

The Yeast Hsp110 Family Member, Sse1, Is an Hsp90 Cochaperone*

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In eukaryotes, production of the diverse repertoire of molecular chaperones during normal growth and in response to stress is governed by the heat shock transcription factor HSF. The *HSC82* and *HSP82* genes, encoding isoforms of the yeast Hsp90 molecular chaperone, were recently identified as targets of the HSF carboxyl-terminal activation domain (CTA), whose expression is required for cell cycle progression during prolonged heat stress conditions. In the present study, we have identified additional target genes of the HSF CTA, which include nearly all of the heat shock-inducible members of the Hsp90 chaperone complex, demonstrating coordinate regulation of these components by HSF. Heat shock induction of *SSE1*, encoding a member of the Hsp110 family of heat shock proteins, was also dependent on the HSF CTA. Disruption of *SSE1* along with *STI1*, encoding an established subunit of the Hsp90 chaperone complex, resulted in a severe synthetic growth phenotype. Sse1 associated with partially purified Hsp90 complexes and deletion of the *SSE1* gene rendered cells susceptible to the Hsp90 inhibitors macbecin and geldanamycin, suggesting functional interaction between Sse1 and Hsp90. Sse1 is required for function of the glucocorticoid receptor, a model substrate of the Hsp90 chaperone machinery, and Hsp90-based repression of HSF under nonstress conditions. Taken together, these data establish Sse1 as an integral new component of the Hsp90 chaperone complex in yeast.

Cells respond to a variety of environmental stresses by inducing the heat shock response, the coordinated synthesis of a set of proteins that protect the cell from damage and facilitate recovery (1, 2). In eukaryotes, this response is primarily governed at the transcriptional level by the heat shock transcription factor (HSF),¹ a highly conserved protein that binds to heat shock elements (HSEs), specific *cis*-acting sequences upstream of genes encoding heat shock proteins (Hsps). HSF is a modular protein composed of a highly conserved amino-termi-

nal DNA binding domain followed by a series of hydrophobic heptad repeats that comprise the trimerization interface for the active factor (3). In addition, all HSF molecules possess a transcriptional activation domain in the carboxyl terminus that shows low sequence conservation. In higher eukaryotes, multiple distinct HSF isoforms exist that display differential specificity for HSE binding and respond to diverse stimuli. In yeast, however, HSF is encoded by a single essential gene, *HSF1*, which possesses both an amino-terminal *trans*-activation domain and a carboxyl-terminal *trans*-activation domain (CTA) (4, 5). The two domains appear to be differentially used for target gene activation, since expression of the copper metallothionein gene *CUP1* in response to heat, oxidative stress, or glucose starvation is abolished when the CTA is deleted, while expression of at least three other heat shock genes, *SSA1*, *SSA3*, and *SSA4*, is largely unaffected (6–8). Deletion of either domain alone has little effect at normal growth temperatures, but cells expressing HSF (1–583), a truncated form of HSF lacking the CTA, are temperature-sensitive for growth at 37 °C (4, 5). The temperature-sensitive phenotype is due to reversible arrest in the G₂/M phase of the cell cycle (9), a phenotype shared by two other mutant alleles of *HSF1*, *mas3* and *hsf1-82* (10, 11). Both HSF (1–583) and *hsf1-82* mutants are defective in expression of the yeast Hsp90 genes *HSP82* and *HSC82*, and temperature-sensitive cell cycle arrest can be partially suppressed by restoring cellular levels of Hsp90, implying a role for this molecular chaperone in cell cycle progression during stress (9, 11). However, depletion of Hsp90 levels during growth at 37 °C in a wild type HSF background results in a mixed population of G₁/S and G₂/M phase-arrested cells (9). Based on the incomplete suppression of HSF (1–583) temperature sensitivity by *HSP82*, and the different cell cycle arrest phenotypes observed with HSF (1–583) or Hsp90-depleted cells, it was proposed that other HSF gene targets might be required for cell division during heat stress in addition to Hsp90 (9).

Hsp90 is a ubiquitous and abundant cytosolic chaperone first characterized in the function and regulation of steroid hormone receptors (12), but the cast of substrate or “client” proteins has grown to include proteins such as the cell cycle regulatory kinase Cdk4 (13), signal transduction kinases such as Raf and Src (14–17), transcription factors such as Hap1 (18), HSF itself (19–21), the tumor suppressor protein p53 (22), and recently the catalytic subunit of the mammalian telomerase complex (23). Hsp90 associates with a number of proteins required for full chaperone activity, and these individual components are well conserved in eukaryotes (12, 24). In yeast, the complex has been demonstrated to include Hsp70 and the cochaperone Ydj1 (25, 26), the Cyp-40 cyclophilin orthologs Cpr6 and Cpr7 (25, 27), the p60 ortholog Sti1 (25, 28), Cdc37 (29), and the p23 ortholog Sba1 (30). The Hsp90 chaperone complex is dynamic, with distinct subunits associating at various stages of the folding/chaperoning process for different client proteins (31). For example, during maturation of the glucocorticoid receptor (GR), early complexes form, which include GR, Hsp90, Hsp70, Ydj1, and p60. After hormone binding, the Hsp70/Ydj1 pair dissoci-

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¹ The abbreviations used are: HSF, heat shock factor; HSP, heat shock protein; HSE, heat shock element; CTA, carboxyl-terminal transcriptional activation domain; HA, hemagglutinin; GR, glucocorticoid receptor; PCR, polymerase chain reaction; SC, synthetic complete; PAGE, polyacrylamide gel electrophoresis.

TABLE I
S. cerevisiae strains used in this study

Strains	Genotype (including plasmids)	Source	Parental strain
PS145	<i>MATa ade2-1 trp1 can1-100 leu2-3, -112 his3-11, -15 ura3 hsfΔ::LEU2</i> Ycp50-GAL1-HSF	Ref. 59	W303
NSY-A	<i>MATa ade2-1 trp1 can1-100 leu2-3, -112 his3-11, -15 ura3 hsfΔ::LEU2</i> pRS314-HSF	Ref. 7	PS145
NSY-B	<i>MATa ade2-1 trp1 can1-100 leu2-3, -112 his3-11, -15 ura3 hsfΔ::LEU2</i> pRS314-HSF(1-583)	Ref. 7	PS145
XLY18	<i>MATa ade2-1 trp1 can1-100 leu2-3, -112 his3-11, -15 ura3 hsfΔ::LEU2 sse1::KAN^R</i> pRS314-HSF	This study	NSY-A
XLY19	<i>MATa ade2-1 trp1 can1-100 leu2-3, -112 his3-11, -15 ura3 hsfΔ::LEU2 sti1::KAN^R</i> pRS314-HSF	This study	NSY-A
XLY24	<i>MATa ade2-1 trp1 can1-100 leu2-3, -112 his3-11, -15 ura3 hsfΔ::LEU2 sse1::KAN^R</i> Ycp50-GAL1-HSF	This study	PS145
XLY25	<i>MATa ade2-1 trp1 can1-100 leu2-3, -112 his3-11, -15 ura3 hsfΔ::LEU2 sti1::KAN^R</i> Ycp50-GAL1-HSF	This study	PS145
DS10	<i>MATa GAL2 his3-11,15 leu2-3,112 lys1 lys2 trpΔ1 ura3-52</i>	Ref. 49	DS10
CN11	<i>MATa GAL2 his3-11,15 leu2-3,112 lys1 lys2 trpΔ1 ura3-52 sti1::HIS3</i>	Ref. 49	DS10
XLY35	<i>MATa GAL2 his3-11,15 leu2-3,112 lys1 lys2 trpΔ1 ura3-52 sse1::KAN^R</i>	This study	DS10
XLY36	<i>MATa GAL2 his3-11,15 leu2-3,112 lys1 lys2 trpΔ1 ura3-52 sti1::HIS3 sse1::KAN^R</i>	This study	DS10
XLY29	<i>MATa his4-539 ura3-52 lys2-801 SUC2 sti1::KAN^R</i>	This study	DTY123
XLY34	<i>MATa ura3-52 lys2-801 ade2-101 trp1-63 his3-200 leu2-1 hsc82::URA3 hsp82::GAL1-HSP82::LEU2 sse1::KAN^R</i> p-T-GPD-HSP82 (TRP+) pRS313-HA-SSE1	This study	5CG2 (60)
XLY30	<i>MATa ura3-52 lys2-801 ade2-101 trp1-63 his3-200 leu2-1 hsc82::URA3 hsp82::GAL1-HSP82::LEU2 sse1::KAN^R</i> p-T-HIS6-GPD-HSP82 (TRP+) pRS313-HA-SSE1	This study	5CG2

ate, and p60 is replaced by the cyclophilin Cyp-40, probably through competition for the single tetratricopeptide repeat binding domain in Hsp90 (32, 33). p23 is frequently found associated with late stage complexes immediately prior to substrate release (34). *In vitro*, many of these purified subunits are capable of maintaining substrate proteins in a folding competent state, which requires the action of the Hsp70 and Ydj1 chaperones to achieve the final folded conformation (29, 35, 36).

The Hsp70 superfamily of molecular chaperones is divided into three subgroups based on sequence homology: the DnaK subfamily, the GRP170/Lhs1 subfamily, and the Hsp110/Sse1 subfamily (37). All share a high level of homology within their amino termini, which includes the ATP-binding domain essential for catalytic folding activity, but diverge within the carboxyl termini, thought to be responsible for interaction with substrate proteins. The founding members of the Hsp110/Sse1 subfamily are the *SSE1* and *SSE2* genes from yeast, which show 76% identity with each other but only 30–40% identity with the DnaK group of Hsp70s (38). The *SSE1* and *SSE2* genes were first identified as calmodulin-binding proteins (38), and *SSE1* was also isolated independently in a genetic study as *MSI3*, a multicopy suppressor of the heat shock-sensitive phenotype of Ras-cAMP pathway hyperactivation (39). However, the true functions of Sse1 and Sse2 in yeast are currently unknown. While heat shock-induced proteins of approximately 110 kDa have long been observed in higher eukaryotes, only recently have Hsp110 members from a variety of organisms including human, plant, nematode and fission yeast been cloned and found to share substantial homology (~40%) to the *SSE1/SSE2* genes (40–45). Interestingly, unlike Hsp70, the Hsp110 class of chaperones can maintain heat-denatured proteins such as firefly luciferase in a folding-competent state (46, 47).

In order to more precisely determine the cause of cell cycle arrest in HSF (1–583) mutant cells, we sought to identify additional gene products whose heat shock-induced expression was abrogated relative to wild type cells. We demonstrate that expression of *SSE1* is dependent upon the HSF CTA. In addition, most of the heat shock-inducible components of the Hsp90 chaperone complex were found to share this requirement, suggesting tight coordinate regulation of their expression. Moreover, *SSE1* displays synthetic genetic interactions with chaperone genes, is physically associated with purified Hsp90 complexes, and is required for Hsp90 activity *in vivo*. Together these findings identify Sse1 as a novel member of the Hsp90 chaperone complex.

EXPERIMENTAL PROCEDURES

Plasmids—In-frame fusion of the triple hemagglutinin (HA) tag to the amino terminus of the *SSE1* gene was accomplished by insertion of annealed oligonucleotides encoding three repeats of the HA epitope 3' of the initiator ATG codon. The fusion gene was cloned into the *HIS3*-based pRS313 vector to generate pRS313-HA-SSE1. For disruption of the *STI1* gene, the oligonucleotides 5'-GAGCTCTTCACTAAAGCTAT-TGAAGTTTCTGAAACTCCAAACCATGTTTATATTCTAACAGGTC-CGCCTGTaagcttctgacgtcag-3' and 5'-TTCATGGCCCTTTGATAGG-TTCTTCTGCGGTTTCGTTACTGGTACCAGGTTGGAATCTTTGTT-GGCTTGCCgcgcactagtgatctga-3' were used to amplify the KanMX2 cassette from the pFA-KanMX2 plasmid (48) by PCR. Uppercase letters represent sequences native to the *STI1* gene. The PCR-amplified fragment contains 72 base pairs of flanking sequences (both 5' and 3') that are homologous to the *STI1* gene. Approximately 4 μg of the PCR product was used to transform *Saccharomyces cerevisiae* cells, and G418-resistant colonies were recovered. The *SSE1* gene was similarly disrupted by a PCR product amplified from the pFA-KanMX2 cassette using oligonucleotides 5'-GATGAGTACTCCATTTGGTTTAGATTTAG-GTAACAATAACTCTGTCCTTGCCGTTGCTAGAAACAGAGGTATaagcttctgacgtcag-3' and 5'-GACCTTCTGGTAATTGAACACAGGTGAT-CTCCAGTTAGCGATTTGTTCTGGAGTGTGTTGGTGGTAACTGTG-gcgcactagtgatctga-3'. The resulting strains carrying the appropriate disruptions were G418-resistant and displayed the expected phenotypes as described previously (48), and the mutated alleles were verified by PCR. p413GPD-rGR and pYRP-GRElacZ (a kind gift from D. McDonnell, Duke University) expressing rat glucocorticoid receptor and a *lacZ* reporter construct containing GR-binding elements were as described (9). YEp-STI1 was a kind gift from Elizabeth Craig (University of Wisconsin) (49), and YEp-SSE1 was from Akio Toh-e (University of Tokyo) (39). pCM64-SSA3-lacZ is as described (7).

Strains and Growth Conditions—*S. cerevisiae* strains used in this study are listed in Table I. Cells were grown in synthetic complete (SC) medium minus the indicated nutrients (SC-x) at 30 °C to midlog phase ($A_{650} = 0.7$ –1.4). For growth dilution series assays, saturated liquid cultures were adjusted to approximately 1×10^6 cells/ml, serially diluted, and plated on SC-x agar plates for incubation at 30 °C or 37 °C as indicated. Sensitivity to the benzoquinoid ansamycins mabcetin and geldanamycin was assayed on YPD plates made by adding the drugs in Me₂SO to melted YPD agar solution to a final concentration of 35 μM. Mabcetin was obtained from the Drug Synthesis and Chemistry branch of NCI, National Institutes of Health. Geldanamycin was obtained from Dr. William Pratt (University of Michigan Medical School, Ann Arbor, MI). *sse1Δ* HSF (1–583) and *sti1Δ* HSF (1–583) cells were generated from XLY24 (*sse1Δ*, GAL1-HSF) and XLY25 (*sti1Δ*, GAL1-HSF) strains, respectively. XLY24 and XLY25 were transformed with HSF(1–583)-expressing plasmids and grown to saturation in galactose-containing medium (to allow for expression of GAL1-HSF). The spot dilution assay was carried out as described above using SC agar plates with glucose as sole carbon source to repress GAL1-HSF production, causing HSF(1–583) to be the only source of HSF. For heat shock treatment, cells were grown at 25 °C to midlog phase, and 5 ml of cells were either kept at 25 °C (control) or at 41 °C (heat shock) for 16 min or 1 h (Fig. 6). Cells

were then washed with ice-cold sterile glass-distilled water and stored at -80°C , prior to extraction of RNA for RNA blotting experiments or proteins for immunoblotting.

³⁵S Protein Labeling—Five ml of cells were grown in SC-Met-Cys to early log phase, harvested, and resuspended in 1 ml of SC-Met-Cys. The cultures were incubated at 23 or 41 $^{\circ}\text{C}$ for 15 min with constant agitation and then transferred to prewarmed Eppendorf microcentrifuge tubes each containing 158 μCi of Tran³⁵S-labelTM (ICN, CA). The tubes were further incubated for another 15 min at 23 or 41 $^{\circ}\text{C}$. Cells were then washed once with ice-cold sterile glass-distilled H_2O , and the pellet was frozen at -80°C . Cell extracts were prepared as described below, and scintillation counting to determine total incorporation of label was carried out on a Beckman LS6500 scintillation counter (Beckman Instruments). Equivalent cpm of extracts were fractionated with SDS-PAGE (8%) using a Hoeffer gel electrophoresis system (SE400 model) at 4 $^{\circ}\text{C}$. The gel was rinsed in glass-distilled H_2O for 15 min, incubated in AutoFluorTM (National Diagnostics, Inc.) for 30 min at room temperature, vacuum-dried, and exposed to Kodak BioMax film at -80°C . A Molecular Dynamics PhosphorImager system was used for quantitative assessment of labeled protein levels.

Immunoblot Analysis—Cell pellets containing between 5 and 15 A_{650} units of cells were resuspended in HEGN buffer (20 mM HEPES, pH 7.9, 1 mM EDTA, 10% glycerol, 50 mM NaCl) or SDS harvest buffer (0.5% SDS, 10 mM Tris-HCl at pH 7.4, 1 mM EDTA), with the following protease inhibitors added: 1 mM PefablocTM (Roche Molecular Biochemicals), 8 $\mu\text{g}/\text{ml}$ aprotinin, 4 $\mu\text{g}/\text{ml}$ pepstatin, 2 $\mu\text{g}/\text{ml}$ leupeptin. After the addition of an equal volume of acid-washed sterile glass beads (450–600 μm), the samples were lysed by rapid agitation at 4 $^{\circ}\text{C}$ in a micro-tube mixer (model MT-360; Tomy Tech, Inc.) at top speed for 2 min for four cycles with 2-min intervals on ice between each round. Extracts were clarified by microcentrifugation at $2500 \times g$ for 6 min at 4 $^{\circ}\text{C}$, and protein concentration was determined by Bradford assay. The extracts were fractionated by SDS-PAGE (8%), and electroblotted to nitrocellulose. Standard immunoblot procedures were used, followed by protein detection using the ECL chemiluminescence system according to the manufacturer's instructions (Amersham Pharmacia Biotech). Monoclonal antibody recognizing phosphoglycerate kinase was purchased from Molecular Probes, Inc. (Eugene, OR), and the following antibodies were generous gifts from the following sources: Anti-Ssa3/Ssa4 from Elizabeth Craig (University of Wisconsin, Madison, WI); anti-hsp90 from Susan Lindquist (University of Chicago); anti-Ydj1 from Avrom Caplan (Mt. Sinai School of Medicine); anti-Sti1 from David Toft (Mayo Graduate School); anti-Cpr7 from Richard Gaber (Northwestern University); anti-Kar2 from James Gaut (University of Michigan Medical School).

Affinity Purification of Hsp90 Complexes—Lysates were prepared from cells (XLY34 and XLY30) grown to midlog phase in SC-His medium at 30 $^{\circ}\text{C}$ in LyB buffer (25), using similar procedures as described above for immunoblot analysis. Isolation of His₆-Hsp82 and associated polypeptides with nickel affinity chromatography was carried out exactly as described (25).

β -Galactosidase Activity Assay for GR Function—Five ml of cells transformed with p413GPD-rGR and pYRP-GRElacZ were grown to midlog phase in SC-Ura-His medium at 30 $^{\circ}\text{C}$, and 5 μl of absolute ethanol (control) or 10 mM DOC (deoxycorticosterone; Sigma) dissolved in ethanol were added to the cell culture (final concentration of 10 μM). The cells were further incubated with shaking at 30 $^{\circ}\text{C}$ for 1 h, harvested, washed once with sterile glass-distilled H_2O , and resuspended in 700 μl of Z buffer (100 mM sodium phosphate, pH 7.0, 10 mM KCl, 1 mM MgSO_4 , 50 mM β -mercaptoethanol). 50 μl of chloroform and 50 μl of 0.1% SDS were added, and the cell suspension was vortex-mixed at top speed for 10 s followed by incubation at 30 $^{\circ}\text{C}$ for 5 min. 200 μl of 4 mg/ml ONPG in Z buffer was added to each sample and incubated at 30 $^{\circ}\text{C}$ for 3–7 min. 350 μl of 1 M Na_2CO_3 was added to stop the reactions. After clarification by centrifugation, the supernatant was diluted, and A_{420} was measured within the linear response range. Specific β -galactosidase activity was calculated as follows: (dilution factor $\times 1000 \times A_{420}) / (A_{650} \times (\text{volume of cells in ml}) \times (\text{time of incubation in min}))$. Data shown are averages of three independent experiments with associated S.D.

RESULTS

The HSF Carboxyl-terminal Activation Domain Regulates Expression of a Subset of the Heat-inducible Components of the Hsp90 Chaperone Complex—Previous work has demonstrated that expression of the two yeast Hsp90 genes *HSC82* and *HSP82* is markedly reduced in a strain expressing the HSF

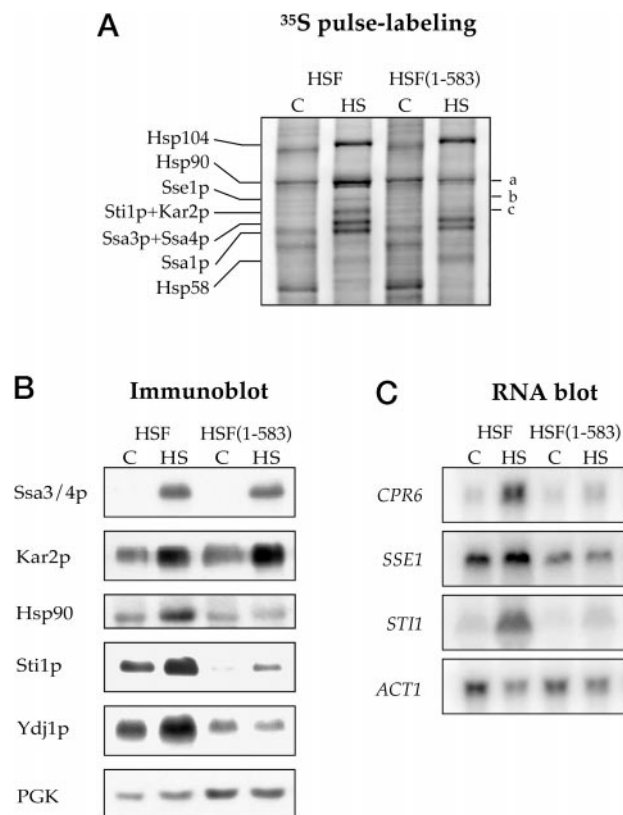


FIG. 1. The HSF CTA regulates expression of the Hsp90 chaperone complex. A, the HSF CTA is required for heat shock-inducible synthesis of a subset of heat shock proteins. Cells harboring wild type HSF or HSF(1–583) were grown in SC-Met-Cys medium at 25 $^{\circ}\text{C}$, maintained at 25 $^{\circ}\text{C}$ (C) or heat-shocked at 41 $^{\circ}\text{C}$ for 15 min (HS), and pulse-labeled with ³⁵S-Trans-labelTM for another 15 min under the same conditions. The cell extracts were fractionated by SDS-PAGE (8%) and exposed to film. Bands a–c correspond to proteins whose heat shock-inducible synthesis is lower in the HSF(1–583) strain. B, the HSF CTA regulates the production of Sti1 and Ydj1 in addition to Hsp90. Immunoblotting was performed on cell extracts prepared from wild type HSF or HSF(1–583) cells grown under heat shock (41 $^{\circ}\text{C}$) or control (25 $^{\circ}\text{C}$) conditions for 1 h. Phosphoglycerate kinase (PGK) is the loading control. C, *CPR6* and *SSE1* gene expression also requires the HSF CTA. mRNA levels of *SSE1* and *STI1* were assessed by RNA blotting, using RNA extracted from wild type HSF or HSF(1–583) cells grown under heat shock (41 $^{\circ}\text{C}$) or control (25 $^{\circ}\text{C}$) conditions for 16 min.

truncation mutant HSF (1–583), which displays a temperature-sensitive growth phenotype characterized by arrest in the G₂/M phase of the cell cycle (9). Two points, however, suggest that loss of Hsp90 is not the sole determinant of this arrest. First, restoration of Hsp90 levels through introduction of the *HSP82* gene on a high copy vector does not lead to growth rates at 37 $^{\circ}\text{C}$ comparable with wild type cells. Second, specific depletion of Hsp90 from cells wild type for HSF results in a mixed population of cells arrested in both the G₁/S and G₂/M phases of the cell cycle at 37 $^{\circ}\text{C}$, in contrast to the fairly uniform G₂/M arrest observed in HSF (1–583). Therefore, we reasoned that there must be additional genes under the specific control of the HSF CTA that are required for growth at higher temperatures and that are poorly expressed in HSF(1–583) cells.

To begin to identify these additional targets, SDS-PAGE protein profiles of ³⁵S pulse-labeled HSF and HSF (1–583) cell extracts was conducted under control and heat shock conditions (Fig. 1A). Heat shock induces the synthesis of a number of known Hsps, whose SDS-PAGE migration patterns have been well documented (50). We were able to faithfully recapitulate these patterns, demonstrating robust heat shock induction of a number of proteins, including Hsp104 and the Hsp70 isoforms

Ssa1, Ssa3, and Ssa4. Three proteins whose heat shock inducibility was diminished in HSF (1–583) cells compared with HSF wild type were tentatively identified (Fig. 1A, bands a–c) by comparison with published heat shock protein profiles (39, 50). Consistent with our previous results, band a was likely to be the unresolved Hsp90 isoforms Hsc82 and Hsp82p, band b corresponded to the Hsp70 homolog Sse1, and band c was consistent with the known positions of the heat shock proteins Kar2 and Sti1, which co-migrated in this SDS-PAGE system. Immunoblotting was carried out to unambiguously ascertain expression levels of these putative targets in wild type or HSF (1–583) cells, as shown in Fig. 1B. Kar2 expression was unchanged in both strains, similar to Ssa3 and Ssa4, whose heat shock induction has been previously demonstrated to be HSF CTA-independent (6, 7). However, both the basal and heat shock-induced expression levels of Sti1, a key component of the Hsp90 chaperone complex, were strongly dependent on the HSF CTA. The finding that two heat shock-inducible components of the Hsp90 chaperone complex, Hsp90 and Sti1, were both regulated by the HSF CTA prompted us to examine other known heat-inducible components of the complex. Immunoblotting revealed that Ydj1 is indeed a third member of the chaperone complex whose expression is contingent on the HSF CTA (Fig. 1B). In addition, it was recently reported that the gene encoding the yeast Cyp-40 homolog Cpr6 was also highly heat-inducible (51); our RNA blot analysis confirmed this heat-inducibility and demonstrated that this induction is abolished in HSF (1–583) cells (Fig. 1C). Expression of the other Cyp-40 homolog Cpr7 is not heat-inducible, nor is its constitutive expression HSF CTA-dependent (data not shown). The expression of two other Hsp90-associated proteins, the p23 homolog Sba1 and the protein kinase cochaperone Cdc37, have been reported not to change upon heat shock. Therefore, all of the heat-inducible components of the Hsp90 chaperone complex, with the exception of the cytosolic Hsp70 proteins, depend on the HSF CTA for expression at elevated temperature. To verify that the reduction in signal intensity of band b in HSF (1–583) cells corresponded to a lack of transcriptional induction of the *SSE1* gene, RNA blotting was used due to lack of antibody against Sse1 and was compared with *STI1* RNA levels (Fig. 1C). Changes in *STI1* transcript levels upon heat shock in wild type and HSF (1–583) cells closely paralleled Sti1 protein expression levels, emphasizing the strict transcriptional control of this gene product. *SSE1* mRNA was moderately induced by heat shock, and this induction was largely abolished in HSF (1–583) cells, concomitant with a reduction in basal expression. Therefore, in addition to the copper metallothionein Cup1, the heat-inducible expression of five additional proteins requires the HSF CTA: Hsp82, Sti1, Ydj1, Cpr6, and Sse1.

SSE1* Displays Genetic Interactions with the Hsp90 Chaperone Complex Subunit *STI1—The composition and function of the Hsp90 chaperone complex is highly conserved from yeast to humans, and it is required for maturation and functional maintenance of a number of key cellular regulatory proteins. Nearly half of its known components show heat-inducible synthesis (Hsp90, Sti1, Ydj1, and Cpr6p), suggesting a higher demand for chaperone function at heat shock temperatures, during which protein structure and function are compromised. The fact that Hsp90, Sti1, Ydj1, and Cpr6 expression are all regulated by the HSF CTA further supports our previous hypothesis that the temperature sensitivity of cells harboring HSF (1–583) is largely due to inadequate levels of the Hsp90 chaperone complex at 37 °C. In keeping with the close functional affiliation of the protein subunits, many of the genes encoding the cochaperones exhibit strong synthetic genetic interactions with *STI1*. For example, although deletion of the *STI1* gene does not cause

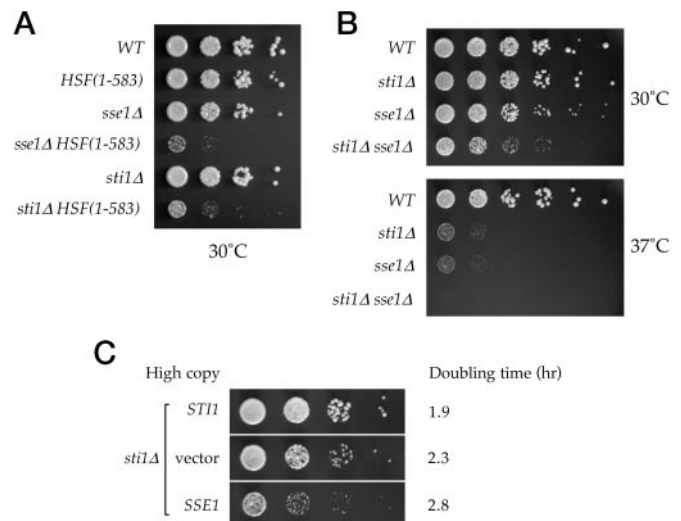


FIG. 2. *SSE1* genetically interacts with *STI1*, encoding a component of the Hsp90 chaperone complex. A, deletion of the *SSE1* gene exhibits a synthetic phenotype with HSF (1–583). Isogenic NSY-A (WT), NSY-B (*HSF* (1–583)), XLY18 (*sse1Δ*), XLY19 (*sti1Δ*) strains and strains harboring either *sse1ΔHSF* (1–583) or *sti1ΔHSF* (1–583) (see “Experimental Procedures”) were grown to saturation. Equivalent cell concentrations were serially diluted and plated on SC agar. The plate was incubated at 30 °C for 2 days and photographed. B, isogenic DS10 (WT), CN11 (*sti1Δ*), XLY35 (*sse1Δ*), and XLY36 (*sti1Δsse1Δ*) strains were grown to saturation, and serial dilutions of the cells were plated on SC agar, incubated for 2 days, and photographed. C, the strain XLY29 (*sti1Δ*) was transformed with YEpSTI1, YEpSSE1, or YEp24 alone. The transformed cells were plated on SC agar plates as in A. Growth rates (doubling times) were derived from the exponential range of replicate growth curves of the corresponding strains in liquid culture at 30 °C.

an obvious growth defect at 30 °C, disruption of *STI1* along with *CPR7*, *SBA1*, or *HSC82* results in severe growth impairment (27, 28, 30). To ascertain if low levels of Hsp90 chaperone activity rendered HSF(1–583) cells sensitive to additional perturbation of the Hsp90 complex, a strain was generated carrying a disruption of the *STI1* gene in the presence of the HSF (1–583) allele. As shown in Fig. 2A, HSF (1–583), *sti1Δ*, or *sse1Δ* mutant strains exhibited relatively normal growth at 30 °C compared with wild type cells; however, the combination of *sti1Δ* and HSF (1–583) resulted in a synthetic phenotype, drastically reducing cell viability and growth rates. The lack of obvious growth defects of HSF(1–583) cells at 30 °C suggests that although HSF CTA truncation lowers basal level expression of Hsp90, Sti1, and Ydj1 (Fig. 1B), the residual chaperone activity is sufficient under these growth conditions. However, complete elimination of Sti1 by gene disruption caused a severe growth defect in HSF (1–583) cells even at 30 °C, consistent with Sti1 playing an important role in Hsp90 chaperone function, especially when Hsp90 levels are limiting. Interestingly, when HSF (1–583) cells were deleted for *SSE1*, a phenotype similar to *sti1Δ* HSF (1–583) was observed (Fig. 2A). Additional evidence for genetic interaction was obtained by combining the *sti1Δ* and *sse1Δ* mutations, as shown in Fig. 2B. While disruption of either gene alone had little effect at 30 °C, a *sti1Δ sse1Δ* strain displayed moderate growth inhibition, which was exacerbated at 37 °C. The synthetic phenotype obtained by combining the *sti1Δ* and *sse1Δ* mutations implies that the presence of at least one of the two proteins is critical for cell growth. However, Sti1 and Sse1 are not functionally redundant, since high copy expression of the *SSE1* gene not only failed to rescue the slight growth disadvantage of *sti1Δ* cells but in fact further debilitated cell growth, as assayed in a dilution series on solid medium and by growth rates in liquid culture (Fig. 2C). These genetic data suggest that like Sti1, Sse1 function may also be

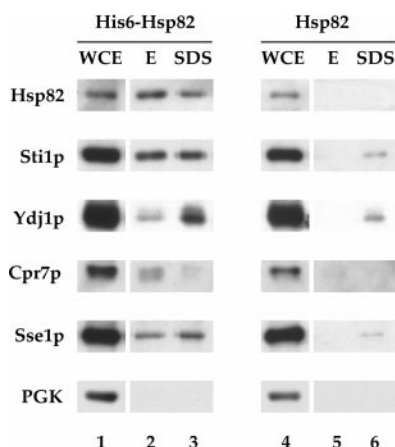


FIG. 3. Sse1 physically associates with the Hsp90 chaperone complex. Cell extracts were prepared from XLY30 (His₆-Hsp82, HA-SSE1) and XLY34 (Hsp82, HA-SSE1) strains grown at 30 °C to midlog phase as described under "Experimental Procedures." The cell extracts were incubated with a Ni²⁺ affinity resin and washed, and the bound fraction was eluted with 150 mM imidazole (E). The posteluted resin was boiled in SDS sample buffer to solubilize remaining proteins (SDS). A portion of the total cell extract was also included to verify the presence of the immunoblotted proteins prior to batch chromatography (WCE). All fractions were resolved by SDS-PAGE (8%) and subjected to immunoblotting with the corresponding antibodies (anti-HA monoclonal Ab 12CA5 was used to visualize HA-Sse1p).

affiliated with the Hsp90 complex or is redundant with Hsp90 function in an alternative pathway.

Sse1 Is a Component of the Hsp90 Complex Required for Chaperone Function—The coincident heat shock regulation and genetic interaction with components of the Hsp90 chaperone complex suggested that Sse1 may directly participate in Hsp90 chaperone complex function. Biochemical purification of Hsp90 and associated proteins from native cell extracts has been successful in identifying new components of the chaperone complex, including Sti1, Cpr6, and Cpr7 (25, 27). The possibility that Sse1 may also be a component of the complex was investigated by affinity chromatography of Hsp90 complexes containing a polyhistidine-tagged Hsp82 (His₆-Hsp82p), previously shown to be fully functional in yeast (25). Cells expressing a plasmid-borne His₆-Hsp82 or an unmodified wild type Hsp82 as the sole source of Hsp90 (the endogenous *HSP82* and *HSC82* genes were deleted) and expressing a triple HA-tagged Sse1 (HA-SSE1) in place of the endogenous *SSE1* gene were used to purify the Hsp90 chaperone complex (HA-Sse1 was demonstrated to be largely functional based on complementation of the *sse1Δ* growth defect, data not shown). A metal chelation affinity matrix was used to selectively isolate His₆-Hsp82 and associated proteins. As shown in Fig. 3, Sti1, Ydj1, and Cpr7, known components of the complex, co-purified with His₆-Hsp82 (lanes 2 and 3), as did HA-Sse1. Selective enrichment of Hsp90-associated proteins was demonstrated by the lack of binding of the abundant cytosolic enzyme phosphoglycerate kinase. A small amount of nonspecific binding of all of the assayed proteins to the affinity matrix alone (lane 6) was observed, which could be eluted with SDS-PAGE sample buffer but not the more specific imidazole elution buffer, and was negligible compared with that observed with the His₆-Hsp82 in lane 3. This specific interaction of Sse1 with Hsp90 in native cell extracts strongly suggested that it may play a functional role with Hsp90 *in vivo*. This hypothesis was tested by examining whether loss of Sse1 affected the ability of cells to tolerate the benzoquinoid ansamycins macbecin and geldanamycin. These closely related compounds have been demonstrated to selectively bind and inhibit the chaperone activity and signal transduction functions of Hsp90 (52, 53). Both drugs strongly

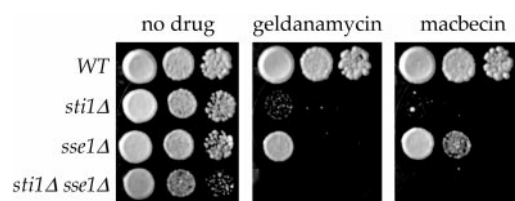


FIG. 4. *sse1Δ* cells are hypersensitive to Hsp90 inhibitors. Equivalent cell concentrations of isogenic DS10 (WT), CN11 (*sti1Δ*), XLY35 (*sse1Δ*), and XLY36 (*sti1Δsse1Δ*) strains were serially diluted and plated on YPD agar containing no drug or containing 35 μM geldanamycin or macbecin. The plates were incubated in the dark at 30 °C for 2 days and photographed.

inhibit Hsp90-dependent steroid hormone receptor activity in yeast (9, 54), and geldanamycin is cytotoxic to cells compromised for Hsp90 function by reduction of Hsp90 levels or deletion of Hsp90-associated cochaperones (9, 51). As demonstrated in Fig. 4, whereas wild type cell growth was unaffected by the presence of either Hsp90 inhibitor, the growth of *sti1Δ* cells, and to a lesser extent *sse1Δ* cells, was strongly inhibited. Residual colony formation observable in *sti1Δ* cells was completely abolished in the *sti1Δ sse1Δ* mutant strain, consistent with the synthetic growth phenotype observed at 37 °C in Fig. 2. Interestingly, geldanamycin was slightly more cytotoxic at the same concentration (35 μM) than macbecin, consistent with earlier findings that the former compound was more efficacious in inhibition of steroid hormone receptor signaling in yeast (54).

To further explore the role of Sse1 in Hsp90 function, we examined whether Sse1 was required for function of the mammalian glucocorticoid receptor (GR) expressed in yeast cells, a benchmark of Hsp90 chaperone activity. β -Galactosidase activity from a *GRE-lacZ* reporter gene was used to assay for the *trans*-activation function of GR upon stimulation with the synthetic hormone deoxycorticosterone (*DOC*) (Fig. 5). A strong hormone-dependent activation of GR was obtained in wild type cells. However, GR activity was markedly reduced in *sse1Δ* cells to an extent indistinguishable from that observed in cells lacking Sti1, which is required for full Hsp90-chaperoned GR activity (28). Importantly, the effect of Sti1 deletion, and presumably Sse1 deletion, on GR activity is mediated solely through Hsp90, as demonstrated by robust hormone-independent transactivation by a GR derivative lacking the Hsp90 interaction domain expressed in *sti1Δ* cells (28). To determine if loss of Sse1 had a functional consequence for a physiologically relevant role of Hsp90 in yeast, the ability of *sse1Δ* cells to restrain the transcriptional activation of heat shock genes by HSF was examined. Recent reports have established that the Hsp90 chaperone complex negatively regulates HSF activity both *in vitro* and *in vivo* (19–21), effectively holding HSF in check under nonstress conditions. Deletion of *SSE1* resulted in a 9-fold derepression of HSF activity under control conditions, demonstrated by a reporter gene consisting of the *lacZ* gene fused to the promoter of the heat-inducible yeast Hsp70 gene *SSA3* (Fig. 6A). In contrast the derepression observed in the *sti1Δ* strain was marginal, consistent with previous reports demonstrating differential effects of chaperone mutations on HSF regulation (19). Mutation of the HSE sequences in the *SSA3-lacZ* reporter abolished both the heat shock induction and basal activity, confirming that HSF was solely responsible for reporter induction (data not shown). In addition, HSF deregulation in the *sse1Δ* strain was also manifest as a dramatic increase in steady state levels of the endogenous Ssa3/Ssa4 proteins as detected by immunoblot analysis (Fig. 6B). These observations indicate that Sse1 is an important component of the Hsp90 chaperone complex required for repression of HSF

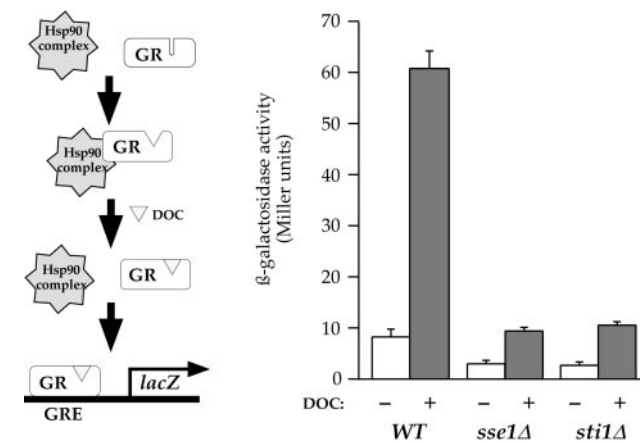


FIG. 5. Sse1 is required for glucocorticoid receptor function. p413GPD-rGR and pYRP-GRElacZ were transformed into isogenic strains NSY-A (WT), XLY18 (*sse1Δ*), and XLY19 (*sti1Δ*). Cells were grown to midlog phase at 30 °C and treated with 10 μ M deoxycorticosterone (DOC) in ethanol (+) or treated with ethanol alone (–) for 1 h, and β -galactosidase activities were measured as described under “Experimental Procedures.” The averages of three independent experiments with associated S.D. values are shown. A diagram depicting critical steps in Hsp90-mediated activation of GR by hormone is shown at left.

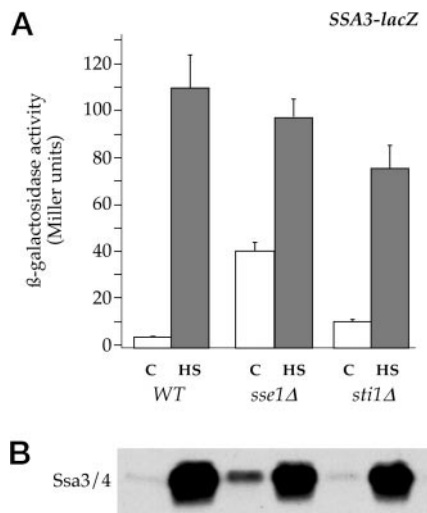


FIG. 6. Sse1 is required for repression of HSF basal activity. A, pCM64-SSA3-lacZ was transformed into isogenic DS10 (WT), CN11 (*sti1Δ*), XLY35 (*sse1Δ*), and XLY36 (*sti1Δsse1Δ*) strains. The cells were grown to midlog phase at 30 °C and maintained at 30 °C or heat-shocked at 39 °C for 1 h, and β -galactosidase activities were measured. B, protein extracts from the same cells as in A were resolved by SDS-PAGE and immunoblotted for Ssa3/Ssa4 protein levels.

basal activity. Taken together, these results provide compelling genetic and biochemical evidence for a functional role for Sse1, a previously uncharacterized heat shock protein, in the Hsp90 chaperone complex. Moreover, production of this complex is coupled with demand in response to thermal stress at the transcriptional level via a dedicated *trans*-activation domain of HSF.

DISCUSSION

This study has revealed an intriguing coordinate regulation of the heat-inducible components of the Hsp90 chaperone complex by the HSF CTA: Hsp82, Sti1, Ydj1, Cpr6, and Sse1. This observed co-regulation strongly supports a hypothesis put forward previously to explain the incomplete suppression of the temperature-sensitive cell cycle arrest phenotype of HSF-(1–583) cells by high copy *HSP82* alone: that other critical targets of the HSF CTA fail to be induced in this strain in response to

heat stress (9). It is also consistent with a proposed higher requirement for Hsp90 chaperone complex function for a select group of proteins at elevated temperatures (55). Co-regulation at the transcriptional level ensures efficient and synchronized increase of chaperone activity in response to stress, preventing a potentially deleterious stoichiometric imbalance of subunits. The observed regulation of the Hsp70 genes provides an interesting exception to this rule. Six genes have been identified in yeast encoding cytosolic Hsp70s, *SSA1*–*SSA4*, *SSB1*, and *SSB2*, and it is not yet clear which of these participate in Hsp90 chaperone complex function. Moreover, while *SSA1*, *SSA3*, and *SSA4* are highly heat shock-inducible, *SSA2*, *SSB1*, and *SSB2* are expressed at relatively high basal levels and are involved in a number of Hsp90-independent processes, such as translation (Ssb) and post-translational translocation (Ssa) of proteins (56, 57). These considerations, together with the apparent insensitivity of *SSA1*, *SSA3*, and *SSA4* heat shock induction to HSF CTA truncation, are consistent with the notion that distinct transcriptional control patterns are dictated by the two HSF transcriptional activation domains.

By both genetic and biochemical criteria, we have identified Sse1 as a novel component of the Hsp90 chaperone complex. *SSE1* along with a close homolog, *SSE2*, are both members of the Hsp70 superfamily of molecular chaperones, distant relatives of the family archetype DnaK, and are the founding members of the Hsp110 subfamily of Hsp70. Members of the Hsp110 family have been identified in multiple species in addition to *S. cerevisiae*, including *Schizosaccharomyces pombe*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Neurospora crassa*, and mammals, and they have been found to be up-regulated by a number of stresses including heat (40–45). In yeast, the *SSE1* gene is expressed at moderately high levels during normal growth and is further induced upon heat shock, while *SSE2* transcripts are undetectable until a more than 20-fold induction following heat shock (38, 39). Disruption of *SSE1* results in a slow growth phenotype at normal temperature, and cell growth at 37 °C is extremely slow or completely stopped (Refs. 38 and 39; this work). In contrast, *sse2Δ* cells display no obvious growth phenotype at 30 or 37 °C, nor does the *sse2Δ* mutation exacerbate the *sse1Δ* growth defect (38). Heat inducibility of *SSE2* mRNA is not appreciably affected by HSF CTA truncation, similar to that of *KAR2* (data not shown). Despite the sequence similarity between *SSE1* and *SSE2*, we believe that by these criteria, Sse1 is likely to be primarily involved in Hsp90 chaperone function, especially at normal growth temperature where *SSE2* is not expressed.

What is the role of Sse1 in protein folding by the Hsp90 complex? Sse1 was first identified as a calmodulin-binding protein (38), and interestingly, murine Hsp90 also binds calmodulin, and the binding domain has been mapped (58), leading to the hypothesis that the calmodulin-binding ability of these two proteins may actually reflect association of the chaperone complex with calmodulin, a calcium-binding protein involved in signal transduction. It has recently been shown that although possessing little protein folding activity by itself, hamster Hsp110 associates with unfolded proteins *in vitro* and blocks aggregation, with substantial renaturation of substrate proteins upon the addition of Hsc70 and Hdj1 (46). Likewise, purified Sse1 was recently reported to maintain denatured firefly luciferase in a folding-competent state prior to the addition of concentrated yeast cytosol (47). Interestingly, the ability to maintain proteins in a folding-competent state is a hallmark of Hsp90 and some Hsp90 cochaperones, including Cyp-40, p23, and Cdc37 (29, 35, 36), unlike the Hsp70/DnaJ chaperones, which do not share this catalytic property. This finding suggests that the Hsp110 subfamily is apparently functionally

distinct from the classical DnaK-like Hsp70 subfamily including mammalian Hsp70, Hsc70, and the yeast Hsp70 proteins and probably plays different roles than Hsp70 in the Hsp90 chaperone complex. Attempts at demonstrating functional complementation of *sse1Δ* cells by mammalian Hsp110 cDNA clones were unsuccessful (data not shown), perhaps suggesting some degree of functional divergence within the eukaryotic Hsp110 family or incomplete conservation of partner protein interactions. Given the sophisticated *in vitro* systems developed for Hsp90 chaperone function, it will be of considerable interest to learn whether Hsp110 homologs in other eukaryotic systems also function within the context of the Hsp90 chaperone complex.

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