Hop, an abundant and conserved protein of unresolved function, binds concomitantly with heat shock protein 70 (Hsp70) and Hsp90, participates with heat shock proteins at an intermediate stage of progesterone receptor assembly, and is required for efficient assembly of mature receptor complexes in vitro. A largely untested hypothesis is that Hop functions as an adaptor that targets Hsp90- to Hsp70-substrate complexes; if true, then loss of either Hsp70 binding or Hsp90 binding by Hop should equally disrupt its ability to promote assembly of mature receptor complexes. To generate Hop mutants that selectively disrupt heat shock protein interactions, highly conserved amino acids in the previously mapped Hsp70 and Hsp90 binding domains of Hop and in a conserved C-terminal domain were targeted for small substitutions and deletions. In co-precipitation assays, these mutants displayed selective loss of association with heat shock proteins. In assays using Hop-depleted rabbit reticulocyte lysate for the cell-free assembly of receptor complexes, none of the Hop mutants inhibited Hsp70 binding to receptor, but all mutants were defective in supporting Hsp90-receptor interactions. Thus, Hop has a novel role in the chaperone machinery as an adaptor that can integrate Hsp70 and Hsp90 interactions.

Hop is an abundant, highly conserved eukaryotic protein that binds the major cytosolic chaperones heat shock protein 70 (Hsp70)1 and Hsp90, either independently or simultaneously (1, 2), but the function of Hop in the chaperone machinery has not been clearly resolved. The role of Hop as a modulator of Hsp70-mediated refolding of misfolded model substrates has been examined in several studies using a minimal in vitro refolding system, but no clear picture of the role of Hop in general protein folding has emerged from these studies. Depending on the exact composition of chaperone components in the refolding reactions, Hop has been shown to inhibit (3), enhance (4), or have no effect (5, 6) on protein refolding by Hsp70 and DnaJ chaperones. It is not readily apparent in these conflicting studies which in vitro condition is more physiologically relevant than others. Of note in each of these studies, Hop by itself displayed no chaperone activity, either in preventing aggregation or in promoting refolding of misfolded proteins. In another report (7), Hop was purported to have a nucleotide exchange-like activity toward Hsp70 that promoted Hsp70 cycling on misfolded substrates. However, Toft and colleagues (4) were unable to confirm this finding, and the apparent nucleotide exchange activity seen in the former study may have been attributable to a minor contamination by DnaJ.

When studying refolding of luciferase in rabbit reticulocyte lysate (RL), Hop was found along with other chaperones in complexes with misfolded luciferase (8). Depletion of Hop from RL was found to retard the refolding of luciferase somewhat more than control depletions, and the difference was largely reversible by addition of purified Hop (4). This argues that Hop may enhance the efficiency of chaperone-mediated refolding, at least with some substrates, but how Hop does this is unresolved.

One of the few specific systems in which Hop is known to function in cells is the assembly and maintenance of steroid receptor complexes (for review, see Ref. 9). Both progesterone (10, 11) and glucocorticoid (12) receptors depend on proper assembly with Hsp90 and other chaperone proteins to maintain high affinity hormone binding at physiological temperatures. Based largely on assembly studies in crude RL, Hop has been shown to participate at an intermediate assembly stage that is obligatory for the maturation of functional progesterone receptor (PR) complexes, even though Hop is not a component of mature complexes (2, 10, 11, 13). Using a purified reconstitution system, Pratt and colleagues (14–16) have shown that Hop is similarly required for the functional maturation of glucocorticoid receptor complexes.

Lending support to the physiological relevance of cell-free receptor assembly studies, the Hsp90 binding drug geldanamycin has similar effects on the function and composition of receptor complexes in either intact cells or RL (11). In both backgrounds, PR complexes were found to quickly lose their progesterone binding ability and were arrested at an intermediate assembly stage at which Hop, Hsp70, and Hsp90 are present.

The yeast Saccharomyces cerevisiae contains a Hop homolog termed Sti1 that is not vital under normal growth conditions but is required for a robust heat shock response (17). Because vertebrate steroid receptors can function in yeast cells, Sti1—yeast were tested for their ability to support steroid signaling (18). Absence of Sti1 did not completely block steroid signaling but did significantly inhibit expression of a glucocorticoid-responsive reporter gene. Moreover, synthetic lethality of combined Sti1 and Hsp90 mutations in yeast support the functions of Sti1 and Hsp90 in a common pathway (18).

We and others have suggested that Hop, functioning essentially as an adaptor, coordinates Hsp70 and Hsp90 interactions during assembly of receptor complexes. Such an adaptor function would be distinct from the known activities of all other chaperone components, so it is important to test this hypothe-

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1 The abbreviations used are: Hsp, heat shock protein; RL, rabbit reticulocyte lysate; PR, progesterone receptor; TPR, tetratricopeptide repeat; WT, wild-type Hop; PAGE, polyacrylamide gel electrophoresis.
sis more rigorously. Previously, truncation mutants of Hop were used to localize its Hsp90 binding domain to a central tetra-tricopeptide repeat (TPR) and its Hsp70-binding domain to an N-terminal TPR (2, 19). TPR repeats are found in many proteins and are generally considered protein interaction sites (20). In the present study, highly conserved sequences within the TPRs of Hop were identified and mutated in an effort to produce Hop forms with minimal structural alterations that are selectively deficient in Hsp70 or Hsp90 binding. In addition to the TPR mutants, the C-terminal region of Hop was also targeted for mutation. This region shares some homology with Hip, another Hsp70-binding protein (21, 22) and a transient factor in PR assembly (10, 11). Point mutations within the homologous region of Hip generated a dominant inhibitor of PR assembly that appeared to act by inhibiting Hop binding to Hsp70-PR complexes (13). All the Hop mutants generated here were tested for their abilities to bind Hsp70 and Hsp90 and to participate in PR assembly reactions. If Hop functions as an adaptor, selective disruption of either Hsp70 or Hsp90 binding by Hop should similarly disrupt PR assembly.

**EXPERIMENTAL PROCEDURES**

**Construction of Hop Mutants**—The in vitro expression plasmid Hop/pSPUTK encoding wild-type human Hop (WT) was used as the starting DNA for generation of mutant cDNAs. Mutant −41–43 was created by polymerase chain reaction amplification of Hop/pSPUTK using oligonucleotide primers (forward, 5′-gatatccggccggccgctctgacagcataag -3′; and reverse, 5′-gatatccggccggccgctctgacagcataag -3′) to generate a mutation that changed codon Leu407 to Phe; this mutation was not corrected. It was found to have a polymerase chain reaction-related artifactual mutation predicted sequences of Hop open reading frames in all mutant constructs were verified by automated sequencing. Mutant Δ382–402 was found to have a polymerase chain reaction-related artifactual mutation that changed codon Leu407 to Phe; this mutation was not corrected. Mutants APAV (Asp492 and Glu494 changed to Ala), APAM (Asp501 and Glu502 changed to Ala), and AP2 (combination of APAV and APAM) were created using the QuickChange site-directed mutagenesis kit (Stratagene). The general scheme involved constructing a plasmid encoding Hop as a Chaperone Adaptor.

**Construction of Bacterial Expression Plasmids and Purification of Recombinant Proteins**—Expression and purification of recombinant Hop protein were facilitated by use of the Impact kit (New England Biolabs). The general scheme involved constructing a plasmid encoding Hop protein were facilitated by use of the Impact kit (New England Biolabs). The general scheme involved constructing a plasmid encoding Hop as a Chaperone Adaptor.

**Binding of Hop Forms to Hsp90 and Hsp70 and Association with PR Complexes**—Hop forms were expressed in vitro (TntK kit, Promega) from pSPUTK plasmids in the presence of [35S]methionine. The generation of radiolabeled protein was monitored by SDS-PAGE and autoradiography. Molar equivalents of each radiolabeled Hop product were added to normal rabbit RL (1:1 lysate from Green Hectares, Oregon, WI) and either Hsp90 or Hsp70 complexes were immunoprecipitated from the mixtures. Mouse monoclonal IgGs H90-10 and BB70, specific for Hsp90 and Hsp70, respectively, were preadsorbed to protein A-Sepharose (Amer- sham Pharmacia Biotech) at the ratio of 1 μg of antibody/1 μl of resin pellet. For each sample, 10 μl of immunoaffinity resin were added to 100 μl of RL containing 5–10 μl of radiolabeled Hop synthesis mixtures. Samples were incubated at 30 °C for 30 min with occasional agitation to suspend resin. Complexes were separated by SDS-PAGE. The gel was Coomassie Blue-stained to visualize total proteins and autoradiographed to visualize radiolabeled Hop forms present in each lane.

To compare association of Hop forms with PR complexes, PR assembly reactions were performed as described previously (13) in the presence of radiolabeled Hop forms. Briefly, PR resin (~0.5 μg of PR on a 10 μl of immunoaffinity resin pel- let) was added to 200 μl RL containing one of the radiolabeled Hop forms, an ATP-regenerating system (4.5 mg of phosphocreatine and 7 units of creatine phosphokinase/ml of RL), and geldanamycin (20 μg/ml final concentration) to enhance recovery of Hop-containing intermediate PR complexes (11). Assembly was allowed to proceed at 30 °C for 30 min, and resin complexes were then washed, separated by SDS-PAGE, and visualized by Coomassie Blue staining and autoradiography.

**PR Assembly in Hop-depleted RL**—To deplete endogenous Hop from RL, mouse monoclonal F5 or a nonspecific mouse IgG was preadsorbed to protein A-Sepharose (150 μg of antibody/40 μl of resin pellet) and added to 1 ml of RL preadjusted to 450 mM NaCl. Immunoadsorption was carried out with gentle rocking at 4 °C for 90 to ~120 min. The supernatants were collected, and immunoadsorptions were repeated one additional time followed by a third immunoadsorption using protein A-Sepharose alone to remove any free antibody remaining. To remove NaCl, depleted RL was dialyzed extensively in Slidea- lyzer cassettes (2000 molecular weight cutoff, Pierce) against 20 mM Hepes, pH 7.4, 100 mM KCl, 10 mM monothioglycerol, and 0.5% Tween 20 and separated by SDS-PAGE. The gel was Coomassie Blue-stained to visualize total proteins and autoradiographed to visualize radiolabeled Hop forms present in each lane.

**Conservation of Hop Function and Structure**—Previous studies (2, 14) have provided evidence that Hop is required for in vitro assembly of steroid receptors with Hsp90. The importance of Hop is illustrated in RL-based assembly reactions (Fig. 1). Immunodepletion of endogenous rabbit Hop prevents Hsp90
binding to PR but has no effect on Hsp70 binding (Fig. 1, compare first two lanes). Hsp90 binding is fully restored by addition of recombinant human Hop or the yeast homolog Sti1 (Fig. 1, final two lanes).

Before proceeding, several points about the in vitro assembly system and interpretation of the resulting PR complexes should be made. First, Hop co-migrates on SDS gels with an unidentified protein that is recovered nonspecifically on immunoaffinity resins, and this band is often visible even when Hop is absent (Fig. 1, note second lane). Second, unless assembly conditions are altered to favor the formation of Hop-containing intermediate PR complexes, e.g. by using geldanamycin, Hop levels in total PR complexes will normally be stoichiometrically low, reflecting the relative stability of mature PR-Hsp90 complexes compared with transient early and intermediate complexes (10, 11, 23).

Finally, the pattern of bands observed in Fig. 1, lane 1, differs somewhat from a typical PR assembly profile (for comparison, see Refs. 10, 11, and 24) in that the level of Hsp70 relative to PR is somewhat elevated and the recovery of Hsp90 is reduced. This pattern suggests that the equilibrium in steady-state assembly dynamics (10) is shifted away from the formation of mature PR complexes in favor of the early complex containing Hsp70. These deviations from the norm are somewhat variable, as will be seen in later figures, but relate to an apparent loss of an unidentified salt-sensitive, dialyzable factor from RL during the immunodepletion procedure. Because Hop-dependent association of Hsp90 with PR is still readily detectable using depleted lysates, we do not believe the validity of our assay for Hop function is significantly compromised.

An adaptor mechanism for Hop in PR assembly would require that Hop maintain its abilities to bind both Hsp90 and Hsp70, and it would follow that disruption of Hop binding to either Hsp would block the ability of Hop to mediate PR assembly. Amino acids directly involved in Hsp binding are probably highly conserved, so DNA sequence data bases were searched for sequences homologous to human Hop, and deduced amino acid sequences were aligned (Fig. 2). Hop homologs were identified from all the major eukaryotic lineages: protists, fungi, plants, and animals. The putative amino acid sequence for the Caenorhabditis elegans homolog was derived from a genomic DNA sequence, and, as judged by the in-frame stop codon, this gene apparently contains a small intron within the TPR2 coding region. The sequence from Leishmania lacks a start codon but probably is nearly complete.

TPR1, which is most strongly implicated in Hsp70 binding, and the larger TPR2 containing the Hsp90 binding site are indicated in Fig. 2 by lightly shaded boxes over the N-terminal 100 amino acids (TPR1) and over the central region (TPR2) of the alignment. Within each TPR, a core of highly conserved
indicated changes. All of the mutants were expressed as full-length products except for the APAM mutation at 501 (mutations at positions 491 and 493 (397–402)). Three mutants were generated in the DP domain: Ala substitutions at amino acids 397–402 (397–402), a substitution of amino acids at positions 388 and 389 (388–389), a substitution of amino acids 387–402 (387–402), and a deletion of these amino acids (387–402). Three mutants were generated in the DP domain: Ala substitutions at amino acids 491 and 493 (APAV), a single Ala substitution at 501 (APAM), and a combination of these two mutants (AP2). All of the mutants were expressed as full-length products except for the indicated changes.

sequence (Fig. 2, darkly shaded boxes) was targeted for mutagenesis. Also, based on the similarity to a functionally important DPEV repeat motif found in Hop (13), three strictly conserved amino acids near the C terminus of Hop (Fig. 2, darkly shaded columns) were targeted for mutation. Several mutant human Hop proteins (diagramed in Fig. 3) were generated for analysis.

In characterizing Hop mutant proteins for a general loss of conformational stability, purified recombinant forms of the TPR1, TPR2, and DP mutants were all compared with WT by gel filtration chromatography (results not shown). All migrated as a single homogenous peak identical to WT, except AP2, which appeared to be approximately twice the size of WT. No evidence of aggregation was observed for any Hop form, indicating that mutant proteins are predominantly in a folded conformation. Comparing AP2 and WT by density gradient centrifugation, the two had identical sedimentation profiles (results not shown). In combination, the gel filtration and centrifugation results indicate that AP2 has a more extended conformation than WT that may reflect domain-domain interactions between the DP region and another Hop region.

Interaction of Hop TPR1 Mutant with Hsp70, Hsp90, and PR Complexes—Radiolabeled WT and mutant ~41–43 proteins were synthesized in vitro, and equal amounts of incorporated radioactivity were added to normal RL before immunoprecipitation of Hsp complexes. Proteins in isolated complexes were separated by SDS-PAGE, Coomassie Blue-stained to reveal total protein, and autoradiographed to detect radiolabeled Hop forms. As seen in the Coomassie Blue-stained panel of Hsp90 complexes (Fig. 4A, upper panel), equal amounts of rabbit Hop and Hsp70 co-precipitated with Hsp90. Autoradiographic results (Fig. 4A, lower panel) indicate that WT and mutant ~41–43 mutant are recovered equally in Hsp90 complexes. Hsp70 precipitates (Fig. 4B) were treated somewhat differently. Misfolded substrates typically bind Hsp70 in a salt-insensitive manner, whereas Hop binding to Hsp70 is dissociated by ionic strengths >0.3 M. The latter is evident when comparing the loss of rabbit Hop and Hsp90 from Hsp70 complexes with high salt washes (Fig. 4B, upper panel). Because mutations in Hop could conceivably generate a misfolded protein, the salt sensitivity of mutant Hop binding was used as an indicator for binding to Hsp70 as a substrate. When washed with low salt buffer (~41–43 recovery in Hsp70 complexes was reduced 80% relative to WT (Fig. 4B, lower panel). High salt washes eliminated recovery of WT, but a small amount of ~41–43 was retained, suggesting that a minor portion of ~41–43 is in a misfolded conformation.

Next, the recovery of Hop forms in PR complexes was compared (Fig. 4C). Geldanamycin was included in assembly reactions to enhance recovery of intermediate PR complexes containing Hop. Mutant ~41–43 was recovered at much lower

![Diagram of Hop mutants](http://www.jbc.org/)
levels in PR complexes than WT, correlating with its decreased association with Hsp70. Because the results in Fig. 4C reflect the relative abilities of radiolabeled human Hop forms to compete with endogenous rabbit Hop for association with PR complexes, it was conceivable that -41–43 may still be competent for supporting PR assembly despite its reduced recovery in PR complexes. To test this further, recombinant -41–43 was prepared and purified for addition to Hop-depleted RL before PR assembly reactions (Fig. 4D). Recombinant WT was able to restore PR-Hsp90 association in depleted RL, but -41–43 was not.

Interaction of Hop TPR2 Mutants with Hsp70, Hsp90, and PR Complexes—Four mutations in a highly conserved region (amino acids 382–402) of Hop TPR2 were generated (see Fig. 3). Earlier results had shown that TPR2 is necessary and sufficient for Hsp90 binding (2, 19), so it was hoped that mutations in this region would selectively disrupt the association of Hop with Hsp90.

Recoveries of mutants -397–402, Δ397–402, and Δ382–402 in Hsp90 complexes (Fig. 5A) were dramatically reduced compared with WT, pointing to the importance of these sequences for Hsp90 binding by Hop. In Hsp70 complexes (Fig. 5B), recoveries of the mutants were reduced by 50–65% relative to WT, suggesting that maximal binding of Hsp70 to Hop occurs when Hop is associated with Hsp90. A small portion of the Hsp70 binding in each case is resistant to salt washes, presumably reflecting limited substrate interactions with Hsp70. Recoveries of Hop TPR2 mutants in PR complexes were dramatically impaired (Fig. 5C), paralleling the poor recovery of each mutant in Hsp90 complexes. Purified, recombinant TPR2 mutant proteins were tested for their abilities to replace endogenous Hop in supporting PR assembly (Fig. 5D), and each of the three mutants lacking Hsp90 binding was equally defective in restoring Hsp90 to PR complexes. The double point mutant -388–389 was mildly defective in its association with Hsp90 and Hsp70 (results not shown), and correspondingly, -388–389 was able to partially restore Hsp90 binding to PR in Hop-depleted RL (Fig. 5D, final lane).

Interaction of Hop DP Mutants with Hsp70, Hsp90, and PR Complexes—In an earlier study of Hip (13), alanine substitutions of the acidic amino acids in two DPEV sequences were found to generate a dominant inhibitory Hip mutant that blocked formation of the intermediate PR complex. The corresponding sites in Hop, a DPEV and a downstream DPAM, are highly conserved (see Fig. 2) and were targeted for mutations (see Fig. 3). Mutations in this region of Hop had no effect on recovery of radiolabeled mutants with Hsp90 (Fig. 6A; APAM not shown). On the other hand, recovery of mutants in Hsp70 complexes was impaired (Fig. 6B). Mutation of the upstream DPEV to APAV or of the downstream DPAM to APAM (results not shown) caused a 50% reduction of mutant binding to Hsp70; however, the combined mutant AP2 was recovered in Hsp70 complexes at only 20% of the level of WT. There was very little residual binding of DP mutants to Hsp70 after salt washes.

As with Hsp70, the recovery of DP mutants in PR complexes was deficient (Fig. 6C). In replacement assays (Fig. 6D), APAV and APAM mutants partially supported Hsp90 association with PR, but the combined AP2 mutant could not. In a further test, recombinant AP2 was added to undepleted RL in 10-fold excess over endogenous Hop, but no inhibition of PR assembly was observed (results not shown). Therefore, AP2 fails to support Hsp90 incorporation into PR complexes but is not a dominant inhibitor of assembly like the corresponding Hip mutant.

Discussion

In this report, we have tested the hypothesis that Hop functions as an adaptor that directs Hsp90 to preexisting Hsp70–PR complexes. A prediction from this hypothesis is that disruption of the ability of Hop to bind either Hsp70 or Hsp90 would have a similar deleterious effect on Hsp90 assembly with PR but not with Hsp70 binding to PR. Accordingly, human Hop mutant proteins were generated after identifying highly conserved sequences that lie within either of the two TPR domains—TPR1, containing the Hsp70 binding site, and TPR2, containing the Hsp90 binding site—or in the C-terminal region of Hop that shares homology with the C terminus of Hip. A 3-amino acid mutation in TPR1 of Hop selectively impairs Hsp70 binding, and this mutant was unable to support cell-free assembly of Hsp90 with PR complexes (Fig. 4). Similarly, each of three mutations in TPR2 abrogated the binding of Hop to Hsp90 and
Hop as a Chaperone Adaptor

Hop is illustrated as a two-domain protein with an N-terminal TPR domain (T) that binds Hsp70 and a C-terminal DPEV repeat domain (D). PR is shown associated with Hsp70. Hop is shown with its N-terminal TPR1 domain (I), central TPR2 domain (II), and C-terminal DP domain (D). Hsp90 (90) is shown as a dimer bound to Hop. See “Discussion” for details.

also failed to support Hsp90 incorporation into PR complexes (Fig. 5). The ability of TPR2 mutants to interact with Hsp70 was reduced somewhat, but it seems likely the significant residual binding to Hsp70 would be sufficient to at least partially restore PR assembly if only Hsp70 interactions were necessary. Finally, mutant AP2 in the C-terminal region of Hop was selectively deficient in Hsp70 binding and also failed to support Hsp90 assembly with PR (Fig. 6). In no case did Hop depletion or substitution by mutant Hop forms impair Hsp70 binding to PR. Each of these findings is consistent with Hop’s functioning as an adaptor that directs Hsp90 to preexisting Hsp70-PR complexes.

The finding that mutant AP2 is defective for Hsp70 binding was unexpected for two reasons. First, the N-terminal TPR1 domain is the likely binding site for Hsp70 (2, 19). Second, similar mutation of the corresponding DPEV repeat in Hop did not disrupt its interactions with Hsp70 (13). In fact, the Hip APAV2 mutant bound Hsp70 in a more stable manner than wild-type Hip. Gel filtration and density gradient centrifugation analyses indicate that Hop mutant AP2 has a significantly altered conformation compared with WT, but AP2 does not behave as a misfolded substrate for Hsp70 (Fig. 6B). It thus appears that mutations in AP2 disrupt an interdomain interaction, perhaps between some portion of TPR1 and DP domains, that is required for the binding of Hop to Hsp70.

Because a previous study had shown that Hip APAV2 arrested PR assembly before the appearance of Hop and Hsp90, a model was proposed in which Hip, through its DPEV repeat, facilitates binding of Hop-Hsp90 to Hsp70 that is preassociated with PR (13). This model has been somewhat modified and extended (Fig. 7) based on the results with Hop mutants. In this model Hsp70 binding to PR stimulates the typical substrate-dependent hydrolisis of Hsp70-bound ATP. Hip associates with the ADP-bound form of Hsp70 (21, 22, 25), and the DPEV motif of Hip may then directly interact with Hop or somehow alter the conformation of Hop that such Hop, already associated with Hsp90, is better able to bind Hsp70 that is preassociated with PR. These collective interactions form the intermediate PR complex shown at the final model stage. Once Hsp90 is locally concentrated on PR complexes, its direct association with PR would be favored and more readily stabilized by the Hsp90 binding partner p23 (26, 27), thus generating a functionally mature PR complex (not shown in Fig. 7).

Hidden by the relative simplicity of the model in Fig. 7 are an array of potential conformational states and allosteric influences that must be considered for a more complete model. For example, Hsp70 domains are conformationally linked in a manner that is influenced by the binding of ATP versus ADP, by the binding of substrate, and likely by the presence of particular Hsp70 partner proteins such as DnaJ (28, 29) or Hip. The conformation of full-length Hsp90 is somehow altered by the presence of ATP versus ADP in its nucleotide binding site (30) in a manner not suggested by limited conformational differences in the isolated ATP binding domain (31). Hsp90 conformation may also be influenced by substrate interactions and binding to any of a variety of partner proteins. Unfortunately, the only detailed structural information currently existing for Hsp70 and Hsp90 are x-ray crystal structures of isolated domains, and only the crudest conformational information exists for Hip, Hop, or any heterocomplex between chaperone components. A full appreciation for the coupling mechanisms that orchestrate chaperone-mediated assembly of steroid receptor complexes would be greatly facilitated by, and may ultimately await, the solution of underlying protein structures.

That Hop functions as an adaptor protein, at least in some systems, is a novel activity among the many chaperone components that have been characterized. Analogous adaptor proteins are common components in cellular signal transduction pathways where different protein kinases and mediators are brought together (32), but adaptors have not been generally recognized in multichaperone pathways. Of all the chaperone components participating in the cell-free assembly of steroid receptor complexes, Hop is the only one that has not been shown by in vitro refolding assays to have independent chaperone activity, either by holding a misfolded protein in a folding competent state or by directly promoting refolding of the protein. Still, Hop may have a concerted modulatory effect on Hsp70 or Hsp90 function in certain situations, as has been suggested by some reports with minimal refolding systems. A precaution, however, in interpreting Hop-Hsp interactions in a minimal assay system is that measurable changes in refolding efficiencies may result from nonadaptive, steric influences by Hop. Interestingly, however, the greatest enhancement by Hop on refolding efficiency in a minimal assay was observed when Hsp90 and Hsp70 were both present in refolding reactions (4), suggesting that the adaptor function of Hop may extend to general protein folding processes mediated by Hsp70 and Hsp90.

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