Domain Requirements of DnaJ-like (Hsp40) Molecular Chaperones in the Activation of a Steroid Hormone Receptor*

(Received for publication, April 15, 1999, and in revised form, August 9, 1999)

Albert E. Fliss‡‡§, Jie Rao‡, Mark W. Melville¶¶, Michael E. Cheetham****, and Avrom J. Caplan‡ ‡ ‡ $ $ From the ‡Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, New York, New York 10029, the ‡Department of Microbiology, University of Washington, Seattle, Washington 98195, and the **Department of Pathology, Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, United Kingdom

DnaJ-like proteins function in association with Hsp70 molecular chaperones to facilitate protein folding. We previously demonstrated that a yeast DnaJ-like protein, Ydj1p, was important for activation of heterologously expressed steroid hormone receptors (Caplan, A. J., Langley, E., Wilson, E. M., and Vidal, J. (1995) J. Biol. Chem. 270, 5251–5257). In the present study, we analyzed Ydj1p function by assaying hormone binding to the human androgen receptor (AR) heterologously expressed in yeast. We analyzed hormone binding in strains that were wild type or deleted for the YDJ1 gene. In the deletion mutant, the AR did not bind hormone to the same extent as the wild type. Introduction of mutant forms of Ydj1p to the deletion strain revealed that the J-domain is necessary but not sufficient for Ydj1p action, and that other domains of the protein are also functionally important. Of three human DnaJ-like proteins introduced into the deletion mutant, only Hdj2, which displays full domain conservation with Ydj1p, suppressed the hormone binding defect of the deletion mutant. By comparison of the domains shared by these three human proteins, and with mutants of Ydj1p that were functional, it was deduced that the cysteine-rich zinc binding domain is important for Hdj2/Ydj1p action in hormone receptor function. A model for the mechanism of DnaJ-like protein action is discussed.

Molecular chaperones function in protein folding by repeated cycles of binding and release from their polypeptide substrates or targets. These cycles are mechanistically related to nucleotide-dependent changes in chaperone protein conformation, which are in turn regulated by proteins called co-chaperones. One of the more intriguing aspects of chaperone biology is that the co-chaperones can also have chaperone-like activity, i.e. they can too bind to unfolded or partially folded polypeptides, prevent them from aggregating, and maintain them in a folding competent conformation (see Refs. 1 and 2 for reviews). This apparent dual function of some co-chaperones is not well understood, although it may be related to the specificity with which general molecular chaperones, such as Hsp70, may act.

Well characterized molecular chaperones, such as Hsp70 and the oligomeric chaperonins Hsp60 and GroEL, typically display very little substrate specificity in vitro. The substrate binding pocket in Hsp70 can accept a wide variety of polypeptide sequences of 7–9 amino acid in length and having a hydrophobic character. Such sequences have been reported to occur in proteins every 36 amino acids (2). How then, does Hsp70 perform some very specific tasks, such as clathrin uncoating (3, 4)? The answer appears to lie in the divergent nature of the co-chaperones, specifically of the class called DnaJ, named after their Escherichia coli orthologue (also called Hsp40). DnaJ-like co-chaperones are all related by the presence of a 70-amino acid helical domain called the J-domain, which is required for these proteins to stimulate Hsp70’s ATPase activity and alter Hsp70 substrate binding. Conversion of Hsp70-ATP to Hsp70-ADP leads to a change in peptide binding affinity that has been correlated variably with tighter peptide binding and also in peptide release (reviewed in Refs. 5 and 6). Beyond the J-domain, dnaJ-like co-chaperones can be highly divergent. For example, auxilin is a protein that functions in association with Hsc70 to perform clathrin uncoating and is localized to the clathrin cage itself, but shares only the J-domain with DnaJ (7). By localizing to the clathrin cage, auxilin recruits Hsc70 to the ideal location to catalyze uncoating. On the other hand, proteins sharing full domain conservation with E. coli dnaJ can be found in both humans and yeast, as can a plethora of other proteins sharing some but not all domains (5). Eukaryotic DnaJ-like proteins sharing full domain conservation with E. coli dnaJ may have a general chaperoning function, since they can facilitate polypeptide folding in association with Hsp70 (8–10). These divergent DnaJ-like proteins have been characterized into three broad categories: type I (sharing full domain conservation with DnaJ; see Ref. 5), type II (sharing a more limited domain conservation by having an N-terminal J-domain and Gly/Phe-rich region, but not the central cysteine-rich zinc binding domain), and type III DnaJ proteins, such as auxilin, that share only a J-domain in common with all other DnaJ-like proteins, and this may reside anywhere in the polypeptide, not just at the N terminus.

Two recent studies (9, 10) have directly compared the ability of type I and type II DnaJ proteins to participate in protein folding events in the presence of Hsp70. In both cases, only the type I DnaJ proteins (DnaJ itself, yeast Ydj1, and human HDJ2), which have the zinc finger domain could function in folding, while the type II DnaJ proteins (HDJ1/Hsp40) failed to do so, although an earlier study had shown that HDJ1 (Hsp40)
DnaJ Co-chaperones in Androgen Receptor Activation

can have some chaperone activity (11). Two domains of type I DnaJ proteins are known to bind peptides, which may explain their role in folding events. One is the cysteine-rich zinc binding domain that is found exclusively in type I DnaJ proteins, and the other is a region found in the C-terminal half of type I and some type II DnaJ proteins (45% similarity; see Refs. 12–14).

Previous studies have shown that Hsp90 and Hsp70 molecular chaperones cooperate in the folding of steroid hormone receptors and in maintaining them in a high affinity hormone binding conformation (reviewed in Ref. 15). DnaJ-like co-chaperones are also involved in this process. Genetic studies, using yeast as a model system, revealed that mutation in a yeast DnaJ-like protein, YDJ1 (a type I DnaJ protein), led to reduced levels of hormone-dependent signaling by androgen receptors (AR),1 glucocorticoid receptors (GR), and estrogen receptors (ER) (16, 17). The role of DnaJ proteins in receptor signaling has also been probed by biochemical methods. Depletion of endogenous DnaJ-like proteins from rabbit reticulocyte lysates inhibits association between GR and Hsp90, but addition of purified Ydj1 protein restored binding (25). In another recent study, association of the progesterone receptor with Hsp90 also required purified Ydj1 (or its human homologue Hdj2) and Hsp70 (26). Together, these studies demonstrated that DnaJ-like proteins are required for proper maturation of steroid hormone receptors. What has remained unclear is whether there is specificity in the action of different DnaJ-like proteins in this process.

In the present study, we used the yeast Saccharomyces cerevisiae as a model system to study the domain organization of Ydj1, and to test the specificity of different DnaJ-like proteins in the activation of heterologously expressed human AR. Previous studies have demonstrated the conserved nature of yeast with respect to folding of steroid hormone receptors, and their dependence on Hsp90 for activity (18). Furthermore, mutation of yeast Hsp90 led to a reduction in hormone-dependent signaling by the receptor (19).

EXPERIMENTAL PROCEDURES

Materials—R1881 was obtained from NEN Life Science Products and hydroxyflutamide from Schering-Plough. Dihydrotestosterone was a gift from Dr. M. McGinnis. The above compounds were solubilized in ethanol and stored at 20°C.

Yeast Strains and Growth Conditions—Yeast cells were cultured in selective media (0.67% yeast nitrogen base, 2% glucose, or 2% galactose plus the appropriate amino acids) using standard procedures. The YDJ1 and Δydj1 parental strains were MYY290 (MATα, leu2, his3, ura3) and MY405 (MATα, leu2, his3, Δydj1:URA3), respectively (the gift of Dr. Mike Yaffe; see Ref. 27), in all cases except the following: The strain containing the Δydj1 double mutant (ΔD6N,K46M) was derived from parental strain A3 (MATα, ura3, leu2, lys2; the gift of Dr. Frank Boschelli). The strain containing Δydj1-G315D was derived from YPH499 (as described in Ref. 17). In most experiments, Δydj1 mutants were expressed in MY405 that was plated on 5-fluoroorotic acid to deselect for the URA3 marker. The resulting strain was used as the parental strain for the remainder of this study. Plasmid transformations were performed by the LiAc procedure as described previously (28). Plasmids containing the AR (pPGKARC) and lacZ reporter gene (pPGKarelacZ) were described previously (29). Plasmids expressing the ydj1–151 mutant, overexpressing SIS1 and expressing HJ1 under GAL1 promoter control have also been described (23). The ydj1–C159Y mutant is genomic, and the strain is isogenic with MYY 290.

Plasmid Constructions—The promoter and open reading frame for ydj1–H34Q was subcloned into the vector pRS315 (CEN/ARS, LEU2) from pYQPD (gift of Dr. Doug Cyr; see Ref. 20). pYQPD was digested with EagI and XhoI, and the fragment was gel-purified and ligated into similarly digested pRS315. The open reading frame for HDJ2 was subcloned into the vector pRS315gal (GAL1 promoter, CEN/ARS, LEU2). pHDJ2 (the kind gift of Dr. T. Mohanakumar) was digested with BamHI and EagI, and the appropriate fragment was gel-purified and ligated into similarly digested pRS315gal (GAL1 promoter, CEN/ARS, LEU2). The araLE2 from pPGKarelacZ was subcloned into a LEU2 vector. pPGKarelacZ was digested with HindIII and SalI, and the appropriate fragment was ligated into similarly cut vector and was termed pRS315araLE2. The plasmid encoding human HDJ1 was constructed by digestion of full-length HDJ1 from plReScript harboring the HDJ1 cDNA with EcoRI, which was ligated into the EcoRI site of pYX223 (Novagen).

β-Galactosidase Activity Assay—Yeast cells were grown to early log phase (A600 = 0.2) and preincubated at either 25°C or 37°C for 1 h prior to the addition of dihydrotestosterone. These cells were incubated for an additional 2 h at the same temperature prior to harvesting, extract preparation, and β-galactosidase activity assays as described previously (16).

Ligand Binding Assays—These assays were performed as described previously (19). Briefly, yeast cells were grown in selective media containing 2% glucose or 2% galactose to early log phase (A600 = 0.2) and 1–ml aliquots were subsequently incubated at either 25°C or 37°C for 30 min. Following this preincubation, cells were incubated with 1H-labeled R1881 (a synthetic androgen, diluted 1:10 or 1:20 in cold R1881 of the same concentration) for an additional 1.5 h at the same temperature. Cells were washed three times with 1 ml of water each and counted in 5 ml of liquid scintillation fluid. Nonspecific bound cpm was calculated by subtracting the cpm obtained from samples incubated with a 100-fold excess of unlabeled R1881 from the samples incubated in the absence of cold R1881. In these strains, nonspecific R1881 binding was always less than 10% of specific binding. Retention of R1881 in cells not expressing the AR was also less than 10% of counts retained by cells expressing the AR. Typically, the counts retained by the wild-type strain constitutively expressing the AR were in the range of 500–1500 cpm at 100 nM R1881, depending on initial dilution with cold hormone of the same concentration.

Western Blot Analysis—The levels of AR and Ydj1p were assayed by Western blot analysis. Yeast lysates were prepared as described previously (16). Lysates (10 μg of total protein) were resolved by SDS-polyacrylamide gel electrophoresis, and the proteins in the gel were subsequently transferred to nitrocellulose (0.45 μm). Filters were incubated overnight at room temperature with TTBS containing 5% nonfat dry milk. Filters were subsequently incubated with primary antibody (diluted in antibody dilution buffer containing 1× phosphate-buffered saline, 3% bovine serum albumin, 0.05% Tween 20) and blocked overnight at room temperature with TTBS containing 5% nonfat dry milk. Filters were subsequently incubated with primary antiserum (diluted in antibody dilution buffer containing 1× phosphate-buffered saline, 3% bovine serum albumin, 0.05% Tween 20, and 0.1% thimerosal) for 1 h, then washed three times for 10 min each in TTBS. Filters were then incubated with horseradish peroxidase-conjugated secondary antibodies (diluted 1:10,000 in antibody dilution buffer) for 1 h and subsequently washed three times for 10 min each in TTBS. Finally, filters were treated with a chemiluminescence reagent (Pierce) and exposed to x-ray film.

RESULTS

Analysis of Ydj1p in Yeast—Previous studies demonstrated that mutations in the YDJ1 gene led to reduced hormone-dependent signaling by the AR, as assessed by transactivation of a target reporter gene (16). However, hormone-independent transactivation of the same reporter by a truncated version of the AR was unaffected. Since the truncated AR was deleted for the hormone binding domain, these results suggested that Ydj1p functioned via the hormone binding domain. Hormone binding studies in the same ydj1–151 mutant showed little change in hormone binding capacity by the AR, however, suggesting that Ydj1p might function downstream of ligand binding in the activation process. To clarify the role of Ydj1p in AR activation, we performed additional hormone binding studies.

1 The abbreviations used are: AR, androgen receptor; ER, estrogen receptor; GR, glucocorticoid receptor; TTBS, Tris-buffered saline with Tween 20.
in a yeast strain that was deleted for the YDJ1 gene. This strain (Δydj1) and an isogenic wild type were transformed by a plasmid that constitutively expressed the AR gene. Hormone binding studies were performed by direct addition of a 3H-labeled androgen, R1881 (methyltrieneolone), to the culture medium of cells in the early log phase of growth. In wild type yeast cells, R1881 was retained in a dose-dependent manner until the binding saturates at 50–100 nM hormone (Fig. 1A). In the cells deleted for YDJ1 (Δydj1), however, there was a sharp decrease in hormone binding by the receptor, even though there were similar levels of AR protein in both Δydj1 and wild type strains (Fig. 1B). Thus, hormone binding by the AR is impaired in cells deleted for YDJ1.

In a previous study, we observed that the anti-androgen hydroxyflutamide became a more potent competitor of R1881 binding in yeast strains expressing a mutant form of Hsp90, and suggest that Ydj1p may function in association with Hsp90 in regulating hormone binding to the AR.

One question not addressed by these experiments was whether Ydj1p was required for folding of the entire AR molecule, or just the hormone binding domain. As mentioned earlier, previous analyses with the ydj1-151 mutant suggested that it acted via the hormone binding domain (16). However, it was possible that the ydj1-151 mutant was still functional for folding of other AR domains. To clarify this matter, we expressed a truncated form of the AR (AR1–660) in wild type and Δydj1 strains that also contained the lacZ gene under control of androgen response elements. AR1–660 lacks a hormone binding domain and functions constitutively in yeast as a transcriptional activator on genes regulated by androgen response elements. AR1–660 lacks a hormone binding domain and functions constitutively in yeast as a transcriptional activator on genes regulated by androgen response elements. Thus, AR1–660 activity is essentially independent of Ydj1p.

We next tested which domains of Ydj1p were important for hormone binding by the AR expressed in yeast. We first performed hormone binding experiments in strains expressing J-domain mutants. The first mutant investigated contained a single point mutation in the J-domain converting His-34 to Gln (ydj1-H34Q). This mutation rendered yeast cells temperature-sensitive for growth and the purified Ydj11-H34Q protein was unable to stimulate Hsp70’s ATPase activity (20). In direct hormone binding experiments, the AR was clearly defective for R1881 binding in the ydj1-H34Q mutant compared with the wild type, although not as defective as the Δydj1 strain (Fig. 3A, filled circles). The same was found in the hydroxyflutamide competition assay, where the level of competition by the drug in the ydj1-H34Q mutant was intermediate between the wild type and Δydj1 strain (Fig. 3B, filled circles). In separate experiments with another J-domain mutant (carrying the double mutation D36N,K46M; see Ref. 21), we also observed a de-
increase in hormone binding by the AR and increased competition by hydroxyflutamide compared with an isogenic wild type strain (data not shown). These results indicate that the J-domain of Ydj1p was important for AR activation, although Ydj1p containing a mutated J-domain was still partially functional. Thus, domains other than the J-domain are important for Ydj1p function in AR activation, although it seems unlikely that a J-domain can function in trans from the rest of the Ydj1 protein.

Using these assays we also re-analyzed the ydj1–151 mutant (16, 23), which has multiple point mutations. The results, however, were difficult to interpret, since there was a mild defect in hormone binding by AR and a decrease in sensitivity to hydroxyflutamide compared with the wild type (see Fig. 3). In a previous analysis (16), we observed similar levels of hormone binding to the AR in wild type and ydj1–151 strains.

One domain of Ydj1p that distinguishes it from other DnaJ proteins like Sis1p is the presence of a zinc binding region that is thought to mediate peptide binding. This region contains canonical repeats of cysteine residues that are known to coordinate zinc in E. coli DnaJ. Mutation in one of these cysteines (ydj1-C159Y) is known to affect Ydj1p function in mitochondrial protein import and to manifest in a temperature-sensitive growth phenotype (27, 30). We analyzed whether this mutation would also affect hormone binding by AR. As shown in Fig. 4, hormone binding by the AR is substantially reduced in the mutant strain compared with the wild type, suggesting that the zinc binding region in addition to the J-domain is necessary for hormone binding by AR. However, the decrease in hormone binding by AR in the ydj1-C159Y mutant did not correlate with the results from hydroxyflutamide competition experiments. In this case, there was no defect in the level of competition by hydroxyflutamide for hormone binding to the AR in the mutant versus the wild type.

The C-terminal end of Ydj1p is farnesylated, and this modification helps to localize the protein to intracellular membranes (24). We addressed the role of this modification in hormone binding by the AR, using a mutant, ydj1-C406S, which cannot be farnesylated. However, there was no decrease in hormone binding by the AR in this mutant compared with the wild type (Fig. 5A). Similar results were observed in experiments that analyzed hormone binding by AR in a strain that expressed E. coli DnaJ, which does not have the C-terminal farnesylation motif but is otherwise very similar to Ydj1p (both are type I DnaJ proteins). As shown in Fig. 5B, similar hormone binding by AR was observed in strains expressing wild type Ydj1p or DnaJ. Together, these data indicate that farnesylation is not essential for Ydj1p function in generating or maintaining AR in a hormone binding-competent conformation.

We also assayed hormone binding by AR in the ydj1-G315D mutant. This mutation was characterized by having synthetic lethality with mutation in a gene encoding Hsp90 (17). Furthermore, a yeast strain having this mutation was defective for signaling by GR and ER, although in a manner different from the other mutations described here. This is because ydj1-G315D appeared to suppress the hormone dependence for GR- and ER-dependent lacZ gene expression, leading to constitutive gene activation. However, the AR bound hormone in this strain and the wild type to similar extents (data not shown).

**Analysis of Human DnaJ-like Proteins in Yeast—**We performed further analysis of the domain organization of DnaJ molecular chaperones by testing for the ability of three different human DnaJ proteins to compensate for the loss of Ydj1p in yeast. These three proteins, Hsj1b (31), Hdj1(Hsp40; Ref. 32), and Hdj2 (33, 34) all have the J-domain but are otherwise quite different from each other. Of the three, Hdj2 is most similar to Ydj1p (50% identical amino acids), with both proteins sharing an identical domain organization, including the prenylated C terminus and the cysteine-rich domain. Neither Hsj1b nor Hdj1 have these domains and are also quite different from each other except for the J-domain and extended Gly/Phe-rich regions. Previous studies with Hsj1b demonstrated that it could partially suppress the slow growth phenotype of ydj1Δ cells but not the temperature-sensitive lethality at 37 °C (Ref. 23; also see Fig. 6). Similar results were observed with expression of Hdj1 in these cells. Hdj2, however, could also suppress the lethality at 37 °C. Thus, these three different DnaJ-like proteins all functioned in yeast but to varying degrees.

We next tested whether any of these proteins could suppress the hormone binding defect of the human AR expressed in Δydj1 yeast. Each of the human DnaJ proteins was expressed
in Dipodascus pseudotropicalis yeast cells that also constitutively expressed the human AR, and in vivo hormone binding studies were performed as described above. The results from these experiments, shown in Fig. 7A, demonstrate that only Hdj2 suppressed the hormone binding defect in the Δydj1 strain. Neither Hdj1 nor Hsj1b could raise the level of hormone binding to the AR in these cells, even though they partially suppressed the Δydj1 growth phenotype (Fig. 6). A similar pattern was observed in the hydroxyflutamide competition experiments (Fig. 7B). In this case, the AR remained supersensitive to hydroxyflutamide in the presence of Hdj1 and Hsj1b, but became resistant in the presence of Hdj2. In both hormone binding and hydroxyflutamide competition experiments, Hdj2 fully suppressed the Δydj1 phenotype to the same level found in wild type cells.

The finding that Hdj2 could fully suppress the AR hormone binding defect in the Δydj1 strain also correlated with results from experiments to test the transactivation potential of the AR. For these studies, the ability of hormone to activate the AR as a transcription factor was measured by expression of a lacZ reporter gene as described. The results from these experiments, shown in Fig. 7C, demonstrate that in wild type yeast cells that also constitutively expressed the human AR, and in vivo hormone binding studies were performed as described above. The results from these experiments, shown in Fig. 7D, demonstrate that only Hdj2 suppressed the hormone binding defect in the Δydj1 strain. Neither Hdj1 nor Hsj1b could raise the level of hormone binding to the AR in these cells, even though they partially suppressed the Δydj1 growth phenotype (Fig. 6). A similar pattern was observed in the hydroxyflutamide competition experiments (Fig. 7E). In this case, the AR remained supersensitive to hydroxyflutamide in the presence of Hdj1 and Hsj1b, but became resistant in the presence of Hdj2. In both hormone binding and hydroxyflutamide competition experiments, Hdj2 fully suppressed the Δydj1 phenotype to the same level found in wild type cells.
reporter gene under control of androgen response elements. Induction of lacZ was dependent on the presence of dihydrotestosterone, which was added to the culture medium of cells in the early log phase of growth. The results (Fig. 8) show that only Hdj2 could substitute for Ydj1p in this assay, and the presence of Hdj1 or Hsj1b had no effect.

**DISCUSSION**

The results shown in this report are consistent with a role for Hsp40 molecular chaperones at an early stage in the activation process of a steroid hormone receptor. Specifically, a subclass of Hsp40 proteins containing the zinc binding domain are necessary for hormone binding by the AR. Deletion of the yeast YDJ1 gene results in loss of hormone binding, but this phenotype was suppressed by expression of other type I DnaJ proteins, such as E. coli DnaJ itself and human Hdj2, both of which have the zinc binding domain. The hormone binding defect was not suppressed upon expression of other Hsp40 proteins such as Hdj1 and Hsj1, or overexpression of Sis1p in a Δydj1 strain. What all of these proteins have in common is that they are type II DnaJ proteins, each having a J-domain and an extended glycine/phenylalanine-rich region but not the zinc binding domain common to type I DnaJ proteins (see Fig. 9). Furthermore, mutation of one cysteine in the zinc binding domain led to a defect in hormone binding by the AR, consistent with this region being important for Ydj1/Hdj2 function in this process. Besides the zinc binding domain, the J-domain was also found to be important for hormone binding by the AR.

Two assays were used in these studies: direct in vivo hormone binding and hydroxyflutamide competition. To a large extent, results from these assays correlated with each other. Decreased levels of hormone binding correlated with supersensitivity of the AR to competition by hydroxyflutamide. However, this was not always so. The major exception was the ydj1-C159Y mutant in which decreased levels of hormone binding by the AR were observed, yet the level of competition by hydroxyflutamide was wild type. The hydroxyflutamide competition assay was first described in studies on the AR in yeast strains that were mutant for Hsp90 (19). The supersensitivity of the AR in these strains was very similar to the supersensitivity of the ydj1 mutant strains described here. Our working hypothesis to account for this change in sensitivity is that the AR adopts a low affinity hormone binding conformation in the absence of molecular chaperones in vivo. This manifests in both reduced hormone binding and in the ability of hydroxyflutamide to compete more effectively. This assumes that the affinity of hydroxyflutamide for the AR is largely unaltered in strains that are mutant for different molecular chaperones. The ability of molecular chaperones to affect the binding of one ligand (R1881) but not another (hydroxyflutamide) suggests that the conformational changes involved are relatively small,

---

**Fig. 7.** Suppression of hormone binding defect in the Δydj1 strain by different human dnaJ-like proteins. A, hormone binding assay in Δydj1 strains expressing HDJ2 (closed squares), HSJ1 (filled circles), HDJ1 (open diamond), or no other gene (open triangle). B, hydroxyflutamide competition assay in the same strains.

**Fig. 8.** lacZ reporter gene assay in yeast strains expressing different human dnaJ-like proteins. Assay was performed after preparing extracts from strains treated with or without 100 nM dihydrotestosterone (DHT). Results are the mean of three independent assays.

**Fig. 9.** Domain organization of DnaJ-like proteins. Scale bar is in amino acids. See text for details.
and that the AR maintains its overall tertiary organization even in the absence of molecular chaperones. Indeed, only the hormone binding appears to be affected by Ydj1p loss of function since AR1–660 functioned normally even in the \(\Delta ydj1\) strain (Fig. 2). The results from the \(ydj1\text{-}C159Y\) mutant, however, were not consistent with this hypothesis. In this case, there was a defect in hormone binding similar to that found in the \(\Delta ydj1\) strain, although the ability of hydroxyflutamide to compete for this binding was wild type. This suggests that the two assays, direct hormone binding and hydroxyflutamide competition, measure different aspects of AR folding, although what these may be remains to be determined. One possibility is that the \(ydj1\text{-}C159Y\) mutant behaves differently from other \(ydj1\) mutants or human type II DnaJ proteins. This may manifest in a wild type phenotype for hydroxyflutamide competition and a \(\Delta ydj1\) mutant phenotype for hormone binding.

Besides the zinc binding domain, the J-domain was also found to be important for folding of the AR hormone binding domain. The J-domain is well characterized region of DnaJ/Hsp40 family members, and functions to catalyze ATP hydrolysis in Hsp70. This action is a regulatory step in the Hsp70 reaction cycle, and in its ADP form, Hsp70 has a more stable interaction with its peptide substrates. In the context of AR activation, the action of Ydj1p/Hdj2 may reflect a mechanism by which Hsp70 becomes more stably associated with the receptor. Initial recruitment of Hsp70 may depend on prior binding of Ydj1/Hdj2 via the zinc binding domain, which is known to bind peptides and assist in Hsp70-mediated folding events (10, 14). Recent studies have demonstrated that Ydj1p binds to the progesterone receptor by direct interaction, and that this binding is necessary for subsequent interaction with Hsp70. Furthermore, both Dittmar et al. (25) and Kosano et al. (26) observed that excess Ydj1p was inhibitory to subsequent maturation of the receptors. Combined with the data presented in this report, a model for the action of Ydj1/Hdj2 may be proposed. In this model, Ydj1/Hdj2 bind to receptors via their cysteine-rich zinc binding regions. Presumably, the hormone binding domains of steroid receptors adopt a relatively unfolded conformation that is recognized by this region of the chaperone. Bound Ydj1/Hdj2 can then interact with Hsp70 in the context of the receptor itself, and may help position Hsp70 accurately for subsequent folding events. Stable association of Hsp70 with the receptor is catalyzed by the J-domain stimulating ATP hydrolysis in Hsp70. As discussed by Kosano et al., excess Ydj1p inhibits folding of the hormone binding domain due to its ability to catalyze Hsp70’s ATPase prior to receptor binding, thus preventing association. Once Ydj1p/Hdj2 has successfully stabilized Hsp70 binding to the receptor, it may itself dissociate and recycle to another receptor molecule.

What remains unclear, however, is the connection between the specific action of Ydj1p or Hdj2 and Hsp90. That such a connection exists is supported by the evidence shown here, that only these Hsp40 subtypes participate in Hsp90-mediated folding of the AR, and that the mutations in genes encoding Ydj1p and Hsp90 display a synthetic lethal phenotype (17). One possibility is that only type I DnaJ proteins containing the Zinc binding region recognize steroid hormone receptors and bind to them with sufficient stability to recruit Hsp70. The co-chaperone listed could then serve to integrate the actions of both Hsp90 and Hsp70 by forming a bridge between them (reviewed in Ref. 39). The alternative is that Hsp90 and Ydj1p or Hdj2 interact with each other, although there is no evidence for a direct physical association.

The role of Ydj1/Hdj2 in this process may be similar to the role of auxilin in clathrin uncoating. In this case, however, auxilin is an integral part of the clathrin cage (7). Indeed, if soluble dnaJ-like proteins are added to a clathrin cage, they will also inhibit uncoating in a manner that is reminiscent of the inhibitory action of excess Ydj1p to hormone binding by steroid receptors (35, 36). Together, these examples may provide a more general hypothesis to explain the action of all DnaJ-like proteins, regardless of their domain organization. This is because different domains of dnaJ-like proteins may simply serve to correctly position Hsp70 on a substrate molecule. Once correctly positioned, the J-domain helps Hsp70 to lock into place. The cysteine-rich zinc binding domains of Ydj1p/Hdj2 (of type I dnaJ proteins) may recognize a particular conformation of an unfolded protein that may help Hsp70 to bind in a place where it can facilitate folding. Other types of DnaJ-like proteins may have a different or more restrictive range of substrates with which they can interact. Of type II proteins, for example, Hdj1 interacts specifically with the protein kinase inhibitor p56Lck and in doing so recruits Hsp70 into a ternary complex (37). Type III DnaJ-like proteins may have their J-domains located elsewhere on the protein besides the N terminus, potentially providing an anchor or localization signal for Hsp70. For example, Sec63 has a J-domain situated between two transmembrane domains, thus providing a highly restricted space for Hsp70 to bind (38).

In conclusion, we have found that folding of a steroid hormone receptor requires a subset of DnaJ-like proteins that all share the central cysteine-rich domain. We propose that this domain is important for targeting Hsp70 to nascent or unfolded receptor molecules. These events probably reflect early steps in Hsp90-dependent steroid receptor maturation.

Acknowledgments—We thank Drs. D. Cyr, F. Boschelli, S. Lindquist, T. Mohanakumar, and M. Yaffe for the gift of yeast strains and plasmids, and Dr. A. Gurevich and B. Bloom for critical reading of the manuscript.

REFERENCES

1. Hartl, F. U. (1996) Nature 381, 571–580
2. Bukau, B., and Horwich, A. L. (1998) Cell 92, 351–366
3. Chappell, T. G., Welch, W. J., Schlossman, D. M., Palter, K. B., Schlesinger, M. J., and Rothman, J. E. (1998) Cell 45, 3–13
4. Ungerwickel, K. E. (1985) EMBO J. 2, 1401–1408
5. Cheetham, M. E., and Caplan, A. J. (1998) Cell Stress Chap. 3, 28–36
6. Cyr, D. M., Langer, T., and Douglas, M. G. (1994) Trends Biochem. Sci. 19, 176–181
7. Ungerwickel, K., Ungerwickel, H., Holstein, S. E. H., Linder, R., Prasad, K., Barouch, W., Martin, B., Greene, L. E., and Eisenberg, E. (1995) Nature 378, 632–635
8. Levy, E. J., McCarty, J., Bukau, B., and Chirico, W. J. (1995) FEBS Lett. 368, 435–440
9. Terrada, K., Kanazawa, M., Bukau, B., and Mori, M. (1997) J. Cell Biol. 139, 1089–1095
10. Lu, Z., and Cyr, D. M. (1998) J. Biol. Chem. 273, 27824–27830
11. Freeman, B. C., and Morimoto, R. I. (1996) EMBO J. 15, 2969–2979
12. Baneki, B., Liberik, K., Wall, D., Wawrzynow, A., Georgopoulos, C., Bertoli, E., Tanfani, F., and Zylicz, M. (1996) J. Biol. Chem. 271, 14840–14848
13. Szabo, A., Korszen, R., Hartl, F. U., and Flanagan, J. (1996) EMBO J. 15, 408–417
14. Lu, Z., and Cyr, D. M. (1998) J. Biol. Chem. 273, 5970–5978
15. Pratt, W. B., and Toft, D. O. (1997) Endocrinol. Rev. 18, 306–360
16. Caplan, A. J., Langley, E., Wilson, E. M., and Vidal, J. (1995) J. Biol. Chem. 270, 5251–5257
17. Kimura, Y., Yahara, I., and Lindquist, S. (1995) Science 268, 1362–1365
18. Caplan, A. J. (1997) Trends Endocrinol. Metab. 8, 271–276
19. Fang, Y., Pliss, A. E., Robbins, D. M., and Caplan, A. J. (1996) J. Biol. Chem. 271, 28707–28712
20. Tsai, S., and Douglas, M. G. (1996) J. Biol. Chem. 271, 9347–9354
21. Dey, B., Caplan, A. J., and Boschelli, F. (1996) Mol. Biol. Cell 7, 91–100
22. Caplan, A. J., and Douglas, M. G. (1991) J. Cell Biol. 114, 609–621
23. Caplan, A. J., Cyr, D. M., and Douglas, M. G. (1992) Cell 71, 1143–1155
24. Caplan, A. J., Tsai, J., Casey, P., and Douglas, M. G. (1992) J. Biol. Chem. 267, 18890–18895
25. Dittmar, K., Banach, J., Galigniana, M., and Pratt, W. B. (1998) J. Biol. Chem. 273, 7358–7366
26. Kosano, H., Stengard, B., Charlesworth, M. C., McMahon, N., and Toft, D. O. (1998) J. Biol. Chem. 273, 32973–32979
27. Atencio, D. P., and Yaffe, M. P. (1999) Mol. Cell. Biol. 19, 283–291
28. Geirn, R. D., Schiestl, R. H., Willems, A. R., and Woods, R. A. (1995) Yeast 11, 353–369
29. Purvis, I. J., Chotai, D., Dykes, C. W., Lubahn, D. B., French, F. S., Wilson, E. M., and Hedden, A. N. (1991) Gene (Amst.) 106, 35–42
30. Atencio, J. (1994) The Characterization of MASS, an N-ethyl Maleimide-
DnaJ Co-chaperones in Androgen Receptor Activation

31. Cheetham, M. E., Brion, J.-P., and Anderton, B. H. (1992) Biochem. J. 284, 469–476
32. Ohtsuka, K. (1993) Biochem. Biophys. Res. Commun. 197, 235–240
33. Chellaiah, A., Davis, A., and Mohanakumar, T. (1993) Biochim. Biophys. Acta 1174, 111–113
34. Oh, S., Iwahori, A., and Kato, S. (1993) Biochim. Biophys. Acta 1174, 114–116
35. Cheetham, M. E., Anderton, B. H., and Jackson, A. P. (1996) Biochem. J. 319, 103–108
36. King, C., Eisenberg, E., and Greene, L. (1997) Biochemistry 36, 4067–4073
37. Melville, M. W., Tan, S.-L., Wambach, M., Song, J., Morimoto, R. I., and Katze, M. G. (1999) J. Biol. Chem. 274, 3797–3803
38. Sadler, I., Chiang, A., Kurihara, T., Rothblatt, J., Way, J., and Silver, P. (1989) J. Cell Biol. 109, 2665–2675
39. Caplan, A. J. (1999) Trends Cell Biol. 9, 262–268