Cdc37 has distinct roles in protein kinase quality control that protect nascent chains from degradation and promote posttranslational maturation

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Cdc37 is a molecular chaperone that functions with Hsp90 to promote protein kinase folding. Analysis of 65 Saccharomyces cerevisiae protein kinases (~50% of the kinome) in a cdc37 mutant strain showed that 51 had decreased abundance compared with levels in the wild-type strain. Several lipid kinases also accumulated in reduced amounts in the cdc37 mutant strain. Results from our pulse-labeling studies showed that Cdc37 protects nascent kinase chains from rapid degradation shortly after synthesis. This degradation phenotype was suppressed when cdc37 mutant cells were grown at reduced temperatures, although this did not lead to a full restoration of kinase activity. We propose that Cdc37 functions at distinct steps in kinase biogenesis that involves protecting nascent chains from rapid degradation followed by its folding function in association with Hsp90. Our studies demonstrate that Cdc37 has a general role in kinome biogenesis.

Introduction

Quality control mechanisms in eukaryotic cells involve molecular chaperone-dependent protein folding and ubiquitin/proteasome-dependent degradation. In addition, environmental stress such as heat shock or endoplasmic reticulum stress leads to a general down-regulation of translation (Wickner et al., 1999; Schneider, 2000; Frydman, 2001; Harding et al., 2002).

Hsp90 is a molecular chaperone that functions in association with many cochaperones to fold transcription factors, protein kinases, and many other clients (Millson et al., 2005; Zhao et al., 2005). Protein kinase folding is collaborative between Hsp90 and the molecular chaperone Cdc37. Cdc37 functions by stabilizing protein kinases or maintaining them in a folding-competent conformation (Kimura et al., 1997). In addition, Cdc37 promotes the assembly of Hsp90–protein kinase complexes (Stepanova et al., 1997), and expression of a dominant-negative form that lacks the Hsp90-binding domain inhibits kinase activation in animal cells (Grammatikakis et al., 1999). Interestingly, similar truncations promote yeast cell viability, suggesting that complex formation between Hsp90 and Cdc37 is not essential for their function in protein kinase maturation in all cases (Lee et al., 2002; Turnbull et al., 2005). Both chaperones interact directly with kinases during folding, and inhibition of Hsp90 with geldanamycin or other ansamycin antibiotics results in the rapid proteasome-dependent degradation of client kinases and transcription factors (MacLean and Picard, 2003; Pearl, 2005; Whitesell and Lindquist, 2005).

Protein kinase folding requires the action of several chaperones and cochaperones in addition to Cdc37 and Hsp90. Like nuclear receptors, initial chaperone interactions with unfolded kinases require Hsp70/40 chaperones to prepare the kinase for interaction with Cdc37 (Arlander et al., 2006). Cdc37 needs to be phosphorylated at Ser13 (mammals) or Ser14/17 (yeast) by casein kinase II to interact with its kinase clients (Bandhakavi et al., 2003; Shao et al., 2003; Miyata and Nishida, 2004). Hsp90 is subsequently recruited into this complex in a manner that is stimulated by the action of Sti1/Hop (Lee et al., 2004; Arlander et al., 2006). A stable ternary complex between Hsp90, Cdc37, and the client kinase then forms. Interestingly, Cdc37 exists in dimeric form in solution but in monomeric form in this ternary complex (Vaughan et al., 2006). How the folding reaction proceeds from this point is unknown.

Although it is clear that Cdc37 is vital for the biogenesis of protein kinases, it remains unknown what proportion of the kinome...
requires this and other chaperones for maturation. The binding site for Cdc37 on client protein kinases has been localized to the N lobe of the catalytic domain (Prince and Matts, 2004; Zhao et al., 2004) via highly conserved sequence motifs that stabilize nucleotide binding. These findings suggest a very general role for Cdc37 in kinome biogenesis because the sequences that mediate chaperone interactions are present in virtually all kinases.

Previous studies have noted that Cdc37 is up-regulated in cancer cells and can act as an oncogene (Stepanova et al., 2000a,b). As such, it may play an important role in regulating the signaling capacity of the cell, especially if it has a broad function across the kinome. To clarify the extent to which Cdc37 controls protein kinase biogenesis, we first adopted a proteomic approach to understand how many different kinases are affected by the loss of Cdc37 function. By analyzing a large proportion of the yeast kinome, we observed that Cdc37 has a general function in maintaining the levels of most but not all kinases. In addition, we characterize a novel function for Cdc37 in protecting nascent kinase chains against rapid degradation by the proteasome.

**Results**

**Cdc37 has a general role in kinome biogenesis**

To establish what proportion of the yeast kinome is dependent on Cdc37 function, we performed a large-scale analysis of

![Figure 1](file)

*Figure 1. Effect of CDC37 mutation on protein kinase levels.* [A] Schematic of the CDC37 locus showing the position where URA3 was inserted as well as the serine 14 to alanine mutation [S14A] in CDC37. Arrows denote the direction of transcription. [B] Four examples of unstable kinases identified with anti-TAP (cells grown at 30°C). Western blots of the same samples with anti-Pgk1 are shown as loading controls. [C] Four examples of kinases that were unchanged after culturing wild type and cdc37 S14A at 30°C. [D] Phylogenetic analysis of protein kinases used in this study. Kinases that accumulated to wild-type levels in the cdc37 S14A mutant are shown in red. Kinases in black accumulated at less than twofold compared with wild-type levels.
kinase steady-state levels in wild-type and cdc37 mutant yeast. This analysis used the tandem affinity-purified (TAP)–tagged yeast library, in which each strain contains a different TAP-tagged gene (Ghaemmaghami et al., 2003). The TAP tag was used as a means of identifying individual kinases after the replacement of wild-type CDC37 with a mutant version known to affect kinase maturation (Bandhakavi et al., 2003). This mutation, serine 14 to alanine (cdc37S14A), precludes phosphorylation at amino acid 14 by casein kinase II. A second phosphorylation site at serine 17 was left intact. Selection of the mutant was made possible by insertion of an auxotrophic marker (URA3) in the intergenic region between cdc37S14A and the downstream gene STB3 (Fig. 1 A). Ura+ transformants were rescreened for a temperature-sensitive lethal growth phenotype at 37°C, which was diagnostic for replacement of the wild-type gene with the cdc37S14A mutant (Dey et al., 1996; Bandhakavi et al., 2003).

A large-scale analysis was performed using TAP-tagged kinases isolated from the library. 65 strains containing different TAP-tagged kinases (~50% of the kinome) were converted to cdc37S14A, and the steady-state levels of each were determined by Western blotting in comparison with the levels found in wild-type strains. Kinase identity was verified by size (predicted size of the protein plus the 21-kD tag) and by PCR analysis of the kinase-encoding gene from individual strains. Steady-state levels of each kinase were assessed by Western blotting in at least two independent transformants. Phosphoglycerate kinase was used as a loading control. Some kinases were not readily observed by direct Western blot analysis of crude extracts as a result of low abundance, and these were instead assayed after a single round of pull down with IgG–Sepharose beads (see Materials and methods).

Of the 65 kinases assayed, 51 displayed reduced steady-state levels by at least twofold in the cdc37S14A mutant strain compared with the wild-type. 14 kinases accumulated to the same levels in both wild-type and cdc37S14A strains (less than a twofold difference; n > 4) when the cells were grown at 30°C. Examples of the primary data are shown in Fig. 1 (B and C), and the combined dataset is presented in Fig. 1 D as a phylogenetic tree. Multiple sequence alignment revealed no strong evolutionary relationships that distinguish those kinases affected by mutation in CDC37 from those that were not. However, three kinases that were unaffected by the cdc37 mutant, Bud32, Ygl059W, and Yil042C, represent outliers based on their phylogenetic distance from others in the group. All three have atypical Mn2+ instead of Mg2+ (Facchin et al., 2002), and Ygl059W and Yil042C are similar to mitochondrial branched-chain α-ketoacid dehydrogenase kinases. The overwhelming majority of the other kinases analyzed have broad distributions in cytosolic or nuclear compartments (55 kinases). Three are localized to vesicle intermediates in the secretory pathway (Akl1, Ire1, and Vps15), and four have ambiguous distributions (Mkk1, Ydl025c, Sat4, and Iks1; Huh et al., 2003).

The binding of Cdc37 is restricted to the N domain of protein kinases, which comprises five β sheets that pack against a single conserved α helix (the αC helix). This structure is also found in lipid kinases (Hanks and Hunter, 1995; Walker et al., 1999). Therefore, we tested whether any lipid kinases required Cdc37 for stability. Strains expressing TAP-tagged Pik1 (PI4 kinase) and Vps34 (a PI3 kinase) strains were converted to cdc37S14A, and their steady-state levels were assayed by Western blotting (Fig. 2). Both had reduced accumulation in the cdc37S14A mutant compared with the wild-type strain. Two other kinases that phosphorylate metabolic intermediates, Guk1 (a guanylate kinase) and Prs5 (a subunit of ribose phosphate diphosphokinase), accumulated to wild-type levels in the cdc37S14A mutant strain. These combined data indicate that most protein kinases have access to and require Cdc37 for biogenesis at normal growth temperatures, whereas a smaller proportion of kinases accumulate to wild-type levels independent of Cdc37.

Protein kinases can function in pathways that have a self-regulatory capacity to control their own cellular abundance. In the pheromone-responsive pathway, kinase components such as Ste11, Ste7, and Fus3 are under transcriptional control that depends on a basal level of pathway activation (Fields et al., 1988; Hagen et al., 1991). Deletion of any of these kinases leads to reduced basal signal transmission, resulting in decreased levels of other proteins within the pathway. Therefore, it is possible that the reduced kinase levels we observed in the cdc37S14A mutant could result from the inhibition of just a single kinase within the pathway, and this leads to further indirect decreases in the levels of other kinases. This is illustrated in Fig. 3, in which deletion of the known chaperone client STE11 (Abbas-Terki et al., 2000) resulted in decreased levels of Fus3, a mitogen-activated protein kinase that is downstream in the pheromone-responsive pathway. However, the mutation of CDC37 results in a further reduction in Fus3 levels. Similar results were observed when BCK1 was deleted and the levels of Slt2 protein kinase (which is downstream in the PKC signaling pathway) were measured. The deletion of BCK1 resulted in reduced levels of Slt2, and these were further reduced in the bck1Δcdc37S14A double mutant (Fig. 3 B). These results show that kinase expression can be affected indirectly by the activity of other kinases but that Cdc37 has additional functions to control kinase abundance.

The results shown in Fig. 3 demonstrate that protein kinases affect the levels of other kinases within a pathway.
In the case of pheromone-responsive and PKC-dependent pathways, this regulation is at a transcriptional level (Fields et al., 1988; Hagen et al., 1991; Jung and Levin, 1999). Accordingly, if Cdc37 affects protein kinase abundance via the indirect method, this should result in changes in kinase gene mRNA levels. To test this hypothesis, we performed a microarray analysis to determine whether the cdc37<sup>S14A</sup> mutation affects kinase gene expression on a general level. However, a comparison of transcriptomes between wild-type and cdc37<sup>S14A</sup> mutants that affect translation, such as ssb1Δ/ssb2Δ (Nelson et al., 1992). Quantitative analysis of kinase mRNA levels in polysomes and 80S ribosomes also failed to reveal any differences between the wild-type and cdc37<sup>S14A</sup> mutant strains.

Further studies addressed whether kinase levels were reduced in the cdc37<sup>S14A</sup> mutant as a result of rapid degradation by the proteasome. MG132 was incubated with cells for 30 min before the pulse labeling. Under these conditions, we observed that Tpk2 levels were increased in the cdc37<sup>S14A</sup> mutant strain compared with DMSO alone and that the kinase appeared as a nonphosphorylated form of Rim11. Similar pulse-labeling studies were performed with Cdc28, the cyclin-dependent kinase that promotes cell cycle progression. Surprisingly, levels

Cdc37 protects nascent chains from rapid degradation

We next investigated the fate of kinases that accumulated at reduced levels in the cdc37<sup>ssb2Δ</sup> mutant, focusing first on Tpk2. Pulse-chase analysis showed that Tpk2 levels were reduced by at least twofold in the cdc37<sup>ssb2Δ</sup> mutant immediately after a 10-min pulse compared with the wild type (Fig. 4 A). Once synthesized, the levels of Tpk2 altered little in either strain over a 2-h period. Pulse labeling of Pho85 and Rim11 resulted in similarly decreased kinase levels in the cdc37<sup>ssb2Δ</sup> mutant (Fig. 4, E and F). Experiments with Cmk2, whose accumulation at steady state was normal in the cdc37<sup>ssb2Δ</sup> mutant, showed that it also accumulated normally under pulse-labeling conditions (Fig. 4 B).

The decrease in Tpk2, Pho85, and Rim11 levels under pulse-labeling conditions could represent the rapid degradation of each kinase or a defect in translation. Degradation by the vacuolar pathway was ruled out by experiments in a pep4<sup>Δ</sup> mutant and incubation of cells with PMSF. Incubation of cells with PMSF or deletion of the gene encoding the vacuolar peptidase Pep4 did not lead to substantial increases in the levels of Tpk2 or Rim11 in cdc37 mutant cells (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200604106/DC1). Translation was assessed by examination of polysome profiles and by quantitation of the levels of kinase mRNA on the polysomes from wild-type and cdc37 mutant cells. However, polysome profiles from wild-type and cdc37<sup>ssb2Δ</sup> strains were identical, suggesting that general defects in translation initiation and elongation do not occur in the cdc37<sup>ssb2Δ</sup> mutant (Fig. S2). Furthermore, the cdc37<sup>ssb2Δ</sup> strain did not display growth hypersensitivity on media containing paromomycin (Fig. S2), which is common for mutants that affect translation, such as ssb1Δ/ssb2Δ (Nelson et al., 1992). Quantitative analysis of kinase mRNA levels in polysomes and 80S ribosomes also failed to reveal any differences between the wild-type and cdc37<sup>S14A</sup> mutant strains.

The effect of MG132 on the levels of several other kinases during pulse labeling was also examined. Pho85, a cyclin-dependent kinase, behaved in a similar manner to Tpk2. The kinase was present in reduced amounts in the cdc37<sup>ssb2Δ</sup> mutant compared with the wild type after a 5-min pulse, and MG132 partially suppressed this defect (Fig. 4 E). Rim11 levels were also reduced in the cdc37<sup>ssb2Δ</sup> mutant, and, in this case, MG132 led to the accumulation of a slower migrating band that is present in all samples but is typically present as a minor component of the immunoprecipitation (Fig. 4 F). This band may represent a nonphosphorylated form of Rim11. Similar pulse-labeling studies were performed with Cdc28, the cyclin-dependent kinase that promotes cell cycle progression. Surprisingly, levels
of Cdc28 were similar in both wild-type and cdc37
mutant strains after pulse labeling (Fig. 4 G) even though we observed greatly reduced levels of the kinase in the cdc37 mutant strain at steady state (Fig. 1 and not depicted). This paradox was resolved by pulse-chase analysis (Fig. 4 G), which shows that Cdc28 is synthesized in the same amount in both wild-type and mutant strains, yet its levels decrease sharply in the cdc37 mutant strain within a 30-min period. As with Rim11, nascent Cdc28 expressed in the mutant cells migrates more slowly in the gel compared with Cdc28 from wild-type cells, as noted previously (Gerber et al., 1995). These findings are consistent with previous studies on Cdc28 biogenesis in a different cdc37 mutant strain (Gerber et al., 1995; Farrell and Morgan, 2000) but contrast with the more rapid degradation observed for Tpk2, Pho85, and Rim11 (Fig. 4, C–E). This could reflect differences in chaperone binding between kinases that will be discussed later.

Cdc37 promotes kinase maturation

Protein folding is a temperature-dependent process, and chaperone dependence for folding is more stringent at higher growth temperatures. We investigated the temperature dependence of kinase accumulation in the cdc37 mutant. Three protein kinases that accumulated at low levels in the cdc37 mutant were chosen for analysis (Rim11, Tpk2, and Yck2) based on their representation of different subgroups of the kinome (Hunter and Plowman, 1997). Notably, the kinases displayed similar stability profiles that were inversely correlated with increasing temperature (Fig. 5 A). Furthermore, each of the kinases accumulated to levels that were similar to those found in the wild-type strain after growth at 26°C (Fig. 5 B). Next, we measured the activity of Tpk2 and Rim11 after growth of the cells at 26°C. The TAP-tagged kinases were assayed for activity after isolation on IgG-Sepharose beads from wild-type and mutant strain lysates. A synthetic peptide substrate was used for Tpk2, and myelin basic protein (MBP) was used for Rim11 (Zhu et al., 2000). The results of these assays (Fig. 5 C) showed that Rim11 and Tpk2 both had reduced activity when isolated from the cdc37 mutant strain compared with wild-type yeast even though similar amounts of kinase were present in the assays.

A further study addressed whether Tpk2, a cAMP-dependent protein kinase, could interact with its negative regulatory subunit Bcy1 in the cdc37 mutant strain (Toda et al., 1987). As with mammalian PKA, inhibitory binding of the regulatory subunit is relieved at high CAMP levels, leading to increased kinase activity (Johnson et al., 1987). An HA-tagged version of Bcy1 was expressed in wild-type and cdc37 mutant strains grown at 26 and 30°C. Similar amounts of HA-Bcy1 were expressed in both wild-type and cdc37 mutant strains grown at both temperatures. TAP-tagged Tpk2 was isolated using IgG-Sepharose bead resin, and the amount of coprecipitating Bcy1 was detected by Western blotting. When the cells were grown at 30°C, there was a decreased yield of TAP-Tpk2 by threefold (Fig. 5 D) as expected because of the decreased kinase levels (Fig. 4). The amount of coprecipitating Bcy1 was decreased by fivefold relative to those amounts isolated from the wild-type strain extracts. When the cells were grown at 26°C, however, similar amounts of TAP-Tpk2 were isolated, but twofold reduced levels of Bcy1 were still bound. This suggests that some population of Tpk2 failed to bind to Bcy1 in the cdc37 mutant, yet this correlated with the decreased activity of the kinase.

To determine whether Cdc37 was required for the activity of kinases that were synthesized normally at 30°C, we investigated Sky1, a constitutively active kinase (Nolen et al., 2001). Sky1 has an extra helix between the βα4 loop is implicated in Sky1 function (Hunter and Plowman, 1997). Notably, the kinases displayed similar stability profiles that were inversely correlated with increasing temperature. Cdc37 promotes kinase maturation.
from either strain, suggesting that it became independent of Cdc37 at the lower growth temperature. Overall, kinases that are stable at 26 or 30°C in the cdc37S14A mutant are less active than those in wild-type strains. Similar findings were made with the kinase Mps1, which is stably expressed in a cdc37 mutant but has decreased activity (Schutz et al., 1997). However, the chaperone dependence for kinase activity is inversely proportional to growth temperature.

Discussion

There is accumulating evidence that Cdc37 has a general role in kinase biogenesis, but there has been no systematic analysis at a kinomic level. In our studies, we addressed this issue by analysis of ~50% of the yeast kinome for dependence on Cdc37. Our results are consistent with Cdc37 having a general role in kinome biogenesis and functioning either during or immediately after translation to protect nascent chains from degradation. In addition, Cdc37 promotes kinase maturation to the folded state.

The finding that Cdc37 plays a general role in kinase biogenesis is consistent with the growing number of kinases that have been characterized to interact with this chaperone (MacLean and Picard, 2003). Two other large-scale screens for Hsp90-interacting proteins also uncovered protein kinases. In a two-hybrid screen with a mutant form of Hsp90 as bait (Millson et al., 2005), 27 kinases interacted with Hsp90 either physically or genetically. Interestingly, the kinases found in these studies did not overlap, indicating that neither screen was saturating.

The results of our studies show that Cdc37 functions immediately after translation to protect nascent kinase chains from degradation. Even after a subminute pulse labeling, we noted that kinase levels were reduced in the cdc37S14A mutant strain (unpublished data). However, proteasome inhibition followed by pulse labeling largely restored Tpk2, Pho85, and Rim11 levels, providing strong support for Cdc37’s role in nascent chain quality control. This phenotype was only observed when MG132 was incubated for brief periods (30 min) in the cells before pulse labeling. Prolonged treatments (2 h) failed to restore Tpk2 levels, although this may result from reduced transcription occurring from long-term proteasome inhibition (unpublished data).

The finding that some kinases require Cdc37 to protect them against rapid degradation shortly after synthesis suggests that the chaperone functions as a conformational sensor at or near to the polysome. The degradation of several kinases during pulse labeling is so fast that we initially considered that translation itself was impaired. However, polysome profiles and measurement of the rate of protein synthesis suggested that Cdc37 was not involved in regulating translation, at least in a general manner (Fig. S2). Also, there is a precedent for the very rapid degradation of ribosomal proteins shortly after synthesis.

Figure 5. Effect of temperature on kinase accumulation and activity. (A) Western blot analysis of Rim11, Tpk2, and Yck2 (arrows) after growth of the relevant TAP-tagged cdc37S14A strains at the indicated temperatures. Asterisks denote Pgk1, which was used as a loading control. (B) Comparison of Rim11 (lanes 1 and 2), Tpk2 (lanes 3 and 4), and Yck2 (lanes 5 and 6) levels by Western blotting in whole cell extracts from wild-type (ser14) and cdc37S14A (ala14) cells grown at 26°C. The asterisk denotes Pgk1. (C) Activity of protein kinases in the cdc37 mutant strain. Activity of Rim11 and Tpk2 after growth of the relevant TAP-tagged wild-type (WT) and cdc37S14A cells at 26°C. The blots above the graph show Western blot analysis of protein levels of the kinases used in the assays. Error bars represent SEM. (D) Binding of HA-Bcy1 to Tpk2 in wild-type (ser14; lanes 1 and 3) and cdc37S14A (ala14; lanes 2 and 4) cells grown at 30 and 26°C as indicated. Top panels show Western blots of Tpk2; middle panels show HA-Bcy1 (Bcy1) after immunoprecipitation (IP) of TAP-Tpk2 using IgG-Sepharose beads; bottom panel shows levels of HA-Bcy1 in cell lysates. Note that two bands appear in the anti-HA Western blots, but only the slower migrating band coimmunoprecipitated with TAP-Tpk2 (not depicted).
studies showing that Cdc37 promotes Cdc28 stability (Gerber a 30-min period. These findings are consistent with previous after pulse labeling, but the kinase was degraded rapidly within the cdc37 mutant strains during pulse labeling was not observed in all cases. The temperature-sensitive phenotype for kinase stability in the cdc37S14A mutant allowed us to further dissect the different roles for this chaperone in kinase biogenesis. For Rim11 and Tpk2, the increase in kinase stability in cdc37S14A when grown at 26°C did not lead to full activity; rather, a twofold decrease in activity was observed. For Tpk2, the decrease in activity of the isolated kinase correlated with decreased binding of its inhibitory subunit Bcy1 in cell lysates. This could be caused by Tpk2 misfolding and/or by increased cAMP levels in the cdc37S14A cells caused by a combined decrease in Tpk2/12/3 activity (Nikawa et al., 1987). These combined results are consistent with decreased kinase activity in cdc37S14A mutant cells even when the triage system for degrading misfolded kinases is suppressed by growth at low temperature.

A previous study supports the hypothesis that kinases can accumulate as misfolded conformers that are not degraded. In this case, the deletion of STT1 or HSC82 resulted in very low v-Src levels, an effect that was suppressed by the overexpression of full-length or truncated forms of Cdc37 (Lee et al., 2002). Although the full-length Cdc37 also restored v-Src activity, the truncated forms promoted stabilization without large increases in activity. Because v-Src is constitutively active in the absence of other kinases, these data indicate that v-Src accumulates in an improperly folded form that is not degraded. The same is likely true for Sky1, which is also constitutively active (Nolen et al., 2001). Furthermore, the accumulation of misfolded proteins upon the deletion of several different chaperone proteins was recently demonstrated (McClellan et al., 2005). In this case, components of the Hsp90 chaperone machinery were implicated in promoting degradation but not in the folding of a heterologously expressed protein in yeast. For kinases, the triage system may operate to clear the cell of misfolded kinases close to their site of synthesis before they have a chance to aggregate. This can be bypassed by growth at low temperature even in the absence of chaperone function (Fig. 5 A). In this case, the effect of low temperature may compensate for decreased chaperone function and allow the kinases to proceed to a later stage of maturation with a greater probability of achieving the native state. We suspect this has physiological relevance because some kinases interact persistently with chaperones even after initial folding.
For these kinases, chaperones are constantly needed to promote the folded state rather than target the polypeptide for degradation.

In conclusion, our findings show that Cdc37 has a general role in protecting nascent kinase chains from degradation in addition to its function in posttranslational maturation. These findings suggest that Cdc37 is a gatekeeper to cellular kinase abundance. Importantly, yeast Cdc37 synthesis is fairly constant compared with other chaperones whose expression is stress regulated, and this may be a means of limiting kinase abundance in cells.

Materials and methods

Strains and plasmids

The TAP-tagged yeast library based on strain S288C was purchased from Open Biosystems. Strain genotype was verified by sizing individual gene products by Western blotting and PCR analysis using one primer specific to the tag and one primer specific to the gene of interest.

The URA3-marked cdc37(D144) mutant (p514AU) was constructed by inserting a 1.24 kb DNA fragment containing the URA3 gene into Bcl1-digested pRS314.cdc37S14A (a gift from C. Glover III, The University of Georgia, Athens, GA). A similar plasmid was constructed using wild-type CDC37 for microarray analysis. A plasmid encoding HA-tagged Bcy1 was a gift from J. Hirsch (Mount Sinai School of Medicine, New York, NY). A strain deleted for TPB1, SSB1 and SSB2 was a gift from E. Craig (University of Wisconsin-Madison, Madison, WI).

The cdc37(D144) mutant strains were constructed as follows. Wild-type cells containing TAP-tagged genes were grown overnight. A DNA fragment containing cdc37(D144::URA3) was prepared for transformation by digesting p514AU with BamHI, HindIII, and KpnI. Transformation was performed using the cdc37(D144::URA3) fragment into the TAP-tagged strains performed using the standard lithium acetate method as previously described (Gietz et al., 1995), which places the wild-type genes in the TAP-tagged strains under study by a modified version of the transformation protocol of Gietz et al. (1995), which included a heat-shock step at 42°C for 20 min followed by washing the cells with water, resuspension in YPD (yeast extract, peptone, glucose), and incubation at 30°C for 2 h before plating.

Microarray analysis

Wild-type S288C yeast were converted to CDC37::URA3 or cdc37(D144::URA3 as described above. Cultures of each strain were grown to log phase, and total RNA was prepared as described previously (Schmitt et al., 1990). A cleanup procedure was used with an RNase kit (Qiagen) and column DNA digestion. Hybridization to S98 chips (Affymetrix, Inc.) and data analysis were performed by the Mount Sinai microarray facility.

Phylogenetic analysis

The analysis shown in Fig. 2 was performed on isolated kinase domains downloaded from http://kinase2.salk.edu. A multiple alignment was performed with ClustalW in the MacVector software package (version 7; Accelrys) followed by phylogenetic analysis using Protist and Neighbor present in the Phylip 3.6 package (http://evolution.genetics.washington.edu/phylip.html). The tree was drawn using Hypertext (Bingham and Sudarsanam, 2000).

Online supplemental material

Supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200604106.DC1.

We thank Drs. Elizabeth Craig, Claiobie Glover III, and Jeannne Hirsch for plasmids/yeast strains, Drs. Tearina Chu and Yuxen Liu for microarray analysis, and John Woolford for use of his gradient fractionator. We also thank Drs. Doug Cyt, Stephen Garrett, Jeannne Hirsch, Bob Matts, Kevin Morano, and David Ron for helpful discussions during the course of this study. We are especially grateful to R. Deshaies for his insight regarding our work.

This study was supported by National Institutes of Health grants GM70596 (to A.J. Caplan) and GM 075061 (to J.L. Brodsky).

Submitted: 18 April 2006
Accepted: 26 December 2006

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