

An Essential Role for the Substrate-binding Region of Hsp40s in *Saccharomyces cerevisiae*

Jill L. Johnson and Elizabeth A. Craig

Department of Biomolecular Chemistry, University of Wisconsin–Madison, Madison, Wisconsin 53706

Abstract. In addition to regulating the ATPase cycle of Hsp70, a second critical role of Hsp40s has been proposed based on in vitro studies: binding to denatured protein substrates, followed by their presentation to Hsp70 for folding. However, the biological importance of this model is challenged by the fact that deletion of the substrate-binding domain of either of the two major Hsp40s of the yeast cytosol, Ydj1 and Sis1, leads to no severe defects, as long as regions necessary for Hsp70 interaction are retained. As an in vivo test of this model, requirements for viability were examined in a strain

having deletions of both Hsp40 genes. Despite limited sequence similarity, the substrate-binding domain of either Sis1 or Ydj1 allowed cell growth, indicating they share overlapping essential functions. Furthermore, the substrate-binding domain must function in cis with a functional Hsp70-interacting domain. We conclude that the ability of cytosolic Hsp40s to bind unfolded protein substrates is an essential function in vivo.

Key words: Ydj1 • Sis1 • molecular chaperone • heat shock protein 70 • yeast

Introduction

Hsp70s and Hsp40s cooperate in a wide range of cellular processes, including protein translation, translocation across membranes, and folding. Hsp70s bind unfolded or partially unfolded polypeptides in an ATP-regulated cycle (Hartl, 1996; Bukau and Horwich, 1998; Mayer et al., 2000). Hsp40s stimulate ATP hydrolysis, promoting the ADP-bound form of Hsp70, which has a higher affinity for unfolded protein substrates. In addition, many Hsp40s also bind unfolded polypeptide substrates and are able to prevent their aggregation independently of Hsp70 action. Based on the results of several in vitro studies, the current model of the cycle of Hsp70 and Hsp40 purports that Hsp40 first binds unfolded protein substrates and then transfers them to Hsp70 for folding (Langer et al., 1992; Liberek et al., 1995; Laufen et al., 1999).

Hsp40s are modular proteins. The signature J domain binds the ATPase domain of Hsp70 and is required for stimulating ATP hydrolysis. In class I and II Hsp40s, the J domain is followed by a region rich in glycine and phenylalanine residues (G/F)¹ (Cheetham and Caplan, 1998).

Class I Hsp40s are distinguished by a cysteine-rich region that has been implicated in substrate binding (Banecki et al., 1996; Szabo et al., 1996). In addition, class I and II Hsp40s contain a poorly conserved COOH terminus that also has been implicated in binding unfolded polypeptides (Lu and Cyr, 1998a,b).

Multiple Hsp40s exist in both prokaryotic and eukaryotic cells. Here, we focus on two cytosolic Hsp40 chaperones of *Saccharomyces cerevisiae*, Ydj1 and Sis1 (see Fig. 1) (Caplan and Douglas, 1991; Luke et al., 1991). Lack of Ydj1, a class I Hsp40, results in a severe growth phenotype, especially at higher temperatures. Sis1 is an essential type II Hsp40. Some functional overlap exists between these two Hsp40s, as overexpression of *SIS1* can partially rescue the growth defect of a *ydj1* disruption strain. However, overexpression of *YDJ1* does not restore viability to an *sis1* disruption strain. Each of these Hsp40s is capable of stimulating the ATPase activity of the abundant essential cytosolic Hsp70, Ssa, and can cooperate with Ssa in refolding denatured luciferase (Lu and Cyr, 1998a,b).

Both Ydj1 and Sis1 can bind unfolded polypeptides. A proteolytic fragment of Ydj1 encompassing amino acids (a.a.) 179–384, which contains half of the cysteine-rich region and most of the COOH terminus, has been shown to bind denatured substrates in vitro (Lu and Cyr, 1998a). The region of Sis1 responsible for binding denatured substrates also maps to the COOH terminus (a.a. 171–352)

Address correspondence to Elizabeth Craig, Department of Biomolecular Chemistry, University of Wisconsin–Madison, 1300 University Ave., Madison, WI 53706. Tel.: (608) 263-7105. Fax: (608) 262-5253. E-mail: ecraig@facstaff.wisc.edu

¹Abbreviations used in this paper: 5-FOA, 5-fluoroorotic acid; a.a., amino acids; ALA, δ -aminolevulinic acid; G/F, glycine and phenylalanine residues.

(see Fig. 1 A), (Lu and Cyr, 1998b). The recent crystal structure of this fragment revealed two similar domains, domain I and domain II, as well as a dimerization domain. Domain I contains a large hydrophobic depression and thus is proposed to contain the peptide-binding site (Sha et al., 2000).

Although it has been demonstrated that Ydj1 and Sis1 can bind substrates *in vitro*, the importance of this function *in vivo* is not clear. In a *ydj1* disruption strain, expression of the first 134 a.a. of Ydj1, containing the J domain, G/F region, and a short spacer region, restores wild-type growth at the optimal growth temperature of 30°C (Johnson and Craig, 2000). Likewise, in a *sis1* disruption strain, a strain expressing only the J plus G/F region of Sis1 (Sis1-121) grows as well as a wild-type strain at 30°C and exhibits only a mild temperature-sensitive growth phenotype (Yan and Craig, 1999). These results suggest that the ability of either Ydj1 or Sis1 to bind substrate is unnecessary. Therefore, we set out to critically test the requirements for Hsp40 function *in vivo*, using the cytosol of yeast as a test. Using a strain having chromosomal deletions of both *YDJ1* and *SIS1*, we found that the COOH terminus of either Ydj1 or Sis1 must be present for viability and thus, despite limited sequence homology, share overlapping essential functions.

Materials and Methods

Yeast Strains

Strain JJ160 (*ydj1::HIS3*) (Johnson and Craig, 2000) was crossed to the isogenic strain WY26 (*sis1::LEU2*) (Yan and Craig, 1999) to yield JJ1146 (*MAT a trp1-1 ura3-1 leu2-3,112 his3-11,15 ade2-1 can1-100 met2-Δ1 lys2-Δ2 ydj1::HIS3 sis1::LEU2*)/pYW17(*SIS1-YCp50*). Strain JJ261 (*MATα ade1-100 Δtrp1 ura3-52 leu2-3,112 ydj1::HIS3 Δ hem1*) was constructed by crossing the nonisogenic strains MH1 (*MAT a, ura3-52 leu2-112 his4-519 ade1-100 Δ hem1*) (Haldi and Guarente, 1989) and the *ydj1::HIS3* disruption strain JJ257 (Johnson and Craig, 2000).

Plasmids and Additional Methods

Wild-type or mutant *YDJ1* was supplied on a low copy *LYS2* plasmid (Sikorski and Boeke, 1991). Wild-type or mutant *SIS1* was supplied on a low copy *TRP1* plasmid (Yan and Craig, 1999). After transformation of indicated plasmids into strain JJ1146, colonies were grown in media containing 5-fluoroorotic acid (5-FOA) (USBiological) to counterselect for *Ycp50-SIS1*. YYS fusions (J plus G/F of Ydj1 fused to the G/M rich region and portions of the COOH terminus of Sis1) have been described (Yan and Craig, 1999). The *sis1-253* truncation mutant was selected in a genetic screen to isolate *sis1* mutants that failed to grow in the absence of the COOH terminus of Ydj1. Sis1-253 levels expressed from a low copy vector were low (data not shown), thus this mutant was expressed on a multicopy vector in these studies. The COOH terminus of Sis1 (a.a. 170–352) was expressed under the control of the GPD promoter in p414GPD (Yan and Craig, 1999) and moved to pRS317 as a Sac1-Sal1 fragment. Levels of Sis170–352 expressed from this plasmid were similar to wild-type Sis1 levels (data not shown). Plasmid Ycplac22-N134/121 was constructed to co-express *ydj1-N134* and *sis1-121* from the same low copy plasmid (Gietz and Sugino, 1988). Western blot analysis of Sis1 proteins using a polyclonal antibody raised against full-length Sis1 was performed as described (Yan and Craig, 1999).

Hap1 Activity in *ydj1* Mutant Strains

To assay Hap1 activity, strain JJ261 expressing wild-type *YDJ1*, *ydj1-104*, or the indicated Ydj1–Sis1 fusion protein was transformed with a *UAS1/CYC1-lacZ* reporter plasmid (Guarente et al., 1984). After overnight incubation at 25°C in the presence of high concentrations of δ-aminolevulinic acid (ALA) (250 μg/ml), β-galactosidase assays were performed as described (Johnson and Craig, 2000).

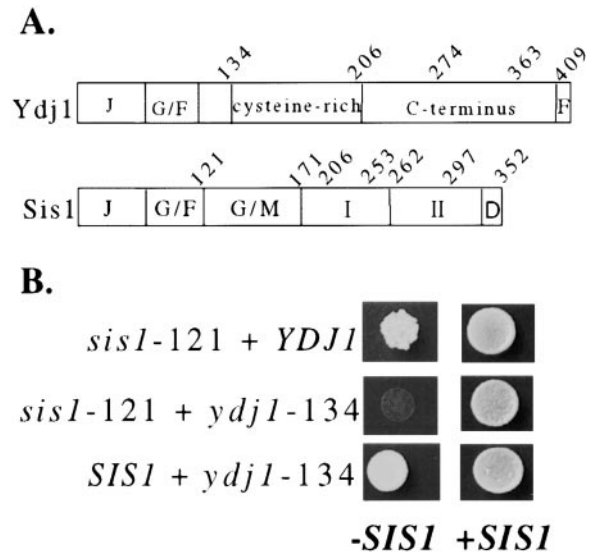


Figure 1. The COOH terminus of either Sis1 or Ydj1 is essential. (A) Domains of Ydj1 and Sis1. Ydj1: J domain (J), G/F region (G/F), cysteine-rich region (cysteine-rich), COOH terminus (C-terminus), and farnesylation signal (F). Sis1: J domain (J), G/F region (G/F), G/M region (G/M), domains I (I) and II (II) of the COOH terminus, and dimerization domain (D). (B) Strain JJ1146 (*sis1 ydj1/YCp50:SIS1*) was transformed with indicated combinations of plasmids containing wild-type *YDJ1* (or *SIS1*) and truncation mutants *ydj1-134* (or *sis1-121*). After overnight incubation at 23°C, equal amounts of cells were plated onto selective media to maintain wild-type *SIS1* (+*SIS1*) or media containing 5-FOA to counterselect for *SIS1* (–*SIS1*). Plates were incubated at 23°C for 5 d.

Results and Discussion

The COOH Terminus of either Ydj1 or Sis1 Is Essential for Viability

The nonessentiality of the substrate-binding regions of Sis1 and Ydj1 *in vivo* contrasts with the proposed critical role of Hsp40s in binding denatured substrates before presentation of those substrates to Hsp70. Two possibilities come to mind. Either binding of unfolded protein substrates is not critical for Hsp40 function in the cytosol, or there is significant functional overlap between Sis1 and Ydj1, and either protein can carry out this function.

To test these ideas, we created a strain that contains chromosomal disruptions of both *YDJ1* and *SIS1*. Using combinations of plasmids expressing mutant and wild-type *YDJ1* and *SIS1* genes, we tested the importance of the COOH-terminal regions of these Hsp40s while maintaining the J plus G/F regions required for interaction with Hsp70. We constructed an *sis1 ydj1* strain that carries a wild-type copy of *SIS1* on a plasmid harboring *URA3* to allow viability. To test for the ability of introduced genes to allow cell growth, transformants were spotted onto plates containing 5-fluoroorotic acid (5-FOA) to select for cells having lost the *URA3*-based plasmid containing *SIS1*. As expected from previous results (Yan and Craig, 1999), a strain carrying a combination of wild-type *YDJ1* and *sis1-121*, encoding the J plus G/F regions, was viable (Fig. 1 B). Similarly, the combination of wild-type *SIS1* plus *ydj1-134*, encoding the J

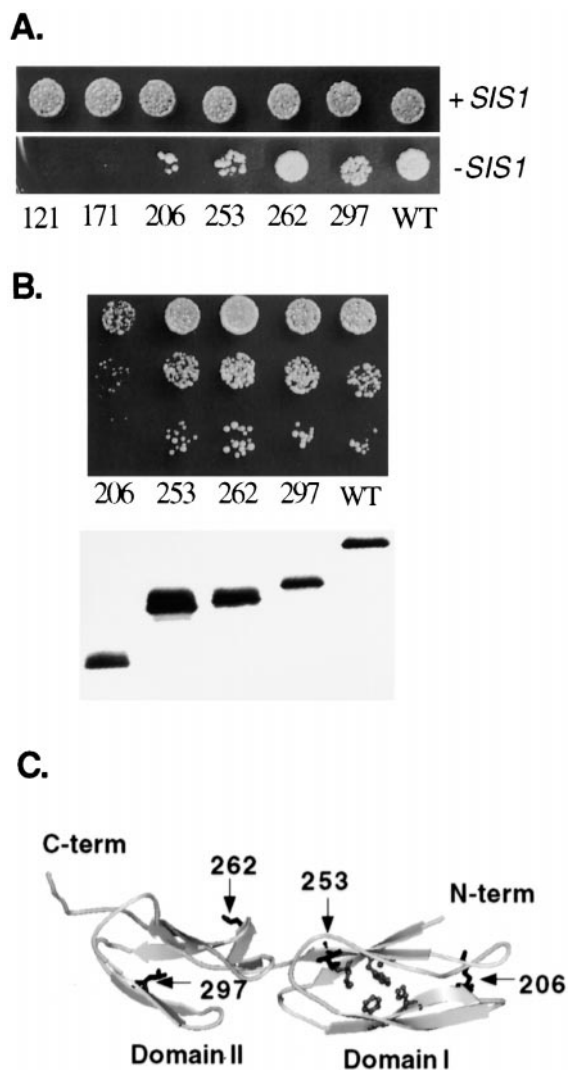


Figure 2. Essential region of the COOH terminus of Sis1. Strain JJ1146 (*ydj1 sis1/YCp50:SIS1*) expressing *ydj1-134* was transformed with wild-type *SIS1* or *sis1* mutants expressing Sis1 fragments of increasing length. (A) After overnight incubation at 23°C, equal amounts of cells were plated onto selective media to maintain wild-type *SIS1* (+*SIS1*) or media containing 5-FOA to counterselect for *SIS1* (-*SIS1*). Plates were grown at 23°C for 5 d. (B) Colonies that appeared on 5-FOA plates were grown overnight, and serial dilutions were grown at 30°C for 2 d on selective media (top). Yeast extracts expressing wild-type or mutant Sis1 proteins were separated on a 15% SDS-acrylamide gel, transferred to nitrocellulose, and immunoblotted with an antibody against Sis1 (bottom). (C) Structure of substrate-binding region of Sis1. Sites of truncation mutants are labeled and marked with arrows. Shaded residues are postulated to interact with bound substrate (Sha et al., 2000). Image was generated using MSI WebLab™ ViewerPro. C-term, COOH terminus; N-term, NH₂ terminus; WT, wild type.

plus G/F plus spacer regions, was viable (Johnson and Craig, 2000). However, the combination of *sis1-121* plus *ydj1-134* did not support viability, even though the regions necessary for Hsp70 interaction were retained. These results indicate that the COOH-terminal substrate-binding region of either Ydj1 or Sis1 is required *in vivo*, suggesting that Ydj1 and Sis1 may perform overlapping functions in the cell.

Localization of the Essential Region in the COOH Terminus of Sis1

Substrate binding is likely the essential function of the COOH terminus of Sis1, as a proteolytic fragment encompassing a.a. 171–352 is capable of binding denatured substrates *in vitro* (Lu and Cyr, 1998b). Recently, the crystal structure of this fragment revealed three domains (Fig. 2 C). Domains I and II have similar folds. However, domain I contains a prominent hydrophobic depression and thus was proposed to contain the substrate-binding site. In addition, the extreme COOH terminus (a.a. 338–352) is a dimerization domain (Sha et al., 2000).

To determine which sequences in the COOH-terminal fragment of Sis1 are essential in the absence of the COOH terminus of Ydj1, *SIS1* truncation mutants were transformed into the *sis1 ydj1* strain expressing *ydj1-134*. When transformants were spotted onto plates containing 5-FOA, colonies appeared in strains expressing *sis1-206*, -253, -262, -297, and wild type (Fig. 2 A). However, when cells picked from these colonies were tested more carefully (Fig. 2 B, upper panel), it became apparent that cells expressing Sis1-206 grew slowly at 30°C, even though this truncation is expressed at wild-type levels (Fig. 2 B, lower panel). Cells expressing Sis1-253, -262, or -297 exhibited wild-type growth rates.

These results clearly indicate that domain II (a.a. 260–336) and the dimerization region (a.a. 338–352) of Sis1 are not required for normal growth at 30°C even in the absence of the COOH terminus of Ydj1. However, domain I (a.a. 180–255) is required, as *sis1-206* cells grow very slowly, and *sis1-171* cells are inviable in this assay. Sha et al. (2000) proposed that, within domain I, residues V184 and L186 from β strand 1, F201, and I203 from β strand 2, and F251 from β strand 5 interact with bound substrate (Fig. 2 C), however, the role of these residues in substrate interaction has not yet been established. Sis1-262 and Sis1-253 contain these residues, whereas Sis1-206 truncates after β strand 2 and thus lacks F251 and β sheets that are probably important in stabilizing the domain. Our results suggest that Sis1-262 contains a functional substrate-binding domain. However, at this time, the only available *in vitro* assay of Sis1 chaperone function measures the ability to bind and refold denatured luciferase in cooperation with Hsp70 (Sha et al., 2000). Since activity in these *in vitro* assays is dependent on the presence of the dimerization domain of Sis1, we are unable to monitor the *in vitro* substrate-binding activity of Sis1-262 in the absence of domain II and the dimerization domain. However, our genetic results demonstrate a critical role of domain I in the absence of the COOH terminus of Ydj1 and are consistent with that role being substrate binding.

Localization of the Essential Regions in the COOH Terminus of Ydj1

Having found the region of Sis1 that is critical in the absence of the COOH terminus of Ydj1, we asked the same question of Ydj1: what portion of Ydj1 is essential in the absence of the COOH terminus of Sis1? The substrate-binding region of Ydj1 has been localized between a.a. 179 and 384 of Ydj1 (Lu and Cyr, 1998a), a fragment that contains part of the cysteine-rich region and most of the COOH terminus (Fig. 1 A). The extreme COOH terminus

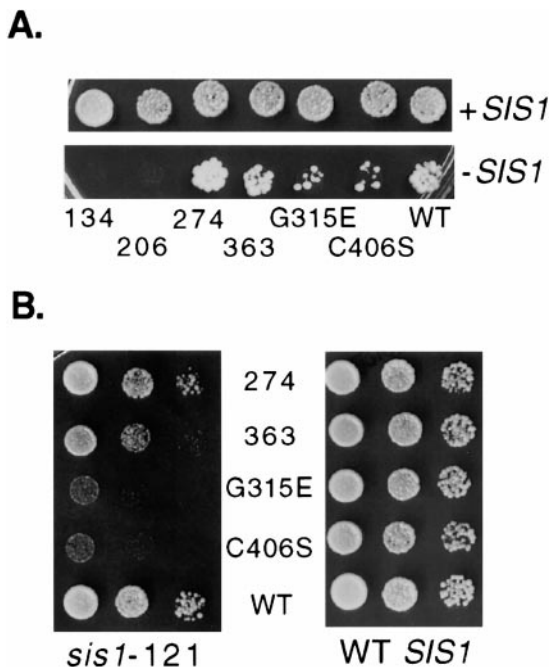


Figure 3. Essential region of the COOH terminus of Ydj1. Strain JJ1146 (*ydj1 sis1/YCp50:SIS1*) expressing *sis1-121* was transformed with wild-type *YDJ1* or the indicated *ydj1* mutants. (A) After overnight incubation at 23°C, equal amounts of cells were plated onto selective media to maintain wild-type *SIS1* (+*SIS1*) or media containing 5-FOA to counterselect for *SIS1* (–*SIS1*). Plates were grown at 23°C for 5 d. (B) Colonies that appeared on 5-FOA plate (*sis1-121*) were grown overnight and serial dilutions were grown at 30°C for 2 d on selective media after 2 d at 30°C in a *ydj1* disruption strain (WT *SIS1*) (right). WT, wild type.

of Ydj1 contains the CAAX box, the site of a farnesylation modification thought to be involved in localization of Ydj1 to membranes (Caplan et al., 1992b). Truncation and point mutants of *YDJ1*, picked because they disrupt function of these regions, were transformed into the *sis1 ydj1* strain that carried wild-type *SIS1* on a *URA3*-based plasmid and also expressed *sis1-121*. The shortest truncation that allowed growth in the absence of full-length *Sis1* was *ydj1-274*, which contains not only the J plus G/F region, but also the cysteine-rich region and 68 a.a. of the COOH terminus (Fig 3 A). When retested for growth at 30°C, it was clear that *ydj1-274* allowed cells to grow nearly as well as wild-type *YDJ1*.

These results are consistent with substrate binding being a critical role of the region distal to the J plus G/F. Mutant DnaJ proteins having alterations in the cysteine-rich region have shown defects in suppression of aggregation of rhodanese and binding to some, but not all, protein substrates (Banecki et al., 1996; Szabo et al., 1996). Therefore, the cysteine-rich region is implicated in binding of at least some protein substrates. However, a fragment containing only the J plus G/F plus cysteine-rich region of Ydj1 (*ydj1-206*) did not support viability when *sis1-121* was the only copy of *SIS1* present. This result suggests that, in addition to the cysteine-rich region, the first 68 a.a. of the COOH terminus are required for the ability to bind substrates in vivo. Thus, our results are in agreement with studies in

which a fragment of Ydj1 encompassing a.a. 179–384 bound denatured substrate (Lu and Cyr, 1998a).

Other mutants that could be recovered on 5-FOA did not grow as well as *ydj1-274* (Fig. 3 B). Although sequences required for substrate binding or other sequences important in allowing proper transient interactions with substrates have not been clearly established, the amino acid substitution G315D led to in vivo defects in binding of glucocorticoid receptor (Kimura et al., 1995). In vitro, G315D was found to be defective both in suppressing rhodanase aggregation and refolding denatured luciferase (Lu and Cyr, 1998a). Consistent with a role in substrate interaction, an amino acid substitution at the same residue, *ydj1-G315E*, exhibited severe growth defects when *sis1-121* was the only *SIS1* gene present (Fig. 3 B). Although the most straightforward interpretation of these results is that G315 and surrounding residues are important for substrate binding, it is also possible that, in light of the robust growth of *ydj1-274*, COOH-terminal sequences can act negatively on more proximal residues, inhibiting their ability to function. Consistent with this idea, the truncation mutant *ydj1-363*, which results in removal of fewer residues from the COOH terminus than *ydj1-274*, and *ydj1-C406S*, which alters the farnesylation signal, grow poorly in the presence of *sis1-121* compared with the truncation *ydj1-274*. The C406S mutation has been shown to result in a decrease in the membrane association of Ydj1 (Caplan et al., 1992b). The associated temperature-sensitive growth defect was attributed to this alteration in cellular localization. However, in other proteins, the farnesylation modification has been shown to not only affect cellular localization but also protein–protein interactions (Sinensky, 2000); so it is possible that farnesylation has additional effects on Ydj1 function. Further studies will be required to determine how the farnesylation modification of Ydj1 affects in vivo chaperone activity.

The COOH Terminus of *Sis1* Can Substitute for the COOH Terminus of Ydj1 In Vivo, but Only in Cis

The results presented above indicate that substrate binding is critical for Hsp40 function. In the current model of interaction between substrates, Hsp40 and Hsp70, substrate first bind the COOH terminus of Hsp40 and then are targeted to Hsp70 via interaction of the J domain with the ATPase domain of Hsp70 (Langer et al., 1992; Liberek et al., 1995; Laufen et al., 1999). The J domain of both Ydj1 and *Sis1* have been shown to be important in vivo, as a single amino acid change in either J domain has severe effects on function. In a *ydj1* disruption strain, an F47L alteration of Ydj1 causes both severe growth defects and defects in the maturation of Hsp90 substrates (Johnson and Craig, 2000). The H34Q mutation of *Sis1* disrupts the HPD motif in the J domain and causes a null phenotype in an *sis1* disruption strain (Yan and Craig, 1999).

We conducted in vivo tests to determine whether a functional J domain and COOH-terminal substrate-binding domain must be part of the same protein (Fig. 4). Growth of an *sis1 ydj1* strain expressing *sis1-121* and *ydj1-134* could not be rescued by either H34Q *Sis1* or F47L Ydj1. Nor did the COOH-terminal fragment of *Sis1* (a.a. 170–352) expressed in trans with J plus G/F allow viability. These results indicate that the COOH terminus of *Sis1*

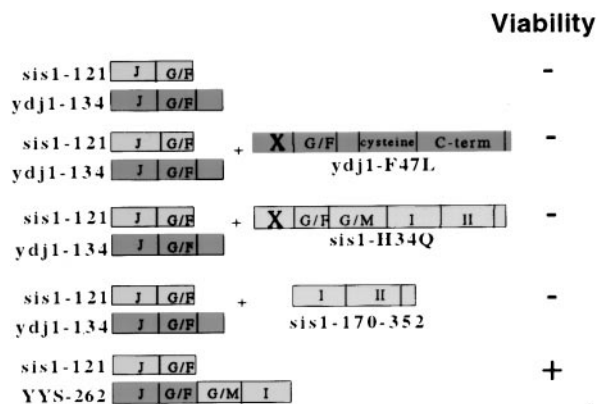


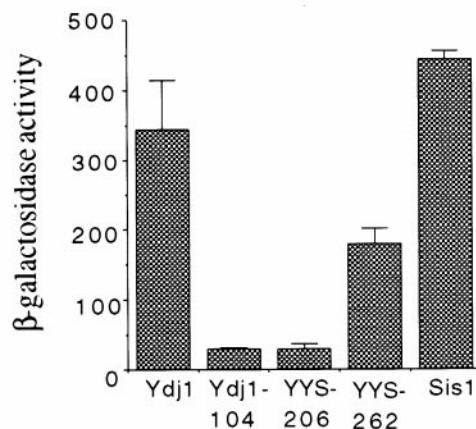
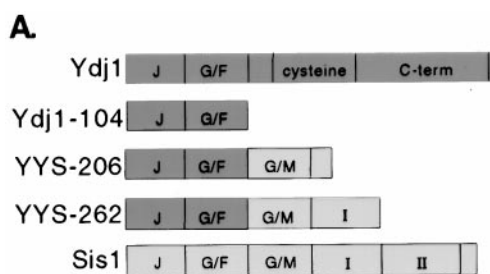
Figure 4. The COOH terminus must cooperate with a functional J domain in cis. Strain JJ1146 (*ydj1 sis1/YCp50:SIS1*) expressing *sis1-121* and *ydj1-134* from Ycplac22-134/121 was transformed with plasmids expressing the indicated *sis1* or *ydj1* mutant. Viability was assayed by plating cells in the presence of 5-FOA to counterselect for wild-type *SIS1*.

must be present in the context of a wild-type J domain to function, indicating that close cooperation between the two domains is essential for function in vivo. However, in support of the idea that the COOH-terminal regions can substitute for one another, a fusion protein (YYS-262) that joins the G/M region and domain I of *Sis1* to the J plus G/F of *Ydj1* (Yan and Craig, 1999) allows viability (Fig. 4).

YYS Fusion Proteins Partially Rescue Hap1 Defect In Vivo

Sis1 and *Ydj1* have important functions in the cell, but few of their specific in vivo roles are known. Little is known about *Sis1* function, although it has been reported to be required for the initiation of translation (Zhong and Arndt, 1993). *Ydj1* cooperates with the Hsp70 *Ssa* in the translocation of preproteins into the ER and mitochondria (Caplan et al., 1992a; Becker et al., 1996). In addition, *Ydj1* has been shown to be required for the in vivo maturation of the heterologous proteins v-src and steroid receptors via the Hsp90 pathway (Kimura et al., 1995; Fliss et al., 1999; Johnson and Craig, 2000), but native substrates have been elusive. However, recent evidence suggests that the transcription factor Hap1, which regulates the expression of *CYC1* and other genes encoding respiratory functions in a heme-dependent manner (Zitomer and Lowry, 1992), is such a substrate. When heme levels are low, *Ydj1* and the yeast Hsp90, Hsp82, bind Hap1, and Hap1 activity is low. When heme levels increase, *Ydj1* and Hsp82 dissociate from Hap1, and its transcriptional activity increases. Hsp82 is believed to play a role in the activation of Hap1, as decreased levels of Hsp82 led to a large decrease in the overall activity of a Hap1 reporter gene construct without affecting Hap1 levels (Zhang et al., 1998).

To determine whether *Ydj1* also plays a role in the activation of Hap1, we examined Hap1 activity in *ydj1* mutant strains containing a *hem1* mutation, which allowed manipulation of heme levels. Varying amounts of the heme precursor, ALA, were added to the media to maintain high or low levels of heme. Hap1 activity was monitored by mea-



B.

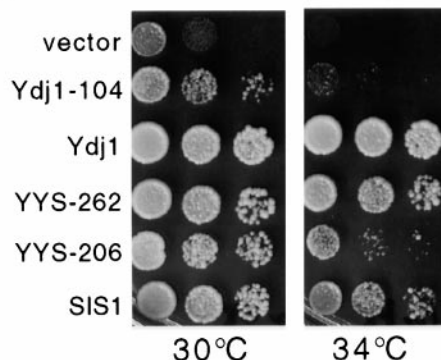


Figure 5. Partial rescue of *ydj1* defects by YYS-262. (A) Hap1 was assayed in the *ydj1 hem1* disruption strain JJ261 expressing full-length *Ydj1*, *Ydj1-104*, YYS-206, YYS-262, or overexpressing wild-type *SIS1*. β-Galactosidase activity of the UAS1/*CYC1-lacZ* reporter construct was measured in cells after overnight incubation at 25°C in the presence of high concentrations of ALA (250 μg/ml). (B) The *ydj1* disruption strain JJ160 was transformed with plasmids expressing the indicated proteins. After overnight incubation at 25°C, 10-fold serial dilutions of cells were plated on rich media. Plates were grown for 2 d at the indicated temperatures.

suring transcription from the UAS1 of the *CYC1* promoter linked to the *lacZ* gene (Guarente et al., 1984). In low levels of ALA (4 μg/ml), strains expressing mutant or wild-type *YDJ1* had similarly low levels of β-galactosidase activity, 1–4 U (data not shown). In the presence of high concentrations of ALA (250 μg/ml), cells expressing wild-type *YDJ1* had dramatically higher activity, 343 U. In contrast, cells expressing the J plus G/F region of *Ydj1* (*ydj1-104*) had only 30 U of activity (Fig. 5 A), indicating that

deletion of the substrate-binding region of Ydj1 causes an activation defect of Hap1, resulting in >10% of the activity observed in wild-type cells.

Since the substrate-binding region of Ydj1 is required for activity of Hap1, we were able to directly test whether the substrate-binding region of Sis1 is able to replace Ydj1 in this function. For this, we used fusion proteins that join the J plus G/F of Ydj1 to the G/M region and domain I of Sis1 (Fig. 5 A). In the absence of *ydj1*, expression of *SIS1* from a high copy plasmid restored wild-type levels of Hap1 activity. In addition, expression of YYS-262 partially restored Hap1 activity (180 β -galactosidase U), whereas cells expressing YYS-206 exhibited the same level of Hap1 activity as *ydj1*-104. Thus, YYS-262 is able to substantially rescue a specific defect, that is loss of Hap1 activity that arises in the absence of the substrate-binding region of Ydj1.

To determine if YYS-262 could more generally carry out Ydj1 functions, we tested its effect on growth of a *ydj1* strain at 30°C and 34°C. As previously reported, the *ydj1*-104 construct partially rescues growth at 30°C but exhibits a severe temperature-sensitive phenotype (Johnson and Craig, 2000). YYS-262 allowed near wild-type levels of growth at 30°C and 34°C (Fig. 5 A), but not 37°C (data not shown). YYS-206 could not support growth at 34°C, supporting evidence from Fig. 2 that this truncation of domain I is not fully functional. Thus, in addition to rescuing the Hap1 defect, domain I of the Sis1 COOH terminus can rescue the growth defect that arises in the absence of the substrate-binding region of Ydj1.

Summary

The COOH termini of Sis1 and Ydj1 share overlapping essential roles. In addition, the J domain of Ydj1 may substitute for the J domain of Sis1 (Yan and Craig, 1999). In contrast, the essential function of the G/F region of Sis1 cannot be replaced by the G/F region of Ydj1 (Yan and Craig, 1999), indicating a unique role of the Sis1 G/F region. Although the substrate binding domains of Sis1 and Ydj1 can substitute for one another under some conditions, they share only 29% sequence identity, compared with 66% sequence identity between the J domains. This difference likely results in some differences in substrate specificity, which may be responsible for the temperature-sensitive phenotype observed in the absence of the substrate-binding region of either Ydj1 or Sis1. Further studies will be needed to understand the specificity of Hsp40 interactions with Hsp70.

We thank L. Guarente for strain MH1 and the UAS1/*CYCI-lacZ* plasmid, and Avrom Caplan (Mount Sinai School of Medicine, New York, NY) for the *ydj1*-C406S mutant. In addition we thank Chris Pfund and Nelson Lopez for critical reading of this manuscript and helpful advice.

This work was supported by National Research Service Award 5F32 GM17139 (to J.L. Johnson) and National Institutes of Health grant 5RO1 GM31107 (to E.A. Craig).

Submitted: 12 December 2000

Revised: 12 December 2000

Accepted: 4 January 2001

References

- Banecki, B., K. Liberek, D. Wall, A. Wawrzynow, C. Georgopoulos, E. Bertoli, F. Tanfani, and M. Zylicz. 1996. Structure-function analysis of the zinc finger region of the DnaJ molecular chaperone. *J. Biol. Chem.* 271:14840–14848.
- Becker, J., W. Walter, W. Yan, and E.A. Craig. 1996. Functional interaction of cytosolic Hsp70 and DnaJ-related protein, Ydj1p, in protein translocation in vivo. *Mol. Cell. Biol.* 16:4378–4386.
- Bukau, B., and A.L. Horwich. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell.* 92:351–366.
- Caplan, A.J., and M.G. Douglas. 1991. Characterization of *YDJ1*: a yeast homologue of the bacterial dnaJ protein. *J. Cell Biol.* 114:609–621.
- Caplan, A.J., D.M. Cyr, and M.G. Douglas. 1992a. YDJ1p facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. *Cell.* 71:1143–1155.
- Caplan, A.J., J. Tsai, P.J. Casey, and M.G. Douglas. 1992b. Farnesylation of YDJ1p is required for function at elevated growth temperatures in *S. cerevisiae*. *J. Biol. Chem.* 267:18890–18895.
- Cheetham, M.E., and A.J. Caplan. 1998. Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chaperones.* 3:28–36.
- Fliss, A.E., J. Rao, M. Melville, M.E. Cheetham, and A.J. Caplan. 1999. Domain requirements of DnaJ-like (Hsp40) molecular chaperones in the activation of a steroid hormone receptor. *J. Biol. Chem.* 274:34045–34052.
- Gietz, R.D., and A. Sugino. 1988. New yeast-Escherichia coli shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. *Gene.* 74:527–534.
- Guarente, L., B. Lalonde, P. Gifford, and E. Alani. 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYCI* gene of *S. cerevisiae*. *Cell.* 36:503–511.
- Haldi, M., and L. Guarente. 1989. N-terminal deletions of a mitochondrial signal sequence in yeast. Targeting information of delta-aminolevulinate synthase is encoded in non-overlapping regions. *J. Biol. Chem.* 264:17107–17112.
- Hartl, F.U. 1996. Molecular chaperones in cellular protein folding. *Nature.* 381: 571–580.
- Johnson, J.L., and E.A. Craig. 2000. A role for the Hsp40 Ydj1 in repression of basal steroid receptor activity in yeast. *Mol. Cell. Biol.* 20:3027–3036.
- Kimura, Y., I. Yahara, and S. Lindquist. 1995. Role of the protein chaperone YDJ1 in establishing Hsp90-mediated signal transduction pathways. *Science.* 268:1362–1365.
- Langer, T., C. Lu, H. Echols, J. Flanagan, M.K. Hayer, and F.U. Hartl. 1992. Successive action of DnaK, DnaJ, and GroEL along the pathway of chaperone-mediated protein folding. *Nature.* 356:683–689.
- Laufen, T., M. Mayer, C. Beisel, D. Klostermeier, A. Mogk, J. Reinstein, and B. Bukau. 1999. Mechanism of regulation of Hsp70 chaperones by DnaJ co-chaperones. *Proc. Natl. Acad. Sci. USA.* 96:5452–5457.
- Liberek, K., D. Wall, and C. Georgopoulos. 1995. The DnaJ chaperone catalytically activates the DnaK chaperone to preferentially bind the sigma 32 heat shock transcriptional regulator. *Proc. Natl. Acad. Sci. USA.* 92:6224–6228.
- Lu, Z., and D.M. Cyr. 1998a. The conserved carboxyl terminus and zinc finger-like domain of the co-chaperone Ydj1 assist Hsp70 in protein folding. *J. Biol. Chem.* 273:5970–5978.
- Lu, Z., and D.M. Cyr. 1998b. Protein folding activity of Hsp70 is modified differentially by the Hsp40 co-chaperones Sis1 and Ydj1. *J. Biol. Chem.* 273: 27824–27830.
- Luke, M., A. Suttin, and K. Arndt. 1991. Characterization of *SIS1*, a *Saccharomyces cerevisiae* homologue of bacterial dnaJ proteins. *J. Cell Biol.* 114:623–638.
- Mayer, M.P., H. Schroder, S. Rudiger, K. Paal, T. Laufen, and B. Bukau. 2000. Multistep mechanism of substrate binding determines chaperone activity of Hsp70. *Nat. Struct. Biol.* 7:586–593.
- Sha, B., S. Lee, and D.M. Cyr. 2000. The crystal structure of the peptide-binding fragment from the yeast Hsp40 protein Sis1. *Struct. Fold. Des.* 8:799–807.
- Sikorski, R.S., and J.D. Boeke. 1991. In vitro mutagenesis and plasmid shuffling: from cloned gene to mutant yeast. *Methods Enzymol.* 194:302–318.
- Sinensky, M. 2000. Recent advances in the study of prenylated proteins. *Biochim. Biophys. Acta.* 1484:93–106.
- Szabo, A., R. Korszun, F.U. Hartl, and J. Flanagan. 1996. A zinc finger-like domain of the molecular chaperone DnaJ is involved in binding to denatured protein substrates. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:408–417.
- Yan, W., and E.A. Craig. 1999. The glycine-phenylalanine-rich region determines the specificity of the yeast Hsp40 Sis1. *Mol. Cell. Biol.* 19:7751–7758.
- Zhang, L., A. Hach, and C. Wang. 1998. Molecular mechanism governing heme signaling in yeast: a higher-order complex mediates heme regulation of the transcriptional activator HAP1. *Mol. Cell. Biol.* 18:3819–3828.
- Zhong, T., and K.T. Arndt. 1993. The yeast *SIS1* protein, a DnaJ homolog, is required for initiation of translation. *Cell.* 73:1175–1186.
- Zitomer, R.S., and C.V. Lowry. 1992. Regulation of gene expression by oxygen in *Saccharomyces cerevisiae*. *Microbiol. Rev.* 56:1–11.