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Identification of *SSF1*, *CNS1*, and *HCH1* as multicopy suppressors of a *Saccharomyces cerevisiae* Hsp90 loss-of-function mutation

(Hsp90/chaperone/yeast/multicopy suppression/signal transduction)

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ABSTRACT **Hsp90 functions in a multicomponent chaperone system to promote the maturation and maintenance of a diverse, but specific, set of target proteins that play key roles in the regulation of cell growth and development. To identify additional components of the Hsp90 chaperone system and its targets, we searched for multicopy suppressors of various temperature-sensitive mutations in the yeast Hsp90 gene, *HSP82*. Three suppressors were isolated for one Hsp90 mutant (glutamate → lysine at amino acid 381). Each exhibited a unique, allele-specific pattern of suppression with other Hsp90 mutants and had unique structural and biological properties. *SSF1* is a member of an essential gene family and functions in the response to mating pheromones. *CNS1* is an essential gene that encodes a component of the Hsp90 chaperone machinery. The role of *HCH1* is unknown; its sequence has no strong homology to any protein of known function. *SSF1* and *CNS1* were weak suppressors, whereas *HCH1* restored wild-type growth rates at all temperatures tested to cells expressing the E381K mutant. Overexpression of *CNS1* or *HCH1*, but not *SSF1*, enhanced the maturation of a heterologous Hsp90 target protein, $p60^{v\text{-}src}$. These results suggest that like Cns1p, Hch1p is a general modulator of Hsp90 chaperone functions, whereas Ssf1p likely is either an Hsp90 target protein or functions in the same pathway as an Hsp90 target protein.**

The structure and function of the Hsp90 family of molecular chaperones has been conserved for over one billion years of evolution. Hsp90 proteins from organisms as diverse as the budding yeast, *Saccharomyces cerevisiae* and humans share 60% amino acid identity. Moreover, the crystal structures of the N-terminal domains of the *S. cerevisiae* and bovine Hsp90 proteins are superimposable (1–3). Hsp90 is essential for viability in all three of the eukaryotic organisms in which it has been tested, *S. cerevisiae* (4), *Drosophila melanogaster* (5), and *Schizosaccharomyces pombe* (6). Both the *Drosophila* and human family members can substitute for the endogenous Hsp90 of *S. cerevisiae* (7–8), and the yeast Hsp90 proteins can effectively chaperone heterologously expressed target proteins from other organisms (8–12). Furthermore, Hsp90 functions as part of a dynamic chaperone complex, the other known components of which are also highly conserved (13–16).

In vitro, Hsp90 exhibits an intrinsic, general chaperone activity, preventing the aggregation of inherently unstable or chemically denatured proteins (17–23). In such assays, Hsp90 is required in large excess and cannot refold denatured proteins. Rather, it maintains these proteins in nonaggregated, folding-competent conformations that can be acted on by other chaperones (18, 20, 21, 23, 24).

In vivo, at normal temperatures, Hsp90 is a highly selective chaperone, with even closely related proteins such as $p60^{c\text{-}src}$ and $p60^{v\text{-}src}$ varying greatly in their dependence on its function (12, 25). Most known Hsp90 target proteins are components of signal transduction pathways, and include steroid hormone receptors, serine/threonine kinases, tyrosine kinases, and transcription factors (13–16). The functions of signaling proteins require them to exist in inactive conformations that are poised for immediate activation. Biochemical and genetic data from many laboratories suggest that Hsp90 helps its target proteins achieve and maintain the inherently unstable conformations that are required for their function (8, 10, 12, 14–16).

Perhaps the most extensively studied chaperone function of Hsp90 is its role in the maturation of the progesterone (PR) and glucocorticoid (GR) receptors, with which Hsp90 forms stoichiometric complexes. Association with Hsp90 and several other cochaperones serves to keep these receptors in inactive conformations that can bind ligand with high affinity (8, 10, 14–16). Studies in reticulocyte lysates indicate that PR maturation involves at least nine proteins and occurs through a cycle of sequential interactions with distinct chaperone complexes, several of which contain Hsp90 (13, 26).

In the yeast *S. cerevisiae*, two nearly identical genes, *HSP82* and *HSC82*, encode Hsp90 proteins. Although Hsp90 is extremely abundant in yeast, its levels can be reduced at least one order of magnitude without affecting cell viability at 25°C. Much higher concentrations are required for growth at higher temperatures (4). Previously, we isolated eight *S. cerevisiae* Hsp90 temperature-sensitive (ts) mutants (10), each of which contains a single amino acid substitution and has a distinct phenotype with respect to cell morphology and growth. As is the case with most ts mutations in heat-shock proteins, seven of the eight are partial loss-of-function mutants: they have reduced activity at all temperatures, and are ts because they cannot fulfill the increased demand for Hsp90 function at high temperatures. One, however, behaves as a classic ts mutant: it has nearly wild-type activity at 25°C, but rapidly loses function on shift to high temperature. This mutant, G170D, contains a substitution of aspartic acid for glycine at amino acid position 170 (10). The instability of G170D at high temperatures is consistent with the location of residue 170 in the hydrophobic core of the N-terminal domain of Hsp90 (1–3).

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Abbreviation: ts, temperature sensitive.

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The unique G170D mutant allowed us to examine the breadth of Hsp90's chaperone functions *in vivo*. In temperature-shift experiments, Hsp90 does not appear to protect proteins from thermal inactivation. Hsp90 does, however, facilitate the refolding of a heterologous heat-damaged test substrate, firefly luciferase (11). Pharmacological inhibition of vertebrate Hsp90 produced similar results (18). These data suggest that the function of Hsp90 during stress is to interact with proteins as they unfold and to maintain them in conformations that are amenable to rapid refolding after the stress has been removed. This function may be related to the above-mentioned general chaperone activity of Hsp90 with unfolded test substrates *in vitro*.

Experiments with G170D also demonstrated that Hsp90 is not required for the *de novo* folding of most cellular proteins in yeast. Rather, Hsp90 functions in the maturation and maintenance of certain target proteins that have difficulty achieving stable structures (10, 11). Presumably, yeast cells require Hsp90 function because one or more Hsp90 target proteins are essential for viability. Given the diversity of mammalian Hsp90 target proteins (13–16) and the distinct phenotypes of different *S. cerevisiae* Hsp90 point mutants (9, 10, 27), it seems likely that the yeast Hsp90 target proteins will include a wide variety of signal transducers that function in many pathways. Indeed, one such target has recently been identified; Ste11p is a MEK kinase involved in several MAP kinase signaling pathways in yeast (ref. 28; B. Errede, personal communication). The diversity of Hsp90 target proteins and their central importance as regulators of growth and development makes Hsp90 a particularly interesting chaperone to study. However, the number and range of its targets and the multicomponent, dynamic nature of the chaperone complexes in which it functions present formidable problems in elucidating its molecular mechanism.

We and others have employed a variety of biochemical methods to isolate and identify proteins that interact with Hsp90. Although these methods have been very successful, they are only applicable to proteins that are fairly abundant and form stable complexes with Hsp90 or its known substrates (13, 15, 16, 29). Genetic screens for enhancers or suppressors of *Hsp90* mutations provide another tool for identifying proteins that function in the same pathway. Indeed, *ydj1*, a member of the dnaJ family of chaperones, was identified in a screen for mutations that exhibit synthetic lethality with G170D. This finding in turn led to the discovery that Ydj1p plays an important role in the maturation of Hsp90 target proteins (30, 31).

Here we continued the search for Hsp90 target proteins and cochaperones, searching for suppressors of yeast Hsp90 ts point mutants. Because reduction of Hsp90 function would affect the activity of several Hsp90 target proteins, it seemed likely that overexpression of a single target would have only subtle effects on cell growth. Furthermore, overexpression of the Hsp90 cochaperone *STI1* suppresses the ts growth phenotype of some Hsp90 mutants, but it exacerbates the growth defects of others (32). Therefore, we searched for suppressors of four phenotypically distinct mutants each containing a single amino acid substitution in a different region of Hsp90 and performed our screens at the lowest possible nonpermissive temperature. This approach allowed us to identify three additional members of the Hsp90 chaperone network, each with unique properties.

MATERIALS AND METHODS

Plasmid and Strain Construction. Integrating plasmids containing wild-type or mutant *HSP82* sequences regulated by the strong constitutive promoter *GPD* were constructed by subcloning the *Cla*I-*Not*I fragments from the corresponding single-copy expression plasmids (10) into pRS303 (34). These plasmids are designated piHGpd/P82 (wild-type *HSP82*), piHGpd/T22I (threonine at amino acid residue 22 replaced by isoleucine), piHGpd/A41V, piHGpd/G81S, piHGpd/T101I, piHGpd/G170D, piHGpd/G313S, piHGpd/E381K and piHGpd/A587T.

Strains iP82a, iT22Ia (T22I), iA41Va (A41V), iG81Sa (G81S), iT101Ia (T101I), iG170Da (G170D), iG313Sa (G313S), iE381Ka (E381K), and iA587Ta (A587T) are derivatives of W303 (*MATa ade2–1 can1–100 his3–12, 16 leu2–3, 112 trp1–1 ura3–1*; R. Rothstein, Columbia University). In these strains, the endogenous Hsp90 genes, *HSC82* and *HSP82* are deleted and wild-type or mutant *HSP82* sequences, regulated by the strong constitutive promoter *GPD*, are integrated into the genome. To create these strains, the integrating plasmids described above were linearized with *Nhe*I and transformed into ΔPCLDa (10). Transformants were grown on 5-fluoroorotic acid (5-FOA) medium to select for those that had lost the wild-type *HSC82* expression plasmid from ΔPCLDa. The ilep1a strain was constructed in a similar manner except that in this case wild-type *HSP82* sequences under the control of a low-expression promoter were integrated into the genome (29).

Yeast and bacterial transformations were obtained by using lithium acetate (35) and calcium chloride (36) methods, respectively, or by using electroporation with a Gene Pulser (Bio-Rad).

Yeast Media. Cells were cultured in SD, SR, or Sgal (20 g/liter glucose, raffinose, or galactose, respectively, with 5 g/liter ammonium sulfate and 1.7 g/liter yeast nitrogen base without amino acids, supplemented with essential amino acids and nucleotides) or 5-FOA (1.7 g/l yeast nitrogen base without amino acids and ammonium sulfate, 1 g/l proline, 20 g/liter glucose, 10 mg/liter uracil, and 600 mg/liter 5-FOA, supplemented with essential amino acids and nucleotides (37). Solid media contained 20 g/liter Difco Bacto agar.

Screen for Multicopy Suppressors of Hsp90 ts Mutants. Hsp90 ts mutant strains T101I, G170D, G313S, and E381K were transformed with 1 μg of a genomic library (gift of Y. Kimura, Tokyo Metropolitan Institute of Medical Science; library was constructed from strain 5CG2 (27) in pTV3 (38), a high copy number plasmid marked with *TRP1*). Transformants that grew on SD minus tryptophan at the lowest temperature that was nonpermissive for growth (36°C for G170D and E381K, 37°C for T101I and G313S) were selected and retested. To ensure that the suppression was caused by the plasmid DNA, plasmids were recovered, transformed into the appropriate strain, and tested for growth at the nonpermissive temperature.

The inserts in the three suppressor plasmids, E20, E26 and E28 were sequenced by the University of Chicago Cancer Research Center DNA Sequencing Facility by using primers specific to vector sequences on either side of the insert. Sequences were compared with those in the yeast genome by using the Saccharomyces Genome Database World Wide Web site (http://genome-www.stanford.edu/Saccharomyces/). All three suppressor plasmids contained multiple ORFs. To identify the specific genes responsible for the suppression, individual ORFs from each suppressor were subcloned into pTV3 and tested for the ability to suppress the growth defect of E381K cells. The original suppressor plasmids E20, E26, and E28 were used in all of the experiments described in this paper.

Assessment of $p60^{v\text{-}src}$ Activity and Accumulation. The galactose-inducible $p60^{v\text{-}src}$ expression plasmid Y316v-src (39) was transformed into E381K cells containing either a plasmid encoding wild-type Hsp82 (pTGpd/P82; ref. 10), an empty vector (pTV3), or one of the three suppressor plasmids E20, E26, or E28. Activity and accumulation of $p60^{v\text{-}src}$ was assessed as described (10).

RESULTS

Isolation of Multicopy Suppressors of Hsp90 ts Mutants. Hsp90 interacts with its target proteins in the context of a dynamic, multicomponent chaperone complex (13–16). Structurally, Hsp90 contains multiple domains that presumably interact with different proteins in different ways. Indeed, Bohen and Yamamoto (9) isolated a mutation in the *S. cerevisae HSP82* gene, E431K, that specifically affects interactions with a single target protein, GR. Therefore, rather than searching for suppressors of general Hsp90 loss-of-function mutations (deletions and promoter rearrangements), we instead searched for suppressors of four phenotypically distinct ts mutants, each containing a single amino acid substitution in a different region of Hsp90 (T101I, G170D, G313S, and E381K).

Because all of these mutations are recessive (10), a large number of the plasmids recovered in a suppressor screen would likely encode one of the two yeast Hsp90 proteins, Hsp82p and Hsc82p. To prevent this, we used a genomic library constructed from a strain in which *HSC82* had been disrupted and *HSP82* had been replaced with a construct containing *HSP82* coding sequences under the control of the galactose-inducible promoter *GAL1* (27). Because *HSP82* and *HSC82* are an essential gene family in yeast, this strain is only viable when grown in galactose media. More importantly, the only wild-type Hsp90 genes present in the genomic library constructed from this strain are under the control of the *GAL1* promoter. By performing our screens in glucose media, which represses the *GAL1* promoter, we avoided recovery of the wild-type Hsp90 genes present in this library.

To ensure that weak suppressors would not be missed, we looked for transformants that could grow at the lowest nonpermissive temperature for each of the *hsp82* point mutants. G170D and E381K were screened at 36°C, whereas T101I and G313S were screened at 37°C. Approximately 5,000 transformants were tested for their ability to suppress each of the four mutants. No suppressors of T101I, G170D, or G313S were isolated. We did, however, identify three plasmids (E20, E26, and E28) that suppressed E381K.

Identification of Suppressor Proteins. Sequence analysis revealed that E20 contained 3.4 kb of chromosome 8 (228060–231468) (Fig. 1). This region encodes only one complete ORF, *SSF1*, but also contains the first 1,104 bp of the 1,631-bp ORF *RRP3* and the first 497 bp of the 842-bp ORF *YHR067w*. E26 contained 4.8 kb of chromosome 2 (546677–551480). This region encodes three complete ORFs, *RIB7*, *RPB5*, and *CNS1*. It also contains the last 528 bp of the 875-bp ORF *SPP381* and the first 422 bp of the 2,096-bp ORF *YBR156c*. E28 contained 6.3 kb of chromosome 14 (105358–111625). This region encodes three complete ORFs, *POP3*, *YNL281w*, and *ERG24*. It also contains the last 1,335 bp of the 1,511-bp ORF *WSC2* and the first 710 bp of the 1,275-bp ORF *YNL279w*.

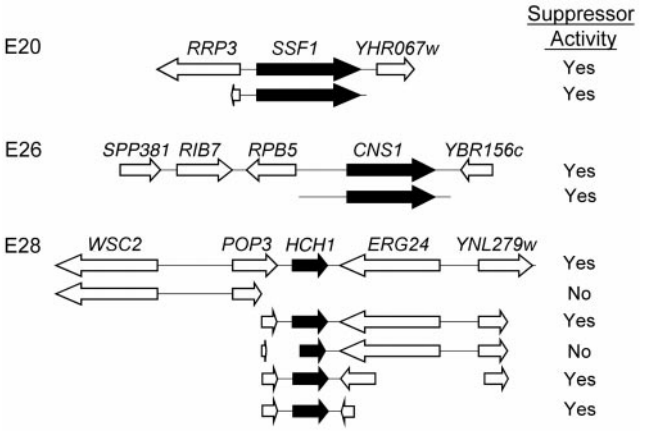

FIG. 1. Maps of individual suppressor plasmids and the fragments tested for suppressor activity. Fragments of (*Top*) E20, (*Middle*) E26, and (*Bottom*) E28 that provided suppressor activity are shown.

The specific genes responsible for the suppression were identified by subcloning individual ORFs from each suppressor (Fig. 1). A *Sca*I-*Kpn*I fragment containing the entire ORF of *SSF1*, 275 bases of upstream sequences (including the first 103 bases of *YHRO65c*) and 16 bases of downstream sequences contained the suppressing function of the E20 plasmid. An *Eco*RI fragment containing the entire *CNS1* ORF, 696 bp of 5′-untranslated sequences and 175 bp of 3′-untranslated sequences possessed the suppressing function of the E26 plasmid. An *Nco*I-*Msc*I fragment containing the entire *YNL281w* (*HCH1–H*igh *C*opy *H*sp90 suppressor) ORF and 398 bp of upstream sequences (including the last 207 bp of *YNL282w*) and 333 bp of downstream sequences (including the last 166 bp of *ERG24*) contained the suppressing activity of plasmid E28. This analysis revealed that the three multicopy suppressors of E381K were *SSF1, CNS1*, and *HCH1*.

***SSF1*, *CNS1*, and *HCH1* Suppress the ts Growth Phenotype of E381K Cells to Different Extents.** E381K cells have a severe growth defect. They grow at half the rate of wild-type yeast at 25°C, even more slowly at intermediate temperatures (34–35°C), and not at all above 35°C (10). The E381K mutation is recessive; introduction of a plasmid encoding wild-type *HSP82* into E381K cells restored normal growth rates at all temperatures tested (25–37°C) (Fig. 2, and data not shown). A plasmid encoding *HCH1* also restored a wild-type growth rate to E381K cells up to 37°C, the highest temperature tested. Plasmids encoding either *SSF1* or *CNS1* increased the maximal growth temperature of E381K cells to 36°C and resulted in faster, but still not wild-type, growth rates at temperatures between 25°C and 35°C. Haploid E381K cells of both mating types responded equally to the suppressors. Diploid E381K cells, however, did not respond as well to the *CNS1* and *HCH1* plasmids, and their growth was completely unaffected by the *SSF1* plasmid (Table 1).

***SSF1*, *CNS1*, and *HCH1* are Allele-Specific Suppressors.** Next, we asked whether overexpression of *SSF1*, *CNS1*, or *HCH1* could suppress the growth defects of the other seven ts point mutants in our collection. We also examined the effect of the suppressors on the growth of cells that express high levels of wild-type *HSP82* and are not ts (strain iP82a) and of cells that express very low levels of wild-type *HSP82* and are therefore ts (strain ilep1a). Growth was assessed at permissive (25°C), semipermissive (34–36°C, depending on the mutant), and nonpermissive (36–37°C, depending on the mutant) temperatures.

The growth of iP82a cells was not affected by any of the suppressors at any temperature tested (Table 2, and data not shown). Introduction of the plasmid encoding *SSF1* enhanced the growth of another mutant, A41V, at semipermissive temperatures, but had no effect on its maximal growth temperature. *SSF1* had no effect on the growth of any of the other six point mutants in our collection, nor did it affect the growth of ilep1a cells. Overexpression of *CNS1* also affected only one other mutant, T22I. The growth rate of T22I cells was increased at 25°C, but remained unchanged at higher temperatures. In marked contrast, overexpression of *HCH1* had profound effects on the growth of most of the mutants in our collection. It reduced the growth rates of four mutants, A41V, G170D, G313S, A587T, and of ilep1a cells. It had no effect on two mutants, G81S and T101I, and suppressed the growth defect of one other mutant, T22I. As with E381K, overexpression of *HCH1* completely suppressed the growth defect of T22I cells at all temperatures tested (Table 2, and data not shown). Thus, each suppressor exhibits a distinct pattern of allele specificity.

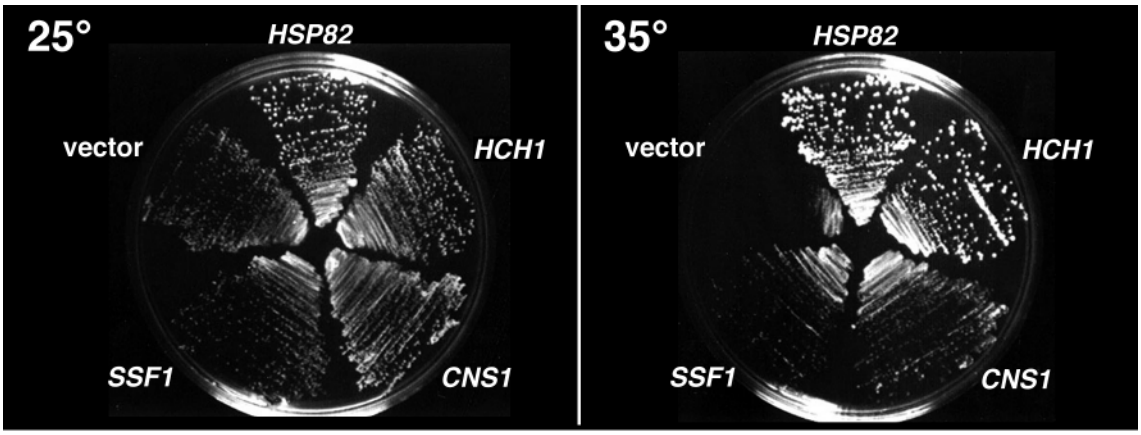

FIG. 2. Effects of *SSF1*, *CNS1*, and *HCH1* on the growth of E381K cells. E381K cells containing an empty vector or a vector expressing *HSP82*, *SSF1*, *CNS1*, or *HCH1* were grown at either 25°C (*Right*) or 35°C (*Left*) for 3 days on SD medium lacking tryptophan.

Overexpression of *CNS1* or *HCH1* Increases the Activity of a Heterologous Target Protein in E381K Cells. The primary function of Hsp90 during normal cell growth appears to be to promote and maintain the activity of a highly selective group of target proteins (11). This function is aided by at least nine other proteins that form the Hsp90 chaperone machine (13–16). Therefore, it seemed likely that the suppressors we isolated were either (*i*) Hsp90 target proteins or proteins that function in the same biochemical pathway as an Hsp90 target protein or (*ii*) proteins that function in or modulate the activity of the dynamic Hsp90 chaperone complex. The biological activities of the former should be affected by Hsp90 mutations; the latter should affect the activity of other Hsp90 target proteins. Because the functions of Cns1p, Ssf1p, and Hch1p are not completely understood, we were unable to directly determine whether their activities are affected by Hsp90 mutations. We could, however, determine whether Cns1p, Ssf1p, or Hch1p affected the capacity of Hsp90 to function in the proper maturation of a known target protein.

To do so we asked whether the overexpression of these proteins enhanced the maturation the oncogenic tyrosine kinase $p60^{v\text{-}src}$. This target protein was chosen for three reasons. First, its maturation is highly dependent on Hsp90 (12). Second, E381K cells have a severe defect in $p60^{v\text{-}src}$ maturation, even at 25°C (10). Third, yeast cells have very low levels of endogenous tyrosine kinase activity, making $p60^{v\text{-}src}$ activity easy to detect by Western blot analysis with an anti-phosphotyrosine antibody.

E381K cells containing either an empty vector, one of the three suppressors, or wild-type *HSP82* were transformed with a *GAL1*-$p60^{v\text{-}src}$ expression plasmid. Tyrosine phosphorylation of total cellular proteins was assessed 6 hours after raffinose-grown cells were shifted to galactose-containing media. As previously described (10, 12), in cells expressing wild-type *HSP82*, $p60^{v\text{-}src}$ promiscuously phosphorylated a broad array of yeast proteins (Fig. 3). As expected, the activity of $p60^{v\text{-}src}$ was severely reduced in E381K cells containing the empty vector. This reduction in activity was accompanied by a decrease in $p60^{v\text{-}src}$ accumulation (Fig. 3), consistent with previous observations that Hsp90 plays a role in both the stability and activation of $p60^{v\text{-}src}$ (10, 12). Introduction of the *SSF1* plasmid into E381K cells had no effect on either $p60^{v\text{-}src}$ activity or accumulation. In marked contrast, overexpression of either *CNS1* or *HCH1* increased $p60^{v\text{-}src}$ activity. In both cases, the increased activity was accompanied by increased accumulation of $p60^{v\text{-}src}$ protein. The ability of *CNS1* and *HCH1* to both promote the maturation of a heterologous Hsp90 target protein in E381K cells and enhance the growth of E381K cells in the absence of $p60^{v\text{-}src}$ indicates they increase general Hsp90 function. This, in turn, strongly suggests that these suppressor proteins are either components of the Hsp90 chaperone machine or modulators of its activity.

Table 1. Suppression of haploid and diploid E381K cells by *SSF1, CNS1*, and *HCH1*

Plasmid	*Mata*	*Matα*	*Mata/α*
Vector	−	−	−
HSP82 (Hsp90)	++++	++++	++++
SSF1	+	+	−
CNS1	+	+	+/−
HCH1	++++	++++	++

DISCUSSION

The chaperone activities of Hsp90 are believed to be essential for the maturation of a specific set of critical target proteins. The identities of these target proteins, however, are just beginning to be elucidated. Moreover, it is by no means clear whether we have yet identified all, or even most, of the cochaperones and modulators of Hsp90 function. In an attempt to identify Hsp90 target proteins and modulators of Hsp90 function in yeast, we searched for genes that, when overexpressed, would suppress the ts growth defect of several distinct Hsp90 mutants. We identified three multicopy suppressors, *SSF1*, *HCH1*, and *CNS1*. Each suppressor produced a distinct pattern of allele specificity and each had a different character. Our data suggest that *SSF1* is likely either to be an Hsp90 target protein or to function in the same pathway as an Hsp90 target protein, whereas *CNS1* and *HCH1* likely are involved in regulating or promoting general Hsp90 chaperone function.

Table 2. Allele-specific effects of *SSF1, CNS1*, and *HCH1* on the growth of Hsp82 mutants

Mutant	*SSF1*	*CNS1*	*HCH1*	*STI1*
E381K	↑	↑	⇑	−
iP82a	−	−	−	
T22I	−	↑	⇑	↑
A41V	↑	−	↓	↓
G81S	−	−	−	−
T101I	−	−	−	↓
G170D	−	−	↓	↑
G313S	−	−	↓	↑
A587T	−	−	↓	↑
ilep1a	−	−	↓	

⇑, Greatly enhanced growth; ↑, somewhat enhanced growth; −, no effect; ↓, inhibited growth.

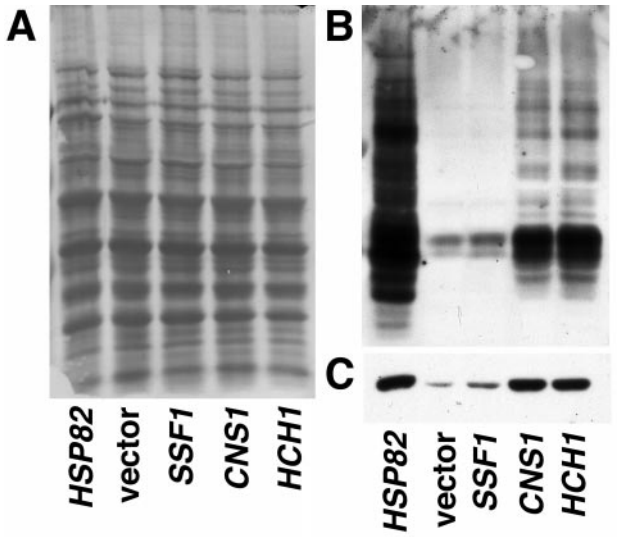

FIG. 3. Effects of *SSF1*, *CNS1*, and *HCH1* on $p60^{v\text{-}src}$ activity in E381K cells. E381K cells expressing an empty vector or a vector expressing *HSP82*, *SSF1*, *CNS1*, or *HCH1* were transformed with a galactose-inducible $p60^{v\text{-}src}$ expression vector. Cells grown to mid-logarithmic phase at 25°C in raffinose-containing medium were shifted to galactose-containing medium for 6 hours to induce expression of $p60^{v\text{-}src}$. Total cellular proteins were isolated, separated on 7.5% SDS/PAGE gels and transferred to Immobilon-P membranes. (*A*) Equal sample loading was confirmed by Coomassie blue staining. (*B*) $p60^{v\text{-}src}$ activity was assessed by reacting the blot with an antibody specific for phosphotyrosine. (*C*) $p60^{v\text{-}src}$ accumulation was assessed by reacting the blot with an antibody specific for $p60^{v\text{-}src}$.

***SSF1*: A Potential Hsp90 Target Protein.** *SSF1* was originally isolated in a screen for proteins that, when overexpressed, increase the mating efficiency of a *ste4* ts mutant (40). A closely related gene, *SSF2,* (the two proteins share 94% amino acid identity), also was identified. Null mutations in either *SSF1* or *SSF2* have no detectable phenotype, but mutations in both genes are lethal (40). Depletion of Ssf gene products from growing cultures significantly impaired the ability of the cells to mate (40). Recently, it was determined that this mating defect is a consequence of a failure to carry out the morphological response to pheromone (41). Thus, Ssf1p has essential functions during both mating and vegetative growth.

Overexpression of *SSF1* did not suppress the defect in $p60^{v\text{-}src}$ maturation that is observed in E381K cells, suggesting that Ssf1p does not affect the general chaperone functions of Hsp90. It is possible, however, that Ssf1p is a cochaperone that affects only a subset of Hsp90 functions. Alternatively, Ssf1p, or another protein that functions in the same biochemical pathway as Ssf1p, may be an Hsp90 target protein. This is consistent with the observations that (*i*) Ssf1p functions in the response to mating pheromones, (*ii*) the suppressor effects of Ssf1p, but not Cns1p and Hch1p, are specific to haploid cells, (*iii*) Hsp90 mutants are defective in pheromone signaling (28), and (*iv*) Ste11p has recently been identified as an Hsp90 target protein (ref. 28; B. Errede, personal communiation). Ste11p is a MEK kinase involved in several MAP kinase-signaling pathways in yeast, including the pheromone response pathway. Thus, overexpression of Ssf1p may suppress the effects of the E381K mutation through effects on one or more of the MAP kinase pathways.

***CNS1*: An Hsp90 Hetercomplex Member.** *CNS1* was identified as an essential ORF on chromonsome 2 (42). Spores containing null mutations in *CNS1* germinate but only undergo 2–3 divisions. Interestingly, the cells in these spores have elongated buds (42), a morphology that is remarkably similar to that of certain Hsp90 ts mutants (27). *CNS1* codes for a 386-aa protein that contains three tetratricopeptide repeat (TPR) domains. Several members of the Hsp90 heterocomplex, including Sti1p and the immunophilins, contain TPR domains, and these domains are required for their interactions with Hsp90 (16, 43–47).

Overexpression of *CNS1* partially suppressed the defect in $p60^{v\text{-}src}$ maturation in E381K cells, suggesting that Cns1p influences the chaperone functions of Hsp90. While this manuscript was in preparation, *CNS1* was reported to be a multicopy suppressor of the slow-growth phenotype of cells lacking the Hsp90 cochaperone *CPR7* (48, 49). In addition to restoring rapid growth to *cpr7* mutant cells, overexpression of *CNS1* rescued their defect in Hsp90 target protein maturation (49). Moreover, Cns1p was found in protein complexes with both Hsp90 and Cpr7 (48, 49). Thus, it seems very likely that *CNS1* is a member of the Hsp90 chaperone machinery.

***HCH1*: A Modulator of Hsp90 Function.** *HCH1* was identified as a 153-aa ORF (*YNL281w)* of unknown function. It shares 37% sequence identity and 55% sequence homology to the N-terminal half of a 350-aa protein encoded by an uncharacterized yeast ORF, *YDR214w*. Otherwise, *HCH1* has no striking homology to any known proteins. Disruption of *HCH1* produced no obvious growth defect, nor did it exhibit an obvious defect in mating or sporulation (B. Scherens, personal communication).

HCH1 was the strongest suppressor identified in our screen. It completely suppressed the growth defect of both E381K and T22I cells. Interestingly, overexpression of *HCH1* exacerbated the growth defect of A41V, G170D, G313S, and A587T cells, and had no effect on G81S and T101I cells. Such allele specificity is reminiscent of, though not identical to, that observed for the Hsp90 cochaperone *STI1* (32). Overexpression of *STI1* enhances the growth of T22I, G170D, G313S, and A587T, reduces the growth of A41V and T101I, and has no effect on G81S and E381K. Mutations in *STI1* inhibit the maturation of diverse Hsp90 target proteins in yeast (32), and Sti1p is found associated with Hsp90 complexes in yeast and mammalian cells (13–16, 32). Thus, for *STI1*, this type of allele-specificity is consistent with Hsp90 functioning at several steps in target protein maturation and with the point mutations affecting different steps—before, during, or after interaction with Sti1p. The same likely is true for *HCH1*. Indeed, overexpression of *HCH1* enhanced the maturation of the heterologous Hsp90 target protein $p60^{v\text{-}src}$ in E381K cells. Although further investigation of *HCH1* and its relative *YDR214w* is required, our data suggest that Hch1p either is a modulator of Hsp90 function or is itself a chaperone that can affect target protein maturation.

Our approach, employing point mutations in different domains of Hsp90 and taking extreme care to ensure that even subtle suppressors would be isolated, has yielded promising candidates for proteins involved in Hsp90 function. Moreover, the patterns of allele-specific suppression uncovered in this study and in earlier work (32) on *STI1* suggest that the various Hsp90 point mutations we have isolated will provide valuable tools for dissecting specific steps in the complex, multicomponent Hsp90 chaperone machinery. Notably, our screens were not saturating: four point mutants were used in this study, but suppressors of only one of them, E381K, were isolated; each suppressor was only obtained once; and finally, we did not isolate *STI1*, a known multicopy suppressor of the G170D mutation (32). It seems likely, therefore, that further screening using these and other Hsp90 point mutants, particularly mutants distributed on different surfaces of each domain, will provide a powerful mechanism for identifying additional Hsp90 target proteins and cofactors. Furthermore, recent work in *Drosophila* demonstrates that different genetic backgrounds contain preexisting genetic variants that sensitize different growth and differentiation pathways to Hsp90 defects (50). Searching for suppressors of Hsp90 point mutations in different genetic backgrounds of *S. cerevisiae* may provide a

richer source of target proteins (and proteins that function in target protein pathways) than a search in any individual strain.

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