The Type I Hsp40 Zinc Finger-like Region Is Required for Hsp70 to Capture Non-native Polypeptides from Ydj1*

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The cytosolic yeast Hsp40 Ydj1 contains a conserved zinc finger-like region (ZFLR), which has two zinc-binding domains (ZBD), that helps regulate and specify Hsp70 function. To investigate the mechanism for Ydj1 ZFLR action, ZBDI and ZBDII mutants were constructed and characterized. ZBDII mutants exhibited temperature-sensitive growth defects, but yeast tolerated mutation of ZBDI. However, ZBDI and ZBDII mutants were defective at facilitating androgen receptor (AR) folding. Defective AR folding was associated with the accumulation of complexes between AR and Ydj1 ZFLR mutants and a reduction in Hsp70-AR complex formation. Purified Ydj1 ZBDI and ZBDII mutants could bind non-native polypeptides but could not deliver luciferase to Hsp70 and were defective at luciferase refolding. Interestingly, the ability of Ydj1 to synergize with Hsp70 to suppress thermally induced protein aggregation was blocked by mutation of ZBDII, but not ZBDI. Hence, ZBDII is required for yeast to survive heat stress because it is essential for Ydj1 to cooperate with Hsp70 to suppress protein aggregation. On the other hand, protein folding is dependent upon the action of both ZBDI and ZBDII because each is required for Hsp70 to capture non-native polypeptides from Ydj1.

Members of the heat shock protein (Hsp) family function with different Hsp40 co-chaperones to facilitate essential aspects of protein metabolism that include ribosome assembly, protein translocation, protein folding, suppression of polypeptide aggregation, β-amyloid fibril assembly, and cell signaling (1–3). The yeast Type I Hsp40 Ydj1 and Type II Hsp40 Sis1 cooperate with cytosolic heat shock protein 70 Ssa1 to facilitate different aspects of cellular protein metabolism (4–8). Study of Ydj1 and Sis1 action serves as a model system to investigate mechanisms for specification of eukaryotic Hsp70 function by Hsp40 co-chaperones (4–8).

Current models for the Hsp70 polypeptide binding and release cycle indicate that Hsp40s interact with non-native polypeptides prior to Hsp70 to prevent their aggregation (9–11). Hsp40-bound substrates are subsequently transferred to Hsp70 in a process that involves Hsp40-dependent hydrolysis of ATP by Hsp70 (3). The mechanism by which Hsp40s function to bind and transfer non-native polypeptides to Hsp70 has been the subject of several studies, but is not well defined (12, 13).

Answers to questions pertaining to the mechanism by which Hsp40s bind and deliver substrates to Hsp70 are complicated by the fact that the Hsp40 family is large and structurally diverse (2). The Hsp40 family can be divided into three different subtypes. All Hsp40s contain a 75-amino acid J domain, which contains the family signature HPD motif that interacts with the ATPase domain of Hsp70 to stimulate ATP hydrolysis (14). Ydj1 is a Type I Hsp40 that is related to Escherichia coli DnaJ and human Hdj2. Type I Hsp40s appear to function as homodimers and contain an amino-terminal J domain that is located adjacent to a glycine and phenylalanine-rich region that is followed by a zinc finger-like region (ZFLR) and conserved carboxyl-terminal domains I and II (CTDI and CTDII). Type II Hsp40s, such as human Hdj1 and yeast Sis1, contain all of the aforementioned subdomains except that ZFLR has been replaced by a glycine and methionine-rich region. Type III Hsp40s contain a J domain and other specialized domains that enable them to interact with highly specific substrates. Hsp70 family members are often co-localized in subcellular compartments with several different members of the Hsp40 family. Thus, multiple Hsp40s can interact with a single Hsp70 to generate specialized Hsp70-Hsp40 pairs that facilitate specific reactions in cellular protein metabolism.

Ydj1 and Sis1 bind non-native polypeptides and utilize this capability in the yeast cytosol to specify reactions catalyzed by Hsp70 (4–8). However, Ydj1 and Sis1 are not functionally equivalent (4, 5) and exhibit major differences in chaperone function (6). In vivo studies have demonstrated that overexpression of Sis1 complements the slow growth phenotype of ydj1Δ, but Ydj1 cannot complement the lethal phenotype of a sis1Δ (4, 5). Purified Ydj1 can function independently of Hsp70 to suppress protein aggregation (11), whereas Sis1 requires the assistance of Hsp70 to perform this function (6). In addition, the Ydj1-Hsp70 chaperone pair refolds denatured luciferase with several-fold greater efficiency than Sis1-Hsp70 (6). When the centrally located polypeptide-binding domains of Ydj1 and Sis1 are swapped to form YSY and SYS, these chimeric Hsp40s exhibit in vivo complementation activity, substrate specificity, and protein folding activity identical to that of Sis1 and Ydj1, respectively (8). In related studies the glycine and phenylalanine regions of Ydj1 and Sis1, which are not identical, were also demonstrated to influence the functional specificity of these Hsp40s (7). Thus, regions within the central portion of Ydj1 and Sis1 play a critical role in specifying the cellular functions of Hsp70 (7, 8).

The centrally located chaperone module of Ydj1 is conserved in all type I Hsp40s and is constructed from residues 102–255.

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§ The abbreviations used are: Hsp, heat shock protein; ZFLR, zinc finger-like region; CTD, carboxyl-terminal domain; ZBD, zinc-binding domain; hAR, human androgen receptor; ELISA, enzyme-like immunoassay; PBS, phosphate-buffered saline.
Regulation of Hsp70 by Hsp40

Ydj1 (102–255) contains the ZFLR and an additional \( \beta \)-sandwich domain that is capable of binding peptide substrates (15). The Ydj1 ZFLR lies adjacent to the \( \beta \)-sandwich domain, is constructed from residues 143–209, and contains 4 repeated Cys-X-X-Cys-X-Gly-X-Gly motifs that function in pairs to form zinc-binding domains (ZBDI and II). The NMR structure of a 79-residue ZFLR fragment from \( E. \) coli DnaJ depicts this domain to have a novel fold with an overall V-shaped extended \( \beta \)-hairpin topology, which is conserved in Ydj1 (15, 16). The calculated surface of the Type I Hsp40 ZFLR has a number of sites that have the potential to be involved in protein–protein interactions, but it does not contain an obvious patch of hydrophobic residues that is often found in the polypeptide binding sites of molecular chaperones (16). Nevertheless, mutations in the ZFLR reduce the chaperone activity of Type I Hsp40s, (17, 18), and amino acid residues within the Hsp40 ZFLR can be cross-linked to model protein substrates (19). These collective data suggest that the ZFLR is required for Type I Hsp40s to cooperate with Hsp70 to facilitate protein folding, but its mode of operation remains obscure.

To investigate the role that the Type I Hsp40 ZFLRs play in the regulation of Hsp70 function we characterized the functional defects exhibited by a set of Ydj1 ZFLR point mutants in which either ZBDI or ZBDII was mutated. Results from \textit{in vitro} complementation studies demonstrated that ZBDII is essential for cells to survive heat stress and is essential for viability in the absence of full-length Sis1. Mutation of the Ydj1 ZFLR led to defects in androgen receptor function that correlated with the accumulation of Ydj1–AR complexes and a corresponding decrease in Hsp70–AR complexes. Purified Ydj1 ZFLR mutants were also found to be defective in refolding chemically denatured luciferase, and this defect was pinpointed to a step in the Hsp40/Hsp70 polypeptide binding and release cycle where Ydj1-bound substrates are transferred to Hsp70. These collective data demonstrated that Type I Hsp40 ZFLR is required for Hsp70 to capture non-native polypeptide from Ydj1.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids**—The following yeast strains were utilized in this study: \textit{MYY405U} (\( \Delta \)ydj1), \textit{MATO}, \textit{leu2}, \textit{his3}, \textit{ydj1:ura3} (20); \textit{JJ1146 (\( \Delta \)yjlsis1), MATa Trp1–1 Ura3–1, 112 his3–11, 15 ade2–1 can–1 100 met2–1 lys2–2 ydj1::HIS3 sisa::LEU2;YpC50-Sis1pRS314-Sis1-l (1–121) (21); CY5701, \textit{J1146 MATa Trp1–1 Ura3–1, 112 his3–11, 15 ade2–1 can–1 100 met2–1 lys2–2 ydj1::HIS3 sis1::LEU2;YpC50-Sis1pRS314-Sis1-l (1–121) (21); CY5701, \textit{J1146 MATa Trp1–1 Ura3–1, 112 his3–11, 15 ade2–1 can–1 100 met2–1 lys2–2 ydj1::HIS3 sis1::LEU2;YpC50-Sis1pRS314-Sis1-l (1–121) (21). YDJ1 ZFLR mutant. Transformants were then cultured under selective conditions (15). The Ydj1 ZFLR mutants were subcloned into pRS412 and pRS315 (22) and pET11a (23, 24). pG1-hAR (human androgen receptor) was described previously (25).

**Cell Viability Assays**—\( \Delta \)ydj1 was transformed with pRS315 that harbored YDJ1 or the indicated YDJ1 ZFLR mutant. Transformants were selected and cultured under selection in synthetic dextrose medium for 2 days at 25 °C. Cells (0.2 \( A_{600 \text{nM}} \)) were then serially diluted (1:10), and 5 \( A_{600 \text{nM}} \) of each dilution was spotted onto a yeast extract, peptone, and dextrose (YPD) plate. YPD plates were photographed after 3 days of incubation at the indicated temperature.

\( \Delta \)sis\( \Delta \)ydj1 that harbored pYpC50-Sis1 and pRS314-Sis1 (1–121) was transformed with pRS412 that harbored YDJ1 or the indicated YDJ1 ZFLR mutant. Transformants were then cultured under selection for 2 days at 25 °C in synthetic dextrose. Then, to drive the loss of pYpC50-Sis1 from cells and make growth dependent upon the chaperone function of Ydj1, aliquots (12 \( A_{600 \text{nM}} \)) of cultures were spotted on synthetic dextrose plates that were supplemented with 5-FOA (26). After 7 days of incubation at 25 °C, plates were photographed.

**Measurement of Ydj1-dependent Binding of Denatured Luciferase by Ydj1**—To investigate the biological importance of the Ydj1 ZFLR, we characterized functional defects exhibited by a set of Ydj1 ZFLR mutants that have point mutations in cytochrome residues located in ZBDI (C143S and C201S) and ZBDII (C162S and C185S) (Fig. 1, A and B). First, we demonstrated that these Ydj1 ZFLR point mutants were prone to aggregation and could accumulate to steady-state levels that were similar to Ydj1 (Fig. 1C).

Next, we examined the importance of ZBDI and ZBDII in cell physiology by determining the ability of Ydj1 ZBDI and ZBDII mutants to support growth of ydj1Δ (Fig. 2A). Ydj1 is not essential for growth, but ydj1Δ exhibits a slow-growth phenotype at the permissive temperature and is inviable at 37 °C (31). When cells were serially diluted and spotted onto selective plates, ZBDII mutants Ydj1 C162S and Ydj1 C185S only supported slow growth at 25 °C and were inviable at 37 °C. In contrast, ZBDI mutants Ydj1 C143S and Ydj1 C201S grew normally at 25 °C and remained viable at 37 °C.

The overexpression of Sis1 can complement growth defects in ydj1Δ (31). Hence, Sis1 that is present in the cytosol of ydj1Δ may suppress some of the functional defects caused by mutation of ZBDI or ZBDII. Therefore, we examined whether or not the

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The Ydj1 ZFLR Is Required for Androgen Receptor Function—To further explore the cellular functions of the Type I Hsp40 ZFLR we examined the ability of the ZBDI mutant Ydj1 C143S and ZBDII mutant Ydj1 C162S to cooperate with Hsp70 to fold a heterologously expressed form of the hAR (25, 32, 33). Mutation of ZBDI or ZBDII did not influence the steady-state concentration of AR, yet when AR was co-expressed with Ydj1 C143S or Ydj1 C162S its ligand binding activity was reduced to a level near that observed in AR (Fig. 3A), yet when AR was co-expressed with Ydj1 ZFLR mutants to support normal AR function we analyzed the ability of the ZFLR mutants to fold AR. Interest-ingly, decreased Hsp70 binding to AR was accompanied by a decrease in AR complex formation. Interest-

function of the Ydj1 ZFLR becomes essential for cell viability when Sis1 is absent from the cytosol. This was accomplished by determination of the complementing activity of Ydj1 ZBDI and ZBDII mutants in ydj1Δsis1Δ (Fig. 2B). Sis1 is an essential gene, but ydj1Δsis1Δ can grow normally if Sis1 (1–121), which contains the J domain and glycine and phenylalanine region but lacks its polypeptide-binding domain, is co-expressed with fully functional Ydj1 (21). ydj1Δsis1Δ was not viable when the plasmid shuffle technique was utilized to exchange Ydj1 ZBDII mutants for Ydj1, but Ydj1 ZBDI mutants could maintain the viability of this strain. Thus, ZBDII in Ydj1 is required for cells to survive heat stress and becomes essential for viability when yeast are forced to depend upon Ydj1 chaperone function for growth.

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from three experiments. Results displayed represent the average permissive growth temperature and quantitated as described under "Experimental Procedures." A number of observations made from the data presented in Fig. 4 suggested that reductions in AR activity and complex formation with Hsp70 result from a specific defect in Ydj1 and is not typically utilized in in vitro studies. Upon dilution from denaturant, chemically denatured luciferase resembles a nascent polypeptide that requires the action of Ydj1 and Hsp70 to reach the native state and is a widely utilized substrate for mechanistic studies on chaperone-assisted protein folding (2, 3).

In protein folding assays, purified Ydj1 C143S and Ydj1 C162S behaved like the J domain mutant, Ydj1 H34Q, and were incapable of cooperating with Hsp70 to reactivate chemically denatured luciferase (Fig. 5A). These luciferase refolding reactions were carried out at an optimal Ydj1:Hsp70 molar ratio of 2:1, and when the Ydj1 ZFLR mutant:Hsp70 molar ratio was increased from 2:1 to 10:1 no increase in luciferase refolding was observed (data not shown). Thus, defects in the ability of Ydj1 ZBDI and ZBDII mutants to support AR folding in vivo correlate well with the inability of purified Ydj1 C143S and Ydj1 C162S to interact with Hsp70 to refold chemically denatured luciferase.

A. Native I.P. with α-AR

B. Western blot of cell extracts

FIG. 3. Assay for androgen receptor (AR) function in ydj1Δ that harbors Ydj1 ZFLR mutants. A, Western blot analysis of AR expression in strains that harbor the indicated Ydj1 ZFLR mutant. B, binding of androgen by AR. The synthetic androgen "H-R1881 (50 nM) was added to liquid cultures of strains that harbored Ydj1 or the indicated Ydj1 ZFLR mutant. Binding of "H-R1881 to AR was assayed at the permissive growth temperature and quantitated as described under “Experimental Procedures.” Results displayed represent the average from three experiments ± S.D.

FIG. 4. Analysis of complex formation between AR and Hsp70 and Ydj1. A, Ydj1Δ that harbored the indicated form of Ydj1 and pG1 harass, which expresses AR under control of the GAL promoter, were cultured in synthetic media that was supplemented with 2% raffinose at 25 °C to an A600 of 0.2. AR expression was then induced by addition of 2% galactose, and cells were grown to an A600 of 1.6 and then harvested. Cells were lysed by agitation with glass beads, and AR was immunoprecipitated from cleared cell extracts with α-AR. The amounts of Ydj1 and Hsp70 that co-precipitated with AR were detected by Western blot with α-Ydj1 and α-Hsp70, respectively (top panel). B, Western blot analysis of Hsp70, Ydj1, AR, and phosphoglycerate kinase (PGK) levels in the total cell extracts that were utilized for the co-immunoprecipitation experiments shown in panel A.

Purified Ydj1 ZBDI and ZBDII Mutants Fail to Promote Polypeptide Binding by Hsp70 and Therefore Exhibit Defects in Protein Folding—If Ydj1 ZBDI mutants can bind protein biogenic intermediates in vivo, then why cannot they promote their proper folding? To address this question, the ability of purified Ydj1 ZBDI and ZBDII mutants to interact with Hsp70 and facilitate the folding of chemically denatured luciferase was examined. Luciferase was chosen as the substrate for these studies instead of AR, because AR is a large metastable protein that is not amenable to purification by standard techniques and is not typically utilized in in vitro studies. Upon dilution from denaturant, chemically denatured luciferase resembles a nascent polypeptide that requires the action of Ydj1 and Hsp70 to reach the native state and is a widely utilized substrate for mechanistic studies on chaperone-assisted protein folding (2, 3).

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To uncover the mechanism for the protein-folding defect exhibited by Ydj1 ZFLR mutants, the ability of Ydj1 C143S and Ydj1 C162S to regulate the ATPase activity of Hsp70 was examined. This is an important question because, to promote substrate binding by Hsp70 and thereby facilitate protein folding, Ydj1 must interact with Hsp70 to stimulate its ATPase activity (34). Ydj1 C143S and Ydj1 C162S were fully active in the stimulation of Hsp70 ATPase activity (Fig. 5B). Hence, the protein folding defects exhibited by these ZFLR mutants cannot be attributed to a defect in their ability to regulate Hsp70 ATPase activity.

To facilitate Hsp70-dependent refolding of denatured lucif-
erase, Ydj1 must bind and then deliver non-native luciferase to the Hsp70 polypeptide-binding domain (29). Hence, to determine whether Ydj1 ZFLR mutants exhibit a defect in this aspect of Ydj1 function we examined the ability of Ydj1 C143S and Ydj1 C162S to bind chemically denatured luciferase (Fig. 5C). Ydj1 C143S and Ydj1 C162S retained their polypeptide binding function and bound a quantity of chemically denatured luciferase that was similar to the quantity that associated with Ydj1. Thus, mutation of ZBDI or ZBDII does not alter the ability of Ydj1 to bind chemically denatured luciferase.

Because Ydj1 ZBDI and ZBDII mutants function to bind luciferase and form complexes with AR (Fig. 4), it appears that the protein folding defect caused by mutation of the ZFLR manifests itself after polypeptide binding by Ydj1. Therefore, function of the ZFLR might be required for the efficient transfer of polypeptides from Ydj1 to Hsp70. If this is the case, Ydj1 ZFLR mutants should exhibit a defect in stimulating polypeptide binding by Hsp70. To investigate this possibility, we developed an ELISA assay to monitor the Ydj1-dependent binding of luciferase by Hsp70 (Fig. 5D). Chemically denatured luciferase was immobilized in the wells of a microtiter plate, and purified Hsp70 was added alone or in combination with Ydj1. The quantity of Hsp70 that bound to the immobilized luciferase was then quantitated via ELISA with α-Hsp70. In the absence of Ydj1, Hsp70 bound a small quantity of luciferase. However, when Ydj1 was included in reactions, 5-fold more Hsp70-luciferase complex formation occurred. The Ydj1-dependent increase in the binding of Hsp70 to luciferase was deemed to be specific because it was ATP-dependent and not supported by Ydj1 H34Q. When Ydj1 C143S or Ydj1 C162S was substituted in reactions for Ydj1, it was incapable of stimulating substrate binding by Hsp70. Thus, function of the ZFLR is required for Ydj1 to promote complex formation between Hsp70 and chemically denatured luciferase.

The inability of Ydj1 ZFLR mutants to promote luciferase binding by Hsp70 appears to be the cause of their inability to cooperate with Hsp70 to refold denatured luciferase. In addition, these data help explain the mechanism behind the in vivo observation that, instead of being bound by Hsp70, AR biogenic intermediates accumulate in complexes with Ydj1 ZFLR mutants. Thus, data from in vivo and in vitro studies suggest that the Type I Hsp40 ZFLR plays an important role in chaperone-assisted protein folding by promoting substrate transfer from Ydj1 to Hsp70.

**Mutation of ZBDI, but Not ZBDII, Interferes with the Ability of Ydj1 to Cooperate with Hsp70 to Suppress Thermally Induced Protein Aggregation—**If mutation of ZBDI and ZBDII hinders the ability of Ydj1 to cooperate with Hsp70 to fold nascent AR and refold chemically denatured luciferase, then why do yeast that harbor Ydj1 ZBDI mutants survive heat stress but Ydj1 ZBDII mutants become inviable (Fig. 2)? Because protein synthesis is inhibited during heat stress, the major cytoprotective function of the Hsp70/Hsp40 system at elevated temperatures is to suppress the aggregation of native proteins that become unfolded (1–3). Therefore, we explored whether the differences in the sensitivity of strains that harbored Ydj1 ZBDI and Ydj1 ZBDII mutants to thermal stress reflected differences in the ability of these proteins to cooperate with Hsp70 to suppress heat-induced protein aggregation.

To accomplish this, the ability of Ydj1, Ydj1 C143S, and Ydj1 C162S to act alone and in combination with Hsp70 to suppress the thermally induced aggregation of native firefly luciferase was compared (Fig. 6). Upon incubation of native luciferase at 42 °C for 10 min, ~90% of it formed large aggregates that could be pelleted from reaction cocktails by centrifugation (Fig. 6B, column 1). When Ydj1 or Hsp70 was added individually to reactions during the heat treatment, luciferase aggregation was suppressed by only 5–10% (Fig. 6B, column 1 versus columns 2 and 6). The combination of Ydj1 and Hsp70 maintained >90% of total luciferase in a soluble state (Fig. 6B, column 1 versus 7). The ability of Ydj1 and Hsp70 to jointly suppress luciferase aggregation required them to functionally interact because Ydj1 H34Q could not substitute for Ydj1 (Fig. 6B, column 1 versus columns 7 and 10). Surprisingly, Ydj1 C143S could function in combination with Hsp70 to suppress luciferase aggregation to the same degree as Ydj1 (Fig. 6B, column 1 versus columns 7 and 8). In contrast, Ydj1 C162S was incapable of cooperating with Hsp70 to prevent luciferase aggregation.

Thus, Ydj1 C162S appears incapable of supporting the growth of yeast at the non-permissive temperature, because loss of ZBDII function prevents it from cooperating with Hsp70 to suppress heat stress-induced protein denaturation. In contrast, the ZBDI mutant Ydj1 C143S is partially functional because it retains its ability to cooperate with Hsp70 to suppress protein aggregation and can thereby protect cells from heat stress.

**DISCUSSION**

The experimental data presented indicate that proper function of the Type I Hsp40 ZFLR is required for Hsp70 to capture non-native proteins from Ydj1. This function of the Ydj1 ZFLR appears to be important for cell physiology because mutation of ZBDII caused ydj1Δsis1Δ to become inviable and prevented ydj1Δ from surviving heat stress. The following observations support the conclusion that the ZFLR facilitates substrate transfer from Ydj1 to Hsp70. First, mutation of the Ydj1 ZFLR caused defects in AR function that were associated with a dramatic decrease in Hsp70-AR complex formation and the accumulation of Ydj1-AR complexes. Second, purified Ydj1 ZFLR mutants were defective at cooperating with Hsp70 to refold chemically denatured luciferase but retained the ability to bind denatured luciferase and stimulate Hsp70 ATPase activity. Finally, Ydj1 ZFLR mutants were incapable of promoting Hsp70-luciferase complex formation.

During the course of these studies we compared the influence that mutation of ZBDI and ZBDII had on Ydj1 function and observed some similarities and differences. Mutation of ZBDI and ZBDII blocked the in vivo and in vitro protein folding activity of Ydj1, and this defect correlated with the inability of ZFLR mutants to promote substrate binding by Hsp70. However, Ydj1 ZBDII mutants were able to support the normal growth of ydj1Δ and ydj1Δsis1Δ and could also protect cells from heat stress, whereas Ydj1 ZBDII mutants could not support the growth of ydj1Δsis1Δ and could not protect ydj1Δ from heat stress. Therefore, the functional defects exhibited by Ydj1 ZBDII mutants were more severe, and Ydj1 ZBDI mutants were partially functional. A function that was retained by Ydj1 ZBDI mutants, but was lost by ZBDII mutants, was the ability to cooperate with Hsp70 to suppress thermally induced protein aggregation. Retention of the ability to cooperate with Hsp70 to suppress thermally induced protein aggregation helps to explain why ZBDI mutants can protect cells from heat stress. An explanation as to why ZBDI mutants were only partially defective in the execution of Ydj1 chaperone function is presented below.

Ydj1 appears to function as a dimer. When the crystal structure of monomeric Ydj1 (110–337) is modeled as dimer it is predicted to form a U-shaped assembly in which the rod-like ZFLRs on adjacent monomers protrude toward each other (15). The distance that separates the ZFLRs on each monomer in the Ydj1 dimer appears short enough to allow for interactions between the β-strands located at the tips of each ZFLR to occur. The formation of intermolecular contacts between adjacent ZFLRs in the Ydj1 dimer may be functionally important be-
cause it would fix the distance between the polypeptide binding pockets found on CTDI. This putative event could be required for Ydj1 to maintain polypeptides in a conformation that can be bound by Hsp70.

Portions of the Ydj1 ZFLR appear to be flexible because the B-factors for amino acid residues that surround ZBDI and ZBDII are as high as 130 (Fig. 1B). Therefore, mutation of either ZBDI or ZBDII could cause defects in Ydj1 function by destabilizing local secondary structure. ZBDI is located at the junction between CTDI and the ZFLR, so the introduction of a single point mutation into ZBDI is predicted to increase the flexibility of this junction but should not dramatically influence the overall ZFLR structure. ZBDII, on the other hand, is located in the middle of the ZFLR and is surrounded by amino acids that have relatively high B-factors (Fig. 1B). Hence, the mutation of ZBDII has the potential to disrupt the rod-like shape of the ZFLR and render it non-functional. If this scenario were proven to be true, then it would explain why mutation of ZBDII is more detrimental to Ydj1 function than mutation of ZBDI.

The ZFLRs from Ydj1 and DnaJ are structurally conserved, and the data we have presented suggest that they also perform related functions (15–18). Zylicz and co-workers (17) have investigated the functional importance of the DnaJ ZFLR by

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**Fig. 6.** Ydj1 ZBDI and ZBDII exhibit differential defects in their ability to cooperate with Hsp70 to suppress luciferase aggregation. A, suppression of luciferase aggregation by Hsp70 and Ydj1. Luciferase (100 nM) was mixed with Hsp70 (2 μM) and the indicated form of Ydj1 (3 μM) and incubated at 42 °C for 10 min. Reaction cocktails were then subjected to centrifugation at 20,000 rpm for 10 min at 4 °C. Total (T) luciferase and luciferase that was present in the supernatant (S) and pellet (P) from equal portions of reaction mixtures were then determined by Western blot with α-luciferase. B, quantitation of luciferase present in the soluble fraction and pellets of reaction mixtures shown in panel A. Western blots were quantitated by laser densitometry; values are presented as percentage of total luciferase present in individual reactions. **Soluble** represents luciferase detected in the supernatant fraction after centrifugation. Aggregated material represents luciferase detected in the pellet after centrifugation.
characterizing defects exhibited by DnaJΔZFLR. DnaJΔZFLR retained its ability to bind DnaJ substrates but exhibited a reduced capacity to prime E. coli Hsp70 (DnaK) for substrate binding (17). However, direct interactions between DnaK and DnaJ were not impaired in DnaJΔZFLR (17). In a recent study, site-directed mutagenesis was carried out on the DnaJ ZFLR in which all four of the cysteines that form ZBDI or ZBDII were mutated (18). Mutation of DnaJ ZBDI impaired the autonomous polypeptide binding activity of DnaJ but had little influence on the growth of E. coli (18). These results differ from data obtained with Ydj1 ZBDI point mutants, because mutation of Ydj1 C143S or Ydj1 C201S had no effect on the polypeptide binding activity of Ydj1. Data obtained with Ydj1 ZBDI and DnaJ ZBDI mutants may differ because mutation of all four residues in DnaJ ZBDI may lead to destabilization of adjacent regions in CTDI that are involved in polypeptide binding (15, 18), whereas the introduction of single point mutations in Ydj1 ZBDI might only influence ZFLR function.

Characterization of DnaJ ZBDII mutants yielded similar results to those exhibited by Ydj1 ZBDII mutants. DnaJ ZBDII mutants grew slowly at 37 °C and were inviable at 43 °C (18). Purified DnaJ ZBDII mutants were unable to cooperate with DnaK to suppress protein aggregation (18). Thus, results we have reported from studies on the function of the Ydj1 ZFLR are in good agreement with functional features of the DnaJ ZFLR. However, the data reported here extend previous knowledge of Type I Hsp40 ZFLR action because we demonstrated for the first time that loss of ZFLR function leads to the accumulation of complexes between Ydj1 and protein folding intermediates and limits the ability of Hsp70 to form complexes with substrates of Ydj1. Thus, it appears that the Type I Hsp40 ZFLR and the J domain act synergistically to enable Hsp70 to capture non-native polypeptides from Ydj1.

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