CNS1 Encodes an Essential p60/Sti1 Homolog in Saccharomyces cerevisiae That Suppresses Cyclophilin 40 Mutations and Interacts with Hsp90

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Cyclophilins are cis-trans-peptidyl-prolyl isomerases that bind to and are inhibited by the immunosuppressant cyclosporin A (CsA). The toxic effects of CsA are mediated by the 18-kDa cyclophilin A protein. A larger cyclophilin of 40 kDa, cyclophilin 40, is a component of Hsp90-steroid receptor complexes and contains two domains, an amino-terminal prolyl isomerase domain and a carboxy-terminal tetratricopeptide repeat (TPR) domain. There are two cyclophilin 40 homologs in the yeast Saccharomyces cerevisiae, encoded by the CPR6 and CPR7 genes. Yeast strains lacking the Cpr7 enzyme are viable but exhibit a slow-growth phenotype. In addition, we show here that cpr7 mutant strains are hypersensitive to the Hsp90 inhibitor geldanamycin. When overexpressed, the TPR domain of Cpr7 alone complements both cpr7 mutant phenotypes, while overexpression of the cyclophilin domain of Cpr7, full-length Cpr6, or human cyclophilin 40 does not. The open reading frame YBR155w, which has moderate identity to the yeast p60 homolog STI1, was isolated as a high-copy-number suppressor of the cpr7 slow-growth phenotype. We show that this Sti1 homolog Cns1 (cyclophilin seven suppressor) is constitutively expressed, essential, and found in protein complexes with both yeast Hsp90 and Cpr7 but not with Cpr6. Cyclosporin A inhibited Cpr7 interactions with Cns1 but not with Hsp90. In summary, our findings identify a novel component of the Hsp90 chaperone complex that shares function with cyclophilin 40 and provide evidence that there are functional differences between two conserved sets of Hsp90 binding proteins in yeast.

Cyclophilin 40 is one of several protein components of the Hsp90 protein complex. Hsp90 has a dual function; it acts as a chaperone after heat shock to help fold denatured proteins and also maintains the activity of signalling proteins under normal conditions. Hsp90 and associated proteins function as large chaperone units that regulate several molecules involved in signal transduction, including oncogenic kinases and members of the steroid receptor family (reviewed in references 2 and 43). These Hsp90 complexes consist of several proteins, including Hsp70, p60, p48, p23, and a large immunophilin, which may be either FKBP52, FKBP54, or cyclophilin 40. Several of these proteins have recently been shown to have chaperone activity in vitro (4, 22, 48).

Interactions between the components of the Hsp90 chaperone complex and their substrates are highly ordered and very dynamic. The order of assembly of these complexes with the progesterone receptor has been determined from reconstitution experiments in cell-free lysates (53, 54). First, Hsp70 binds the progesterone receptor, forming an early complex. Next, the progesterone receptor is found in an intermediate complex containing Hsp90, Hsp70, and p60. The trimeric Hsp90-Hsp70-p60 complex is soon displaced from the progesterone receptor by a preformed Hsp90-immunophilin-p23 complex. In this mature complex, the progesterone receptor is maintained in a state competent to bind hormone. If the receptor does not bind steroid, it is released from the mature complex and starts the association-dissociation cycle again. Recently, it has been shown that if the Hsp90 substrate is locked in a complex with Hsp90 and is not released, it is targeted for degradation by the proteasome (48). This study used the Hsp90 inhibitor geldanamycin, an antiproliferative agent that may find use as a novel chemotherapeutic agent. It has been previously suggested that geldanamycin blocks the binding of p23 to the Hsp90-immunophilin complex (59), which may improperly stabilize interactions between this complex and target proteins, thus stimulating degradation. Two recent studies show that geldanamycin inhibits binding of a yeast p23 homolog to yeast Hsp90 (1, 19).

By Hsp90 affinity chromatography and heterologous coexpression of the steroid receptor and a reporter gene under control of a steroid response element in the yeast Saccharomyces cerevisiae, it was shown that the Hsp90 complex is biochemically and functionally conserved in S. cerevisiae (6, 8, 25, 36, 37, 42). Previous studies and the recent completion of the yeast genome sequencing project have identified genes encoding other proteins found in Hsp90 complexes. There are two Hsp90 homologs in yeast, HSP82 and HSC82; HSC82 is expressed constitutively at high level and is moderately induced by heat shock, whereas HSP82 is expressed constitutively at a much lower level but is much more strongly induced by heat shock (3). Yeast strains require at least one copy of either HSP82 or HSC82 for viability. STI1, the yeast p60 homolog, physically and genetically interacts with HSP90, and mutations in STI1 affect HSP90 functions in vivo (9, 16, 39). In addition, several HSP70 homologs are found in S. cerevisiae (38). A yeast p23 homolog, Sba1, has also recently been identified (1, 19). There are two cyclophilin 40 genes in yeast, CPR6 and CPR7 (8, 15–17, 57). The Cpr6 and Cpr7 cyclophilins share 47 and 35% identity with human cyclophilin 40, respectively, and 41% identity with each other. All of the cyclophilin 40 homologs have in common an amino-terminal peptidyl-prolyl isomerase...
domain and a carboxy-terminal tetratricopeptide repeat (TPR) domain. TPR domains are loosely conserved repeats of roughly 34 amino acids that are found in several proteins that interact with Hsp90; the TPR domain of cytoplasm 40 mediates its binding to Hsp90 (13, 16, 41, 44). Yeast strains lacking cpr6 or cpr7, alone or in combination, are viable. cpr7 mutant strains, however, exhibit a slow-growth phenotype, while cpr6 mutant strains do not (15–17, 57).

Here we have further characterized the yeast cytoplasm 40 homologs. We find that cpr7 mutant strains are hypersensitive to the Hsp90 inhibitor geldanamycin (59). Mutant forms of the cytoplasm 40 homolg 40 were analyzed to determine the unique features required for function in vegetative growth and geldanamycin resistance. The TPR domain of cpr7 alone, when overexpressed, restores normal growth rate and Geldana-

Gene and cpr6 mutant strains. Nos1 complement both the slow growth and the geldanamycin sensitivity of both cpr7 single-mutant and cpr6 cpr7 double-mutant strains. Nos1 is required for viability in yeast, and the lethality of a Nos1 null mutant strain is not rescued by overexpression of STI1, CPR6, CPR7, HSP90, or any other genes implicated in Hsp90 functions. Unlike its homolog STI1, Nos1 is not transcriptionally regulated by heat shock. Finally, we show that the Nos1 protein is found in protein-protein complexes containing yeast Hsp90 and the yeast cytoplasm 40 homolog Cpr7 but not the Cpr6 cytoplasm 40. Taken together, our findings and previous studies reveal that the components of the Hsp90-associated chaperone machinery are duplicated in yeast and that one partner of each pair is heat inducible and nonessential (Hsc82, CPR6, and STI1) whereas the other partner is constitutive and often more important for vegetative growth (Hsc82, CPR7, and Nos1).

MATERIALS AND METHODS

Media and strains. Media were prepared as described in reference 49. Media were prepared as described in reference 49. Media containing galactose (NCIH, National Cancer Institute) was prepared by adding a sterile stock of geldanamycin in dimethyl sulfoxide to autoclaved medium containing geldanamycin (National Cancer Institute) was prepared by

Construction of Cpr7 deletion mutant and Cpr6-Cpr7 fusion proteins. The ΔCYP Cpr7, ΔTPR Cpr7, and Cpr6-Cpr7 hybrid proteins were engineered and expressed with Cpr7 and 3′ untranslated regions by PCR overlap mutagenesis as described in reference 28 using the following primers for ΔCPR7, 5′-GGCGAGTGGAGGAAAGAAATTTTGGTC-3′ and 5′-GGCGAGCTCCCGCTTGTTGATTG-3′; for ΔTPR Cpr7, 5′-AACAAGTTAATTTACATCTAAGTGCTGTTAAGTAAC-3′ and 5′-GAGGTTGATTAAGTTGATATGTTG-3′; for Cpr6-Cpr7, 5′-CTCAGTACGCGGTTGGAGTGGT-3′ and 5′-AAGCGGCGAGCAGTCTATATACTA-3′; for ΔCPR7 and Cpr6 Cpr7, 5′-CGTTGGATCCCTGGGAACATCG-3′ and 5′-TGGGTCGAGCTGTTGAAACTATGTTG-3′; for ΔTPR Cpr7, 5′-ATTCTGAAAGGTGTCAGCAACATATCCGCTATGTCGCCAGCAGCACCCTGCCCAACAAATTCATCTATACGTCATCGAATGTTG-3′ and 5′-TTGGGTTATTTAATCT-3′. The resulting PCR product was gap-repaired with pRS316 (50) and cloned into the corresponding sites of both pRS316 (50) and YEp195 (24). The human cytoplasm 40 gene 40 was fused to the 5′ and 3′ untranslated regions of Cpr7 as gap repair as described elsewhere (47). The resulting PCR product was gap-repaired with pRS316 (50) and cloned into the corresponding sites of both pRS316 (50) and YEp195 (24), respectively.

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Cpr6 were generously provided by Susan Lindquist and Didier Picard, respectively. Mouse polyclonal antisera to yeast Hsc82 was generously provided by Avrom Caplan. Antisera against human cyclophilin 40 was purchased from Affinity Bioreagents. Avrom Caplan. Antisera against HA (Boehringer Mannheim) or mouse polyclonal antisera against yeast Hsc82 (provided by Avrom Caplan).

RESULTS

The TPR domain of the yeast cyclophilin 40 homolog Cpr7 is critical for function. To determine which domains of the yeast cyclophilin 40 homolog Cpr7 are important for function, we engineered a series of deletion and fusion proteins and tested whether these restore normal growth in a cpr7 null mutant strain when expressed from either a low-copy-number CEN plasmid or a high-copy-number 2μ plasmid. As expected, expression of the wild-type Cpr7 gene from either a CEN or 2μ plasmid complemented the slow-growth defect of the cpr7 mutation, restoring colony size to the wild-type level (Fig. 1). Interestingly, overexpression of the Cpr7 TPR domain alone from a 2μ plasmid (but not from a CEN plasmid) was sufficient to complement the cpr7 slow-growth mutant phenotype and restore colony size to wild-type (Fig. 1). In contrast, the Cpr7 cyclophilin domain failed to complement the cpr7 mutation, even when overexpressed (Fig. 1). These findings are in accord with a recent report by others (18). Expression of the yeast Cpr6 cyclophilin homolog, or human cyclophilin 40, also failed to complement the cpr7 mutation (Fig. 1). Western blot analysis with specific antisera confirmed that both Cpr6 and human cyclophilin 40 were expressed (data not shown). Finally, we note that when the Cpr7 TPR domain was fused to the cyclophilin domain of either Cpr7 (wild-type protein) or Cpr6 (Cpr6-7 hybrid protein), complementation was observed even with expression from a CEN plasmid. When overexpressed in a wild-type background, none of the cyclophilin 40 deletion or fusion proteins had any dominant negative effects on growth rate (data not shown).

To test whether the TPR domain of Cpr7 required the presence of the Cpr6 protein to function, we repeated the preceding experiments in a cpr6 cpr7 double-mutant strain. When overexpressed, the TPR domain of Cpr7 still complemented the growth defect of the cpr6 cpr7 mutant strain (Fig. 2). In addition, the Cpr7 TPR domain alone restored normal growth in a cpr1 cpr6 cpr7 triple-mutant strain (CPR1 encodes the yeast cytoplasmic cyclophilin A); thus, no cytoplasmic cyclophilin domain is required for the TPR domain to complement the slow-growth defect of cpr7 (data not shown). Together, these findings indicate that it is the TPR domain of Cpr7 that is most critical for its in vivo function.

**cpr7 mutants are sensitive to the Hsp90 inhibitor geldanamycin.** Geldanamycin is a potent antitumor drug whose target is Hsp90 (59). We found that cpr7 mutant strains, and also cpr6 and cpr6 cpr7 mutant strains, are hypersensitive to geldanamycin, indicating that in the absence of the yeast cyclophilin 40 homologs the cell is sensitive to perturbations in Hsp90 func-
tion (Fig. 3). These findings suggest that Cpr7 and Hsp90 normally interact, either physically, functionally, or both, in accord with previous genetic analyses that revealed a synthetic lethal interaction between yeast **hsp90** and **cpr7** mutations (16).

We tested whether Cpr6, human cyclophilin 40, or any of the Cpr7 deletion proteins could restore growth to a **cpr7** (or **cpr6** **cpr7**) mutant strain on medium containing 20 μg of geldanamycin per ml. As in the growth rate studies, the Cpr6-Cpr7 fusion protein and the overexpressed Cpr7 TPR domain alone (but not Cpr6 or human cyclophilin 40) complemented the geldanamycin-sensitive phenotype of a **cpr7** mutant strain (Fig. 1 and 2). This result provides further evidence that the slow-growth phenotype of **cpr7** mutant strains is linked to defects in Hsp90 function.

The overexpressed Cpr7 TPR domain only partially restored normal growth on medium containing geldanamycin in a **cpr6** **cpr7** double-mutant strain (Fig. 2). In addition, the Cpr6-Cpr7 fusion protein must be overexpressed to complement the geldanamycin sensitivity in the **cpr6** **cpr7** double-mutant strain, while in the **cpr7** single mutant the fusion protein in low copy number was sufficient for full function. These results suggest that Cpr6 and Cpr7, though not functionally redundant, may to some extent overlap in function.

**CPR6** expression is heat induced, whereas **CPR7** expression is not. Because several other proteins found in Hsp90 complexes are inducible by heat shock, we tested whether Cpr6 or Cpr7 expression is regulated by heat shock via Northern blot analysis. **CPR6** was induced 3.3-fold after 5 min at 37°C (Fig. 4A), in accord with previous studies that have shown that Cpr6 protein levels are induced fourfold after heat shock at 39°C (57). In contrast, expression of the **CPR7** gene was not induced by heat shock (Fig. 4B). In accord with these findings, the **CPR6** gene promoter contains consensus heat shock response elements (57), whereas the **CPR7** gene promoter does not. **cpr6** and **cpr7** single-mutant and **cpr6** **cpr7** double-mutant strains

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**FIG. 2.** The TPR domain does not require the presence of Cpr6 to functionally replace Cpr7. The **Δcpr6 Δcpr7** mutant strain KDY66.5a was transformed with 2μm or **CEN URA3** plasmids expressing wild-type Cpr7, the TPR domain of Cpr7 (ΔCYP), the cyclophilin domain of Cpr7 (ΔTPR), wild-type Cpr6, a hybrid protein containing the cyclophilin domain of Cpr6 fused to the TPR domain of Cpr7 (Cpr6/7), or human cyclophilin 40 (hCYP40). Transformants were grown on synthetic dextrose media lacking uracil (SD − URA) or on YPD medium containing 20 μg of geldanamycin per ml (YPD + GA) for 48 h at 30°C. Criteria used to establish complementation of the **Δcpr6 Δcpr7** mutant strain were as described in the legend to Fig. 1. Findings presented are representative of several similar experiments. Open, solid, and hatched boxes, Cpr7, Cpr6, and human cyclophilin 40 protein sequences, respectively; +, wild-type colony size; +/−, smaller colony size and poorer growth; R, R/S, and S, drug resistant, partially drug resistant, and drug sensitive, respectively.

**FIG. 3.** Yeast mutants lacking cyclophilin 40 homologs are hypersensitive to the Hsp90 inhibitor geldanamycin. Isogenic wild-type (JK93da), **Δcpr6** (KDY46), **Δcpr7** (KDY65), and **Δcpr6 Δcpr7** (KDY66.5a) mutant yeast strains were grown overnight in YPD medium, diluted to equal OD, and 10-fold serially diluted; 5-μl portions were plated on YPD medium containing no drug or 20 μg of geldanamycin per ml and incubated for 48 h at 30°C. Approximate numbers of cells plated are indicated to the right.
were not more sensitive to heat shock at 45 or 48°C than the isogenic wild-type strain (data not shown).

cpr7 mutants are not suppressed by overexpression of known Hsp90-interacting proteins Ppt1, Cdc37, Cdc23, Ubc4, and Sun2. We tested whether the yeast Hsp90 homologs can function as high-copy-number suppressors of the cpr7 slow-growth phenotype. Overexpression of Hsc82 or Hsp82 did not complement the slow-growth phenotype of a cpr7 mutant strain (data not shown). We also tested the following proteins that interact with Hsp90 or may be involved in Hsp90 functions: Ppt1, a serine/threonine phosphatase with four TPR domains that copurifies with the glucocorticoid receptor (10); Cdc37, the p60/Sti1 homolog (20% identity and 39% similarity [Fig. 5]); another group has also independently identified Cns1, a novel component of the Cpr7-Hsp90 complex, a 26S proteasome (33). None of these proteins restored normal growth to a cpr7 mutant strain when overexpressed from a 2µm high-copy-number plasmid (data not shown).

Identification of a multicopy suppressor of cpr7 mutations as the p60/Sti1 homolog CNS1. To identify the target(s) or novel components of the Cpr7-Hsp90 complex, a 2µm URA3 yeast genomic library was screened for genes that, when overexpressed, suppress the Δcpr7 slow-growth phenotype. We screened ~55,000 Ura + transformants on synthetic medium lacking uracil and identified eight potential suppressors. The plasmids containing the putative suppressor clones were rescued from the Δcpr7 mutant strain, amplified in E. coli, and retransformed into both a single-mutant and a double-mutant strain. Seven of the eight rescued plasmids complemented the slow growth of both the cpr7 single and cpr6 double-mutant strains and were further analyzed. By subcloning and sequencing, we determined that one of these clones contained the CPR7 gene, as expected. The remaining six clones were overlapping genomic sequences; all contained YBR155w, a previously uncharacterized open reading frame (29); Cdc23, which contains several TPR domains that interact with Hsp90 or may be involved in Hsp90 function (14, 51); Ubc4, a ubiquitin-conjugating enzyme and is involved in ubiquitination and degradation of B-type mitotic cyclins (14, 51); Cdc23, which contains several TPR domains that copurifies with the glucocorticoid receptor (10); Cdc37, the p50 component found in several Hsp90-kinase complexes (20, 32); Cdc23, which contains several TPR domains and is involved in ubiquitination and degradation of B-type mitotic cyclins (14, 51); Ubc4, a ubiquitin-conjugating enzyme with TPR domains which, when mutated, is synthetically lethal with TPR domains (32); and Sun2, a component of the 26S proteasome (33). None of these proteins restored normal growth to a cpr7 mutant strain when overexpressed from a 2µm high-copy-number plasmid (data not shown).

CNS1 open reading frame was replaced by the G418 resistance gene in a wild-type diploid strain. The resulting heterozygous CNS1/cns1-1::G418 strain was sporulated and dissected, and 18 of 19 tetrads yielded two viable and two inviable segregants (a representative sample is shown in Fig. 6). All of the viable segregants were found to be G418 sensitive, consistent with the cosegregation of lethality and the Δcns1-1::G418 allele (data not shown).

The CNS1/cns1-1::G418 heterozygous diploid was transformed with high-copy-number 2µm plasmids alone (vector) or containing the HA epitope-tagged CNS1, wild-type STI1, or wild-type HSP82 gene. The transformed diploid strains were then sporulated and dissected; representative samples of the segregants are illustrated.
formed with a plasmid containing the wild-type CNS1 and URA3 genes, sporulated, and dissected. The majority of the tetrads showed four viable and no inviable segregants (Fig. 6), which consisted of two G418-sensitive, 5-fluoro-orotic acid-resistant and two G418-resistant, 5-fluoro-orotic acid-sensitive segregants (data not shown). These findings indicate that CNS1 is an essential gene and that reintroduction of the wild-type CNS1 gene restores viability in the Δcns1::G418 mutant strain. Overexpression of CPR7, STI1, HSP82, HSC82, CDC23, UBC4, PPT1, or SUN2 did not restore viability of the Δcns1::G418 mutant (Fig. 6 and data not shown). In addition, overexpression of CNS1 did not suppress the conditional synthetic lethality exhibited by a Δsti1 mutation in combination with an hsp82 mutation (data not shown).

It was previously shown that STE11 is induced by heat shock (39). We examined CNS1 gene expression during heat shock by Northern analysis and found that transcription of the CNS1 gene was not induced at elevated temperatures (data not shown).

Cns1 is in protein complexes containing Hsc82 and Cpr7 but not Cpr6. To examine physical interactions between Hsp90, Cns1, and cyclophilin 40, Cns1 was tagged with the HA epitope at its amino terminus (see Materials and Methods). The HA-tagged form of Cns1 complemented the lethality of a Δcns1::G418 null mutant (Fig. 6). Total-cell lysate was prepared from a wild-type yeast strain containing the HA-tagged Cns1. Cns1 was then immunoprecipitated with anti-HA antibodies coupled to Sepharose beads, and immunoprecipitates were analyzed by Western blotting. Hsc82 coimmunoprecipitated with the HA-Cns1 protein (Fig. 7A), indicating that Cns1 and Hsc82 are present in protein-protein complexes and may directly interact. This observation and interpretation would be in accord with previous findings that Hsp90 is directly physically associated with the p60/Sti1 protein that shares sequence identity with Cns1. In contrast to Hsc82, the Cpr6 protein was not present in Cns1 immunoprecipitates (Fig. 7B). This observation would again be in accord with previous observations that Hsp90 can exist in distinct complexes with p60/Sti1 and Cpr6 in yeast (9) and mammalian cells (41, 44).

To examine interactions between cyclophilin 40, Cns1, and Cpr7 by a different approach, Cpr7 was fused to GST and expressed in bacteria, and GST-Cpr7 was adsorbed to glutathione-Sepharose beads. The resulting Cpr7 affinity matrix was then incubated with yeast total-cell extracts containing HA-Cns1. Interestingly, the HA-Cns1 protein interacted with the GST-Cpr7 fusion protein (Fig. 8A and B). Because Cpr6 was not detected in the Cns1 immunoprecipitate, this finding suggests that Cpr7 is distinguished from Cpr6 by its ability to interact, directly or indirectly, with the Cns1 protein. Western blot analysis revealed that Hsc82 was also specifically bound to the GST-Cpr7 affinity matrix (Fig. 8C), in accord with previous findings (16). Interestingly, CsA disrupted the Cpr7-Cns1 interaction (Fig. 8B), suggesting that the cyclophilin domain of Cpr7 may participate in binding of Cns1 to Cpr7. CsA did not inhibit Hsc82 binding to Cpr7 (Fig. 8C), in accord with previous findings that the TPR domain of Cpr7 is sufficient for binding to Hsc82 (16). Finally, the Hsp90 inhibitor geldanamycin had no effect on binding of either HA-Cns1 or Hsc82 to the GST-Cpr7 affinity matrix (Fig. 8B and C).

DISCUSSION

In this study, we have further analyzed the structures and functions of components of the Hsp90-associated chaperone machinery. We performed a structure-function analysis of the yeast cyclophilin 40 homologs Cpr6 and Cpr7 that revealed the conserved TPR domain of Cpr7 is critical for function, demonstrated that yeast mutants lacking Cpr7 are hypersensitive to the Hsp90 inhibitor geldanamycin, and identified Cns1, a novel essential p60/Sti1 homolog that associates with Hsp90 and Cpr7.

The cyclophilin 40 proteins of yeast and mammals contain two conserved domains, an amino-terminal cyclophilin prolyl isomerase domain and a carboxy-terminal TPR domain (15–17, 57). We have found that the TPR domain of Cpr7 is critical for in vivo function, whereas the cyclophilin domain is largely dispensable. The TPR domain is known to be the critical domain for cyclophilin 40-Hsp90 interactions in both yeast and mammals (16, 41, 44). Because the TPR domain can complement in vivo and is the critical domain for Hsp90 interactions, Cpr7 and Hsp90 likely interact under normal physiological conditions via the Cpr7 TPR domain. While this report was in...
preparation, another group reported similar findings that the Cpr7 TPR domain is important for function (18).

We also find that yeast mutants lacking the cyclophilin 40 homolog Cpr7 are uniquely hypersensitive to the antitumor agent geldanamycin. Given previous studies that Cpr7 and the yeast hsp90 homologs genetically interact and that geldanamycin binds to and perturbs Hsp90 function in mammalian cells and in yeast (1, 19, 26, 48, 55, 59), our findings provide additional evidence that Hsp90 function is compromised in cpr7 mutant strains. In addition, our findings open the door to a genetic dissection of geldanamycin action in yeast. Previous studies have revealed that Hsp90 and its associated partner proteins have been conserved, both in structure and in function, from yeast to mammals (3, 8, 9, 25, 39, 42); thus, our findings should be generally applicable to understanding Hsp90, cyclophilin 40, and p60/Sti1 functions in mammalian systems.

Our studies have also identified a previously uncharacterized open reading frame as a multicopy suppressor of the Δcpr7 mutation. The product of this suppressor gene, Cns1, shares limited sequence identity with Sti1, the yeast homolog of the mammalian p60 protein, which genetically and physically interacts with the yeast Hsp90 homologs. We have shown that Cns1 is found in protein complexes that contain Hsc82 and Cpr7. Others have shown that the Cns1 homologs, Sti1 in yeast and p60 in mammals, are also components of Hsp90 complexes (9, 53). It has also been shown that p60 and cyclophilin 40 are present in distinct complexes with Hsp90, p60 and cyclophilin compete, via their TPR domains, for Hsp90 binding and do not bind to each other (16, 41, 44). We have found, however, that the yeast Cns1 protein is present in complexes that contain the Cpr7 cyclophilin 40 homolog but not the Cpr6 cyclophilin. There are several different interpretations and implications of this result. First, there may be subtle differences between the constitution of yeast and mammalian Hsp90 complexes. Studies that support a similarity between the protein content of yeast and mammalian Hsp90 complexes examined the presence of only Cpr6 and Sti1 (9). Second, because our experiments involved incubation of yeast extracts containing HA-Cns1 with bacterially expressed Cpr7 protein, Cns1 and Cpr7 need not directly interact and could, for example, be present in a ternary Cns1-Hsc82-Cpr7 complex in which Cns1 and Cpr7 are not in direct contact. However, our finding that cyclosporin A inhibits formation of Cpr7-Cns1 complexes, but not of Cpr7-Hsc82 complexes, suggests that Cpr7 directly interacts with both Cns1 and Hsc82. Finally, although the p60 homologs Sti1 and Cns1 are related, the level of sequence identity is low, the two genes are differentially regulated, and Cns1 is essential whereas Sti1 is not. Thus, Cns1 may have functions quite distinct from those of Sti1 that could involve direct protein-protein interactions with both Hsp90 and the cyclophilin 40 homolog Cpr7, whereas p60 and Sti1 have evolved to compete with cyclophilin 40 homologs for Hsp90 binding. Further study will be required to address these issues in detail.

Several of the proteins in Hsp90 complexes are encoded by two differentially regulated genes. For instance, the yeast homologs of Hsp90 (HSC82 and HSP82), Hsp70 (SSA1, SSA2, SSA3, and SSA4), cyclophilin 40 (CPR6 and CPR7), and p60 (STI1 and CNS1) are each encoded by at least two genes that are regulated differentially at the transcriptional level, with one partner constitutively expressed and the other induced by heat shock (3, 38, 39, 57). Perhaps under normal conditions, expression of the constitutively expressed homolog is sufficient for physiological functions but growth at elevated temperatures requires higher levels of protein. Thus, it is more efficient to induce transcription of just one homolog. This is likely to be the case for the Hsp82-Hsc82 pair, which share 97% identity at the amino acid level and have seemingly overlapping functions. For more divergent sets such as Cpr6-Cpr7 (41% identity) and Sti1-Cns1 (20% identity and 39% similarity), the homologs may have partially overlapping but also unique functions. It is interesting that for the cyclophilin 40 and p60 homologs, the constitutively expressed gene has the more dramatic phenotype when mutated compared to the effects of mutating the heat-regulated homolog. One hypothesis consistent with these observations is that the stress-regulated homolog is less important during normal growth conditions. For instance, normally the constitutively expressed protein may have very transient interactions but under stressed conditions, chaperone-like interactions may persist, requiring a larger pool of protein; thus, the stress-regulated protein is induced. Alternatively, perhaps the range of substrates is broadened under stress conditions, which would also require an increase in chaperone protein levels. Examining Hsp90 complexes under stressed conditions by using reagents that detect specific homologs would help to address these alternative hypotheses.

Although our multicopy suppressor screen was exhaustive, just one suppressor of the Δcpr7 mutant phenotype was identified. Potential targets of the Hsp90 complex, however, were not identified in this high-copy-number suppressor screen. Perhaps targets were not isolated because there may be several, critical substrates for the Hsp90 complex, and overexpression of any one is not sufficient to restore a normal level of growth in the Δcpr7 mutant strain.

Possible functions and targets of cyclophilin 40 homologs have recently been identified by other studies. First, the Schizosaccharomyces pombe cyclophilin 40 homolog Wis2 was identified as a multicopy suppressor of a cdc25 wee1 win1 triple mutant, suggesting that the Wis2 cyclophilin may be involved in progression from the G2 phase to mitosis (58). Second, mammalian cyclophilin 40 has been shown to bind to and negatively regulate DNA binding by the c-Myb transcription factor (34). While the in vivo significance of this observation remains to be explored, an interesting finding was that the cyclophilin domain was required for inhibition of c-myb DNA binding activity; this is in contrast to our finding that the Cpr7 cyclophilin domain is not critical for in vivo function in yeast but may be in accord with our observation that the cyclophilin domain may be involved in high-affinity binding of Cpr7 to Cns1. Finally, in the cases of both Wis2 and c-Myb, a role for Hsp90 or other Hsp90-associated proteins remains to be elucidated.

Why does overexpression of the CNS1 gene suppress the cpr7 mutation? One model is that CNS1 overexpression makes formation of an initial Hsp90 complex (Hsp70-Hsp90-Cns1) more efficient. Alternatively, when overexpressed, Cns1 may substitute for Cpr7 in the mature Hsp90 complex (Hsp90-p23 immunophilin). Finally, our findings suggest that Cpr7 and Cns1 may be present simultaneously in the same Hsp90 complexes, and thus overexpression of one component might compensate for the loss of a different component of the complex. It is especially intriguing that while the components of the Hsp90 complex are duplicated, some components are significantly divergent. In this regard, Cns1 is quite divergent from its homolog Sti1, and further studies will be required to further address the unique or shared features of these distinct Hsp90-associated components.

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REFERENCES
1. Bohen, S. P. 1998. Genetic and biochemical analysis of p23 and ansamycin 
antibiotics in the function of Hsp90-dependent signaling proteins. Mol. Cell. 
Biol. 18:4323–4330.
2. Bohen, S. P., and K. R. Yamamoto. 1994. Modulation of steroid receptor 
signal transduction by heat shock proteins, p. 313–334. In R. I. Morimoto, A. 
Tissieres, and C. Georgopoulos (ed.). The biology of heat shock proteins and 
molecular chaperones. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
3. Borkovich, K., F. W. Farrelly, D. B. Finkelstein, J. Taulien, and S. Lindquist. 
1989. hsp82 is an essential protein that is required in higher concentrations 
for growth of cells at higher temperatures. Mol. Cell. Biol. 9:3919–3930.
4. Bose, S., T. Weikl, H. Bujal, and J. Buchner. 1996. Chaperone function of 
Hsp90-associated proteins. Science 274:1715–1717.
5. Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. 
Stinchcombe, K. Struhl, and R. W. Davis. 1979. Sterile host yeasts (SHY): a 
eukaryotic system of biological containment for recombinant DNA experi-
ments. Gene 7:319–327.
6. Caplan, A. J. 1997. Yeast molecular chaperones and the mechanism of 
steroid hormone action. Trends Endocrinol. Metab. 8:271–276.
7. Chen, M.-S., N. C. Hemmingsen, R. S. Mats, R. Ye, D. Fiorentino, and J. Heit-
man. 1994. Immune complexes interact with calcin in the absence of exog-
eous immunosuppressive ligands. EMBO J. 13:5944–5957.
8. Chang, H.-C. J., and S. Lindquist. 1994. Conservation of Hsp90 macromo-
lecule complexes in Saccharomyces cerevisiae. J. Biol. Chem. 269:24983– 
24988.
9. Chang, H.-C. J., D. F. Nathan, and S. Lindquist. 1997. In vivo analysis of the 
Hsp90 co-chaperone Sti1 (p60). Mol. Cell. Biol. 17:3121–3129.
10. Chen, M.-S., A. M. Silverstein, W. B. Pratt, and M. Chinkers. 1996. The 
tetratricopeptide repeat domain of protein phosphatase 5 mediates binding 
to glucocorticoid receptor heterocomplexes and acts as a dominant negative 
molecule. J. Biol. Chem. 271:32315–32320.
11. Cherry, J. M., C. Adler, C. Ball, S. Dwight, S. Chervitz, G. Juvik, T. Roe, S. 
Owens-Grillo, J. K., L. F. Stancato, K. Hoffmann, W. B. Pratt, and P. 
Mak, P., D. P. McDonnell, N. L. Weigel, W. T. Schrader, and B. W. O'Malley. 
1989. Expression of functional chicken oviduct progesterone receptors in 
yeast (Saccharomyces cerevisiae). J. Biol. Chem. 284:21613–21618.
12. Metzger, D., J. H. White, and P. Chambon. 1988. The human oestrogen 
receptor functions in yeast. Nature 334:31–36.
13. Miao, B., J. Davis, and E. A. Craig. 1997. The Hsp70 family—an overview 
p. 3–13. In M.-J. Getting (ed.). Guidebook to molecular chaperones and 
protein-folding catalysts. Oxford University Press, New York, N.Y.
14. Nicolet, C. M., and E. A. Craig. 1989. Isolation and characterization of 
STI1, a stress-inducible gene from Saccharomyces cerevisiae. Mol. Cell. Biol. 
9:3638–3646.
15. Perr-Wray, T. L. 1998. Yeast transformation: a model system for the study of 
recombination. Proc. Natl. Acad. Sci. USA 95:8032–8037.
16. Owens-Grillo, J. K., L. F. Stancato, K. Hoffmann, W. B. Pratt, and P. 
Krishna. 1996. Binding of immunophilins to the 90 kDa heat shock protein 
(hsp90) via a tetratricopeptide repeat domain is a conserved protein inter-
action in plants. Biochemistry 35:4249–4255.
17. Picard, D., B. Khurseved, M. J. Garabedian, M. G. Forlin, S. Lindquist, and 
K. R. Yamamoto. 1990. Reduced levels of hsp90 compromise steroid recep-
tor action in vivo. Nature 348:166–168.
18. Pratt, W. B. 1993. The role of heat shock proteins in regulating the function, 
folding, and trafficking of the glucocorticoid receptor. J. Biol. Chem. 268: 
21455–21458.
19. Ratajczak, T., and A. Carrello. 1996. Cyclophillin (40–40), mapping of its 
hsp90 binding domain and evidence that FKBP52 copurifies with Cyc-40 for 
hsp90 binding. J. Biol. Chem. 271:2961–2965.
20. Rothstein, R. 1991. Targeting, disruption, replacement, and allele rescue: 
integrative DNA transformation in yeast. Methods Enzymol. 194:261–301.
21. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a 
laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold 
Spring Harbor, N.Y.
22. Schmid, M. E., T. A. Brown, and B. L. Trumpower. 1990. A rapid and simple 
method for preparation of RNA from Saccharomyces cerevisiae. Nucleic 
Acids Res. 18:3091–3092.
23. Schneider, C., L. Sepp-Lorenzino, E. Nimmegsen, O. Ouerfelli, S. Dan-
ishfsky, N. Rosen, and F. U. Hartl. 1996. Pharmacologic shifting of a 
balance between protein regulatory activities of the cyclophilin Cyp40 and 
degradation mediated by Hsp90. Proc. Natl. Acad. Sci. USA 93:14536–14541.
24. Sherman, F. 1991. Getting started with yeast. Methods Enzymol. 194:3–21.
25. Sikorski, R. S., and P. Hieter. 1989. A system of shuttle vectors and yeast 
host strains designed for efficient manipulation of DNA in Saccharomyces 
cephalodes. Genetics 122:19–27.
51. Sikorski, R. S., W. A. Michaud, and P. Hieter. 1993. p62cdc23 of Saccharomyces cerevisiae: a nuclear tetrapricopeptide repeat protein with two mutable domains. Mol. Cell. Biol. 13:1212–1221.

52. Smith, D. B., and K. S. Johnson. 1988. Single-step purification of polypeptides expressed in E. coli as fusions with glutathione S-transferase. Gene 67:31–40.

53. Smith, D. F. 1993. Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes. Mol. Endocrinol. 7:1418–1429.

54. Smith, D. F., L. Whitesell, S. C. Nair, S. Chen, V. Prapapanich, and R. A. Rimerman. 1995. Progesterone receptor structure and function altered by geldanamycin, an Hsp90-binding agent. Mol. Cell. Biol. 15:6804–6812.

55. Stebbins, C. E., A. A. Rasso, C. Schneider, N. Rosen, F. U. Hartl, and N. P. Pavletich. 1997. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 89:239–250.

56. Wach, A., A. Brachat, R. Pohlmann, and P. Philippsen. 1994. New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast 10:1793–1808.

57. Warth, R., P.-A. Briand, and D. Picard. 1997. Functional analysis of the yeast 40 kDa cyclophilin Cyp40 and its role for viability and steroid receptor regulation. Biol. Chem. 378:381–391.

58. Weisman, R., J. Creanor, and P. Fantes. 1996. A multicopy suppressor of a cell cycle defect in S. pombe encodes a heat shock-inducible 40 kDa cyclophilin-like protein. EMBO J. 15:447–456.

59. Whitesell, L., E. G. Mimnaugh, B. D. Costa, C. E. Myers, and L. M. Neckers. 1994. Inhibition of heat shock protein HSP90-pp60-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc. Natl. Acad. Sci. USA 91:8324–8328.