

The molecular chaperone Cdc37 is required for Ste11 function and pheromone-induced cell cycle arrest

Toufik Abbas-Terki, Olivier Donzé, Didier Picard*

Département de Biologie Cellulaire, Université de Genève, Sciences III, 30 quai Ernest-Ansermet, CH-1211 Genève 4, Switzerland

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Abstract The molecular chaperone Cdc37 is thought to act in part as a targeting subunit of the heat-shock protein 90 (Hsp90) chaperone complex. We demonstrate here that Cdc37 is required for activity of the kinase Ste11 in budding yeast. A *cdc37* mutant strain is defective in Ste11-mediated pheromone signaling and in accumulation and functional maturation of the constitutively active Ste11 version Ste11ΔN. Moreover, Cdc37, Ste11ΔN and Hsp90 coprecipitate pairwise. Thus, Hsp90 and Cdc37 may transiently associate with Ste11 to promote proper folding and/or association with additional regulatory factors. Our results establish Ste11 as the first endogenous Cdc37 client protein in yeast.

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Key words: Molecular chaperone; Pheromone signaling; Ste11; Cdc37; Hsp90; *Saccharomyces cerevisiae*

1. Introduction

cdc37 was initially discovered in the budding yeast *Saccharomyces cerevisiae* as a temperature-sensitive cell division cycle (Cdc) mutation that arrests cells at Start in G1 at the non-permissive temperature [1]. Why it does that and why the null mutation is lethal [1,2] remains poorly understood. Genetic interactions have been found between *CDC37* and the genes encoding the p34^{Cdc2}-type cell cycle kinase homologs Cdc2 in *Drosophila* [3] and Cdc28 in yeast [2,4], the kinases Mps1 [5] and Kin28 [6], and the isoforms Hsc82 and Hsp82 of the molecular chaperone heat-shock protein 90 (Hsp90) [7] in yeast. Whereas biochemical interactions have not been reported for these four proteins, the reduced levels and/or activities of Mps1 and Cdc28 in yeast strains with *cdc37* mutations might account for the observed defects in spindle pole body duplication [5] and cell cycle progression [2].

In contrast to *Drosophila* and yeast, the vertebrate homolog p50^{Cdc37} has been discovered by virtue of it being a 50 kDa component of multisubunit complexes with pp60^{v-src} and other members of the Src kinase family [8–14], the kinases Cdk4 [15,16], Cdk6 [17] and Raf-1 [18,19]. These complexes also contain Hsp90, which binds Cdc37 directly [20]. In vitro, yeast Cdc37 has been demonstrated to be a molecular chaperone [7]. Thus, work with a variety of organisms over the last

decade has led to the view that Cdc37 is a molecular chaperone that may have some specificity for protein kinases and perhaps as a co-chaperone also direct Hsp90 to these substrates (reviewed in [21–23]).

Yeast Cdc37 diverges from mammalian and *Drosophila* p50^{Cdc37}/Cdc37 quite considerably. Overall, it shares only about 20% sequence identity at the amino acid level with human Cdc37 with the highest conservation in the N-terminal 50 amino acids [15]. Several observations suggest that at least some Cdc37 functions may be conserved between yeast and multicellular organisms: (i) *Drosophila CDC37* can complement a *cdc37* yeast strain [3]; (ii) yeast *CDC37* is required for activity of vertebrate pp60^{v-src} expressed in yeast [24], and (iii) its overexpression can rescue pp60^{v-src} activity in a *hsp90* mutant strain [7].

p50^{Cdc37}/Cdc37 is required for sevenless signaling in *Drosophila* [3], and in mammalian cells for Raf-1 function and thus for growth factor signaling to mitogen-activated protein kinase (MAPK) [25]. We therefore wondered whether the Cdc37 function in MAPK pathways is conserved in the budding yeast despite the sequence divergence. We set out to examine the pheromone signaling pathway since it is one of those MAPK pathways that are dependent on the Raf equivalent Ste11 (reviewed in [26,27]), which we have previously shown to require the Cdc37 co-chaperone Hsp90 [28]. Binding of mating pheromone to transmembrane receptors elicits a series of events including the sequential activation of the kinases Ste11, Ste7 and Fus3, leading to morphological changes, a cell cycle arrest in G1 and the expression of specific genes in preparation for mating. For the first time, we provide combined genetic and biochemical evidence for an endogenous Cdc37 function in yeast.

2. Materials and methods

2.1. Plasmids

The wild-type *CDC37* open reading frame was obtained by PCR amplification of yeast genomic DNA using the following oligos: 5'-CCTGGATCCAAGTCAAAAATGGCCATTGATTACTCT-3' and 5'-TTTGAGCTCTAGATGCACGCTGCACCAGTAAATAGCTA-3'. The *Bam*HI-*Sac*I cut PCR product and a *Sac*I-*Bgl*II fragment from plasmid pG/HER [29] were introduced into the *Bam*HI site of the yeast expression vector pG-1 [30] to yield the plasmid pG1/Cdc37. The PCR product was also introduced into the *Bam*HI-*Sac*I of plasmid pSP73 (Promega). To obtain construct pGEX2/CDC37 for expression of a glutathione-S-transferase (GST)/Cdc37 fusion protein, *Bam*HI-*Eco*RI and *Eco*RI-*Eco*RI fragments from pSP73/CDC37 were introduced into *Bam*HI-*Eco*RI sites of the bacterial expression vector pGEX2T (Pharmacia). The recombinant pYes/GST.Cdc37 for galactose-inducible expression of the same fusion protein in yeast contains the GST coding sequences as a *Sac*I-*Bam*HI fragment linked to the above-mentioned *CDC37* fragments in pYES2.0 (Invitrogen). Recombinant pYesADE2/HA-Ste11 is identical to plasmid pYes/HA-

*Corresponding author. Fax: (41)-22-702 6442.
E-mail: picard@cellbio.unige.ch

Abbreviations: Cdc, cell cycle division; GST, glutathione-S-transferase; Hsp90, heat-shock protein 90; MAPK, mitogen-activated protein kinase

Ste11 [28] except that the expression vector pYesADE2 (a gift from M. Strubin, University of Geneva) contains a substitution of the marker *ADE2* for *URA3*. Plasmids pYes/Ste11ΔN.GST, pYes/Ste11ΔN, p2U/GST-2 and p2U/Hsp82.GST have been described previously [28,31].

2.2. Yeast strains

Strain DP007 was made by mating of strain 8A7 (*MATα cdc37-34 leu2 lys2 trp1 ura3*) ([24]) with wild-type strain RMY326 (*MATα his3 leu2-3,112 trp1-1 ura3-52*). After sporulation and tetrad dissection, spores were tested for the *cdc37-34* phenotype (temperature sensitivity). A *MATα cdc37-34* spore was selected as DP007.

2.3. Growth arrest induced by α -factor or by Ste11ΔN expression

To monitor the cell cycle arrest in response to α -factor, cells were diluted to a density of 1.2×10^7 cells/ml and streaked or spotted onto YEPD plates containing 10 mM Na-citrate pH 4.3 and, where indicated, 10 μ M α -factor (Bachem, Switzerland), and incubated at 32°C. For the Ste11ΔN-induced growth arrest, the two strains, RMY326 and DP007, were transformed with plasmids pYES2.0, pYes/Ste11ΔN or pYes/Ste11ΔN.GST, and single colonies were streaked on plates containing glucose or galactose as carbon sources. The plates were incubated at 30°C for several days.

2.4. GST pull-down experiments

GST pull-down experiments were performed as follows: yeast cells (strains 8A7 or RMY326 with plasmids pYes/Ste11ΔN.GST, p2U/GST-2, p2U/Hsp82.GST, or pYes/GST.Cdc37 and pYesADE2/HA-Ste11), where appropriate induced with galactose by overnight incubation, were washed once with water containing 1 mM dithiothreitol (DTT) and 1 mM PMSF, and once with TEG (25 mM Tris-HCl pH 7.4, 15 mM EGTA, 10% glycerol, 1 mM DTT, 1 mM PMSF, 3 μ g/ml chymostatin, 1.5 μ g/ml pepstatin A, 0.75 μ g/ml leupeptin, 3.8 μ g/ml antipain) containing 150 mM NaCl. Cell pellets were then resuspended in a small volume of the same buffer and broken with glass beads by two 30 s pulses at maximum speed in a Mini-BeadBeater-8 (Biospec, Bartlesville) at 4°C. After centrifugation at 15'000 rpm in a table top centrifuge at 4°C, the supernatant was quantitated and adjusted to 0.1% Triton X-100. Glutathione-Sepharose beads (Pharmacia) were added to the extracts, tumbled for 30 min at 4°C, washed three times with TEG containing 150 mM NaCl, 0.1% Triton X-100 and twice with TEG with 0.1% Triton X-100. Bound proteins were eluted with 7.5 mM reduced glutathione in 50 mM Tris-HCl pH 8.0, concentrated by TCA precipitation, resuspended in SDS-sample buffer and separated by 10% SDS-PAGE.

The recombinant proteins GST and GST-Cdc37 were expressed in the *Escherichia coli* strain XL1-Blue (Stratagene) and extracted in a phosphate-buffered saline buffer (PBS) with 1 mM DTT, 1 mM PMSF, 3 μ g/ml chymostatin, 1.5 μ g/ml pepstatin A, 0.75 μ g/ml leupeptin and 3.8 μ g/ml antipain by sonication after induction of protein synthesis with 1 mM of isopropylthio- β -galactoside. After centrifugation at 15'000 rpm in a table top centrifuge at 4°C, the supernatants were adjusted to 0.1% Triton X-100, glutathione beads were added and the samples were tumbled for 30 min at 4°C. The beads (with about 20 μ g of GST.Cdc37 or 80 μ g of GST) were washed three times with PBS and once with TEG. 3 mg of yeast protein extract from wild-type strain RMY326 was added to each sample and incubated for 2 h at 4°C with agitation. The beads were then washed three times with TEG, bound proteins were eluted and processed as described above.

2.5. Immunoblot experiments

After SDS-PAGE and transfer of proteins to nitrocellulose membranes, the membranes were blocked with Tris-buffered saline, 0.05% Tween-20 (TBST) containing 5% (w/v) milk powder and probed with appropriate antibodies in TBST+milk powder at room temperature for 1 h. Mouse anti-GST monoclonal (Santa Cruz), chicken anti-Hsp82 [29] and mouse monoclonal anti-Cdc37 antibodies (a gift from A.J. Caplan, Mount Sinai, New York, USA) were all diluted 1:1000. Mouse monoclonal anti-HA antibody (a gift from K. Matter, University of Geneva) was diluted 1:100. Membranes were washed three times for 10 min with TBST. The secondary antibodies were alkaline phosphatase-conjugated anti-chicken (Promega) and horseradish peroxidase-conjugated anti-mouse antibodies (Cappel). They were incubated in TBST+milk powder at room temperature for 1 h.

After three washes with TBST, the blots were developed either with the NBT/BCIP reagent for alkaline phosphatase or with the enhanced chemiluminescence reagent (Pierce) for horseradish peroxidase.

2.6. In vitro protein kinase assays

After the GST pull-down, the beads with Ste11ΔN.GST expressed in the *cdc37* strain 8A7, complemented or not with pG1/Cdc37, were incubated with 10 μ Ci of [γ -³²P]ATP, at a final concentration of 20 μ M ATP in kinase buffer (20 mM Tris-HCl pH 7.5, 50 mM KCl, 10 mM MgCl₂ and 0.5% glycerol) for 20 min at 30°C. After washing the beads several times with kinase buffer, bound proteins were resuspended with SDS-sample buffer and separated by SDS-PAGE. After transfer to a nitrocellulose membrane, an autoradiography was carried out, and then an immunoblotting experiment with an anti-GST antibody. Autoradiograph and nitrocellulose filter were scanned to quantitate the bands with the software ScanAnalysis (Biosoft, Cambridge, UK).

3. Results

3.1. Cdc37 required for Ste11-mediated signaling

Several temperature-sensitive *cdc37* alleles have been isolated [1,2,24], which facilitate the functional analysis of this essential gene. We tested different *cdc37* strains for their ability to arrest growth in response to the mating pheromone α -factor. Whereas the *cdc37-1* allele supports the pheromone-induced growth arrest at permissive temperature (data not shown), the more severely defective *cdc37-34* allele does not (Fig. 1A). The former result is consistent with the observation that mating and induction of the expression of the pheromone-induced *FUS1* gene are normal in strains with the *cdc37-1* allele [32]. The failure of the *cdc37-34* strain DP007 to respond to α -factor is a specific defect since it can be suppressed by the introduction of the wild-type *CDC37* gene. Note that this signaling defect correlates with impaired, albeit not completely defective, mating (data not shown).

In a first attempt towards determining the step(s) of the pheromone pathway (see Fig. 4) that is dependent on Cdc37, we assayed growth arrest induced by a constitutively active Ste11 mutant expressed under the control of a galactose-inducible promoter. It has been shown that the deletion of the amino-terminal regulatory domain of Ste11 (Ste11ΔN) results in constitutive activation of this kinase [33]. When the expression of Ste11ΔN is induced by plating the cells on galactose, their growth is inhibited. Since Ste11 is also involved in several other signaling pathways [26,27], the growth inhibitory effect results from the combined activation of the pheromone and osmoregulatory pathways [34]. Fig. 1B shows that Ste11ΔN fused to the N-terminus of GST (Ste11ΔN.GST) is as efficient as the unfused version at eliciting this response in a wild-type strain. This tagged version of Ste11ΔN greatly facilitates the biochemical analysis (see below), and it is therefore used in most subsequent experiments. The strain DP007 with the *cdc37-34* allele fails to be growth-arrested by Ste11ΔN.GST unless it is complemented by the wild-type *CDC37* gene (Fig. 1C). The same pattern was observed with the original *cdc37-34* strain 8A7, which has the opposite mating type (*MATα*) (data not shown). Thus, these experiments showed that the requirement for Cdc37 is independent of mating type and possibly at the level of Ste11 or downstream of it.

3.2. Accumulation and maturation of Ste11 are defective in a *cdc37-34* background

We have previously reported that the accumulation of Ste11

is reduced in strains in which the molecular chaperone Hsp90 is altered genetically [28]; this effect can be mimicked pharmacologically by blocking Hsp90 function with the benzoquinone ansamycin Macbecin I (data not shown). Moreover, the vertebrate tyrosine kinase pp60^{v-src} is produced at lower levels and is less active upon expression in a *cdc37-34* yeast strain at permissive temperature [24]. We therefore decided to examine the levels and the activity of Ste11ΔN.GST expressed in strain 8A7 with and without wild-type *CDC37*. Fig. 2A shows that the steady-state level of Ste11ΔN.GST expressed from a galactose-inducible promoter is reduced in the mutant strain. The levels of other proteins, for example of endogenous Hsp82 or of β-galactosidase also expressed from a galactose-inducible promoter, were not affected by the mutation (data not shown). Since Ste11ΔN.GST is expressed from a heterologous promoter, this suggests that it might be the degradation of Ste11ΔN.GST that is increased in the *cdc37-34* strain.

We next performed an in vitro kinase assay with Ste11ΔN.GST pulled down from yeast extracts with glutathione beads (Fig. 2B). Phosphorylation of Ste11ΔN.GST is

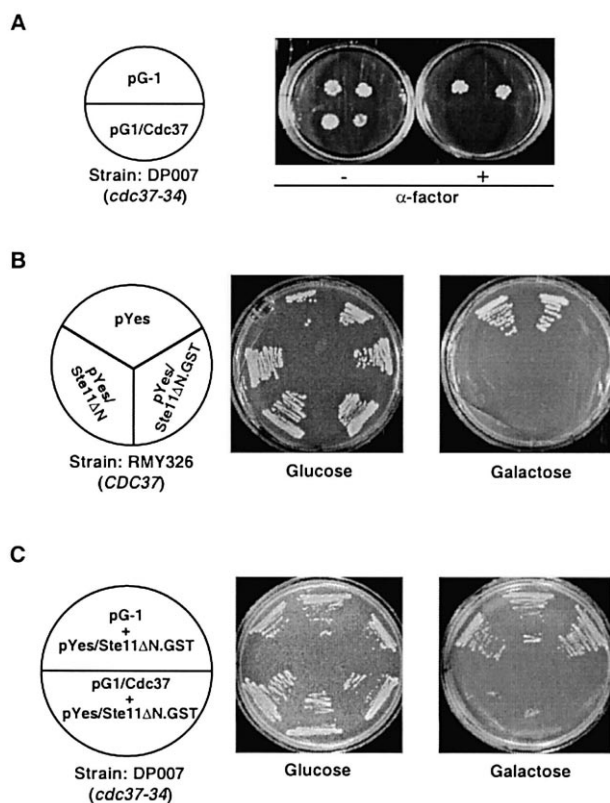


Fig. 1. *CDC37* is required for growth arrest induced by pheromone and by the constitutive kinase Ste11ΔN. (A) Pheromone-induced growth arrest is defective in a *cdc37* yeast strain. Transformants of strain DP007 (*MATa cdc37-34*), containing either the episomic expression vector for wild-type Cdc37 (pG1/Cdc37) or the empty expression vector pG-1, were streaked on plates without or with 10 μM of α-factor, and incubated at 32°C for 3 and 5 days, respectively. (B) A GST fusion protein of Ste11ΔN (Ste11ΔN.GST) induces growth arrest as well as Ste11ΔN. Transformants of the wild-type strain RMY326 with galactose-inducible expression of Ste11ΔN or Ste11ΔN.GST were plated on glucose and galactose plates (repressed and induced conditions, respectively) and grown for 4 days at 30°C. (C) Ste11ΔN.GST fails to induce growth arrest in the *cdc37* strain DP007. Vectors and assays were as in (A) and (B); plates were incubated at 30°C for 5 days. In all three experiments, 2–3 independent transformants were tested.

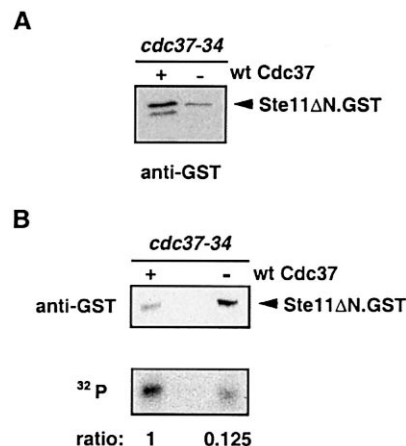


Fig. 2. Ste11ΔN.GST protein levels and Ste11 phosphorylation are defective in a *cdc37* strain. (A) Ste11ΔN.GST protein levels: Ste11ΔN.GST expression was induced by galactose in the *cdc37* strain 8A7 complemented or not with wild-type Cdc37 (vector pG1/Cdc37). The same amounts of protein extract (30 μg) were analyzed by immunoblotting with an anti-GST antibody; equal loading was confirmed by Ponceau red staining of the membrane. (B) Ste11 phosphorylation: after a pull-down of Ste11ΔN.GST from extracts prepared from the same yeast transformants, the purified proteins on the beads were subjected to an in vitro kinase assay. After SDS-PAGE and transfer to a nitrocellulose membrane, an autoradiography was performed prior to detection of Ste11ΔN.GST by immunoblotting with an anti-GST antibody. Note that 4-fold more pulled down Ste11ΔN.GST was loaded for the sample in the second lane (-wt Cdc37) to facilitate quantitation of the reduced ³²P incorporation.

reduced 8-fold in the absence of wild-type Cdc37. Thus, both accumulation of Ste11ΔN.GST and relative phosphorylation of what does accumulate are reduced in the mutant strain. These findings are consistent with the idea that Cdc37 is required for the accumulation and maturation not only of the exogenously expressed Ste11ΔN.GST, but also of the endogenous wild-type Ste11 protein, and that these defects are responsible for the signaling defect described above.

3.3. Pairwise complexes of Cdc37, Ste11 and Hsp90

Several lines of evidence indicated that Ste11 might associate with both Cdc37 and Hsp90. We had previously shown that Ste11 forms complexes with Hsp90 in yeast [28], and the vertebrate Ste11 equivalent Raf-1 had been demonstrated to form complexes with both Cdc37 and Hsp90 [25]. We therefore examined the presence of pairwise complexes in wild-type cells with GST pull-down experiments. Fig. 3A shows that Ste11ΔN.GST expressed in yeast is associated with endogenous Cdc37 whereas GST only pulls down background levels. In the converse experiment, a GST-Cdc37 fusion protein, but not GST alone, pulls down exogenously expressed epitope-tagged full-length Ste11 (Fig. 3B). This demonstrates that the association of Cdc37 and Ste11 is not restricted to the N-terminally truncated Ste11ΔN. Moreover, the yeast Hsp90 isoform Hsp82, expressed as a GST fusion protein in yeast, but not GST alone, pulls down endogenous Cdc37 (Fig. 3C). To characterize the latter interaction further, we examined whether yeast Hsp90 could be retained by a recombinant GST-Cdc37 fusion protein from a total yeast extract. Note that such an N-terminally tagged Cdc37 can be fully functional as the same fusion protein is able to complement a *cdc37-34* strain at non-permissive temperature (data not

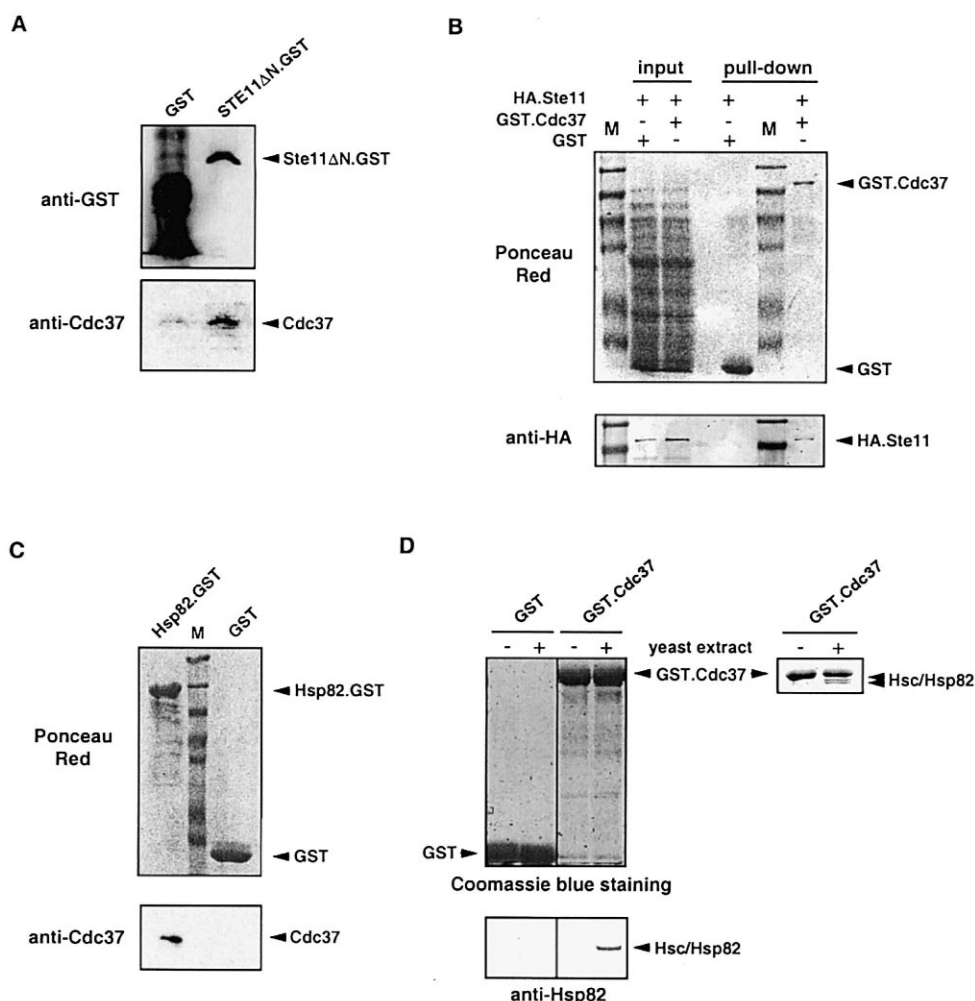


Fig. 3. Biochemical interactions between Cdc37, Ste11 and Hsp90. (A) Ste11ΔN.GST pulls down Cdc37. A GST pull-down experiment was performed with yeast extracts from the wild-type strain RMY326 expressing GST or Ste11ΔN.GST (induced with galactose). GST and Ste11ΔN.GST, and endogenous Cdc37 were revealed by immunoblotting the same membrane with anti-GST and anti-Cdc37 antibodies, respectively. (B) GST.Cdc37 pulls down HA-tagged full-length Ste11. Expression of HA-Ste11 and GST.Cdc37 or GST was induced in RMY326 transformants with galactose. 50 μg of total yeast protein extract was loaded in lanes labelled 'input'; lanes labelled 'pull-down' contain proteins retained by a GST pull-down experiment with 2.4 mg of total yeast protein extract. After transfer, the membrane was stained with Ponceau red to reveal total protein and subsequently immunoblotted with an anti-HA antibody. M, molecular weight marker with bands at 180, 116, 84, 58, 48, 36 and 26 kDa. (C) Hsp82.GST pulls down endogenous Cdc37. The GST pull-down experiment was done as above except that Hsp82.GST and GST were expressed in medium with glucose as carbon source. (D) Hsp90 is retained by recombinant GST.Cdc37 but not by GST. The recombinant purified GST proteins were incubated with 3 mg of total yeast protein extract (lanes marked +). Retained proteins were revealed by Coomassie blue staining (top panel) and immunoblotting with anti-Hsp82 antibodies (bottom panel). The Hsp90 band corresponds to the two yeast isoforms Hsc82 and Hsp82; it resolves into a double band visible by Coomassie staining below the GST.Cdc37 input upon more extensive electrophoresis of a smaller amount of total protein (small panel on the right).

shown). As can be seen in Fig. 3D, GST.Cdc37 specifically pulls out the two Hsp90 isoforms Hsp82 and Hsc82. Since no other band of similar intensity is visible in the Coomassie-stained gel, we tentatively conclude that the interaction between Cdc37 and yeast Hsp90 is direct. Due to the severely reduced levels in a *cdc37-34* strain of both the mutant Cdc37 itself [35] and its client Ste11ΔN.GST (see above), we have not been able to assess the effects of the mutation on all relevant pairwise complexes although Ste11-Hsp90 complexes still seem to form (data not shown).

4. Discussion

We have shown that *CDC37* is required for pheromone signaling, growth arrest induced by a constitutive Ste11 ki-

nase, and accumulation and activity of Ste11. Moreover, we have demonstrated that Cdc37 and Ste11 form complexes. Taken together, these results argue very strongly that Ste11 function depends on Cdc37. Whether all signaling pathways that require Ste11 [26,27] are defective remains to be determined. However, this seems highly likely in view of the low level and activity of Ste11 in a *cdc37* mutant strain. Hence, Cdc37 might be required for pheromone signaling as well as the osmoregulatory response and the response to low nitrogen levels.

Several observations support the conclusion that Ste11 is a direct target of Cdc37: (i) two different epitope-tagged versions of Ste11 associate with Cdc37; (ii) accumulation and phosphorylation of a Ste11-GST fusion protein are reduced; (iii) Hsp90, another molecular chaperone that is required for

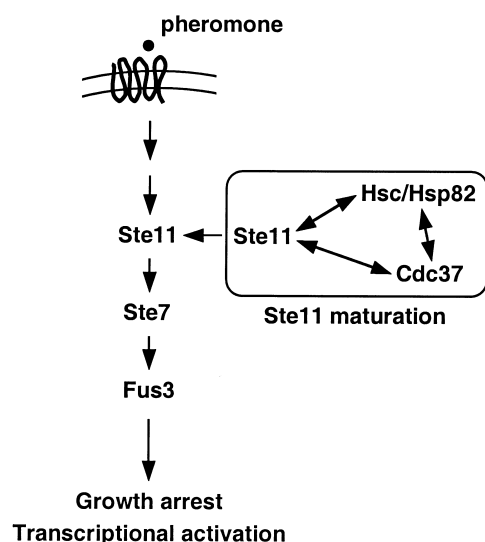


Fig. 4. Site of action of Hsp90 and Cdc37 in the pheromone signaling pathway. To simplify the scheme, only a subset of all signaling components is shown. Double-headed arrows within the box indicate demonstrated pairwise complexes.

Ste11 function [28], associates with Cdc37. These results parallel those obtained in the vertebrate system. The vertebrate Ste11 equivalent Raf-1, with its catalytic domain, binds directly to human p50^{Cdc37} [20]. Interaction of Raf-1 with Hsp90 appears to be both direct and indirect through the formation of a ternary complex with p50^{Cdc37} [25]. Raf-1 function and signaling are blocked by a truncated version of p50^{Cdc37} that is unable to recruit Raf-1 into a ternary complex with Hsp90 [25]. Although we have been able to demonstrate pairwise associations of Cdc37, Ste11 and Hsp90 in the yeast system (Fig. 4), novel approaches need to be developed to determine the stoichiometry and dynamics of these complexes, in general, and whether the three proteins form a ternary complex, in particular.

Our findings are consistent with the idea developed for the metazoan systems [25] that the two molecular chaperones Hsp90 and Cdc37 coordinately, and perhaps only transiently, intervene in the folding of newly synthesized Ste11 and/or in directing additional cofactors to Ste11. When Cdc37 or Hsp90 functions are defective, Ste11 may not properly fold or associate with other factors. As a consequence, it does not become functional and is eventually degraded more rapidly (see also [28]). Allele-specific effects, such as those observed with Ste11 (see above and [28]), have been reported for pp60^{V-src} expressed in *cdc37* [24] and *hsp90* [7,36–38] mutant yeast strains. Indeed, the *cdc37-34* allele was selected for as a mutation that relieves the toxicity of overexpressed pp60^{V-src} in yeast. Decreases in accumulation and specific activity of pp60^{V-src} both contribute to reduce the toxicity in *cdc37-34* cells even at permissive temperature [24]. In contrast, *cdc37-1* is a milder allele that is associated with resistance to both pp60^{V-src} [24] and Ste11ΔN at permissive temperature. We have recently discovered that the eIF2α kinase Gcn2 is an Hsp90 substrate and that the general amino acid control, a Gcn2-dependent response to limiting levels of amino acids, is defective in both *cdc37-1* and *cdc37-34* strains [39]. Whereas reduced accumulation and functional ‘maturation’ was also reported for

Cdc28 in a *cdc37-1* mutant strain [2], Cdc37 defects may not lead to increased degradation of all Cdc37 client proteins. Mps1, another putative Cdc37 client protein in yeast, accumulates to the same levels but its kinase activity is markedly reduced [5].

It is interesting to compare Cdc37 with Hsp90. They are both essential for viability, biochemically and genetically associated with a whole panel of kinases, and they have very similar chaperone activities in vitro (reviewed in [21–23]). However, they cannot replace each other in yeast or in *Drosophila* except perhaps for a subset of substrates such as pp60^{V-src} expressed in yeast [7]. Whereas Hsp90 is very highly conserved with 60% sequence identity between human and yeast, Cdc37 is not (only 20%). It is conceivable that higher order structural elements of the Cdc37-Hsp90 interface are conserved. Although Cdc37 appears to bind to Hsp90 directly both in vertebrates [20] and in yeast (see above), Cdc37 and Hsp90 certainly do not always come together. Hsp90, the more abundant partner, associates with several other cofactors whose binding to Hsp90 sterically prevents the binding of p50^{Cdc37} [20]. As a result, Hsp90 in association with these other cofactors is likely to have a distinct set of client proteins. It remains to be seen whether the converse is true as well, whether there are Cdc37 clients that do not need Hsp90. Unlike Ste11 [28] and Gcn2 [39], Cdc28, Kin28 and Mps1, although genetically linked to Cdc37, have not been shown to form complexes with either Cdc37 or Hsp90 in yeast [2,5,6]. To explore further the similarities and differences between Cdc37 and Hsp90, it will be interesting to determine whether *SSF1*, *HCH1* and *CNS1*, which have been discovered as allele-specific suppressors of temperature-sensitive *hsp90* mutations [40], are able to suppress *cdc37* mutations. It is noteworthy, though, that *CDC37* itself is the only gene that we have been able to recover as a high-copy suppressor of *cdc37* (data not shown). More proteins that interact with Cdc37 need to be identified to assess whether it acts ‘solely’ as one of several Hsp90 co-chaperones.

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