdc37 is essential for the ability of the latter to maintain the activity of multiple protein kinases involved in diverse cellular functions. This reveals a previously unsuspected role for CKII in regulating the activity of diverse protein kinases (via Cdc37 phosphorylation).

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Media—**S. cerevisiae strains used in this study are listed in Table I. Yeast strains were grown in rich glucose medium (YPD: 1% yeast extract, 2% peptone, and 2% glucose) or in supplemented minimal medium (18) at different temperatures as indicated in figure legends. Eviction of URA3-marked plasmids was accomplished by plating cells on supplemented minimal medium containing 0.75 mg/ml 5-fluoroorotic acid (Diagnostic Chemicals Ltd.). Sporulation was carried out in liquid sporulation medium (1% potassium acetate, 0.1%
yeast extract, and 0.5% glucose). Geldanamycin (GA),1 purchased from Sigma, was dissolved in dimethyl sulfoxide and added to warm medium soon after autoclaving. Escherichia coli strain DH5α (Clontech) was grown in Luria broth containing 50 µg/ml ampicillin.

**Multicopy Suppression Screen**—The temperature-sensitive strain YDH13 (Table I) was transformed via the spheroplast method (18) with 10–20 µg of an *S. cerevisiae* genomic library in multicopy vector YEpl4 (19) and plated on CM medium lacking uracil and leucine. Plates were incubated at 33 °C for 24 h to allow accumulation of gene products expressed from YEpl4 and then placed at 35 °C (2 °C above the maximum permissive temperature of YDH13). YEpl4-based suppressor plasmids were isolated by transforming *E. coli* strain SK1108 to ampicillin resistance with DNA-containing extracts prepared from each suppressor strain. That suppression was retained in plasmid-linked YEp24 plasmids was confirmed by retransformation of YDH13, and the relevant gene was identified by similarly testing various subclones. Among the genes identified in the screen was CDC37. The correct assignment in this case was confirmed by constructing a frameshift mutation (an additional GATC sequence) at the BglII site in the CDC37 open reading frame. This construct is predicted to encode a severely truncated product of 120 residues and fails to suppress YDH13.

**Construction of CDC37 Phosphorylation Site Mutants**—Site-directed mutations in CDC37 were prepared by the general method of Deng and Nickoloff (20). A 3.3 kb BamHI/KpnI fragment containing the gene was subcloned into pUC19 to serve as a template. cdc37-S14,17A was prepared using the "Transformer" site-directed mutagenesis kit (Clontech) according to instructions from the manufacturer; cdc37-S14A, cdc37-S17A, and cdc37-S14,17E were prepared using the equivalent Chameleon site-directed mutagenesis kit (Stratagene). The four mutagenic oligonucleotides were: cdc37-S14,17A, 5'-GGTAAAATTTTGCTAGGGATGATGTCTCTGCAGG-3'; cdc37-S14A, 5'-GGTAAAATTTTGCTAGGGATGATGTCTGCAGG-3'; cdc37-S14,17E, 5'-GGTAAAATTTTGCTAGGGATGATGTCTCTGCAGG-3'; and the reverse primer used was 5'-GAATATCAACTTGATGGATGATGTCGAGG-3'. The nucleotide changes for each mutation are underlined. The entire coding region of each mutant was sequenced to confirm that only the desired mutation was introduced. For expression in yeast, the wild-type and each mutant (BamHI/KpnI fragment) were subcloned into BamHI/KpnI-digested single-copy vectors pRS314 (TRP1) and pRS316 (URA3) (22) and multicopy vector pRS426 (URA3) (23).

Strains carrying the above mutations as the only CDC37 allele were constructed as follows. CDC37 (in pRS316) was introduced into a diploid yeast strain (gift from David Morgan) carrying a LEU2-marked deletion in one copy of the CDC37 gene (7). The resultant strain was sporulated, and haploid progeny that were auxotrophic for leucine and histidine were selected. Tyr-phosphorylated Fpr3 was detected by Western blotting as described below.

**RESULTS**

**Isolation of Cdc37 as a Multicopy Suppressor of cha2-13 Mutants**—To identify genes which interact genetically with CKII in *S. cerevisiae*, we utilized strain YDH13 (Table I) to screen for multicopy suppressors of the temperature-sensitive cha2 allele, cha2-13 (28). The screen was carried out at the minimum restrictive temperature of 35 °C, and one of the suppressor plasmids identified encoded the protein kinase-specific (co-)chaperone, Cdc37 (Fig. 1A). Multicopy CDC37 also suppressed the slow growth rate at permissive temperature and flocculation displayed by YDH13 (data not shown). CDC37 suppressed four other cha2 alleles (data not shown), as well as two alleles of the *CKI1* gene, encoding the α subunit of *S. cerevisiae* CKII.2

Because Cdc37 has been shown to act as a chaperone either by itself (17) or in concert with Hsp90 (13), we examined the effect(s) of GA, an Hsp90-specific inhibitor, on cha2-13 mutants. GA is a member of the benzoquinoid ansamycin family of antibiotics that binds Hsp90 within a conserved pocket that constitutes the nucleotide binding site of Hsp90, and inhibits Hsp90 ATPase activity (29–31). GA has been shown to indirectly inhibit a variety of onecogenic tyrosine kinases and other

---

1. The abbreviation used is: GA, geldanamycin.
2. A. Rethinaswamy and C. V. C. Glover, unpublished data.
Table I  

| Strain          | Genotype                             | Source |
|-----------------|--------------------------------------|--------|
| YDH6            | MATα ade2-101 his3-200 leu2ΔΔ1 lys2-801 trp1-ΔΔ1 ura3-52 cka1-ΔΔ1: HIS3  
 |                 |                                      | (28)   |
| YDH13           | MATα ade2-101 his3-200 leu2ΔΔ1 lys2-801 trp1-ΔΔ1 ura3-52 cka1-ΔΔ1: HIS3  
 |                 |                                      | (28)   |
| YPH499          | MATα ade2-101 his3-200 leu2ΔΔ1 lys2-801 trp1-ΔΔ1 ura3-52                      | (22)   |
| SR672-1         | MATα cyh2 gal2 MAL ura1 cdc3-1       | YGSC   |
| YRM14.0         | MATα his3-200 leu2ΔΔ1 trp1-ΔΔ1 ura3-52 cdc3-1                               |        |
| YSB11           | MATα leu2 ura3 trp1 cdc3-ΔΔ1        | This study |
| YSB12           | MATα leu2 ura3 trp1 cdc3-ΔΔ1        | This study |
| YSB13           | MATα leu2 ura3 trp1 cdc3-ΔΔ1        | This study |
| YSB14           | MATα leu2 ura3 trp1 cdc3-ΔΔ1        | This study |
| YSB15           | MATα leu2 ura3 trp1 cdc3-ΔΔ1        | This study |
| YSB40           | MATα leu2 ura3 trp1 cdc3-ΔΔ1        | This study |
| YSB41           | MATα leu2 ura3 trp1 cdc3-ΔΔ1        | This study |
| YSB48           | MATα ade2-101 his3-200 leu2ΔΔ1 lys2-801 trp1-ΔΔ1 ura3-52 cka1-ΔΔ1: HIS3  
 |                 |                                      | This study |
| YSB49           | MATα ade2-101 his3-200 leu2ΔΔ1 lys2-801 trp1-ΔΔ1 ura3-52 cka1-ΔΔ1: HIS3  
 |                 |                                      | This study |
| YSB72           | MATα ade2-1 trp1 can1-100 leu2-3,112 his3-1,15 ura3 hsf1ΔΔΔ: LEU2  
 |                 |                                      |        |
| NSY-B           | MATα ade2-1 trp1 can1-100 leu2-3,112 his3-1,15 ura3 hsf1ΔΔΔ: LEU2  
 |                 |                                      |        |
| WX257-4c        | MATα his3-200 leu2-3,112 ura3-52    | (10)   |
| WX241-17a       | MATα his3-200 leu2-3,112 ura3-52    | (10)   |
| CMY633          | MATα ade2-101 his3-200 leu2-3,112 ura3-52 cdc2-109 [YPE2 CDC28]            | (47)   |
| CMY677          | MATα ade2-101 his3-200 leu2-3,112 ura3-52 cdc2-109                         | (47)   |
| GF1067          | MATα his3 leu2 trp1 ura3            | (9)    |
| GF2092          | MATα his3 leu2 trp1 ura3 kin28-tk3  | (9)    |

Notes:
- Yeast Genetics Stock Center.
- All strains were transformed with pRS315 KANMX4 and pRS314 HPHMX4.

This study

Substrates of Hsp90 including mineralocorticoid receptor and glucocorticoid receptor (32-36). Refolding of denatured firefly luciferase by the Hsp90 chaperone complex is also inhibited by treatment with ansamycins (37). GA has been shown to inhibit Hsp90 function in vitro in yeast as well (25). In contrast to human cell lines, which are sensitive to GA at nanomolar concentrations, the growth of wild-type yeast is unimpaired even at concentrations of 2 mM GA (25). We have also found similar results with strains wild-type for CKII activity (data not shown).

As shown in Fig. 1B, cka2-13 mutants were sensitive to 35 °C and GA at 23 °C (permissive temperature). Moreover, this sensitivity was suppressible by CDC37 overexpression. As might be expected, overexpression of the yeast homologs of Hsp90, HSC82 or HSP82 (which are the targets of GA in yeast; Refs. 25 and 31), also suppressed the GA sensitivity of cka2-13 mutants. A mutant allele of the yeast heat shock factor, hsf1ΔΔΔ, was marginally sensitive to GA at this temperature as a positive control) (25), whereas yeast that are wild-type for CKII were unaffected. CDC37 overexpression suppressed the GA sensitivity of cka2-13 mutants at temperatures from 21 to 34 °C (lowest and highest temperatures tested; data not shown).

The minimum restrictive temperature for cka2-13 mutants is 35 °C, and the fact that they do not grow even at 21 °C in the presence of GA emphasizes the importance of Hsp90 function for the survival of cka2-13 mutants. Consistent with the reported effects of GA on protein kinases in mammalian cells, treatment of cka2-13 cells with GA was found to cause decreased steady-state levels of CKII-dependent phosphorylation of Pfr3 (Fig. 1C; see below for a discussion of this assay). We therefore conclude that, upon prolonged incubation of cka2-13 cells with GA, CKII activity drops below a critical threshold causing these cells to die.

Cdc37 and Not Hsp90 Is the Limiting Factor in Maintenance of CKII Function—CDC37 overexpression has been shown to suppress the temperature-sensitivity of Hsp90 mutants in an allele-specific manner (17), raising the possibility that Cdc37 enhances Hsp90 function in vivo in S. cerevisiae. Alternatively or in addition, Cdc37 might function independently of Hsp90 to protect one or more critical targets from denaturation in an hsp90 mutant background. Given the GA sensitivity of cka2-13 mutants, we sought to determine whether suppression of cka2-13 by CDC37 was mediated via Hsp90. To explore the general relationship between Cdc37 and Hsp90 in yeast, we made use of the previously reported observation that hsf1ΔΔΔ cells are sensitive to GA because of reduced Hsp90 expression (25, 38). If Cdc37 enhances Hsp90 function, CDC37 overexpression might be expected to suppress the GA sensitivity of an hsf1ΔΔΔ mutant. As shown in Fig. 2A, overexpression of CDC37 did not enhance the ability of hsf1ΔΔΔ mutants to grow in the presence of GA at either 25 or 30 °C. This result suggests that CDC37 overexpression does not enhance Hsp90 function (or expression) in vivo. To determine whether the ability of CDC37 to suppress the GA sensitivity of cka2-13 mutants might be mediated via an increase in Hsp90 function, we tested whether direct overexpression of HSC82 or HSP82 could rescue the temperature sensitivity of a cka2-13 strain. As shown in Fig. 2B, neither gene is able to suppress this mutant under conditions where CDC37 is an effective suppressor.

The above results make it unlikely that CDC37 overexpression suppresses the GA sensitivity of a cka2-13 strain by enhancing Hsp90 function. One possibility is that Cdc37 is itself an independent chaperone of CKII, a conclusion consistent with the ability of purified Cdc37 to protect CKII activity in vitro (17). However, this interpretation does not explain the fact that cka2-13 mutants are sensitive to GA (Fig. 1B), which implies that Hsp90 function is required for CKII activity. An alternative possibility is thus that Hsp90 and Cdc37 are both required for CKII activity, possibly as a functional complex, but that Cdc37 is the limiting component (overexpression of Cdc37 but not Hsp90 suppresses the temperature sensitivity of a cka2-13 strain). A similar conclusion has been reached regarding the nature of the Cdc37/Hsp90 relationship with a temperature-sensitive mutant of the Hck kinase in human cells (39). Because our results were consistent with the possibility that Cdc37 is the limiting factor in the maintenance of CKII function, we decided to characterize further the nature of the CKII/Cdc37 relationship.
Cdc37 is a physiological substrate of CKII—Alignment of Cdc37 sequences from diverse organisms revealed an evolutionarily conserved CKII phosphorylation motif near the N terminus of the protein (see Fig. 4A). All available Cdc37 sequences conserve a potential CKII phosphorylation site at Ser-14 in S. cerevisiae numbering, and the fungal sequences share a second potential site at Ser-17. Residue 17 represents the important +3 determinant of the Ser-14 site, and this is potentially satisfied by a phospho-Ser residue in the fungal species or by a glutamate in the others. Consistent with these facts, yeast Cdc37 has been shown to be a substrate of mammalian CKII in vitro (17).

The above data suggest that Cdc37 may be an in vivo substrate of CKII. To test this possibility, we examined Cdc37 phosphorylation in vivo via metabolic labeling and immunoprecipitation in wild-type versus CKII-deficient strains. Western blotting revealed that these strains had comparable Cdc37 levels (data not shown). As shown in Fig. 3 (lanes 1–4), Cdc37 is radiolabeled in vivo in wild-type cells and also in a cka1Δ CKA2 strain, at both permissive and non-permissive temperature. In contrast, incorporation into Cdc37 is strongly diminished in a cka1Δ cka2Δ strain at permissive temperature, and essentially abolished at non-permissive temperature (Fig. 3, lanes 5 and 6). The simplest interpretation of these data is that Cdc37 is a direct substrate of CKII in vivo.

To confirm the importance of the CKII phosphorylation site...
motif on Cdc37, we compared the in vivo phosphorylation of wild-type Cdc37 with that of a Cdc37-S14,17A mutation (see below). As shown in Fig. 3 (lanes 7 and 8), in vivo labeling of the mutant is dramatically reduced compared with that of wild-type Cdc37. Although we cannot formally exclude the possibility of a conformational change that affects CKII phosphorylation elsewhere on the molecule, these results strongly suggest that Ser-14 and/or Ser-17 constitute a major site of Cdc37 phosphorylation in vivo.

CKII Phosphorylation Site Mutants of CDC37—To probe the functional significance of Cdc37 phosphorylation by CKII, mutant alleles encoding non-phosphorylatable (cdc37-S14,17A), semi-phosphorylatable (cdc37-S14A and cdc37-S17A), and quasi-phosphorylated (cdc37-S14,17E) derivatives of Cdc37 were constructed (Fig. 4A) and expressed from their native promoters in a Cdc37 null background. Western blotting with anti-Cdc37 antibodies revealed that all of the mutants are expressed at levels comparable to that of wild-type (data not shown). The cdc37-S14,17A strain was the most severely affected, displaying an extremely slow growth rate (doubling time of 10 h versus 90 min for the isogenic wild-type control) and an elongated, constructed (Fig. 4, lanes 8 and 9) morphology of Cdc37 mutant strains. Strains harboring the indicated Cdc37 alleles were grown at 23 °C to mid-log phase, and photographs of cells were taken on a Zeiss IM 35 epifluorescence microscope fitted with Nomarski optics. S672-1, carrying the C-terminal truncation allele cdc37-1, is included for comparison.

All of the CKII phosphorylation site alleles were recessive to wild-type (data not shown), indicating that they represent loss-of-function mutations.

Because of the known relationship between Cdc37 and Hsp90, we also examined the growth of CKII phosphorylation site mutants of Cdc37 in the presence of the Hsp90 inhibitor GA. As shown in Fig. 5, a cdc37-S14A mutant is sensitive to GA (Fig. 5, vector), and this sensitivity is suppressed by overexpression of wild-type Cdc37 as well as by cdc37-S17A. This mutant is also weakly suppressed by overexpression of cdc37-S14A itself, indicating dosage sensitivity of this mutant allele. In contrast, overexpression of cdc37-S14,17A not only fails to rescue the GA sensitivity of the cdc37-S14A mutant but results in a dominant-negative effect. These data confirm the importance of the CKII sites for Cdc37 function in vivo and support a potential interaction between Cdc37 and Hsp90 in yeast. They also corroborate the conclusions that Ser-14 is the more important of the two sites but that both are required for optimal function.

CDC37 and CKII Constitute a Positive Feedback Loop—The ability of CDC37 overexpression to suppress the temperature and GA sensitivity of a cko2-13 mutant (Fig. 1, A and B) prompted us to examine CKII activity in a strain with impaired Cdc37 function using the phosphorylation state of Fpr3, a well characterized substrate of CKII in S. cerevisiae, as an indicator of CKII activity in vivo (5). Fpr3 is the only known substrate of CKII phosphorylated on tyrosine (in addition to serine and probably threonine), and phosphorylation of Fpr3 (at Tyr-184) by CKII can thus be conveniently monitored by Western blotting of whole cell extracts with anti-phosphotyrosine antibody. Phosphorylation of Fpr3 at Tyr-184 is completely dependent upon functional CKII in vivo, but accumulation of Tyr-phosphorylated Fpr3 requires prior deletion of PTP1, which encodes a tyrosine phosphatase active against this site (5). PTP1 deletions had no effect on the growth, morphology, or temperature sensitivity of the strains used to monitor CKII activity (data not shown).

As shown in Fig. 6A, Tyr-184-phosphorylated Fpr3 represented a major band in a Western blot of a whole-cell extract prepared from a CDC37 ptp1 strain. An increase in the intensity of this band was observed upon overexpression of Drosophila CKII, as described previously (5). Relative to the CDC37 strain, extracts made from cdc37-S14A or cdc37-S17A cells had a lower steady state level of phosphorylated Fpr3, either with
D. melanogaster with empty vector (pBM272) or a plasmid expressing both subunits of antibody 4G10. A parallel blot was probed with anti-Fpr3.

The genetic and biochemical results described here support the idea that CKII and Cdc37 exist in a positive feedback loop wherein CKII activates Cdc37 by phosphorylation, which then can activate/maintain CKII. Because Cdc37 is required at the G1 and G2/M phases of the cell cycle (7, 8), we asked whether CKII activity might peak during these same stages. To monitor CKII activity during the cell cycle, we arrested a strain that is wild-type for CKII in the G1, S, and G2/M phases of the cell cycle (7, 8), and another group has previously reported that CKII activity is most likely inhibited during S phase in human cells (using a substrate other than Fpr3) (40). Failure to phosphorylate Cdc37 could therefore contribute to the previously reported G1 and G2/M arrest of temperature-sensitive CKII mutants (28).

The CKII/CDC37 Feedback Loop Helps Maintain the Activity of Multiple Protein Kinases—The CKII phosphorylation sites on Cdc37 are important for its ability to activate/maintain CKII activity (Fig. 6B). Because Cdc37 functions as a chaperone for a number of protein kinase catalytic subunits, we wished to determine whether phosphorylation of Cdc37 by CKII was important for these substrates as well. Several potential Cdc37 clients have been identified by genetic studies in S. cerevisiae, including Kin28 (9), Mps1 (10), and Cdc28 (7). Because Hsp90 and Cdc37 often share similar kinase substrates (17), we reasoned that temperature-sensitive mutants of Kin28, Mps1, and Cdc28 might be sensitive to GA in a fashion similar to the cka2-13 strain (Fig. 1B).

The normal restrictive temperature for mps1-1, kin28-ts3, and cdc28-109 is reported to be 30 °C, 35 °C, and 37 °C, respec-
tested (nor was its temperature sensitivity suppressible by CDC37 overexpression (Fig. 7) at a variety of temperatures
peaks at the same stages of the cell cycle that Cdc37 function
kinases also represent substrates of the positive feedback loop,
and Cdc28 (the yeast cell cycle engine) (7). At least two other
II with capping enzymes during transcription of genes) (9, 42),
RNA polymerase II that mediates the interaction of polymerase
spindle checkpoint) (10), Kin28 (a C-terminal domain kinase of
promotes the activity of additional protein kinases including,
the positive feedback loop between these two proteins also

Cdc37 are evolutionarily conserved, a similar mechanism that
kinases involved in diverse functions.
3 S. Bandhakavi and C. V. C. Glover, unpublished observations.

the GA sensitivity of cdc28-109 was not suppressed by
CDC37 overexpression (Fig. 7) at a variety of temperatures
tested (nor was its temperature sensitivity suppressible by
CDC37 overexpression; data not shown). However, we consider
it unlikely that the CKII phosphorylation site mutants of
CDC37 have adequate Cdc28 function because these mutants are
partially suppressed by Cdc28 overexpression as well as by
deletion of SWE1, which inhibits the mitotic form of Cdc28. 3
Moreover, cdc37-1 mutants have been shown to be limiting for
Cdc28 function previously (7, 12), and cdc37-1 mutants are
synthetically lethal when combined with the cdc28-109 mutation
(41). Cdc28 function thus is clearly required for Cdc28.
However, in contrast to the other kinase mutants tested, Cdc37
function might not be limiting in cdc28-109 mutants.

DISCUSSION
We have presented biochemical and genetic evidence for a
positive feedback loop between CKII and Cdc37. According to
our model (see Fig. 8), CKII phosphorylates and activates
Cdc37, which in turn promotes/maintains the activity of CKII.
This model is supported by the following observations: 1) CDC37
functions as a multicopy suppressor of temperature-
sensitive CKII alleles; 2) loss-of-function mutations in CDC37
result in reduced CKII activity toward a known physiological
substrate, Fpr3, in vivo; 3) CKII phosphorylates Cdc37 in vivo
at Ser-14 and/or Ser-17 (or at a site affected by mutation of
these residues); 4) replacement of Ser-14 and Ser-17 with alanine
results in severe phenotypic deficits that are partially
reversed in the corresponding glutamate mutant; and 5) CKII
phosphorylation site mutants of Cdc37 fail to suppress the GA
sensitivity of a cdc2-13 strain. Additionally, CKII activity
peaks at the same stages of the cell cycle that Cdc37 function
has been shown to be essential by several groups.

In addition to augmenting the activity of Cdc37 and CKII,
the positive feedback loop between these two proteins also
promotes the activity of additional protein kinases including,
Mps1 (required for spindle pole duplication as well as the
spindle checkpoint) (10), Kin28 (a C-terminal domain kinase of
RNA polymerase II that mediates the interaction of polymerase
II with capping enzymes during transcription of genes) (9, 42),
and Cdc28 (the yeast cell cycle engine) (7). At least two other
kinases also represent substrates of the positive feedback loop,
because the cdc37-34 allele (encoding a Ser-14 to leucine
replacement) (14) has been shown to be defective in the matura-
tion/activation of Ste11 (a MAP kinase involved in α-factor
signaling in yeast) (11) as well as the mammalian oncoprotein
v-Src (when expressed in yeast) (8). Additional Cdc37 clients
such as Cak1 (12) may be dependent upon CKII-mediated
phosphorylation of Cdc37 as well, but have not been tested
explicitly. By regulating Cdc37 phosphorylation, CKII plays an
important role in promoting the activity of multiple cellular
kinases involved in diverse functions.

Because CKII, Cdc37, and the CKII phosphorylation site on
Cdc37 are evolutionarily conserved, a similar mechanism that
positively regulates multiple kinases (including possible or-
thologs of yeast kinase clients of the CKII/Cdc37 feedback loop
shown in Fig. 8) may be in place in higher organisms as well.
Such a model also gives us a deeper appreciation of the molec-
ular basis for the pleiotropic nature of CKII since, by phospho-
ylating and activating Cdc37 (which represents only one of the
many CKII substrates identified so far), it can play a role in
a diverse signal cascades regulated by Cdc37 clients.
Cdc37 overexpression has been shown to induce tumors in
mice (43, 44). Although Cdc37 seems to cooperate with c-Myc
and cyclin D1 in producing tumors, the biochemical mecha-
nisms underlying its action most likely include multiple kinase
substrates of Cdc37 that work in concert to promote prolifera-
tion. Proto-oncogenic kinases that Cdc37 has been shown to
interact with include v-Src (8), Raf-1 (16), and CDK4 (13). CKII
overexpression also produces tumors in mice (45, 46), and the
mechanism for this might involve its ability to activate Cdc37,
which can then activate a host of other oncogenic kinases. It
would be interesting to see whether mice overexpressing non-
phosphorylatable Cdc37 would remain able to cooperate with
c-Myc or cyclin D1 in promoting oncogenesis.

How does phosphorylation of Cdc37 by CKII regulate its
function? At least two possibilities exist. Phosphorylation
might regulate the interaction of Cdc37 with Hsp90 and/or
other co-chaperones. Alternatively, it might affect the interac-
tion(s) between Cdc37 and its protein kinase clients or other
substrates. We have been unable to detect protein-protein in-
teractions between yeast Cdc37 and any other protein kinase
using a two-hybrid system, and others have had trouble isolat-
ing proteins that interact with yeast Cdc37 as well (12). How-
ever, a physical interaction between Cdc37 and Ste11 has been
reported in yeast (11), and the highly conserved N-terminal
half of Cdc37 (which contains the conserved CKII phosphor-
ylation site) has been shown to interact with at least one protein
kinase, Raf-1, in mammalian cells (16). Based on the latter
observation, we suspect that the CKII phosphorylation-defi-
cient mutants of CDC37 might be impaired in interactions with
target protein kinases. Consistent with such a possibility, over-
expression of the N-terminal half of yeast Cdc37 has a domi-
nant negative effect in a cdc37-S14A but not a Cdc37 strain.3

Because a feedback loop between CKII/Cdc37 regulates sev-
eral other proteins as well and because CKII activity (against
Fpr3) shows a cell cycle dependence, we suspect that there
might also exist regulators (activators/inhibitors) of the feed-
back loop. Such gene(s) might also be responsible for modula-
tion of CKII activity during the cell cycle.

Acknowledgments—We gratefully acknowledge Avrom Caplan,
David Morgan, Steve Reed, and Jeremy Thorner for antibodies and Carl
Mann, Gerard Faye, Kevin Morano, Mark Winey, and Peter Phillips
for strains, plasmids, and reagents.

REFERENCES
1. Allende, J. E., and Allende, C. C. (1995) FASEB J. 9, 313–323
2. Pinna, L. A., and Meggio, F. (1997) Prog. Cell Cycle Res. 3, 77–97
3. Glover, C. V. C., III (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1545–1550
4. Guerra, B., and Issinger, O. G. (1999) Electrophoresis 20, 391–408
5. Wilson, L. K., Dhillon, N., Thorner, J., and Martin, G. S. (1997) J. Biol. Chem.
272, 12961–12967
6. Reed, S. I. (1980) Genetics 95, 561–577
7. Gerber, M. R., Farrell, A., Deshaies, R. J., Herskowitz, I., and Morgan, D. O.
(1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6511–6515
8. Dey, B., Lighthoby, J. J., and Boschelli, F. (1996) Mol. Biol. Cell 7, 1405–1417
9. Vayal, J. G., Simon, M., Dubois, M. F., Bensaude, O., Facca, C., and Faye, G.
(1996) J. Mol. Biol. 249, 535–544
10. Schutz, S. R., Giddings, T. J., Jr., Steiner, E., and Winey, M. (1997) J. Cell
Biol. 136, 969–982
11. Abbas-Terki, T., Donze, O., and Picard, D. (2000) FEBS Lett. 487, 111–116
12. Farrell, A., and Morgan, D. O. (2000) Mol. Cell. Biol. 20, 749–754
13. Stepanova, L., Leng, X., Parker, S. B., and Harper, J. W. (1996) Genes Dev. 10,
1491–1502
14. Pliss, A. E., Fang, Y., Boschelli, F., and Caplan, A. J. (1997) Mol. Biol. Cell 8,
2501–2509
15. Silverstein, A. M., Gramatikakis, N., Cochran, B. H., Chinkers, M., and
Pratt, W. B. (1998) J. Biol. Chem. 273, 20990–20995
Cdc37 and CKII in S. cerevisiae

16. Grammatikakis, N., Lin, J. H., Grammatikakis, A., Tsichlis, P. N., and Cochran, B. H. (1999) Mol. Cell. Biol. 19, 1661–1672
17. Kimura, Y., Rutherford, S. L., Miyata, Y., Yahara, I., Freeman, B. C., Yue, L., Morimoto, R. I., and Lindquist, S. (1997) Genes Dev. 11, 1775–1785
18. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) in Current Protocols in Molecular Biology, Greene Publishing Associates/Wiley-Interscience, New York
19. Carlson, M., and Boitstein, D. (1982) Cell 28, 145–154
20. Strathern, J. N., and Higgins, D. R. (1991) Methods Enzymol. 194, 319–329
21. Deng, W. P., and Nickoloff, J. A. (1992) Anal. Biochem. 200, 81–88
22. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 19–27
23. Christianson, T. W., Sikorski, R. S., Dante, M., Shero, J. H., and Hieter, P. (1992) Gene (Amst.) 110, 119–122
24. Padmanabha, R., Chen-Wu, J. L., Hanna, D. E., and Glover, C. V. (1990) Mol. Cell. Biol. 10, 4089–4099
25. Morano, K. A., Santoro, N., Koch, K. A., and Thiele, D. J. (1999) Mol. Cell. Biol. 19, 402–411
26. Cardenas, M. E., Dang, Q., Glover, C. V., and Gasser, S. M. (1992) EMBO J. 11, 1785–1796
27. Wach, A., Brachat, A., Pohlmann, R., and Philipp, P. (1994) Yeast 10, 1793–1808
28. Hanna, D. E., Rethinaswamy, A., and Glover, C. V. (1995) J. Biol. Chem. 270, 25905–25914
29. Prodl, R., Roe, S. M., O'Brien, R., Ladbury, J. E., Piper, P. W., and Pearl, P. W., and Pearl, L. H. (1997) Cell 90, 65–75
30. Stebbins, C. E., Russo, A. A., Schneider, C., Rosen, N., Hartl, F. U., and Pavletich, N. P. (1997) Cell 89, 239–250
31. Panaretou, B., Prodl, C., Roe, S. M., O'Brien, R., Ladbury, J. E., and Pearl, P. W., and Pearl, L. H. (1998) EMBO J. 17, 4829–4836
32. Uchida, Y., Murakami, Y., Mizuno, S., and Kawai, S. (1988) Virology 164, 294–298
33. Whitesell, L., Minnaugh, E. G., De Costa, B., Myers, C. E., and Neckers, L. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8324–8328
34. Bamberger, C. M., Wald, M., Bamberger, A. M., and Schulte, H. M. (1997) Mol. Cell. Endocrinol. 131, 233–240
35. Pratt, W. B., and Toft, D. O. (1997) Endocr. Rev. 18, 306–360
36. Segatz, B., and Gehring, U. (1997) J. Biol. Chem. 272, 18694–18701
37. Schneider, C., Sepp-Lorenzino, L., Nimmegten, E., Ouerrfelli, O., Danishefsky, S., Rosen, N., and Hartl, F. U. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14536–14541
38. Morano, K. A., and Thiele, D. J. (1999) EMBO J. 18, 5953–5956
39. Scholz, G., Hartson, S. D., Cartledge, K., Hall, N., Shao, J., Dunn, A. R., and Matts, B. L. (2000) Mol. Cell. Biol. 20, 6984–6995
40. Marshall, A. R., and Russo, G. L. (1994) Cell. Mol. Biol. Res. 40, 513–517
41. Ferguson, J., Ho, J. Y., Peterson, T. A., and Reed, S. I. (1986) Nucleic Acids Res. 14, 6681–6697
42. Schroeder, S. C., Schwer, B., Shuman, S., and Bentley, D. (2000) Genes Dev. 14, 2435–2440
43. Stepanova, L., Finegold, M., DeMayo, F., Schmidt, E. V., and Harper, J. W. (2000) Mol. Cell. Biol. 20, 4462–4473
44. Stepanova, L., Yang, G., DeMayo, F., Wheeler, T. M., Finegold, M., Thompson, T. C., and Harper, J. W. (2000) Oncogene 19, 2186–2193
45. Seldin, D. C., and Leder, P. (1995) Science 267, 894–897
46. Kellner, M. A., Seldin, D. C., and Leder, P. (1996) EMBO J. 15, 5160–5166
47. Zarzov, P., Bouchier, H., and Mann, C. (1997) J. Cell Sci. 110, 1879–1891