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The Common Tetratricopeptide Repeat Acceptor Site for Steroid Receptor-associated Immunophilins and Hop Is Located in the Dimerization Domain of Hsp90*

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Structurally related tetratricopeptide repeat motifs in steroid receptor-associated immunophilins and the STI1 homolog, Hop, mediate the interaction with a common cellular target, hsp90. We have identified the binding domain in hsp90 for cyclophilin 40 (CyP40) using a two-hybrid system screen of a mouse cDNA library. All isolated clones encoded the intact carboxyl terminus of hsp90 and overlapped with a common region corresponding to amino acids 558–724 of murine hsp84. The interaction was confirmed *in vitro* with bacterially expressed CyP40 and deletion mutants of hsp90β and was delineated further to a 124-residue COOH-terminal segment of hsp90. Deletion of the conserved MEEVD sequence at the extreme carboxyl terminus of hsp90 precludes interaction with CyP40, signifying an important role for this motif in hsp90 function. We show that CyP40 and Hop display similar interaction profiles with hsp90 truncation mutants and present evidence for the direct competition of Hop and FK506-binding protein 52 with CyP40 for binding to the hsp90 COOH-terminal region. Our results are consistent with a common tetratricopeptide repeat interaction site for Hop and steroid receptor-associated immunophilins within a discrete COOH-terminal domain of hsp90. This region of hsp90 mediates ATP-independent chaperone activity, overlaps the hsp90 dimerization domain, and includes structural elements important for steroid receptor interaction.

Peptidyl-prolyl isomerases are cellular proteins that can mediate changes in protein conformation by catalyzing *cis-trans* isomerization about amino acid-proline peptide bonds (1–3). Immunophilins represent the predominant group within the rapidly growing peptidyl-prolyl isomerase protein family that includes the cyclophilins and FK506-binding proteins (FKBPs)[1] identified as cellular targets for the immunosuppressant drugs cyclosporin A and FK506, respectively (4, 5). An overlap of ligand binding and catalytic domains in immunophilins results in an inhibition of isomerase activity in response to immunosuppressant drug interaction (4, 5).

Cyclophilin 40 (CyP40) was first isolated and identified in association with the unactivated estrogen receptor (6) and shares structural and sequence homology with FKBP52, previously described as a common component of aposteroid receptor complexes (7, 8). A third mammalian immunophilin, FKBP51, with significant identity to CyP40 and FKBP52, has recently been identified in the progesterone receptor complex (9). The structural similarity among CyP40, FKBP51, and FKBP52 is characterized by an amino-terminal immunophilin-like domain and a conserved carboxyl-terminal tetratricopeptide repeat (TPR) domain that mediates protein interaction (6, 9–12). Within steroid receptor complexes the immunophilins bind competitively to heat shock protein hsp90 to form distinct immunophilin-hsp90-receptor complexes (9, 13, 14). Conserved structural features that determine immunophilin interaction with an identical site in hsp90 include the TPR domain together with adjacent subregions located at the amino- and carboxyl-terminal ends of the TPR domain, respectively (14, 15). Despite the overall similarities between these immunophilins, there is accumulating evidence that they possess unique structural elements allowing distinct interactions with hsp90, leading to differential responses of receptor function (9, 15, 16).

The dynamic assembly of steroid receptors to a high affinity hormone binding conformation requires the cooperative interaction of the major chaperones hsp70 and hsp90 (17–24; for review, see Ref. 25). Newly synthesized receptor undergoes sequential refolding through reactions mediated by hsp70, in concert with regulatory influences from the co-chaperones hsp40, Hip, and Hop, leading to the formation of receptor-hsp70-hsp90 complexes (23, 26–29). The conformation state and chaperone function of hsp70 and hsp90 are controlled by nucleotide binding (29–38). Hop provides the essential link between hsp70 and hsp90 and binds the chaperones simultaneously through favored interactions with ADP-bound forms of both proteins (37). In what is likely to be a highly dynamic setting, Hop enhances the efficient transfer of receptor substrate from hsp70 to hsp90 (28). After dissociation of Hop and hsp70, ATP-dependent conversion of hsp90 to the ATP-bound conformer promotes recruitment of p23 and one of the immunophilins to generate mature receptor complexes (23–25, 28).

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[1] The abbreviations used are: FKBPs, FK506-binding proteins; CyP40, 40-kDa cyclophilin; bCyP40, bovine CyP40; TPR, tetratricopeptide repeat; PCR, polymerase chain reaction; GST, glutathione *S*-transferase; WT, wild-type; IPTG, isopropyl β-D-thiogalactopyranoside; PAGE, polyacrylamide gel electrophoresis.

Although a precise role for p23 and the immunophilins in receptor complexes has yet to be defined, evidence that these accessory proteins are capable of independent chaperone activity (39, 40) raises the possibility that they might modulate receptor function either directly or indirectly via hsp90.

The majority of accessory proteins that participate in the chaperone-mediated assembly of steroid receptors possess structurally related TPR motifs that mediate their interaction with hsp90 and/or hsp70 (11–14, 22, 41, 42). Thus, in addition to the large immunophilins, TPR units have been identified in the hsp70-binding protein Hip (29), and six repeats are present in the chaperone cofactor Hop (41, 43). Distinct TPR domains located in the NH_2- and COOH-terminal regions direct the simultaneous interaction of Hop with hsp70 and hsp90, respectively (22, 41). The ability of Hop to compete effectively with the immunophilins for hsp90 binding (44), together with similar observations with PP5, a serine protein phosphatase recently identified as an additional component of the unactivated glucocorticoid receptor (42), have led to suggestions of a common TPR interaction site within hsp90 (44).

Genetic studies in yeast have confirmed that hsp90 is an essential component for the activity of diverse intracellular signaling molecules including steroid receptors and regulatory tyrosine kinases involved in cell cycle control (for review, see Ref. 25). A role for CyP40 in hsp90 regulatory function has been demonstrated by studies in which deletion of Cpr7, a *Saccharomyces cerevisiae* homolog of CyP40, resulted in greatly reduced activities of both glucocorticoid and estrogen receptors and $pp60^{v\text{-}src}$ kinase expressed in yeast (45).

To date one of our approaches to determining the function of CyP40 and its partner immunophilins has been to study their *in vitro* interaction with the known chaperone components of steroid receptor complexes. In this way we have identified hsp90 (14) and hsp70[2] as major cellular targets for CyP40 interaction. As an extension of this approach, we have begun to use the yeast two-hybrid system (46) to search for additional protein targets for CyP40. The modular domain structure of the large immunophilins (6, 12, 47) makes it attractive to use these domains separately as probes for protein interaction. In this way the immunophilin domain of FKBP52 has been used successfully in a yeast two-hybrid screen to isolate FAP48, a novel protein that may represent a common natural ligand for FKBPs (48).

Here, we show that use of the yeast-based genetic assay, in combination with the COOH-terminal TPR domain of CyP40 (amino acids 185–370), led predominantly to the isolation of interacting clones that corresponded to hsp90 cDNAs encoding the COOH-terminal dimerization domain of hsp90. *In vitro* binding assays with deletion mutants of this region located the interaction site for CyP40 to a 124-residue COOH-terminal segment of hsp90 and confirmed that this region incorporates the common TPR binding site for the immunophilins and Hop. Deletion of the acidic EEVD motif, conserved at the carboxyl terminus of hsp90 proteins, precluded CyP40 and Hop interaction, consistent with a regulatory role of this microdomain in hsp90 function.

EXPERIMENTAL PROCEDURES

Plasmids—pBTM116-CyP40 185–370 contains the carboxyl half of CyP40 fused in-frame to the DNA binding domain of LexA (amino acids 1–202). The COOH-terminal portion of human CyP40, encoding residues 185–370, was amplified by PCR using primers that introduced an *Eco*RI site at the 5′-end and a termination codon with an *Eco*RI site at the 3′-end. Subcloning of this fragment into pGEM 3Z (Promega) at the *Eco*RI restriction enzyme site allowed confirmation of sequence integrity by automated sequence analysis (Applied Biosystems). The *Eco*RI fragment was then ligated into the corresponding restriction site in the bait vector pBTM116 (49), in-frame with the LexA DNA binding domain to give pBTM116-CyP40 185–370.

The plasmid for the protein GST-bCyP40 WT has been described (14). For untagged wild-type bovine CyP40, a bCyP40 cDNA template was amplified by PCR using sequence-specific oligonucleotide 5′- and 3′-primers containing *Nde*I and *Bam*HI restriction enzyme sites, respectively. A TGA stop codon was placed immediately before the *Bam*HI site. The PCR fragment was ligated into pGEM-T (Promega), and the sequence integrity of both ends of the insert in an isolated clone was confirmed by automated sequence analysis (Applied Biosystems). An *Xho*I to *Bcl*I fragment excised from a wild-type bCyP40 cDNA was replaced to eliminate PCR-generated errors within this region. A full-length bCyP40 fragment produced by *Nde*I and *Bam*HI digestion was then cloned into the pET-11 plasmid vector. The pET-11-bCyP40 WT expression plasmid was transformed into the *E. coli* expression host BL21(DE3). Overexpression of wild-type bovine CyP40 was induced by 0.4 mM IPTG, and lysates of recombinant CyP40 were prepared as described below. Expression plasmids for human hsp90β (50), FKBP52 (10), and Hop were kindly provided as gifts by C. T. Walsh, D. A. Peattie, and D. F. Smith, respectively. An identical cloning strategy was used to generate NH_2-terminal His-tagged expression plasmids for hsp90β deletion mutants incorporating codons 530–724, 581–724, 600–724, 530–700, and 530–719. Each construct was PCR amplified from pET-15b-hsp90β (50) full-length cDNA template using *Taq* DNA polymerase with primers that introduced an *Nde*I site at the 5′-end and a termination codon with an *Nde*I site at the 3′-end. The PCR fragment was ligated into pGEM-T and was then excised with *Nde*I digestion from a suitable clone confirmed for sequence fidelity. The gel-purified fragments were cloned into the pET-28a(+) vector that had been linearized with *Nde*I to give the expression plasmids pET-28a(+) 530–724β, pET-28a(+) 581–724β, pET-28a(+) 600–724β, pET-28a(+) 530–700β, and pET-28a(+) 530–719β.

The expression plasmid pET-28a(+) 589–732α was prepared for the NH_2-terminal His-tagged hsp90α deletion mutant encompassing residues 589–732. The plasmid was derived by PCR from a full-length human hsp90α cDNA template (51) (kindly provided K. Yokayama) using *Pfu* DNA polymerase (Stratagene) and specific oligonucleotide primers with built-in *Nhe*I (5′-end) and *Bam*HI (3′-end) restriction sites. The *Bam*HI site was preceded by a termination codon. Subcloning of the blunt ended PCR fragment into *Sma*I-digested pGEM 3Z vector allowed confirmation of sequence integrity. The fragment was excised with *Nhe*I and *Bam*HI and ligated into vector DNA to give pET-28a(+) 589–732α.

Two-hybrid Screening—Two-hybrid system reagents including the mouse embryo cDNA library in pVP16, the *S. cerevisiae* reporter strain L40, and screening methodology were as described previously (49, 52). Briefly, *S. cerevisiae* L40 cells were transformed sequentially with pBTM116-CyP40 185–370 and the library, and the yeast transformants were plated on histidine-deficient medium. Of the 40×10^6 yeast transformants screened, some 300 were identified as histidine prototrophs of which 150 were selected for assessment of β-galactosidase activity. 28 His^+,$LacZ^+$ colonies were grown in liquid medium deficient in leucine and were used to generate crude plasmid DNA preparations. These were transformed into competent *Escherichia coli* HB101 cells allowing selection of colonies containing library hybrid plasmids. Purified minipreparations of the resultant clones were transformed into L40 yeast cells simultaneously with pBTM116-CyP40 185–370 bait plasmid, and the transformants were assayed for β-galactosidase activity. To reduce the risk of false positives, parallel transformations were performed with individual library plasmids alone or in combination with pBTM116,[3] pBTM116-ARL-E1, and pBTM-LYN as negative controls. cDNA inserts in library pVP16 plasmids, isolated from clones considered to be true positives, were sequenced using both a sense (5′-GAGTTTGAGCAGATGTTTA-3′) and antisense M13 universal primer (Applied Biosystems). 10 murine hsp84-related cDNAs (53) were recovered from the true positive clones.

Protein Expression and Purification—The expression plasmid for GST-bCyP40 WT fusion protein was transformed into *E. coli* strain XL-1 Blue, and the protein was purified from lysates of bacterial cultures by affinity chromatography on glutathione-agarose (Amersham Pharmacia Biotech) as detailed previously (14). The expression plasmid for FKBP52 was transformed in *E. coli* pVX28 (10). All other expression constructs used in this study were transformed into *E. coli* BL21(DE3). Recombinant CyP40, Hop, FKBP52, and hsp90β proteins were overex-

[2] A. Carrello, manuscript in preparation.

[3] E. Ingley, manuscript in preparation.

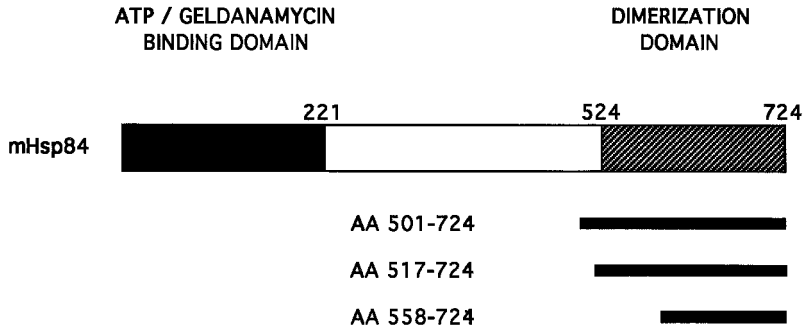

FIG. 1. **Murine (mhsp84) isolates identified by two-hybrid genetic screen with the COOH-terminal domain of CyP40.** Hsp90 functional domains for ATP/geldanamycin binding (*black*) (43, 44, 70) and dimerization (*shaded*) (56) are shown schematically. *Numbers* refer to amino acid positions. CyP40 interaction isolates, denoted by amino acids (*AA*) 501–724, 517–724, and 558–724, are represented as *narrow bars* (*black*).

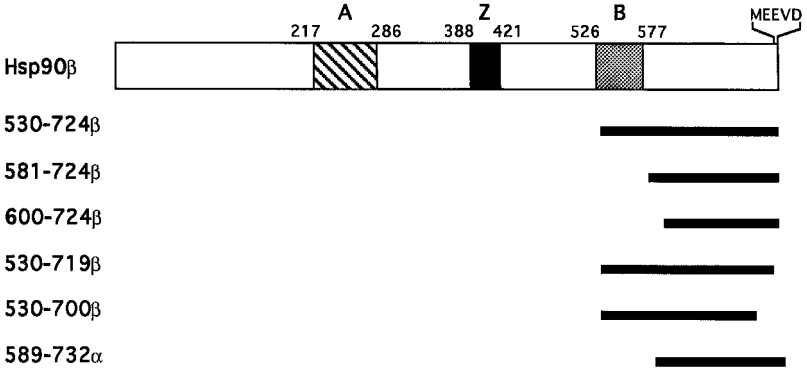

FIG. 2. **Structural domains of hsp90β and hsp90 COOH-terminal deletion mutants.** Structural elements for hsp90 include regions A, B, and Z (57–59) and the MEEVD sequence motif at the extreme COOH terminus (54). COOH-terminal truncation mutants for hsp90α and β isoforms are represented as *narrow bars* (*black*).

pressed in bacterial cultures by induction with IPTG. Lysates were prepared by sonicating bacterial cells in lysing buffer (CyP40, Hop: 10 mM Tris, pH 7.3, containing 100 mM KCl, 1 mM dithiothreitol, 0.2% v/v Triton X-100, and 1 mg/ml lysozyme; FKBP52 and hsp90α and β: 50 mM disodium hydrogen orthophosphate, pH 7.4, containing 300 mM NaCl, 10 mM imidazole, 1 mM dithiothreitol, 0.2% v/v Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 1 mg/ml lysozyme) and were cleared of particulate material by ultracentrifugation (100,000 × *g* for 30 min at 4 °C).

For expression of His-tagged hsp90α and β deletion mutants, bacterial cells harboring these clones were pelleted from overnight cultures (150 ml) by centrifugation. After resuspension in 1.5 liters of fresh 2 YT medium containing 200 μg/ml kanamycin, the bacteria were incubated with shaking for a further 1 h at 37 °C. Protein expression was induced with 1 mM IPTG over a 4-h period. Centrifugation gave a bacterial pellet that was resuspended in 20 ml of lysing buffer (as described for FKBP52, hsp90β). The freeze-thawed bacteria were sonicated on ice, and the lysates were recovered by ultracentrifugation as already described. All His-tagged recombinant proteins were purified from crude lysates by chromatography on Ni-NTA-agarose (Qiagen). Unbound proteins were removed from the chelate-agarose gel (0.5 ml) by 10 successive washes with 50 mM disodium hydrogen orthophosphate, pH 7.4 buffer, containing 300 mM NaCl, 10 mM imidazole, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. The first wash also contained 1% v/v Triton X-100. Gel charged with immobilized His-tagged proteins was stored at 4 °C for use in protein binding studies, or the absorbed protein was recovered by elution with the same buffer (1 ml) containing 500 mM imidazole. Recovered proteins were assessed for purity by SDS-PAGE with Coomassie Blue staining, and after equilibration by dialysis against binding buffer (10 mM Tris pH 7.3, containing 100 mM KCl and 1 mM dithiothreitol) they were stored in 200-μl aliquots at −70 °C until required.

Protein Binding Studies—Direct binding studies between wild-type hsp90β and truncated hsp90 mutants with recombinant CyP40 and Hop were conducted in parallel. Briefly, chelate-agarose gels containing immobilized hsp90-related proteins were equalized for protein content by diluting up to 3-fold with Sepharose 4B. The diluted gels were prepared in duplicate 50-μl aliquots, and each set of duplicates was rotated for 3 h at 4 °C with IPTG-induced bacterial lysates for CyP40 (150 μl) or Hop (200 μl). Both lysates had been equilibrated previously in binding buffer plus 35 mM imidazole. The inclusion of imidazole to 35 mM concentration was found to be effective in limiting nonspecific protein interaction with control chelate-agarose gel devoid of hsp90-related proteins. The gels were subjected to replicate washes (8 × 500 μl) with binding buffer plus 35 mM imidazole, the first 5 washes being supplemented with 0.2% v/v Triton X-100. Gel-retained proteins were recovered by boiling in 40 μl of 2 × SDS-PAGE sample buffer and then analyzed by SDS-PAGE on 12.5% w/v polyacrylamide gel. Proteins were visualized by Coomassie Blue staining.

In a reciprocal binding study, glutathione-agarose containing absorbed GST-bCyP40 WT fusion protein was diluted 5-fold with Sepharose 4B, and 40-μl aliquots of the diluted gel were rotated separately for 3 h at 4 °C with 30 μg of the purified hsp90 truncated mutants equilibrated in binding buffer (500 μl) containing 0.2% Triton X-100. After pelleting by microcentrifugation, the gels were washed repeatedly as already described, boiled in 2 × SDS-PAGE sample buffer (40 μl), and then examined for protein retention by SDS-PAGE.

To determine the binding preference of CyP40 for hsp90α *versus* hsp90β, the diluted glutathione-agarose gel containing GST-bCyP40 WT fusion protein was suspended in 500 μl of binding buffer plus 0.2% v/v Triton X-100, together with 30 μg of protein consisting of either purified hsp90 530–724β or hsp90 589–732α alone or prepared in ratios of 1:1, 5:1, and 1:5, respectively. After rotation for 3 h at 4 °C the gels were washed free of unbound protein, boiled in 2 × SDS-PAGE sample buffer (40 μl), and assessed for retention of the hsp90α and β mutants by SDS-PAGE.

The ability of Hop and FKBP52 to compete with CyP40 for binding to the truncated mutant hsp90 581–724β was determined as follows. 0-, 20-, 50-, 100-, 200-, and 400-μl aliquots of induced Hop bacterial lysate and 0-, 10-, 20-, 50-, 100-, and 200-μl aliquots of an extract containing purified FKBP52 (concentration 0.54 μg/μl), were supplemented with 30 μg of purified hsp90 581–724β protein and the mixtures were brought to 500-μl total volume with binding buffer containing 0.2% v/v Triton X-100. After a 3-h preincubation period at 4 °C, the mixtures were added to separate 40-μl aliquots of Sepharose 4B-diluted glutathione-agarose containing immobilized GST-bCyP40 WT fusion protein. After rotation at 4 °C for a further 3 h the gels were washed as described, boiled with 40 μl of 2 × SDS-PAGE sample buffer, and analyzed for hsp90 581–724β protein retention by SDS-PAGE with Coomassie Blue staining. Protein was quantitated by densitometric scanning using Image Quant (Molecular Dynamics) software.

RESULTS

Isolation of Murine Hsp84 cDNAs Encoding a Distinct Interaction Domain for CyP40—We used a modification (49, 52) of the yeast two-hybrid system (46) to isolate cDNA clones encoding proteins that bind to the COOH-terminal half of CyP40. This segment of CyP40 incorporates the TPR domain flanked by acidic and basic subdomains at the NH_2- and COOH-terminal ends, respectively (14). The CyP40 COOH-terminal domain was fused to the LexA DNA binding domain, and the resulting

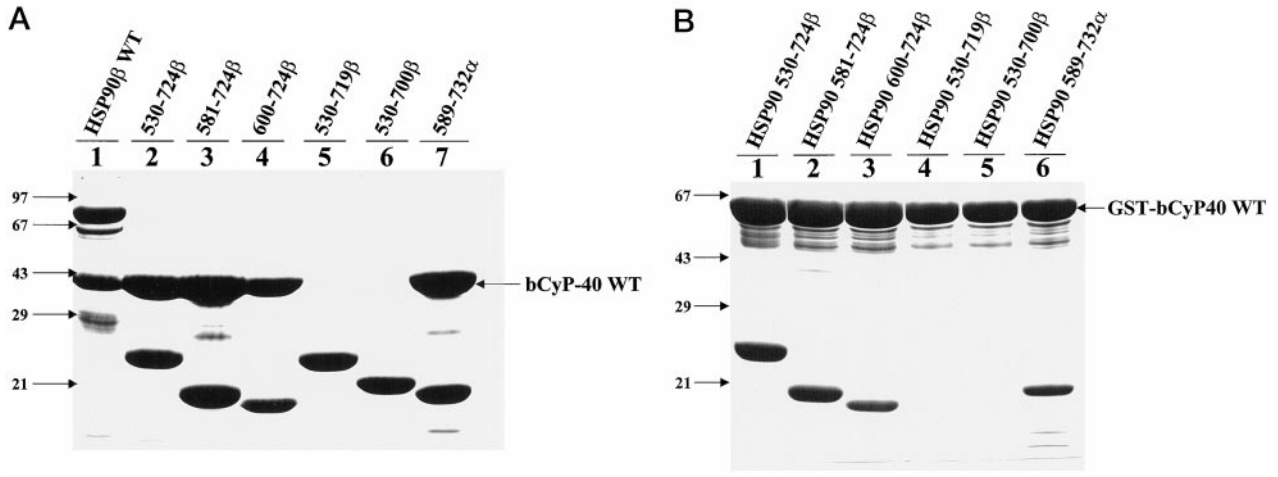

FIG. 3. **Binding properties of wild-type hsp90β and hsp90α and β COOH-terminal truncation mutants to CyP40.** *Panel A*, chelate-agarose gels (50 μl) charged with His-tagged hsp90-related proteins were mixed with rotation for 3 h at 4 °C with IPTG-induced bacterial lysate (150 μl) for full-length bCyP40 in binding buffer containing 35 mM imidazole. After centrifugation, the gels were washed repeatedly with the same buffer, initially supplemented with 0.2% v/v Triton X-100. Gel-retained proteins were recovered with SDS-PAGE sample buffer and analyzed on a 12.5% w/v polyacrylamide gel followed by Coomassie Blue staining. Protein molecular weight markers (Amersham Pharmacia Biotech) are shown on the *left* side. *Panel B*, glutathione-agarose gels (40 μl) charged with GST-bCyP40 WT fusion protein were rotated separately for 3 h at 4 °C with purified hsp90 truncated mutants (30 μg) in binding buffer (500 μl) containing 0.2% v/v Triton X-100. After microcentrifugation and replicate washing, gel-bound protein was recovered with SDS-PAGE sample buffer and analyzed as described for *panel A*.

bait hybrid was used to screen a mouse embryo library of hybrid proteins between the nuclear localized VP16 acidic activation domain and random cDNA fragments. Size selection for short cDNAs (~ 500 nucleotides) facilitated rapid sequence analysis and isolation of distinct protein interaction domains within full-length polypeptides (52). Coexpression of the bait and target hybrids into the yeast host *S. cerevisiae* L40 that contains two integrated reporter constructs, the yeast *HIS3* gene and the bacterial *lac*Z gene, allowed the isolation of cDNAs coding for putative CyP40-interacting proteins. With this approach, approximately 40×10^6 yeast transformants were screened. More than 300 histidine-positive prototrophs were detected, of which 150 were assayed qualitatively for the presence of β-galactosidase activity. Transactivation of both reporter constructs was observed in 28 transformants, from which the library plasmids were isolated and subjected to false positive analysis. These clones all interacted more strongly with the CyP40 bait fusion protein than with several LexA DNA-binding fusion controls. Sequence analysis of their cDNA inserts revealed the presence of 10 incomplete murine hsp84 COOH-terminal clones, which fell into three distinct groups. Seven inserts encoding residues 517–724 belonged to the most predominant group. The remaining cDNAs encoded residues 501–724 (two clones) and 558–724 (one clone), respectively (Fig. 1). These results strongly suggested that the COOH terminus of hsp90 mediates binding to CyP40.

The Interaction Domain for CyP40 Is Located within the 124-residue Carboxyl-terminal Region of Hsp90, and Deletion from the Carboxyl Terminus of Hsp90 Interferes with CyP40 Recognition—The mouse hsp84 sequence of those clones found to interact most frequently with CyP40 differs from the equivalent region of human hsp90β (53, 54) at only five residues. Functional domains defined within hsp90 include a common binding site for ATP/ADP and the antitumor agent geldanamycin in the NH_2 terminus (35, 36, 55) and a COOH-terminal region that mediates hsp90 dimerization (56) (Fig. 1). Structural elements include highly charged α-helical regions A and B and region Z, which resembles a heptad repeat domain characteristic of leucine zippers (57–59) (Fig. 2). These structural domains have been predicted to play a role in hsp90-protein interaction (57–59).

Our results with the yeast two-hybrid system determined that the interaction domain for CyP40 was confined to a 200-residue COOH-terminal region of hsp90 corresponding to the dimerization domain of the protein (Fig. 1). To confirm the ability of this region to mediate CyP40 binding *in vitro*, we first prepared the truncated deletion mutant hsp90 530–724β incorporating an NH_2-terminal His-tag (Fig. 2). Pull-down assays in which this protein was immobilized on Ni-NTA-agarose and then exposed to bacterial lysate containing wild-type bCyP40 showed avid retention of the cyclophilin (Fig. 3*A*, *lane 2*). Moreover, in mapping studies (not shown) conducted with a series of GST-fusion proteins incorporating wild-type bCyP40 and several CyP40 deletion mutants, the hsp90 530–724β protein displayed an interaction pattern identical to that described previously for full-length hsp90β (14). This COOH-terminal segment of hsp90 therefore appears to contain all of the essential elements for CyP40-hsp90 interaction.

We then prepared sequential NH_2-terminal and COOH-terminal deletions of this original construct to delineate further the interaction domain for CyP40 (Fig. 2). The design of these additional mutants was guided by information from available reports (57–59) describing the microdomain structure within the hsp90 COOH-terminal region (Fig. 2). For example, the construct hsp90 581–724β was prepared to assess the role of the hydrophilic region B in the hsp90-CyP40 interaction. The EEVD motif, which is conserved at the extreme COOH terminus in both the hsp70 and hsp90 molecular chaperone families (54, 60), has been shown to have an important regulatory role in hsp70 function (60). Preparation of the hsp90 530–719β construct was aimed at testing the influence of this regulatory motif on hsp90 recognition of CyP40. Although hsp90α and β appear to be equivalent in the context of steroid receptor and immunophilin interaction (61), we also prepared hsp90 589–732α to allow a direct comparison with hsp90 581–724β for CyP40 binding efficiency.

The ability of these truncated proteins to bind CyP40 was examined in pull-down assays in which chelate-agarose charged with equal amounts of wild-type hsp90β and the recombinant deletion mutants was incubated with bacterial lysates containing wild-type CyP40. Our binding profiles showed that the hsp90 530–724β, 581–724β, and 600–724β proteins

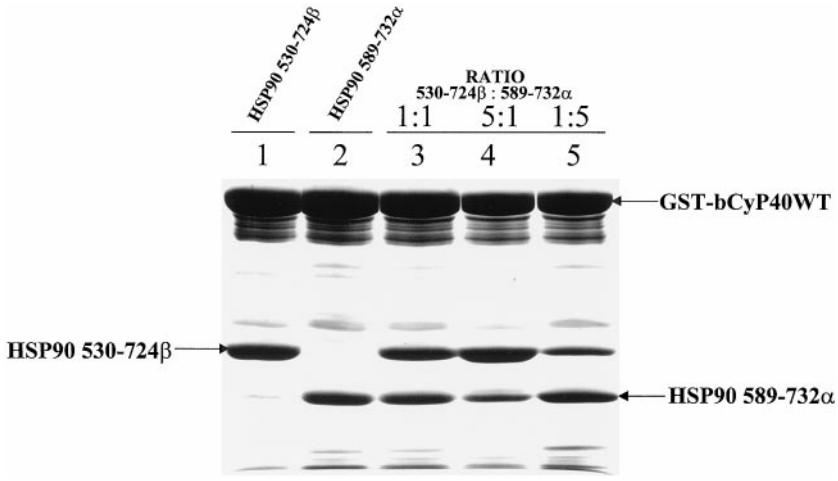

FIG. 4. **CyP40 does not show a binding preference for hsp90α or β isoforms.** Glutathione-agarose gels charged with GST-bCyP40 WT fusion protein were incubated as described in the Fig. 3*B* legend with 30 μg of purified hsp90 530–724β or hsp90 589–732α alone or prepared in ratios of 1:1, 5:1, and 1:5, respectively. After replicate washing to remove unbound protein, the gels were boiled in SDS-PAGE sample buffer, and recovered proteins were analyzed by SDS-PAGE.

(Fig. 3*A*, *lanes 2*, *3*, and *4*) bound CyP40 with high efficiency. Removal of the 24-residue segment at the extreme COOH terminus of hsp90 (construct 530–700β) completely abolished CyP40 binding (Fig. 3*A*, *lane 6*). Remarkably, the mutant 530–719β, in which the last five residues MEEVD of hsp90β are deleted, was also unable to interact with CyP40 (Fig. 3*A*, *lane 5*). The above results were corroborated by a reciprocal study in which the hsp90 deletion mutants were incubated separately with GST-bCyP40 WT fusion protein immobilized on glutathione-agarose (Fig. 3*B*). Our results located the binding site for CyP40 to a 124-residue COOH-terminal region of hsp90 and suggested that the presence of the EEVD motif at the extreme COOH terminus of hsp90 is critical for CyP40-hsp90 interaction.

The results of Fig. 3, *A* and *B*, also indicated that the 581–724β and 589–732α deletion mutants were capable of efficient interaction with wild-type CyP40. A more rigorous examination of the possible preference of CyP40 for one or other of the hsp90α or β isoforms was conducted in a separate study. Analysis was facilitated by the use of chelate-agarose-purified hsp90 530–724β and hsp90 589–732α deletion mutants, which allowed SDS-PAGE discrimination on the basis of size. Fig. 4 shows the results of a binding study in which GST-bCyP40 WT, immobilized on glutathione-agarose, was exposed to extracts containing either recombinant hsp90 530–724β (*lane 1*) or hsp90 589–732α (*lane 2*) alone or to mixtures in which the ratios of the respective isoforms were 1:1 (*lane 3*), 5:1 (*lane 4*), and 1:5 (*lane 5*). It was apparent from the observed binding profiles that CyP40 does not display an affinity for one hsp90 isoform over the other.

CyP40, FKBP52, and Hop Interact with Hsp90 via a Common Domain within the Hsp90 Carboxyl Terminus—Evidence that Hop and FKBP52 compete directly with CyP40 for hsp90 binding has led to proposals that these proteins might target a common or overlapping site within hsp90 (44). It was of interest therefore to test the ability of Hop and FKBP52 to bind to the COOH-terminal region of hsp90 which we had determined to be important for CyP40-hsp90 interaction. Fig. 5 shows that incubation of our panel of hsp90 deletion mutants, immobilized on chelate-agarose, with Hop bacterial lysates produced a retention profile that closely resembled the pattern observed with CyP40 (Fig. 3*A*). The result is consistent with the presence of common binding elements for CyP40 and Hop within a discrete carboxyl-terminal domain of hsp90. We next examined the ability of FKBP52 and Hop to compete with CyP40 for binding to hsp90 581–724β. Extracts containing the purified hsp90 deletion construct were preincubated with increasing amounts of purified FKBP52 or Hop bacterial lysate. After addition of glutathione-agarose charged with GST-bCyP40 WT fusion protein, the mixtures were incubated for a further period, and the gels were then assessed for retention of hsp90 581–724β by SDS-PAGE. Fig. 6 shows that increasing concentrations of FKBP52 caused a progressive reduction in the relative amount of the hsp90 deletion mutant retained by gel-immobilized CyP40. Similar competitive binding was observed with Hop (not shown). Together the results confirmed that CyP40, FKBP52, and Hop interact directly with a common domain in hsp90 581–724β.

DISCUSSION

A search for cellular protein targets for the CyP40 COOH-terminal protein-interaction domain (amino acids 185–370), using the yeast two-hybrid method, resulted in the cloning of cDNAs encoding hsp90 with an intact carboxyl terminus. The overlapping region common to these cDNAs corresponded to amino acids 558–724 of human hsp90β. To confirm this interaction *in vitro*, the deletion mutant hsp90 530–724β, containing an NH_2-terminal histidine tag, was immobilized on chelate-agarose gel and shown to retain wild-type bCyP40 specifically. Pull-down assays with recombinant hsp90 530–724β and GST-CyP40 deletion mutants on glutathione-agarose revealed a binding profile similar to that determined previously for full-length hsp90 (14), suggesting that the COOH-terminal hsp90 segment contains the essential binding elements for CyP40-hsp90 interaction.

Binding analyses with sequential NH_2-terminal deletion mutants of the hsp90 530–724β protein showed the recognition site for CyP40 to be located within a 124-residue, COOH-terminal region of hsp90. A deletion mutant truncated from the COOH-terminal end by deleting the pentapeptide MEEVD was unable to bind CyP40, highlighting the critical importance of this conserved motif for CyP40-hsp90 interaction. Under the same conditions, the interaction profile of Hop for the hsp90 derivatives closely matched that displayed by CyP40. This, together with evidence of direct competition of Hop and FKBP52 with CyP40 for binding to hsp90 581–724β protein, supports the existence of a common TPR interaction site for Hop and the immunophilins within a discrete COOH-terminal domain of hsp90. It is possible, however, that additional regions in hsp90, lying outside of this general TPR acceptor site, contribute to stabilize the interaction between hsp90 and its cochaperone partners (16). Our data extend the findings of recent reports by Chen *et al.* (16) and by Young *et al.* (63) that Hop and the steroid receptor-associated immunophilins bind to the COOH-terminal region of hsp90.

After a comprehensive assessment of the effect of mutations

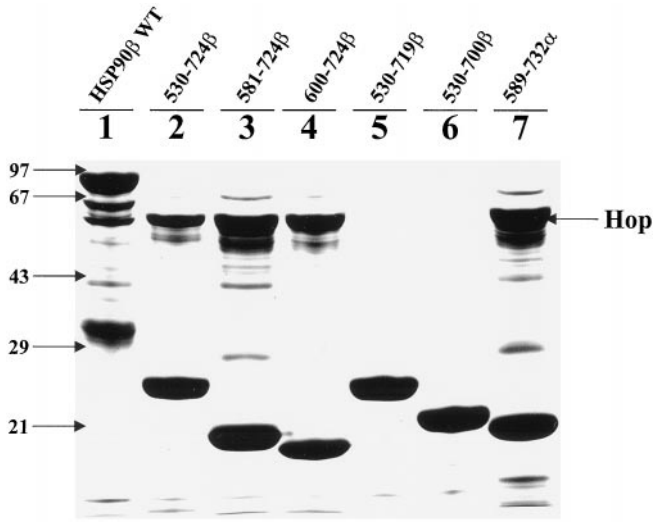

FIG. 5. **Interaction properties of wild-type hsp90β and hsp90α and β COOH-terminal truncation mutants with Hop.** Chelate-agarose gels (50 μl) charged with His-tagged hsp90-related proteins were rotated for 3 h at 4 °C with IPTG-induced bacterial lysate (200 μl) for Hop in binding buffer containing 35 mM imidazole. After microcentrifugation and replicate washing, the gels were assessed for bound Hop by SDS-PAGE as described in the Fig. 3*A* legend.

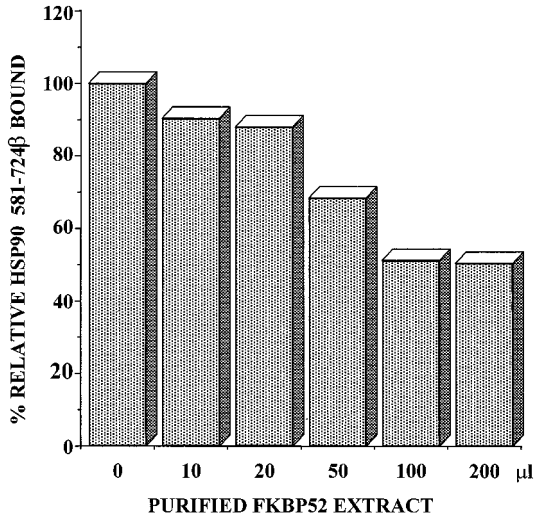

FIG. 6. **FKBP52 competition with CyP40 for binding to hsp90 581–724β.** Aliquots (0, 10, 20, 50, 100, and 200 μl) of purified FKBP52 extract (FKBP52 concentration 0.54 μg/μl) were supplemented with purified hsp90 581–724β protein (30 μg) and were equalized to 500 μl with binding buffer plus 0.2% v/v Triton X-100. After brief mixing, the samples were preincubated for 3 h at 4 °C and then added to separate 40-μl aliquots of glutathione-agarose charged with GST-bCyP40 WT fusion protein. After rotation at 4 °C for a further 3 h, the gels were washed as in Fig. 3*B* and analyzed for retained hsp90 581–724β by SDS-PAGE as already described.

throughout chicken hsp90α on hsp90 interaction with accessory proteins, Chen *et al.* (16) have been able to provide detailed comparisons between the hsp90 binding requirements for Hop, the TPR-containing immunophilins, and p23. Replacement of the EEVD motif by AAVD, at the extreme COOH terminus of hsp90, reduced hsp90 interactions for all of the TPR proteins, with binding being all but eliminated for CyP40 and Hop (16). The interactions of hsp90 with FKBP52 and FKBP51 were less sensitive to this mutation, with significant binding levels being maintained (16). Our study, in which the hsp90 mutant deleted in the MEEVD peptide failed to recognize both CyP40 and Hop, is consistent with the findings of Chen *et al.* (16). It is possible that, as in hsp70 (60), the conserved EEVD motif might contribute to the overall conformation of the hsp90 protein and have a role in the intramolecular regulation of hsp90 function.

Our study has demonstrated conclusively that CyP40 does not have a binding preference for either hsp90α or β. From heat-induced dissociation experiments with FKBP52·hsp90 complexes, Czar *et al.* (61) noted previously that neither of the isoforms is dissociated selectively, suggesting that FKBP52 may bind equivalently with each hsp90 subtype. The isoforms are produced in equal amounts in higher eukaryotes (64) and share an 86% homology, differing at 99 residues in their amino acid sequences (54). A glutamate-rich segment within the NH_2-terminal sequence distinguishes the hsp90α class from hsp90β homologs (54). Heat shock induces a more profound increase in hsp90α expression compared with hsp90β, and only hsp90α is induced by adenovirus E1A (65). Despite these differences, virtually identical hydropathy plots for the isoforms indicate a high degree of structural similarity (54). It is of interest, however, that the hsp90 species associated with chicken steroid receptors (66) and that isolated with the bovine estrogen receptor (67) have both been identified as α isoforms.

We have examined the 124-residue COOH-terminal sequence that incorporates the TPR acceptor site in hsp90 to identify structural elements with potential to mediate hsp90 interaction with TPR proteins. Charge distribution within this domain is highly conserved between human hsp90α and β (54) and in chicken hsp90α (66) and mouse hsp84 (53). Taking this charge distribution into account, we divided the binding region in hsp90β into two subdomains (Fig. 7). A highly acidic region is located at the carboxyl terminus (amino acids 691–724, 1 basic and 15 acidic residues) and includes the EEVD motif, deletion of which abrogates hsp90-TPR protein binding. An essentially neutral subdomain (amino acids 600–690, 16 basic and 12 acidic residues), which is characterized by a repeated helix-coil-helix secondary structure (57), contains a concentration of charged residues between amino acids 623–653 (9 basic and 7 acidic residues) (54). This hydrophilic domain sits adjacent to a hydrophobic region (amino acids 657–673 in human hsp90β, equivalent to amino acids 661–677 in chicken hsp90α) which is necessary for hsp90 dimerization (68). Chen *et al.* (16) have also shown that deletion of this 16-residue segment causes a marked reduction in the binding of hsp90 to Hop and the steroid receptor-associated immunophilins. Removal of this critical domain disrupts a predicted α-helical structure (57), possibly resulting in conformational changes unfavorable for dimerization and TPR-protein interaction.

Several points of evidence suggest that the interaction site for Hop and the TPR immunophilins lies within the dimerization domain of hsp90. Dimerization is an intrinsic property of hsp90, required for its biological function in intact cells (69). Nemoto *et al.* (56, 70) have demonstrated that the ability of hsp90 to dimerize and to form higher order oligomers resides in the COOH-terminal 200 amino acids. Furthermore, the same workers have proposed that dimerization of hsp90α is mediated by the interaction of one subdomain (defined by residues 542–615) with a different subregion (residues 621–698) of a second hsp90 subunit (56, 71). Such a mechanism is compatible with the observed sensitivity of hsp90-TPR protein interaction to deletions within these specific regions (16).

Evidence suggests that within steroid receptor heterocomplexes the stoichiometry is defined by single molecules of receptor, p23 and one of the immunophilins together with dimeric hsp90 (61). Although we have not determined whether our hsp90β mutants exist as dimers or monomers, the protein hsp90 600–724β is largely deficient in sequences thought necessary for dimerization (56, 71) and is therefore likely to be monomeric. Efficient binding of both CyP40 and Hop to this

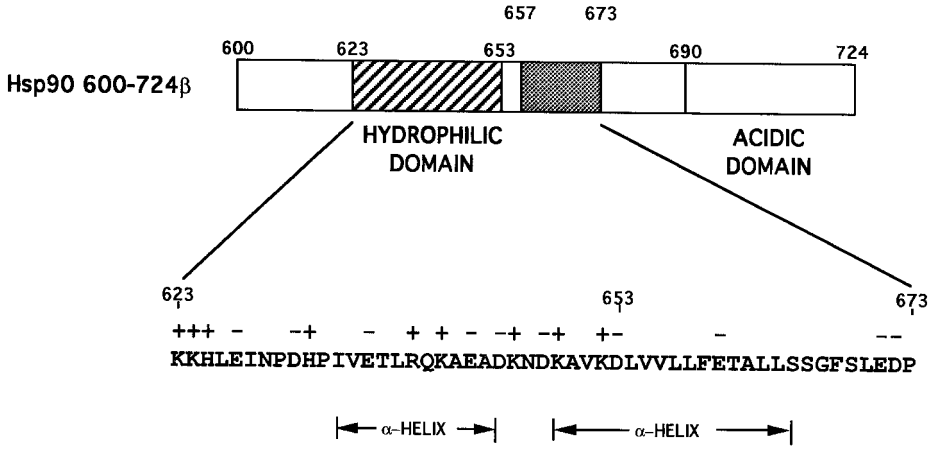

FIG. 7. **Structure of the TPR interaction domain in hsp90.** The TPR acceptor site of hsp90β is located within a COOH-terminal region spanning amino acid residues 600–724. Acidic and basic residues are *highlighted* by negative and positive charges, respectively. Within this sequence exists an acidic domain, defined by residues 690–724, which includes the EEVD sequence motif at the extreme COOH terminus. A 50-residue segment (623–673) incorporates a hydrophilic domain adjacent to a hydrophobic region (residues 653–673) and includes two predicted α-helical microdomains (57).

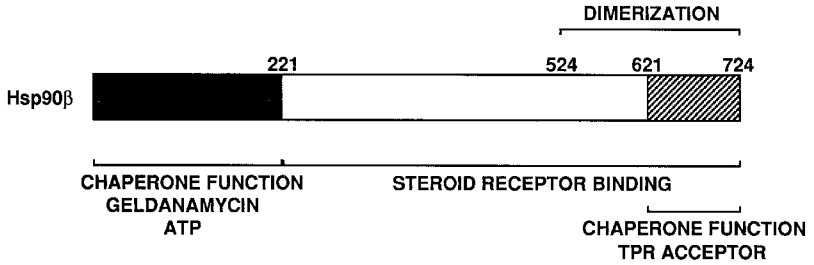

FIG. 8. **Hsp90 functional domains.** The NH_2-terminal sequence (residues 1–221) incorporates a chaperone function (74, 75) and binding sites for geldanamycin and ATP (35, 36, 55). A dimerization domain (residues 524–724) exists at the COOH-terminal end of hsp90 (56). This overlaps a region (residues 621–724) that elicits a chaperone function (74, 75) and the ability to interact with TPR proteins. A large segment (residues 221–724) includes elements that mediate association with steroid receptors (64, 68, 76, 77).

mutant suggests that both proteins are capable of interacting with a monomer of hsp90.

Shaknovich *et al.* (72) provided initial evidence that the COOH-terminal domain of hsp90 might have a biological function beyond that of dimer formation. They described the conversion of the basic helix-loop-helix transcription factor MyoD to a form with enhanced DNA binding activity through a transient interaction with a recombinant protein containing the COOH-terminal 194 residues of hsp90 (72). This conformational activation of MyoD occurred without the requirement of ATP (72) and was extended to other basic helix-loop-helix proteins (73). A recent analysis of human hsp90α NH_2-terminal (residues 9–236) and COOH-terminal (residues 629–732) domains has confirmed independent chaperone activities within these sites (74, 75). In full-length hsp90, the sites appear to contribute independently to chaperone activity, differing in substrate specificity and nucleotide dependence (74, 75). The presence of chaperone and dimerization functions within a discrete hsp90 COOH-terminal domain, which also mediates interactions with TPR cochaperone proteins, is intriguing. It is possible then that hsp90 partner proteins, such as Hop and the TPR-containing immunophilins, might have an important modulating role in hsp90 function (38).

Data from mutational analyses of hsp90 binding to steroid receptors are consistent with the COOH-terminal half of hsp90 being involved in receptor interaction and in the maintenance of receptor biological function (64). Certain regions within hsp90 appear to have a differential effect on hsp90-steroid receptor interaction and on the activity of individual receptor systems (59, 68, 76, 77), highlighting the diversity among the receptors. Deletion of the acidic region A (residues 221–290) precluded the interaction of chicken hsp90α with the glucocorticoid receptor (59), but recognition of the progesterone (76) and estrogen receptors (68) was unaffected. Critical regions for hsp90-progesterone receptor binding are located between residues 381 and 441, and 601 and 677 of chicken hsp90α (76). The first incorporates the leucine heptad repeat region Z (59), and the second overlaps the hsp90 dimerization domain (56). Deletions that compromise the *in vivo* interaction of hsp90 with the estrogen receptor include those that correspond to region Z and the charged region B (residues 521–567). Additional elements implicated in hsp90 association with the estrogen receptor are contained between residues 601 and 620, and 698 and 728 (68). The latter defines the acidic subdomain at the carboxyl terminus of hsp90 (57, 66). Its deletion results in the loss of hsp90 dimerization (68). Deletion of the hydrophobic sequence between residues 661 and 677 also disrupts hsp90 dimer formation, but this segment appears to be dispensable for hsp90-estrogen receptor interaction (68).

Hsp90 functions as a dimer and is divisible into three functional domains (Fig. 8). The NH_2-terminal domain harbors a common site for ATP binding and interaction with geldanamycin and has a chaperone activity that is modulated by both agents (35, 36, 55, 74, 75). An ATP-independent chaperone function exists in the COOH-terminal domain, a region that also appears to be critically important for hsp90 dimerization (56, 68, 70, 71) and binding of client proteins such as steroid receptors (64, 68, 76, 77). Our results show that this domain is also a target for the TPR-containing immunophilins and Hop. A central charged domain may provide additional elements that contribute to stabilizing the dimeric form of hsp90 (36) as well as interactions with steroid receptors (57–59) and hsp90 partner proteins (16). Identification of an ATP binding site within hsp90 has led to proposals that in response to ATP binding and hydrolysis, hsp90 may undergo changes in conformation in a manner analogous to hsp70 (36). Indeed, evidence suggests that these conformational changes are translated throughout the hsp90 protein (34–38) and may involve the MEEVD peptide at the hsp90 COOH terminus (16). Thus only the ATP-bound state of hsp90 interacts with p23 (34). Nucleotide ex-

change to the ADP-bound form stabilizes a hydrophobic surface in hsp90 (34, 35) which may facilitate interaction with protein substrates (34–38). Hop binds preferentially to ADP-bound hsp90 and blocks ATP-dependent conversion to a form capable of interaction with p23 (37).

It is possible that the interaction between hsp90 and different target substrates is governed by different cochaperones (38). The three immunophilins CyP40, FKBP51, and FKBP52 then might modulate the function of hsp90 either by altering hsp90 conformation or by influencing ATP binding and the ability of hsp90 to recognize and interact with unfolded substrate proteins (34, 38). A precedent for such controlling influences over hsp90 has been set by the ability of CyP40 to inhibit c-Myb DNA binding activity via a mechanism that requires both the CyP40 protein interaction domain and its peptidyl-prolyl isomerase function (62). In an alternate model, hsp90 may provide a scaffold for TPR proteins, allowing them to locate close to hsp90 chaperone substrates. From such a position the immunophilins CyP40, FKBP51, and FKBP52 might act directly on steroid receptors to modulate receptor activity through an independent chaperone function (39, 40).

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REFERENCES

1. Fischer, G., Wittmann-Lebold, B., Lang, K., Kiefhaber, T., and Schmid, F. X. (1989) *Nature* **337,** 476–478
2. Kay, J. E. (1996) *Biochem. J.* **314,** 361–385
3. Marks, A. R. (1996) *Physiol. Rev.* **76,** 631–649
4. Schreiber, S. L. (1991) *Science* **251,** 283–287
5. Fruman, D. A., Burakoff, S. L., and Bierer, B. E. (1994) *FASEB J.* **8,** 391–400
6. Ratajczak, T., Carrello, A. Mark, P. J., Warner, B. J., Simpson, R. J., Moritz, R. L., and House A. K. (1993) *J. Biol. Chem.* **268,** 13187–13192
7. Tai, P.-K. K., Maeda, Y., Nakao, K., Wakim, N. G., Duhring, J. L., and Faber, L. E. (1986) *Biochemistry* **251,** 5269–5275
8. Renoir, J.-M., Radanyi, C., Faber, L. E., and Bauliéu, E.-E. (1990) *J. Biol. Chem.* **265,** 10740–10745
9. Nair, S. C., Rimerman, R. A., Toran, E. J., Chen, S., Prapapanich, V., Butts, R. N., and Smith, D. F. (1997) *Mol. Cell. Biol.* **17,** 594–603
10. Peattie, D. A., Harding, M. W., Fleming, M. A., De Cenzo, M. T., Lippke, J. A. Livingston, D. J., and Benasutti, M. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 10974–10978
11. Hoffman, K., and Handschumacher, R. E. (1995) *Biochem. J.* **307,** 5–8
12. Radanyi, C., Chambraud, B., and Bauliéu, E.-E. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 11197–11201
13. Owens-Grillo, J. K., Hoffmann, K., Hutchison, K. A., Yem, A. W., Deibel, M. R., Jr., Handschumacher, RE., and Pratt, W. B. (1995) *J. Biol. Chem.* **270,** 20479–20484
14. Ratajczak, T., and Carrello, A. (1996) *J. Biol. Chem.* **271,** 2961–1965
15. Barent, R. L., Nair, S. C., Carr, D. C., Ruan, Y., Rimerman, R. A., Fulton, J., Zhang, Y., and Smith D. F. (1998) *Mol. Endocrinol.* **12,** 342–354
16. Chen, S., Sullivan, W. P., Toft, D. O., and Smith, D. F. (1998) *Cell Stress Chaperones* **3,** 118–129
17. Smith, D. F. (1993) *Mol. Endocrinol.* **7,** 1418–1429
18. Smith, D. F., and Toft, D. O. (1993) *Mol. Endocrinol.* **7,** 4–11
19. Hutchinson, K. A., Dittmar, K. D., Czar, M. J., and Pratt, W. B. (1994) *J. Biol. Chem.* **269,** 5043–5049
20. Johnson, J. L., and Toft, D. O. (1995) *Mol. Endocrinol.* **9,** 670–678
21. Smith, D. F., Whitesell, L., Nair, S. C., Chen, S., Prapapanich, V., and Rimerman, R. (1995) *Mol. Cell. Biol.* **15,** 6804–6812
22. Chen, S., Prapapanich, V., Rimerman, R. A., Honoré, B., and Smith D. F. (1996) *Mol. Endocrinol.* **10,** 682–693
23. Prapapanich, V., Chen, S., Nair, S. C., Rimerman, R. A., and Smith, D. F. (1996) *Mol. Endocrinol.* **10,** 420–431
24. Dittmar, K. D., and Pratt, W. B. (1997) *J. Biol. Chem.* **272,** 13047–13054
25. Pratt, W. B., and Toft, D. O. (1997) *Endocr. Rev.* **18,** 306–360
26. Kimura, Y., Yahara, I., and Lindquist, S. (1995) *Science* **268,** 1362–1365
27. Bohen, S. P., Kralli, A., and Yamamoto, K. R. (1995) *Science* **268,** 1303–1304
28. Frydman, J., and Höhfeld, J. (1997) *Trends Biochem. Sci.* **22,** 87–92
29. Höhfeld, J., Minami, Y., and Hartl, F.-U. (1995) *Cell* **83,** 589–598
30. Cyr, D. M., Langer, T., and Douglas, M. G. (1994) *Trends Biochem. Sci.* **19,** 176–181
31. Hartl, F.-U. (1996) *Nature* **381,** 571–580
32. Minami, Y., Höhfeld, J., Ohtsuka, K., and Hartl, F.-U. (1996) *J. Biol. Chem.* **271,** 19617–19624
33. Csermely, P., Kajtar, J., Hollosi, M., Jalsovszky, G., Holly, S., Jahn, C. R., Gergely, P., Jr., Söti, C., Mihaly, K., and Somogyi, J. (1993) *J. Biol. Chem.* **268,** 1901–1907
34. Sullivan, W., Stensgard, B., Caucutt, G., Bartha, B., McMahon, N., Alnemri, E. S., Litwack, G., and Toft, D. (1997) *J. Biol. Chem.* **272,** 8007–8012
35. Grenert, J. P., Sullivan, W. P., Fadden, P., Haystead, T. A. J., Clark, J., Mimnaugh, E., Krutzsch, H., Ochel, H.-J., Schulte, T. W., Sausville, E., Neckers, L. M., and Toft, D. O. (1997) *J. Biol. Chem.* **272,** 23843–23850
36. Prodromou, C., Roe, S. M., O'Brien, R., Ladbury, J. E., Piper, P. W., and Pearl, L. H. (1997) *Cell* **90,** 65–75
37. Johnson, B. D., Schumacher, R. J., Ross, E. D., and Toft, D. O. (1998) *J. Biol. Chem.* **273,** 3679–3686
38. Beissinger, M., and Buchner, J. (1998) *Biol. Chem. Hoppe-Seyler* **379,** 245–259
39. Bose, S., Weikl, T., Bügl, H., and Buchner, J. (1996) *Science* **274,** 1715–1717
40. Freeman, B. C., Toft, D. O., and Morimoto, R. I. (1996) *Science* **274,** 1718–1720
41. Lässle, M., Blatch, G. L. Kundra, V., Takatori, T., and Zetter, B. R. (1997) *J. Biol. Chem.* **272,** 1876–1884
42. Chen, M.-S., Silverstein, A. M., Pratt, W. B., and Chinkers, M. (1996) *J. Biol. Chem.* **271,** 32315–32320
43. Honoré, B., Leffers, H., Madsen, P., Rasmussen, H. H., Vandekerckhove, J., and Celis, J. E. (1992) *J. Biol. Chem.* **267,** 8485–8491
44. Owens-Grillo, J. K., Czar, M. J., Hutchison, K. A., Hoffmann, K., Perdew, G. H., and Pratt, W. B. (1996) *J. Biol. Chem.* **271,** 13468–13475
45. Duina, A. A., Chang, H.-C. J., Marsh, J. A., Lindquist, S., and Gaber, R. F. (1996) *Science* **274,** 1713–1715
46. Fields, S., and Song, O. (1989) *Nature* **340,** 245–246
47. Renoir, J.-M., Mercier-Bodard, C., Hoffman, K., Le Bihan, S., Ning, Y.-M., Sanchez, E. R., Handschumacher, R. E., and Bauliéu, E.-E. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 4977–4981
48. Chambraud, B., Radanyi, C., Camonis, J. H., Shazand, K., Rajkowski, K., and Bauliéu, E.-E. (1996) *J. Biol. Chem.* **271,** 32923–32929
49. Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993) *Cell* **74,** 205–214
50. Shi, Y., Brown, E. D., and Walsh, C. T. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 2767–2771
51. Yamazaki, M., Tashiro, H., Yokoyama, K., and Soeda, E. (1990) *Agric. Biol. Chem.* **54,** 3163–3170
52. Tsai, S., Bartelmez, S., Sitnicka, E., and Collis, S. (1994) *Genes Dev.* **8,** 2831–2841
53. Moore, S. K., Kozak, C., Robinson, E. A., Ullrich, S. J., and Appella, E. (1987) *Gene* (*Amst.*) **56,** 29–40
54. Hickey, E., Brandon, S. E., Smale, G., Lloyd, D., and Weber, L. A. (1989) *Mol. Cell. Biol.* **9,** 2615–2626
55. Stebbins, C. E., Russo, A. A., Schneider, C., Rosen, N., Hartl, F. U., and Pavletich, N. P. (1997) *Cell* **89,** 239–250
56. Nemoto, T., Ohara-Nemoto, Y., Ota, M., Takagi, T., and Yokoyama, K. (1995) *Eur. J. Biochem.* **233,** 1–8
57. Binart, N., Chambraud, B., Levin, J. M. Garnier, J., and Bauliéu, E.-E. (1989) *J. Steroid Biochem.* **34,** 369–374
58. Schwartz, J. A., and Mizukami, H. (1991) *Med. Hypoth.* **35,** 140–145
59. Cadespond, F., Binart, N., Chambraud, B., Jibard, N., Schweizer-Groyer, G., Segard-Maurel, I., and Bauliéu, E.-E. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 10434–10438
60. Freeman, B. C., Myers, M. P., Schumacher, R., and Morimoto, R. I. (1995) *EMBO J.* **14,** 2281–2292
61. Czar, M. J., Owens-Grillo, J. K., Dittmar, K. D., Hutchison, K. A., Zacharek, A. M., Leach, K. L., Deibel, M. R., Jr., and Pratt, W. B. (1994) *J. Biol. Chem.* **269,** 11155–11161
62. Leverson, J. D., and Ness, S. A. (1998) *Mol. Cell* **1,** 203–211
63. Young, J. C., Obermann, W. M. J., and Hartl, F. U. (1998) *J. Biol. Chem.* **273,** 18007–18010
64. Jakob, U., and Buchner, J. (1994) *Trends Biol. Sci.* **19,** 205–211
65. Simon, M. C., Kitchener, K., Kao, H. T., Hickey, E., Weber, L., Voellmy, R., Heintz, N., and Nevins, J. R. (1987) *Mol. Cell. Biol.* **7,** 2884–2890
66. Binart, N., Chambraud, B., Dumas, B., Rowlands, D. A., Bigogne, C., Levin, J. M., Garnier, J., Bauliéu, E.-E., and Catelli, M.-G. (1989) *Biochem. Biophys. Res. Commun.* **159,** 140–147
67. Ratajczak, T., Brockway, M. J., Hähnel, R., Moritz, R. L., and Simpson, R. J. (1988) *Biochem. Biophys. Res. Commun.* **151,** 1156–1163
68. Meng, X., Devin, J., Sullivan, W. P., Toft, D. O., Bauliéu, E.-E., and Catelli, M.-G. (1996) *J. Cell Sci.* **109,** 1677–1687
69. Minami, Y., Kimura, Y., Kawasaki, H., Suzuki, K., and Yahara, I. (1994) *Mol. Cell. Biol.* **14,** 1459–1464
70. Nemoto, T., and Sato, N. (1998) *Biochem. J.* **330,** 989–995
71. Nemoto, T., Sato, N., Iwanari, H., Yamashita, H., and Takagi, T. (1997) *J. Biol. Chem.* **272,** 26179–26187
72. Shaknovich, R., Shue, G., and Kohtz, D. S. (1992) *Mol. Cell. Biol.* **12,** 5059–5068
73. Shue, G., and Kohtz, D. S. (1994) *J. Biol. Chem.* **269,** 2707–2711
74. Young, J. C., Schneider, C., and Hartl, F. U. (1997) *FEBS Lett.* **418,** 139–143
75. Scheibel, T., Weikl, T., and Buchner, J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 1495–1499
76. Sullivan, W. P., and Toft, D. O. (1993) *J. Biol. Chem.* **268,** 20373–20379
77. Binart, N., Lombes, M., and Bauliéu, E.-E. (1995) *Biochem. J.* **311,** 797–804

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