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Novel interaction of the Hsp90 chaperone machine with Ssl2, an essential DNA helicase in *Saccharomyces cerevisiae*

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Abstract

Hsp90 is an essential molecular chaperone that is critical for the activity of diverse cellular proteins. Hsp90 functions with a number of co-chaperone proteins, including Sti1/Hop. We conducted a genetic screen in *Saccharomyces cerevisiae* to isolate mutations that exhibit enhanced growth defects in the absence of *STI1*. We obtained mutations in genes encoding components of the Hsp90 chaperone machine, *HSC82*, *CPR7* and *YDJ1*, and two essential genes, *SSL2* and *UTP21*, not previously linked to Hsp90. Ssl2, the yeast homologue of XPB, is an ATP-dependent DNA helicase that is a component of the TFIIH multiprotein complex and has dual functions in transcription and DNA repair. In order to determine whether Ssl2 function is dependent on Hsp90, we further examined the interaction between Ssl2 and Hsp90. Multiple mutant alleles of *SSL2* exhibited a pronounced growth defect when co-expressed with a mutant allele of Hsp90. In addition, isolation of Ssl2 protein resulted in the co-purification of Hsp90 and Sti1, suggesting that Ssl2 and Hsp90 are in the same protein complexes in vivo. These results suggest a novel role for Hsp90 in the essential cellular functions of transcription and DNA repair.

Keywords

Sti1; Ssl2/XPB; TFIIH; Hop; Utp21

Introduction

The molecular chaperone 90-kDa heat-shock protein (Hsp90) plays a critical role in the regulation of numerous signal transduction pathways by promoting the proper folding of interacting client proteins (Pearl and Prodromou 2001; Young et al. 2001; Pratt and Toft 2003). Hsp90 interacts with a number of oncogenic signaling proteins, including Akt, Raf-1, Bcr-Abl, mutant p53 and HER-2/Erb2 (Pratt and Toft 2003). Hsp90 has attracted considerable attention as a target for cancer chemotherapy because many client proteins become targets for proteasomal degradation in the absence of Hsp90 interaction. The naturally occurring Hsp90 inhibitor geldanamycin binds and inhibits the ATP-dependent chaperone activities of Hsp90 and causes the degradation of many Hsp90 client proteins. A geldanamycin derivative exhibits anti-tumor activity and is currently in clinical trials (Neckers 2002; Isaacs et al. 2003; Workman 2003).

Hsp90 is believed to associate with a limited set of proteins rather than acting as a general cytosolic chaperone, since widespread in vivo aggregation of newly synthesized proteins is not observed in the absence of Hsp90 function (Nathan et al. 1997). Hsp90 client proteins do not

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share any obvious sequence or structure homology; and the mechanism by which Hsp90 binds, recognizes and promotes activity of client proteins is largely unknown (Pearl and Prodromou 2001; Young et al. 2001; Pratt and Toft 2003). Hsp90 stabilizes some client proteins, maintaining them in a conformation that is poised to respond to an activation signal, such as ligand binding or phosphorylation (Mayer and Bukau 1999; Pratt and Toft 2003). Hsp90 may also function in the assembly and disassembly of multiprotein complexes, such as transcriptional complexes (Liu and DeFranco 1999; Freeman and Yamamoto 2002), the proteasome (Imai et al. 2003) and the kinetochore complex (Bansal et al. 2004).

Hsp90 interacts with client proteins in an ordered ATP-dependent pathway that is regulated by multiple co-chaperone proteins. Five proteins are essential for efficient maturation of Hsp90 client proteins in a purified system (Pratt and Toft 2003): Hsp70, Hsp40/Ydj1, Hsp90, Hop/Sti1 and p23/Sba1. These proteins cooperate in an ordered pathway that involves sequential ATP-dependent interactions of the client protein with the chaperones Hsp70 and Hsp90 (Smith 1993; Morishima et al. 2000). The co-chaperone proteins Hsp40/Ydj1, Hop/Sti1 and p23/Sba1 regulate the function of Hsp70 and/or Hsp90 (Pratt and Toft 2003). A current model of Hsp90 interaction with client proteins purports that Hsp40/Ydj1 and Hsp70 interact with the client first (Hernandez et al. 2002), followed by Hop/Sti1. Hop/Sti1 can simultaneously bind Hsp70 and Hsp90 and thus serves to recruit Hsp90 (Chen et al. 1996). Hop/Sti1 release allows ATP binding and this is accompanied by Hsp90–client interaction and dimerization of the amino-terminal ATPase domain of Hsp90, which is stabilized by the co-chaperone p23 (Young and Hartl 2000; Prodromou and Pearl 2003).

Because Hop/Sti1 can simultaneously interact with both Hsp70 and Hsp90 through separate tetratricopeptide repeat (TPR) domains, Hop/Sti1 was proposed to have a critical function in the transfer of client proteins from Hsp70 to Hsp90 (Chen and Smith 1998; Scheufler et al. 2000). Recent evidence indicates that Sti1 also regulates the ATPase activity of both Hsp90 and Hsp70 (Prodromou et al. 1999; Richter et al. 2003; Wegele et al. 2003). However, its *in vivo* functions are not well known. Deletion of *STI1* causes only mild growth defects at high (37°C) or very low (18°C) temperatures (Nicolet and Craig 1989), but yeast strains containing a *STI1* deletion have defects in the maturation of Hsp90 clients, such as the heterologous glucocorticoid receptor and v-src (Chang et al. 1997), the native yeast transcription factor HSF (Liu et al. 1999) and protein kinase Stell (Lee et al. 2004). In addition, deletion of *STI1* in combination with mutations or deletions in genes encoding yeast Hsp90 or Hsp90 co-chaperones (Chang et al. 1997; Fang et al. 1998; Liu et al. 1999; Abbas-Terki et al. 2002) produced enhanced growth defects, indicating these proteins work in the same cellular pathway.

Only a handful of native yeast Hsp90 client proteins have been identified (Johnson and Craig 2002) and in light of increasing interest in Hsp90 as a target for chemotherapy it is critical to understand the diverse cellular roles of Hsp90 and the mechanism by which Hsp90 mediates the folding of client proteins. To learn more about the *in vivo* functions of Sti1 within the Hsp90 pathway, we conducted a genetic screen to find proteins that functionally interact with Sti1. In accordance with previous studies, we isolated mutations in one of the genes encoding Hsp90, as well as known Hsp90 co-chaperones. We also isolated mutations in two essential genes, *UTP21* and *SSL2*, that have not previously been linked to Hsp90. Utp21 is part of a large protein complex required for ribosome biogenesis (Dragon et al. 2002; Schafer et al. 2003; Dosil and Bustelo 2004). Ssl2 is a DNA helicase required for transcription and DNA repair (Park et al. 1992; Feaver et al. 1993; Sweder and Hanawalt 1994; van Brabant et al. 2000). In this report, we present evidence that Ssl2 requires Hsp90 for *in vivo* function. We show that multiple alleles of *ssl2* have genetic interactions with *STI1* and the two genes that encode yeast Hsp90, *HSC82* and *HSP82*. In addition we show that deletion of *STI1* enhances the ultraviolet radiation (UV) sensitivity of some *ssl2* mutant strains and that Hsp90 and Sti1 are in the same protein

complexes as Ssl2. These results suggest that Hsp90 and Sti1 mediate the function of Ssl2, and provide evidence that Hsp90 is required for the essential cellular functions of DNA repair and transcription.

Materials and methods

Strains, media and growth assays

All *Saccharomyces cerevisiae* strains are isogenic to W303 (Thomas and Rothstein 1989) and are listed in Table 1. Standard yeast genetic methods were employed (Ito et al. 1983; Sherman et al. 1986). Yeast were transformed by lithium acetate methods (Schiestl and Gietz 1989). Yeast cells were grown in either yeast extract/peptone/dextrose (YPD; 1% Bacto yeast extract, 2% peptone, 2% dextrose) or defined synthetic complete medium supplemented with 2% dextrose. Growth was examined by spotting ten-fold serial dilutions of yeast cultures onto appropriate media, followed by incubation for 2 days at 23, 30 or 37°C. To assess UV sensitivity, ten-fold serial dilution of cells were plated onto YPD plates and exposed to UVC at the indicated dose (J/m²) in a UVC lightbox (254 nm, Stratlinker; Stratagene, Calif.) prior to incubation in the dark at 30°C for 2 days.

Plasmids

A 2.1-kb *Mun1-EcoR1* fragment of *Yep24-STI1* (Nicolet and Craig 1989) containing the complete *STI1* gene was subcloned into pRS315 (Sikorski and Hieter 1989). pRS316 *ADE3STI1* contained a 3.3-kb *Bst1107-Nhe1* fragment of *ADE3* (Carr-Schmid et al. 2002) and the 2.1-kb *Mun1-EcoR1* fragment of *Yep24-STI1*. *Ycp50-CPR7*, *Ycp50-UTP21* and *Ycp50-SSL2* were isolated from a yeast genomic DNA library, as described by Rose et al. (1987). *SSL2* was subcloned into pRS315 or pRS317 (Sikorski and Boeke 1991) as a 4.6-kb *BglIII-HindIII* fragment. Plasmids *Yep24-HSC82*, *Yep24-HSP82*, pTGPD-Hsp82 and pTGPD-Hsp82-G313S were generous gifts of Susan Lindquist (Borkovich et al. 1989; Nathan and Lindquist 1995). Wild-type *HSP82* and *hsp82-G313S* were expressed from the glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter on a low-copy *TRP1*-marked plasmid. *HSC82* was cloned into a low-copy *HIS3*-marked plasmid (pRS313) as a *BamHI-SacI* fragment. The *hsc82-G309S* mutation was constructed by mutagenic PCR and was homologous to the *hsp82-G313S* mutation (Nathan and Lindquist 1995). Plasmids expressing amino-terminal 6×-His-tagged Hsc82 or Hsc82-G309S under control of the GPD promoter (Mumberg et al. 1995) were also used.

Identification of mutations that have synthetic growth defects with *sti1*

Mutations that require *STI1* for growth were identified using an *ade2 ade3* color-sectoring assay (Bender and Pringle 1991). A *sti1* strain (JJ47) carrying pRS316 *ADE3STI1* was grown overnight at 30°C in uracil dropout medium to an optical density at 600 nm of 0.6. Cells were washed with water, diluted, plated and UV-irradiated to a 24–29% survival rate. After incubation for 8–10 days at 25°C, approximately 33,000 colonies were assayed and red colonies that lacked obvious sectoring (Sect[−]) were restreaked onto YPD. False positives were eliminated using 5-fluoroorotic acid (5-FOA) counterscreening to identify cells that had lost the pRS316 *ADE3STI1* plasmid. Mutations that displayed synthetic growth defects with *sti1* were identified by transformation with pRS315-*STI1*, which resulted in a Sect⁺ phenotype and conferred resistance to 5-FOA. Candidate strains were mated to *sti1* strain JJ51 to determine whether the mutation was recessive and then sporulated to determine whether one gene was responsible for both the sectoring and temperature-sensitive phenotypes of the resultant strains. For complementation analysis, after mating to JJ51, a suitable mutant spore (α *met2 LYS2*) from each candidate strain was selected for mating to the original candidate strain (a *MET2 lys2*). Fourteen candidate strains passed these controls and were placed into 12 complementation groups. In the absence of *STI1* (upon 5-FOA counterscreening), each

candidate strain exhibited a lethal phenotype or synthetic growth defect. The 14 mutations comprised 12 complementation groups and all mutations were recessive, as they all sectorized and displayed wild-type growth once crossed to JJ51.

The genes mutated in four *STII*-synthetic temperature-sensitive candidates were identified by transformation with plasmids encoding components of the Hsp90 pathway. The slow-growth defects of SL414 and SL760 were rescued by a plasmid expressing *YDJ1*; and the 37°C growth defect of SL43 and SL495 was rescued by a plasmid expressing *HSC82*. The presence of genomic mutations in *YDJ1* and *HSC82* was confirmed by complementation analysis with confirmed *HSC82 STII* and *STII YDJ1* disruption strains (data not shown).

The genes mutated in three *STII*-synthetic temperature-sensitive candidates were identified by complementation with a wild-type yeast DNA centromeric library (Rose et al. 1987). The SL769, SL42 and SL182 strains lacking pRS316 *ADE3STII* were transformed with a centromeric *URA3*-based yeast genomic library and plasmids that enabled growth at 37°C were isolated. Sequencing and subcloning of these library plasmids resulted in identification of the wild-type gene able to restore growth at 37°C. The 37°C growth defect of SL769 was rescued by a plasmid encoding *CPR7*, the 37°C growth defect of SL42 was rescued by a plasmid encoding *UTP21* and the 37°C growth defect of SL182 was rescued by a plasmid encoding *SSL2*.

Yeast strain construction

STII was disrupted in strain CP2 by insertion of the *TRP1* gene at the *BglII* site (amino acid 175) of *STII*, to yield the heterozygous diploid strain JJ4. Strain JJ4 was sporulated and dissected to obtain JJ47 and JJ51. *SSL2* was disrupted by insertion of the *MET2* gene into the *PspOM1-SwaI* sites of *SSL2*, removing amino acids 253–794. The *ssl2::MET2* construct was linearized with *MscI-SspI* and transformed into PJ53 (Yan and Craig 1999). Upon sporulation and tetrad analysis, only two viable (*met*[−]) spores were evident, consistent with the lethal phenotype of an *ssl2* disruption (Park et al. 1992). The heterozygous diploid strain was transformed with Ycp50-*SSL2* and sporulated to yield strain JJ546 (*ssl2/Ycp50-SSL2*), which was subsequently mated to JJ97 (*sti1*) to obtain JJ830 (*ssl2 sti1*).

Strains CLD82 (*hsc82*) and PLD82 (*hsp82*) were generous gifts of Susan Lindquist (Borkovich et al. 1989) and strain YF223 (*sba1*; Fang et al. 1998) was a generous gift of Avrom Caplan. CLD82 α and PLD82 α were crossed to PJ53 derivatives (*met2 lys2*) to obtain JJ665 (*hsc82*) and JJ824 (*hsp82*). Crosses of other isogenic strains were used to obtain JJ808 (*sba1 sti1*). To obtain an *hsc82 hsp82* strain, JJ665 was mated to JJ824 and the resultant diploid was transformed with Yep24-*HSP82* (gift of Susan Lindquist). After sporulation, tetrads displaying 2:2 LEU⁺/leu[−] growth, indicating co-segregation of *hsc82::LEU2* and *hsp82::LEU2*, were selected. The viability of JJ816 (*hsc82 hsp82/Yep24-HSP82*) was dependent on the Yep24-*HSP82* plasmid, confirming the genotype.

To look at the genetic interaction between *HSC82*, *HSP82* and *SSL2*, *hsc82* and *hsp82* strains were separately crossed to *ssl2* strains, to obtain JJ840 (α *hsc82 ssl2/Ycp50-SSL2*) and JJ842 (α *hsp82 ssl2/Ycp50-SSL2*). These strains were mated and the diploid was either transformed with pRS313-*HSC82* or pRS313-*hsc82-G309S* and dissected to obtain strains JJ835 and JJ839, or transformed with pRS314-*HSP82* or pRS314-*HSP82-G313S* and dissected to obtain strains JJ869 and JJ870.

SSL2 mutation plasmids

The *ssl2-182* mutant allele was rescued by gap repair (Rothstein 1991). A centromeric *SSL2* plasmid was digested with *NcoI-NdeI*, removing amino acids 59–749. This “gapped” plasmid

was transformed into SL182 and transformants containing the rescued mutant allele were isolated and sequenced completely. The *ssl2* mutation in SL182 was a single nucleotide alteration that changed leucine 691 to an isoleucine. Mutagenic PCR primers were used to construct the *ssl2-DEAD* alleles (V490A, H491D; Gulyas and Donahue 1992) and the *ssl2-ts24* alleles (V552I, E556K; Qiu et al. 1993). The *ssl2-XP* allele was constructed by filling-in the *Nde*I site in the *SSL2* coding sequence, producing a premature stop codon after amino acid 749. Each of these mutants was sequenced completely and cloned into pRS315 and pRS317 as *Bgl*II-*Hind*III fragments. Primer sequences are available upon request.

Affinity isolation of Flag-Ssl2

SSL2 was cloned into the pCal-N-Flag vector to insert an amino-terminal calmodulin-binding domain and Flag epitope (pCal-N-Flag; Stratagene). Cal-N-Flag-*SSL2* was subcloned into pRS423 *TEF* (Mumberg et al. 1995), a multicopy plasmid containing a promoter for constitutive expression in yeast. When transformed into a *ssl2* disruption strain (JJ546), pRS423 *TEF*-Flag-*SSL2* was able to support wild-type levels of growth at 30°C and near-wild-type levels of growth at 37°C (not shown). Cal-N-Flag-Ssl2 was isolated from yeast lysates using an anti-Flag affinity resin (Sigma, St. Louis, Mo.) essentially as described by Kimura et al. (1995). Briefly, 50 ml of exponentially growing cells expressing Flag-Ssl2 or wild-type Ssl2 were harvested in lysis buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 50 mM KCl, 20 mM Na₂MoO₄, 10 mM MgCl₂, 20% glycerol, with added complete protease inhibitor tablets; Roche, Penzberg, Germany). Cells were disrupted by vortexing in the presence of glass beads and centrifuged to remove cell debris. Cell lysate was precleared by incubation with Protein-A-Sepharose beads (Amersham), followed by incubation with anti-Flag M2 affinity resin (Sigma). Samples were analyzed by SDS-PAGE, followed by immunoblot analysis using antibodies against Sti1 (generously provided by Dr. David Toft), a polyclonal antibody that recognizes both Hsc82 and Hsp82 (Borkovich et al. 1989) and produced in this laboratory, or using antibodies against the calmodulin-binding peptide (Upstate).

Interaction of Flag-Ssl2 with Hsp90 in rabbit reticulocyte lysate

Radiolabeled (³⁵S)-Flag-Ssl2 was synthesized from the Cal-N-Flag-Ssl2 vector using the Quick T7 TNT coupled in vitro transcription and translation system (Promega, Madison, Wis.). After synthesis, 9 µl of the synthesis reaction were added to 100 µl of untreated reticulocyte lysate (Promega) and incubated with 10 µl anti-Flag affinity resin (Sigma), Protein-A-Sepharose beads (Amersham), or Protein-A-Sepharose beads bound to either D7α antibody against Hsp90 (a gift from David Toft; Brugge et al. 1983) or F5 antibody against Hop (mammalian Sti1, a gift from David Smith; Smith et al. 1993) for 30 min at 30°C, followed by 1 h at 4°C to immunoprecipitate protein complexes. Resin-bound complexes were washed, resuspended in sample buffer, resolved on a 10% SDS-PAGE gel and analyzed by autoradiography.

Results

Sti1 synthetic lethal screen identifies mutations that exhibit *STI1*-dependent growth

Hop/Sti1 is proposed to play a critical role in the Hsp90 pathway (Chen and Smith 1998), but deletion of *STI1* causes only mild growth defects at high (37°C) or very low (18°C) temperatures (Nicolet and Craig 1989). In order to learn more about the in vivo functions of *STI1*, we conducted a *STI1* synthetic lethal screen. We anticipated that this screen would result in the identification of (a) proteins with functions redundant with Sti1, (b) proteins in the same pathway as Sti1 or (c) proteins that require Sti1 for folding. We constructed a *sti1* strain (JJ47) that uses a red/white sectoring assay to monitor the presence of a plasmid expressing *STI1* (Bender and Pringle 1991). In wild-type cells, loss of *STI1* does not cause a significant growth defect; and when strain JJ47 is placed on non-selective medium (YPD), loss of the *URA3*-based

plasmid pRS316 *ADE3STII* results in white sectors in an otherwise red colony. Random mutations were introduced into strain JJ47 by UV exposure and colonies that failed to produce white sectors were characterized further. We used the red/white sectoring assay in combination with counter-screening in the presence of 5-FOA to isolate 14 candidate strains that exhibited a non-sectoring phenotype along with either a lethal phenotype or exaggerated growth defects in the absence of *STII*.

We identified the chromosomal mutation in seven of these strains and these results are summarized in Table 2. Three of the mutations that exhibit enhanced growth defects in the absence of *STII* are mutations in proteins that are known to function in the Hsp90 pathway and have previously been shown to have a genetic interaction with *STII*. Two mutations are in *HSC82* (Borkovich et al. 1989), which encodes the constitutively expressed isoform of yeast Hsp90. The combination of a *hsc82* deletion with a *sti1* deletion has previously been shown to impart a severe growth defect (Chang et al. 1997). An additional mutation is in *CPR7*, a Hsp90 co-chaperone (Duina et al. 1996). Like *Sti1*, *Cpr7* contains TPR domains and may compete for the same binding site on Hsp90 (Pratt and Toft 2003).

Two additional mutations are in *YDJ1*, a Hsp40 required for Hsp90 function (Kimura et al. 1995; Dey et al. 1996). In the presence of *STII*, both SL414 and SL760 exhibit slow growth at 30°C and are inviable at 37°C, similar to a *ydj1*-null strain (Caplan and Douglas 1991; Atencio and Yaffe 1992). Both SL414 and SL760 are inviable in the absence of *STII* (data not shown). We mated a *ydj1*-disruption strain (Johnson and Craig 2000) with a *sti1* strain and found that the combination of a *ydj1* deletion with a *sti1* deletion is inviable (data not shown). Although *Ydj1* and *Sti1* have previously been shown to function with Hsp90, this is the first report of the synthetic lethal interaction between *STII* and *YDJ1*.

The additional two mutations are in genes not previously linked to Hsp90 or *Sti1*. The mutation in SL42 is in the essential gene *UTP21*, which encodes a protein that is part of a large nucleolar complex required for RNA biogenesis (Dragon et al. 2002; Schafer et al. 2003; Dosil and Bustelo 2004). In the presence of *STII*, SL42 exhibits a slight temperature defect at 37°C. In the absence of *STII*, SL42 exhibits slow growth at 23°C and 30°C and is inviable at 37°C (data not shown). The other mutation is in *SSL2*, which encodes a DNA helicase. In the remainder of this report we describe the genetic and physical interaction between *Ssl2* and the Hsp90 molecular chaperone machine.

***STII* has a genetic interaction with *SSL2*, the yeast homologue of the XPB helicase**

Strain SL182 exhibited a non-sectoring phenotype and a temperature-sensitive growth phenotype that was enhanced in the absence of *STII* (Fig. 1b). SL182 was transformed with a yeast genomic library and fast-growing colonies were selected for growth at 37°C. A plasmid encoding *SSL2* as the only complete open reading frame was able to fully rescue the growth defect of strain SL182 (data not shown). *Ssl2* is an ATP-dependent DNA helicase that is part of TFIIH, a multiprotein complex that functions in basal transcription by RNA polymerase II and nucleotide excision repair (NER; Feaver et al. 1993; van Brabant et al. 2000). NER promotes the removal of DNA lesions caused by UV or DNA-damaging agents such as cisplatin. Mutations in the human homologue of *SSL2*, XPB (Gulyas and Donahue 1992; Park et al. 1992), lead to the inherited disorders xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome, which are characterized by increased UV hypersensitivity and are associated with a wide range of developmental abnormalities. Patients with xeroderma pigmentosum also experience a high incidence of UV-related skin cancers, probably due to the inability to repair damaged DNA.

SSL2 encodes an 843-amino-acid protein that contains seven domains conserved within the superfamily of DNA and RNA helicases, including the walker type A nucleotide-binding motif

sequences (domains I, IA) and helicase domains II–VI (Fig. 1a; van Brabant et al. 2000). Purified Ssl2 exhibits single-stranded DNA-dependent ATPase and DNA helicase activities (Guzder et al. 1994). In order to determine the nature of the mutation in *ssl2-182*, we rescued the mutant *ssl2* allele by gap repair (Rothstein 1991). Ssl2-182 contains a single amino acid alteration in the conserved helicase motif VI, in which leucine residue at position 691 is changed to isoleucine. This leucine residue is conserved between humans, mice, *S. cerevisiae*, *Schizosaccharomyces pombe* and *Drosophila* (van Brabant et al. 2000).

To establish that the L691I mutation in *ssl2* is the cause of the slow-growth phenotype observed in the *ssl2-182* strain, we constructed a strain in which the chromosomal copy of *SSL2* was disrupted (JJ546, *ssl2*). Because *SSL2* is essential, this strain harbored a wild-type copy of *SSL2* on a plasmid (Ycp50-*SSL2*). In order to determine whether *ssl2-L691I* exhibits *STII*-dependent growth, we also constructed a strain in which both *SSL2* and *STII* were disrupted (JJ830, *ssl2sti1/Ycp50-SSL2*). Wild-type *SSL2* or *ssl2-L691I* was transformed into the *ssl2* and *sti1ssl2* strains and the plasmid containing wild-type *SSL2* was cured by growing transformants in the presence of 5-FOA. Colonies that grew on 5-FOA were grown overnight in selective medium and equal numbers of cells were serially diluted ten-fold, spotted onto rich medium (YPD plates), and grown for 2 days at the indicated temperature. In the presence of wild-type *SSL2*, deletion of *STII* resulted in only a minor growth defect at 37°C (Fig. 1c). The synthetic growth phenotype observed for the original *ssl2-182* isolate was recapitulated in strains expressing a plasmid-borne *ssl2-L691I* allele. *ssl2-L691I* exhibited growth defects at 23 and 37°C that were exacerbated in the absence of *STII*. This growth pattern was indistinguishable from the original SL182 strain in the presence and absence of *STII* (Fig. 1b).

Structural information about Ssl2 is unavailable, but it is possible that alteration of residue L691 causes local structural disturbances that result in the *STII*-dependence. To further establish that Stl1 is required for the function of Ssl2, we extended our analysis to additional *ssl2* mutant alleles. The *ssl2-XP* allele (amino acids 1–749) is a truncation of Ssl2 that was made to mimic the mutated form of XPB found in a patient with xeroderma pigmentosa. Yeast expressing a truncated form of Ssl2 exhibit extreme UV sensitivity (Gulyas and Donahue 1992; Park et al. 1992), a deficiency in the removal of cyclobutane pyrimidine dimers from the overall genome and a defect in transcription-coupled repair (Sweder and Hanawalt 1994). We also examined mutations that have been found to be defective in transcription, rather than DNA repair. In the *ssl2-DEAD* allele, the DEVH residues of conserved helicase motif II are converted to the DEAD residues common to RNA helicases (van Brabant et al. 2000). This alteration results in a temperature-sensitive phenotype but does not result in increased UV sensitivity (Gulyas and Donahue 1992). A third mutation, *ssl2-ts24*, contains two point mutations, V552I and E556K. This mutation exhibits temperature-sensitive growth and defects in RNA polymerase II-mediated transcription in vivo, but does not display increased UV sensitivity (Qiu et al. 1993).

Using PCR mutagenesis, we constructed *ssl2-XP*, *ssl2-DEAD* and *ssl2-ts24* mutant alleles. Using the plasmid-shuffling technique described above, we compared the growth of *ssl2-DEAD*, *ssl2-XP* and *ssl2-ts24* in the presence and absence of *STII* (Fig. 2b). As expected from prior studies, in the presence of *STII*, the *SSL2-XP*, *SSL2-DEAD* and *SSL2-ts24* strains exhibited wild-type growth at 30°C and decreased growth at 23°C and 37°C (Gulyas and Donahue 1992; Qiu et al. 1993). In the absence of *STII*, each of the three *ssl2* alleles exhibited enhanced growth defects at 23°C and the *ssl2-DEAD* allele also exhibited a slightly enhanced growth defect at 37°C. These results indicate that multiple alleles of *SSL2* exhibit *STII*-dependent growth and thus it is not likely that the *STII*-dependent growth of *ssl2-182* is due to a structural defect limited to that particular allele.

***ssl2* alleles exhibit Hsp90-dependent growth**

In *Saccharomyces cerevisiae*, Hsp90 is encoded by two genes that are believed to have near-identical functions, the constitutively expressed *HSC82* and the heatinducible *HSP82*. Deletion of either gene results in a reduced total Hsp90 protein level but only mild growth defects at 37°C (Borkovich et al. 1989). Consistent with earlier reports that loss of *STI1* results in derepression of the heat shock response (Nicolet and Craig 1989; Liu et al. 1999), we observed a slight heat-shock response in the *STI1* disruption strain compared to a wild-type strain, as judged by levels of the heat-shock proteins Hsp104, Ssa3/Ssa4 and Hsc82/Hsp82 (data not shown). This suggested that overexpression of Hsp90 might alleviate defects of the *ssl2 sti1* strains, masking a synthetic growth effect at 30°C. Both to determine how the Hsp90 level affects the growth of *ssl2* alleles and to determine whether *SSL2* has a genetic interaction with *HSP82* and/or *HSC82*, we examined the growth of *ssl2* mutants in *hsc82 ssl2* and *hsp82 ssl2* strains. Upon deletion of either *HSC82* or *HSP82*, slightly enhanced growth defects of the *ssl2* alleles were observed at 23°C or 37°C, but no effect on growth at 30°C was observed. Thus, despite higher Hsc82/Hsp82 levels in the *ssl2 sti1* strains compared with the *hsc82 ssl2* and *hsp82 ssl2* strains (data not shown), synthetic growth effects were observed only at temperatures above and below the optimal growth temperature of 30°C.

To further examine the effect of alteration in the Hsp90 chaperone on Ssl2 function, we examined mutations that have more dramatic effects on Hsp90 function. A number of mutations in *HSP82* have been found to cause temperature-sensitive growth and specific defects in the activity of Hsp90 client proteins when expressed as the only Hsp90 in the cell (Bohen and Yamamoto 1993; Kimura et al. 1994; Nathan and Lindquist 1995). One of these mutants, Hsp82-G313S, or the similar mutant Hsp82-G313N, has been found to cause reductions in the activity of Hsp90 client proteins expressed in yeast (Bohen and Yamamoto 1993; Nathan and Lindquist 1995). We examined the genetic interaction of *SSL2* with *hsp82-G313S* and the homologous *hsc82-G309S*. To minimize differences due to expression levels, wild-type and mutant Hsc82 or Hsp82 were expressed under the control of the constitutive GPD promoter, and based on immunoblot analysis, all proteins were expressed at a similar level (data not shown). When expressed in a strain in which the chromosomal copies of both *HSC82* and *HSP82* were disrupted (a generous gift of Susan Lindquist), *hsc82-G309S* exhibited near-wild-type growth at 30°C and was inviable at 37°C, similar to the phenotype observed for *hsp82-G313S* (Nathan and Lindquist 1995). *hsp82-G313S* exhibited a slightly stronger growth defect than *hsc82-G309S* at the intermediate temperature of 34°C (data not shown).

We created a strain containing chromosomal deletions of *hsc82*, *hsp82* and *ssl2*, allowing us to look at the combination of mutant alleles of *SSL2* with mutant alleles of Hsp90. Plasmids expressing wild-type or mutant *SSL2* were transformed into the *hsc82 hsp82 ssl2/Ycp50-SSL2* strain expressing either wild-type *HSC82*, *hsc82-G309S*, wild-type *HSP82* or *hsp82-G313S*. The plasmid expressing wild-type *SSL2* was then cured by plating transformants onto medium containing 5-FOA. Resultant colonies were grown overnight and serial dilutions were plated onto YPD and grown for 2 days at 30°C. The strains expressing either *hsc82-G309S* or *hsp82-G313S* and wild-type *SSL2* exhibited near-wild-type growth at 30°C (Fig. 3). However, in the presence of the *hsc82-G309S* mutation, all four *ssl2* mutant alleles displayed reduced growth at 30°C, indicating a strong synthetic growth defect between *SSL2* and *HSC82*. Similarly, in the presence of the *hsp82-G313S* mutation, all four *ssl2* mutant alleles displayed a sharply reduced growth at 30°C (right panel). Each of the *SSL2* alleles exhibited near-wild-type growth in the presence of either *HSC82* or *HSP82*, indicating that the two isoforms can functionally complement each other in these strains. The synthetic effects of the *hsp82-G313S* allele appeared slightly stronger than that of the *hsc82-G309S* allele. Whether this difference was due to the slightly stronger growth defects of *hsc82 hsp82* strains expressing

hsp82-G313S versus *hsc82-G309S* or differences in how Hsc82 and Hsp82 functionally interact with Ssl2 remains to be determined.

***STI1* deletion increases sensitivity of *ssl2* mutants to UV exposure**

One method used to measure the effect of *SSL2* mutation on NER activity is to determine the cellular response to DNA damage (van Brabant et al. 2000). Cells with an intact NER pathway exhibit greater viability after UV exposure compared to cells with mutations in components in the NER pathway. To determine whether deletion of *STI1* affects the function of Ssl2 in NER, we examined the viability of *SSL2* alleles expressed in the *ssl2* or *ssl2sti1* strains upon exposure to increasing doses of UVC (Fig. 4). Ten-fold serial dilutions of isogenic yeast strains were plated onto YPD plates and exposed to UVC at the indicated dose (J/m^2) in a UVC lightbox at 254 nm and then incubated in the dark at 30°C for 2 days. In accordance with previous results, we observed that wild-type cells were fairly resistant to UVC doses of up to 60 J/m^2 , but that cells expressing *ssl2-XP* were hypersensitive to doses as low as 10 J/m^2 (Gulyas and Donahue 1992; Fig. 4; data not shown). Deletion of *STI1* did not noticeably affect the UV sensitivity of cells expressing wild-type *SSL2*. Next we examined the UV sensitivity of *ssl2-L691I*, *ssl2-DEAD* and *ssl2-ts24* in the presence and absence of *STI1*. As found previously, in the presence of *STI1*, the *ssl2-DEAD* and *ssl2-ts24* mutations did not exhibit the severe UV-sensitivity phenotype observed for *ssl2-XP* (Gulyas and Donahue 1992; Qiu et al. 1993), although in this assay they did appear to be slightly more sensitive than cells expressing wild-type *SSL2*. The UV sensitivity of both *ssl2-L691I* and *ssl2-DEAD* increased in the absence of *STI1*, although deletion of *STI1* had little effect on the survival of strains expressing *ssl2-ts24*. These results indicate that deletion of *STI1* affects the ability of some *ssl2* alleles to withstand UV exposure and suggest that Sti1 modulates the function of Ssl2 in DNA repair. We examined whether the UV sensitivity of *ssl2* alleles was enhanced when present in combination with mutant *hsc82* or *hsp82* alleles as the only Hsp90 in the cell. However, the two alleles that exhibit enhanced UV sensitivity in the absence of *STI1*, *ssl2-L691I* and *ssl2-DEAD*, exhibited very strong growth defects when combined with the mutant *hsc82* and *hsp82* alleles (Fig. 3); and thus it was difficult to quantify enhanced growth defects upon UV exposure. As observed in the case of deletion of *STI1*, the UV sensitivity of the *ssl2-ts24* allele was not significantly affected by mutation of *hsc82* or *hsp82* (data not shown).

Strains with mutations in the Hsp90 pathway exhibit increased sensitivity to UV

If Hsp90 and Sti1 modulate the activity of wild-type Ssl2, mutations that disrupt the function of the Hsp90 pathway should lead to growth phenotypes similar to that of the *ssl2* mutant strains. To determine whether the activity of wild-type Ssl2 is modulated by Sti1 and/or Hsp90, we examined the UV sensitivity of strains that express wild-type *SSL2* but contain mutations in the Hsp90 pathway. We expressed wild-type *HSC82*, *hsc82-G309S*, wild-type *HSP82* or *hsp82-G313S* in a strain containing chromosomal deletions of *hsc82* and *hsp82* (JJ816) and assayed their UV sensitivity upon exposure to increasing doses of UVC (Fig. 5). Both the *hsc82-G309S* and *hsp82-G313S* strains exhibited enhanced UV sensitivity, compared with the wild-type strain. Consistent with the stronger synthetic growth defects observed with *hsp82-G313S* and *ssl2* mutant alleles (Fig. 3), the *hsp82-G313S* strain exhibited a slightly stronger UV sensitivity than the *hsc82-G309S* strain.

As previously shown (Fig. 4), deletion of *STI1* did not result in increased UV sensitivity, compared with wild-type cells. We examined the UV sensitivity of a strain lacking the gene encoding the Hsp90 co-chaperone Sba1 (*sba1*; the yeast p23) and a strain lacking both *SBA1* and *STI1*, since this strain exhibits greater growth defects at high and low temperature than either the *sba1* or *sti1* strains alone (Fang et al. 1998). Like the *sti1* strain, the *sba1* strain did not exhibit enhanced UV sensitivity (Fig. 5). However, the *sti1 sba1* strain displayed enhanced sensitivity to 30 J/m^2 and 60 J/m^2 UVC, compared with a wild-type strain. These results suggest

that additional components in the Hsp90 molecular machine may also modulate the function of Ssl2.

Sti1 and Hsp90 interact with Ssl2 in vivo

In order to determine whether Ssl2 physically interacts with Sti1 and Hsp90 in vivo, we constructed a version of Ssl2 containing an amino-terminal calmodulin-binding domain and a Flag epitope. The Flag-Ssl2 expressed in *ssl2* yeast under a constitutive yeast promoter (Mumberg et al. 1995) was able to support wild-type levels of growth at 30°C and near-wild-type levels of growth at 37°C (not shown). Flag-Ssl2 was isolated from yeast lysates using an anti-Flag affinity resin. As shown in Fig. 6 (left panel, lanes 1, 2) yeast lysates from a *ssl2* strain expressing wild-type Ssl2 or Flag-Ssl2 were separated by SDS-PAGE and immunoblotted with an antibody that recognized the Flag epitope. Low levels of Flag-Ssl2 are observed in lane 2. The anti-Flag resin was very effective in the isolation of these low levels of Flag-Ssl2 from the lysate, as a predominant band of the expected size (105 kDa; Guzder et al. 1994) was retained only in cells expressing Flag-Ssl2 (lane 3). Nonspecific binding of a protein (Fig. 6, left panel, asterisk) that migrated faster than Flag-Ssl2 was observed in both lanes 3 and 4, the latter of which did not express Flag-Ssl2. Identical samples were probed for the presence of Hsc82/Hsp82 or Sti1 using antibodies specific for those proteins. Hsc82/Hsp82 and Sti1 were retained on the anti-Flag affinity resin only in the presence of Flag-Ssl2 (lane 3), indicating that Sti1 and Hsc82/Hsp82 are in the same protein complexes as Flag-Ssl2 in vivo.

The reverse experiment to show that Flag-Ssl2 co-immunoprecipitates with anti-Hsc82/Hsp82 antibodies was not possible because the anti-Hsc82/Hsp82 antisera does not immunoprecipitate Hsc82/Hsp82 with high affinity; and Hsc82/Hsp82 is present at approximately 500-fold excess relative to Ssl2 (Ghaemmamghami et al. 2003). As an alternative approach, we examined whether Flag-Ssl2 synthesized in rabbit reticulocyte lysate interacts with mammalian Hsp90. Rabbit reticulocyte lysate is commonly used to examine the interaction of Hsp90 with client proteins and has been used to study the interaction of Hsp90 with diverse client proteins, including steroid receptors, the duck hepatitis reverse transcriptase and the Raf1 kinase (Pratt and Toft 2003). We synthesized radiolabeled Flag-Ssl2 in rabbit reticulocyte lysates and determined whether it co-immunoprecipitated with mammalian Hsp90 or Hop (mammalian Sti1), using antibodies specific for those proteins. As shown in Fig. 6 (right panel), Flag-Ssl2 was selectively immunoprecipitated with an anti-Flag antibody (lane 4). Flag-Ssl2 was also recovered in complexes isolated with an antibody specific for Hsp90 (lane 5). Lower levels of Flag-Ssl2 co-immunoprecipitated with an antibody to Hop (lane 6), but no Flag-Ssl2 was recovered in the control (lane 3) lacking antibody. These results demonstrate that Flag-Ssl2 interacts with rabbit Hsp90, providing additional evidence that Ssl2 and Hsp90 are present in the same protein complexes.

Discussion

Hsp90 is an essential molecular chaperone that mediates the folding of diverse cellular proteins. Hsp90 inhibitors are in clinical trials as anti-cancer agents (Neckers 2002; Isaacs et al. 2003; Workman 2003) and thus it is critical to identify proteins that require Hsp90 for their function. The list of mammalian Hsp90 client proteins is growing rapidly (Pearl and Prodromou 2001; Young et al. 2001; Pratt and Toft 2003). However, only a handful of native yeast client proteins have been identified, such as the transcription factors Hsf1, Hap1 and Mal63 (Duina et al. 1998; Zhang et al. 1998; Bali et al. 2003), the kinases Ste11 and Gcn2 (Louvion et al. 1998; Donze and Picard 1999) and the catalytic subunit of calcineurin, Cna2 (Imai and Yahara 2000). Our results suggest that the DNA helicase Ssl2, which is essential for the viability of

S. cerevisiae and is conserved in humans, may require the Hsp90 molecular chaperone machine for function.

Here we present evidence that Ssl2 is in the same complexes as Hsp90 and Sti1. Ssl2 was also found to interact with Sti1 in a previous genome-wide study (Ho et al. 2002), although the functional significance or specificity of the interaction was not addressed in that study. In preliminary studies, we found that Hsc82/Hsp82 is present in Ssl2 complexes in the absence of *STI1* (data not shown), suggesting that *STI1* is not essential for the Hsc82/Hsp82 interaction. In a similar manner, deletion of *STI1* did not prevent the interaction between Hsc82/Hsp82 and the glucocorticoid receptor (Chang et al. 1997); and thus the *in vivo* functions of Sti1 remain unclear. One of the intriguing aspects of a functional interaction of Ssl2 with Hsp90 is that Ssl2 functions as part of a large protein complex, TFIIF, which has ten protein subunits (Schultz et al. 2000; van Brabant et al. 2000; Giglia-Mari et al. 2004; Ranish et al. 2004). It is interesting to note that we also isolated a mutation in the gene that encodes Utp21, which also functions as part of a large protein complex involved in ribosome biogenesis (Dragon et al. 2002; Schafer et al. 2003; Dosil and Bustelo 2004). Although traditionally thought of as a chaperone that stabilizes proteins in a conformation poised to respond to a signal such as ligand binding, Hsp90 has been proposed to have a role in the assembly and disassembly of transcriptional complexes (Freeman and Yamamoto 2002) and the proteasome (Imai et al. 2003). A recent report helps to bridge the conceptual gap between these different functions of Hsp90 (Bansal et al. 2004). Hsp90 interacts with the Sgt1 protein and this interaction may be required to promote the conformation of Sgt1 that allows Skp1 binding, a critical step in the assembly of the yeast kinetochore. Thus, one possible function of Hsp90 and co-chaperones may be to mediate the interaction of Ssl2 with other TFIIF subunits during assembly or disassembly of the complex. An alternative explanation is that Hsp90 assists the folding of newly synthesized Ssl2 prior to or during its interaction with larger protein complexes. Additional experiments are required to distinguish between these possibilities.

The most exciting aspect of our results is the implication that Hsp90 is involved in the function of Ssl2, a component of TFIIF that is essential for transcription by RNA polymerase II and DNA repair. Our genetic evidence indicates that *ssl2* mutant strains are very sensitive to the loss of Hsp90 function, suggesting that Ssl2 requires Hsp90 for full activity. Previous studies have shown that the mutant alleles of *ssl2* we tested have different effects on transcription and DNA repair (Gulyas and Donahue 1992; Park et al. 1992; Guzder et al. 1994; Sweder and Hanawalt 1994), yet all of the mutant alleles exhibit Hsp90-dependent growth, suggesting that Ssl2 activities in both transcription and DNA repair may be dependent on Hsp90. We do not yet know whether mutation of Hsp90 affects the expression level of wild-type or mutant Ssl2. However, the *ssl2-L691I* and *ssl2-DEAD* mutants exhibited more severe synthetic defects than *ssl2-ts24*, suggesting that not all alleles are equally affected by mutation of the Hsp90 molecular chaperone machine. Intriguingly, this is not the first genetic evidence linking Sti1 to TFIIF. Kin28 is a cyclin-dependent kinase that is part of TFIIF. A prior synthetic lethal screen with a temperature-sensitive allele of *KIN28* resulted in the isolation of mutations in both *STI1* and *CDC37*, which encodes an additional Hsp90 co-chaperone (Valay et al. 1995; Pratt and Toft 2003). Further experiments are needed to specifically determine whether Hsp90 is required for Ssl2 to associate with TFIIF and how Hsp90 mutation affects TFIIF functions in both transcription and DNA repair.

Our finding that Hsp90 mutant strains are sensitive to UV exposure and that deletion of *STI1* enhances the UV sensitivity of *ssl2* mutant strains suggests that Hsp90 may mediate the function of Ssl2 in NER. As a test to determine whether the UV sensitivity of Hsp90 mutant strains reflects NER defects, as opposed to a general stress response caused by misfolding of proteins caused by UV-induced oxidative damage, we tested the sensitivity of Hsp90 mutant strains to hydrogen peroxide, which is commonly used to generate oxidative stress (Toledano

et al. 2003). The strains expressing *hsc82-G309S* or *hsc82-G313S* as the only Hsp90 in the cell did not exhibit enhanced sensitivity to 1 mM hydrogen peroxide, compared with a wild-type strain (data not shown). Experiments to demonstrate whether Hsp90 mutant strains have defects in NER activity are underway. In addition, the DNA-damaging agent cisplatin has been found to directly bind Hsp90 and inhibit its chaperoning activity (Itoh et al. 1999; Soti et al. 2002; Rosenhagen et al. 2003), suggesting this drug may affect NER in multiple ways. These connections between Hsp90 and NER coincide with recent evidence that Hsp90 inhibiting drugs sensitize tumor cells to radiation (Bisht et al. 2003; Machida et al. 2003; Russell et al. 2003; Camphausen and Tofilon 2004) and suggest a cellular mechanism that underlies this phenomenon. Future work will clarify the role of Hsp90 in Ssl2 function in transcription and DNA repair and will help elucidate the diverse cellular functions of this essential molecular chaperone.

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Fig. 1.

SL182 contains a mutation in *SSL2*. **a** Domain structure of Ssl2. Ssl2 contains six conserved helicase motifs (shown in *black*). The location of the single amino acid mutation (*ssl2-L691I*) in strain SL182 is indicated. **b** Growth of *sti1* screening strain JJ47 and original SL182 candidate strain with (+*STI1*) or without the pRS316 *ADE3STI1* plasmid. **c** Growth of *ssl2-L691I* mutant allele in *ssl2* strain (JJ546) or *ssl2sti1* strain (JJ830). A plasmid expressing wild-type (WT) or mutant *ssl2* alleles was transformed into the indicated strains and transformants were grown in the presence of 5-FOA to counterselect for *YCp50-SSL2*. Indicated yeast strains were serially diluted ten-fold, spotted onto YPD and grown for 2 days at the indicated temperature

Fig. 2.

Additional *ssl2* alleles exhibit *STI1*-dependent growth. **a** The location of additional *ssl2* alleles described in the text are indicated. **b** Growth of indicated *ssl2* mutant alleles in *ssl2* strain (JJ546) or *ssl2 sti1* strain (JJ830). Mutant *ssl2* alleles were transformed into the indicated strains and transformants were grown in the presence of 5-FOA to counterselect for *YCP50-SSL2*. Indicated yeast strains were serially diluted ten-fold, spotted onto YPD and grown for 2 days at the indicated temperature

Fig. 3.

The *ssl2* alleles exhibit Hsp90-dependent growth. *Left panel* *hsc82 hsp82 ssl2/Ycp50-SSL2* strains expressing *HSC82* (JJ835;+) or *hsc82-G309S* (JJ839;-) were transformed with *ssl2* alleles and plated onto medium containing 5-FOA to counterselect for *Ycp50-SSL2*. *Right panel* *hsc82 hsp82 ssl2/Ycp50-SSL2* strains expressing *HSP82* (JJ869;+) or *hsp82-G313S* (JJ870;-) were transformed with *ssl2* alleles and plated onto medium containing 5-FOA to counterselect for *Ycp50-SSL2*. Transformants were grown overnight, serially diluted ten-fold, spotted onto YPD and grown for 2 days at 30°C

Fig. 4.

Deletion of *STII* enhances the sensitivity of some *ssl2* strains to UVC. Mutant *ssl2* alleles were transformed into strain JJ546 (*STII*+) or JJ830(*STII*-) and transformants were grown in the presence of 5-FOA to counterselect for *YCp50-SSL2*. The indicated yeast strains were serially diluted ten-fold, spotted onto YPD, exposed to the indicated doses of UVC and grown for 2 days in the dark at 30°C

Fig. 5. Strains with mutations in the Hsp90 chaperone machine exhibit enhanced sensitivity to UVC. The indicated yeast strains were serially diluted ten-fold, spotted onto YPD, exposed to the indicated doses of UVC and grown for 2 days in the dark at 30°C. *Top panel* Strain JJ546 expressing WT *SSL2* or *ssl2-XP*, *middle panel* strain JJ816 (*hsc82 hsp82*) expressing WT *HSC82*, *hsc82-G309S*, WT *HSP82* or *hsp82-G313S*, *bottom panel* WT (PJ43-1B), *sti1* (JJ97), *sba1* (YF223) and *sba1sti1* (JJ808)

Fig. 6.

Interaction of Flag-Ssl2 with Hsp90. *Left panel* Affinity isolation of Flag-Ssl2 out of yeast lysates. *Lanes 1, 2* Yeast lysates from *ssl2* strains expressing wild-type Ssl2 (-) or Flag-Ssl2 (+) were analyzed by SDS-PAGE and immunoblot analysis. *Lanes 3, 4* Proteins retained on the anti-Flag affinity resin were resuspended in sample buffer and analyzed by SDS-PAGE and immunoblot analysis: *lane 3* Flag-Ssl2 (+) cells expressing Cal-N-Flag Ssl2, *lane 4* cells expressing wild-type Ssl2 (-). Immunoblot analysis was performed using antibodies that recognized Flag-Ssl2 (α -Flag), Hsc82/Hsp82 (α -Hsp90) or Sti1. *Right panel* Interaction of Flag-Ssl2 with Hsp90 in rabbit reticulocyte lysate. 35 S-labeled Flag-Ssl2 was synthesized in rabbit reticulocyte lysates (RL). *Lane 1* RL from control in vitro synthesis reaction with the pCAL-N-Flag vector. *Lane 2* RL showing the migration of synthesized radiolabeled Flag-Ssl2. Equal amounts of Flag-Ssl2 were added to fresh RL prior to immunoprecipitation of protein complexes with antibodies specific for: *lane 3* no antibody, *lane 4* α -Flag, *lane 5* α -Hsp90, *lane 6* α -Hop. Samples were separated by SDS-PAGE and autoradiographed to detect radiolabeled Flag-Ssl2

Table 1

Strains used in this study

Strain	Genotype	Source or reference
W303-1b	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100</i>	Thomas and Rothstein (1989)
PJ53	MATa/MATa <i>ade2-1/ade2-1 ura3-1/ura3-1 lys2-Δ 2/lys2-Δ leu2-3,112/leu2-3,112 trp1-1/trp1-1 met2-Δ 1/met2-Δ 1 his3-11,15/his3-11,15 can1-100/can1-100</i>	Yan and Craig (1999)
PJ43-1B	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 met2-Δ 1 his3-11,15 lys2-Δ 2 can1-100</i>	James and Craig
CP1	MATa <i>ade2-1 ade3-Δ 1 ura3-1 lys2-Δ 2 leu2-3,112 trp1-1 MET2 his3-11,15 can1-100</i>	Carr-Schmid (2002)
CP2	MATa/MATa <i>ade2-1/ade2-1 ade3-Δ 1/ADE3 ura3-1/ura3-1 lys2-Δ 2/LYS2 leu2-3,112/leu2-3,112 trp1-1/trp1-1 met2-Δ 1/MET2 his3-11,15/his3-11,15 can1-100/can1-100</i>	Pfund and Craig
JJ4	MATa/MATa <i>ade2-1/ade2-1 ade3-Δ 1/ADE3 ura3-1/ura3-1 lys2-Δ 2/LYS2 leu2-3,112/leu2-3,112 trp1-1/trp1-1 met2-Δ 1/MET2 his3-11,15/his3-11,15 can1-100/can1-100 sti1::TRP1/STI1</i>	This study
JJ47	MATa <i>ade2-1 ade3-Δ 1 ura3-1 lys2-Δ 2 leu2-3,112 trp1-1 MET2 his3-11,15 can1-100 sti1::TRP1</i>	This study
JJ51	MATa <i>ade2-1 ade3-Δ 1 ura3-1 LYS2 leu2-3,112 trp1-1 met2-Δ 1 his3-11,15 can1-100 sti1::TRP1</i>	This study
SL182	MATa <i>ade2-1 ade3-Δ 1 ura3-1 lys2-Δ 2 leu2-3,112 trp1-1 MET2 his3-11,15 can1-100 sti1::TRP1 ssl2-L6911 [pRS316-ADE3STI1]</i>	This study
JJ97	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 met2-Δ 1 his3-11,15 lys2-Δ 2 can1-100 sti1::TRP1</i>	This study
JJ160	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 met2-Δ 1 his3-11,15 lys2-Δ 2 can1-100 ydj1::HIS3</i>	Johnson and Craig (2000)
CLD82	MATa <i>ade2-2 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 hsc82::LEU2</i>	Borkovich et al. (1989)
JJ665	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsc82::LEU2</i>	This study
JJ546	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 ssl2::MET2/Ycp50-SSL2</i>	This study
JJ830	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 sti1::TPR1 ssl2::MET2/Ycp50-SSL2</i>	This study
PLD82	MATa <i>ade2-2 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 hsp82::LEU2</i>	Borkovich et al. (1989)
JJ824	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsp82::LEU2</i>	This study
JJ816	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsp82::LEU2 hsc82::LEU2/Yep24-HSP82</i>	This study
JJ835	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsp82::LEU2 hsc82::LEU2/pRS313-His-HSC82 ssl2::MET2/Ycp50-SSL2</i>	This study
JJ839	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsp82::LEU2 hsc82::LEU2/pRS313-His-hsc82-G309S ssl2::MET2/Ycp50-SSL2</i>	This study
YF223	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 sba1::URA3</i>	Fang et al. (1998)
JJ808	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 sba1::URA3 sti1::TRP1</i>	This study
JJ840	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsc82::LEU2 ssl2::MET2/Ycp50-SSL2</i>	This study
JJ842	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsp82::LEU2 ssl2::MET2/Ycp50-SSL2</i>	This study
JJ869	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsp82::LEU2 hsc82::LEU2/pRS314-HSP82 ssl2::MET2/Ycp50-SSL2</i>	This study
JJ870	MATa <i>ade2-1 ura3-1 leu2-3,112 trp1-1 his3-11,15 can1-100 met2-Δ 1 lys2-Δ 2 hsp82::LEU2 hsc82::LEU2/pRS314-hsp82-G313S ssl2::MET2/Ycp50-SSL2</i>	This study

Table 2*STH* synthetic mutations

Mutant strain	Location of mutation	Previously known genetic interaction?
SL43, SL495	<i>HSC82</i>	Yes (Chang et al. 1997)
SL414, SL760	<i>YDJ1</i>	No
SL769	<i>CPR7</i>	Yes (Duina et al. 1996)
SL42	<i>UTP21</i>	No
SL182	<i>SSL2</i>	No