The Hsp Organizer Protein Hop Enhances the Rate of but Is Not Essential for Glucocorticoid Receptor Folding by the Multiprotein Hsp90-based Chaperone System*

(Received for publication, August 30, 1999, and in revised form, December 20, 1999)

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A system consisting of five purified proteins: Hsp90, Hsp70, Hop, Hsp40, and p23, acts as a machinery for assembly of glucocorticoid receptor (GR)-Hsp90 heterocomplexes. Hop binds independently to Hsp90 and to Hsp70 to form a Hsp90-Hop-Hsp70-Hsp40 complex that is sufficient to convert the GR to its steroid binding form, and this four-protein complex will form stable GR-Hsp90 heterocomplexes if p23 is added to the system (Dittmar, K. D., Banach, M., Galigniana, M. D., and Pratt, W. B. (1998) J. Biol. Chem. 273, 7358–7366). Hop has been considered essential for the formation of receptor-Hsp90 heterocomplexes and GR folding. Here we use Hsp90 and Hsp70 purified free of all traces of Hop and Hsp40 to show that Hop is not required for GR-Hsp90 heterocomplex assembly and activation of steroid binding activity. Rather, Hop enhances the rate of the process. We also show that Hsp40 is not essential for GR folding by the five-protein system but enhances a process that occurs less effectively when it is not present. By carrying out assembly in the presence of radiolabeled steroid to bind to the GR as soon as it is converted to the steroid binding state, we show that the folding change is brought about by only two essential components, Hsp90 and Hsp70, and that Hop, Hsp40, and p23 act as nonessential co-chaperones.

The steroid receptors are recovered from cells as multiprotein heterocomplexes containing a dimer of Hsp90, substoichiometric amounts of Hsp70, an acidic 23-kDa protein, p23, and a tetratricopeptide repeat domain protein, such as immunophilin or protein phosphatase 5 (for review see Refs. 1 and 2). The steroid receptor-Hsp90 heterocomplexes can be formed under cell-free conditions by incubating the immunoadsorbed proteins with reticulocyte lysate (3, 4). Inasmuch as the glucocorticoid receptor (GR) must be associated with Hsp90 for it to have steroid binding activity (5), incubation of Hsp90-free GR with reticulocyte lysate results in generation of steroid binding activity in direct proportion to the number of GR-Hsp90 heterocomplexes that are assembled (6). Hsp90 binds directly to the ligand-binding domain (LBD) of the GR (1), and complexing with Hsp90 also opens up both thiol moieties (7) and trypsin cleavage sites (8, 9) in the LBD to attack by a thiol-derivatizing agent and the protease. These biochemical observations, coupled with data derived from GR mutants (10), support the idea (6, 11) that the Hsp90 heterocomplex assembly system in reticulocyte lysate directs an ATP-dependent partial unfolding of the GR LBD, thus opening the hydrophobic steroid-binding cleft to access by steroid.

The heterocomplex assembly system has been reconstituted (12–16), and five proteins, including Hsp90, Hsp70, Hop (60-kDa Hsp organizer protein), Hsp40, and p23, participate in the ATP/Mg2+-dependent and K+-dependent assembly process (for review of heterocomplex assembly see Refs. 17 and 18). Besides Hsp90 itself, the only component of this Hsp90-based chaperone system proven to be essential for both GR-Hsp90 heterocomplex assembly and unfolding of the steroid-binding cleft in the GR LBD is Hsp70 (12, 16, 19). The peptide binding activity of Hsp70 is coupled to the binding of ADP versus ATP (for review see Ref. 20), and it possesses an intrinsic ATPase activity that is stimulated by Hsp40, the vertebrate homolog of the bacterial DnaJ protein. The ADP-bound conformation of Hsp70 has a high affinity for hydrophobic substrates, and Hsp40 (provided as the purified yeast homolog, YDJ-1) potentiates GR-Hsp90 heterocomplex assembly when it is present in the purified system at about one-twentieth the concentration of Hsp70 (15). It has not been clear whether Hsp40 is essential for GR folding by the five-protein system or whether it enhances a process that occurs, albeit less effectively, when it is not present (15). In this work, we show that Hsp40 is not obligatory for the folding change that results in steroid binding activity.

Like Hsp70, Hsp90 possesses a nucleotide-binding site in its amino terminus that acts as an ATP/ADP switch domain that regulates its conformation, with the ADP-bound conformation possessing high affinity for hydrophobic substrate (21, 22). p23 is a unique, ubiquitous 23-kDa protein (23) that binds to the ATP-dependent conformation of Hsp90 and stabilizes it in that conformation (21). Receptor-bound Hsp90 must achieve the ATP-bound conformation for the generation of hormone binding activity (24), and p23 binds to and stabilizes preformed steroid-binding GR-Hsp90 heterocomplexes in an ATP-independent manner (14). Thus, p23 is not essential for GR folding to the hormone-binding state, rather it stabilizes the GR-Hsp90 heterocomplex once the ATP-dependent conformation of Hsp90 has been achieved (14). The fact that p23 is not essential for the folding change is consistent with the fact that deletion of the p23 gene in yeast does not ablate GR action (25).

The final player in the five-protein assembly system was identified as a 60-kDa rabbit protein intermediate in receptor-Hsp90 heterocomplex assembly (26) that was found to...
be the homolog of a human protein cloned by Honore et al. (27) and the yeast heat shock protein Sti1 (28). Chen et al. (29) showed that this protein binds independently to Hsp90 via a central tetratricopeptide repeat domain and to Hsp70 via an amino-terminal tetratricopeptide repeat domain to form a Hsp90-p60-Hsp70 complex. The 60-kDa protein is now called Hop for Hsp organizer protein. Hop binds preferentially to the ADP-bound conformations of Hsp70 and Hsp90 (30). In progesterone receptor-Hsp90 assembly studies in reticulocyte lysate, Hop has been shown to participate at an intermediate assembly stage that is considered to be obligatory for the formation of functional progesterone receptor-Hsp90 heterocomplexes (29, 31–34).

In our studies with the purified five-protein system, we have also considered Hop to be obligatory for assembly of GR-Hsp90 heterocomplexes and GR folding to the steroid binding state (12). Indeed, the first step in assembly appears to be the formation of a Hsp90-Hop-Hsp70-Hsp40 complex that acts as a machinery to generate steroid binding activity (13, 15, 35). These complexes can be prepared simply by mixing purified components, or they can be immunoadsorbed from reticulocyte lysate with a monoclonal antibody against Hop (15). When mixed with immunoadsorbed, Hsp90-free GR, the immunoadsorbed Hsp90-Hop-Hsp70-Hsp40 complex converts the GR to its steroid binding form in an ATP/Mg$^{2+}$-dependent and K$^{-}$-dependent manner (13, 15).

To properly study the mechanism by which the Hsp90-based chaperone machinery is able to open the steroid-binding cleft to access by ligand, we must first determine the components that are obligatory to the process. Here we use the purified assembly system to demonstrate that Hop is not essential for GR folding. Rather Hop enhances the rate of a folding change carried out by only two essential components: Hsp90 and Hsp70.

**Experimental Procedures**

**Materials**

[6,7-$^{3}$H]triamcinolone acetonide (38 Ci/mmol) and 125I-conjugated goat anti-mouse and anti-rabbit IgGs were obtained from NEN Life Science Products. Untreated rabbit reticulocyte lysate was purchased from Green Hectares (Oregon, WI). Protein A-Sepharose and goat anti-mouse horseradish peroxidase conjugates were from Sigma, and donkey anti-rabbit IgG was from Pierce. The BuGR2 mononclonal IgG antibody against the GR was from Affinity Bioreagents (Golden, CO). The ACS8 monoclonal IgG against Hsp90, the N2TF3-4 anti-72/73-kDa Hsp monoclonal IgG (anti-Hsp70), and anti-Hsp40 rabbit polyclonal antibody were from StressGen (Victoria, BC, Canada). The JJ3 monoclonal IgG against p23 and Escherichia coli expressing human p23 were gifts from Dr. David Toft (The Mayo Clinic). E. coli expressing YDJ-1 was a gift from Dr. Avrom Caplan (Mount Sinai School of Medicine). The DS14F5 monoclonal IgG against Hop and E. coli expressing Hop were kindly provided by Dr. David F. Smith (University of Nebraska Medical School). Hybridoma cells producing the FIGR monoclonal IgG against the GR were generously provided by Dr. Jack Bodwell (Dartmouth Medical School). The baculovirus for mouse GR was kindly provided by Dr. Edwin Sanchez (Medical College of Ohio). Geldanamycin was obtained from the Drug Synthesis and Chemistry Branch of the Developmental Therapeutics Program, National Cancer Institute, National Institutes of Health.

**Methods**

**Expression of Mouse GR in SF9 Cells—**SF9 cells were grown in SFM900 II serum-free medium (Life Technologies, Inc.) supplemented with Cytomax (Kemp Biotechnology, Rockville, MD) in suspension cultures maintained at 27 °C with continuous shaking (150 rpm). Cultures were infected in log phase of growth with recombinant baculovirus at a multiplicity of infection of 3.0. Cultures were supplemented with 0.1% glucose at infection and 24 h post-infection as described by Srinivasan et al. (36). Cells were harvested, washed in Hanks’ balanced saline solution, resuspended in 1.5 volumes of buffer (10 mM Hepes, pH 7.5, 1 mM EDTA, 20 mM molybdate, 1 mM phenylmethylsulfonyl fluoride), and ruptured by Dounce homogenization. The lysate was then centrifuged at 100,000 × g for 30 min, and the supernatant was collected, aliquoted, flash frozen, and stored at −70 °C.

**Immunoadsorption of GR from SF9 Cell Cytosol—**Receptors were immunoadsorbed from 50-μl aliquots of SF9 cytosol by rotating for 30 min at 4 °C with 14 μl of protein A-Sepharose prebound for 7 μl of FITG ascites suspended in 200 μl of TEG (10 mM TES, pH 7.6, 50 mM NaCl, 4 mM EDTA, 10% glycerol). Prior to incubation with reticulocyte lysate or various mixtures of purified proteins as noted, immunoadsorbed receptors were stripped of associated Hsp90 by incubating the immunopellet an additional 2 h at 4 °C with 300 μl of 0.5 M KCl in TEG. The pellets were then washed three times with 1 ml of TEG followed by a second wash with 1 ml of Hepes buffer (10 mM Hepes, pH 7.4). In the experiment of Fig. 7B, the stripped immunopellets were incubated for 20 min at 30 °C with 50 μl of HKD buffer (10 mM Hepes, 100 mM KCl, 5 mM dithiothreitol, pH 7.35) and 5 μl of an ATP-regenerating system (50 mM ATP, 250 mM creatine phosphate, 20 mM MgOAc, 100 units/ml creatine phosphokinase). The pellets were then washed twice with 1 ml of TEG and once with 1 ml of Hepes buffer. This additional procedure of incubating the GR at 30 °C with ATP reduces the extent of receptor reactivation by about one-half but eliminates any trace activation by purified Hsp90.

**Immunoadsorption of Hop from Reticulocyte Lysate—**For immunodepletion of Hop, 60-μl aliquots of reticulocyte lysate were immunoadsorbed to 10 μl of protein A-Sepharose prebound with DS14F5 antibody against Hop or with nonimmune mouse IgG. The samples were then rotated at 4 °C for 2 h, and immunopellets were washed three times with 1 ml of TEG buffer. After two rounds of immunoadsorption, Hop was undetectable by Western blot analysis in the supernatant fraction, and a 50-μl portion of the extracted lysate was used for reconstitution of the GR/Hsp90 heterocomplex.

**Glucocorticoid Receptor Heterocomplex Reconstitution—**FIGR immunopellets containing GR stripped of chaperones were incubated with 50 μl of rabbit reticulocyte lysate or with various mixtures of proteins (20 μg of purified Hsp90, 15 μg of purified Hop-free and Hsp40-free Hsp70, 0.6 μg of purified human Hsp 6, 6 μg of purified p23, 0.4 μg of purified YDJ-1) and adjusted to 50 μl with HKD buffer containing 20 mM sodium molybdate and 5 μl of the ATP-regenerating system. The assay mixtures were incubated for 20 min at 30 °C with suspension of the pellets by shaking the tubes every 2 min. At the end of the incubation, the pellets were washed twice with 1 ml of ice-cold TEGM buffer (TEG with 20 mM sodium molybdate) and assayed for steroid binding capacity and, in some experiments, for receptor-associated proteins.

In the geldanamycin experiment of Fig. 5, an aliquot of a 10 mM stock solution of geldanamycin dissolved in Me$_2$SO was diluted 100-fold into distilled water at room temperature, and this solution was added to one-tenth final volume to the heterocomplex assembly incubation. This yields a final concentration of 10 μM geldanamycin in 0.1% Me$_2$SO (control samples received 0.1% Me$_2$SO). It is important to note that the geldanamycin solution must be added to the incubation system immediately after it is diluted, because geldanamycin precipitates from the diluted solution such that 70–80% of the activity is lost by 4 h after it is diluted into water.

**Assay of Steroid Binding Capacity—**Immune pellets to be assayed for steroid binding were incubated overnight at 4 °C in 50 μl HEM buffer plus 50 mM [3H]triamcinolone acetonide. Samples were then washed three times with 1 ml of TEGM and counted by liquid scintillation spectrometry. The steroid binding is expressed as counts/min of 1H[3H]triamcinolone acetonide-bound FIGFGR immunopellet prepared from 50 μl of SF9 cytosol.

**Western Blotting—**To GR and associated proteins, immune pellets were resolved on 10% SDS-polyacrylamide gels and transferred to Immobilon-P membranes. The membranes were probed with 0.25 μg/ml BuGR for GR, 1 μg/ml AC88 for Hsp90, 1 μg/ml N27F3-4 for Hsp70, 0.1% DS14F5 mouse ascites for Hop, 0.5% anti-Hsp40, or 0.1% JJ3 mouse ascites for p23. The immunoblots were then incubated a second time with the appropriate 125I-conjugated or horseradish peroxidase-conjugated counter antibody to visualize the immunoreactive bands.

**Protein Purification—**Hsp90 and Hsp70 were purified from rabbit reticulocyte lysate by sequential chromatography on DE52, hydroxylapatite (ATP-agarase), and a new precolumn (32). The first step of chromatography on DE52 the fractions containing Hsp90 (identified by immunoblotting) are combined such that Hop is excluded (19). Hsp40 is eliminated by conservative pooling of Hsp90 fractions in the DE52 and hydroxylapatite eluates as described previously (15). The low salt eluting fractions from DE52, which are pooled to form fraction pool A (shown in Ref. 19), contain Hsp70, Hop, and Hsp40. When DE52 pool
Hop Acceleration of Glucocorticoid Receptor Folding

**FIG. 1.** Elimination of Hop from rabbit reticulocyte lysate does not ablate its ability to generate GR steroid binding activity. A, immunodepletion of Hop from lysate. Protein A-Sepharose pellets pre-bound with nonimmune IgG (NI) or with the DS14F5 monoclonal against Hop (I) were incubated with 60 μl of rabbit reticulocyte lysate for 2 h at 4 °C. The supernatants were removed and incubated a second time with antibody-bound pellets. The pellets were then washed three times with 1 ml of TEGM buffer, and the combined immune pellets and a portion (~20%) of the supernatant (super) after the second extraction were assayed for Hop by immunoblotting. B, reconstitution of steroid binding activity with Hop-depleted lysate. Stripped GR immune pellets (Str) were incubated 20 min at 30 °C with reticulocyte lysate incubated with nonimmune IgG (RL(NI)) or lysate depleted of Hop with the F5 antibody (RL(F5)). The immunopellets were then washed and incubated with 50 nM [3H]triamcinolone acetonide to determine steroid binding activity. A is chromatographed on hydroxylapatite, the Hsp70 is completely separated from Hsp40 as we have shown previously (15). The Hsp70 fraction pool from hydroxylapatite is adsorbed to ATP-agarose, and the adsorbed Hsp70 is eluted with ATP followed by Amicon filtration and dialysis. Trace amounts of Hop are present in this final Hsp70 preparation with a 10-μl protein A-Sepharose pellet prebound with DS14F5 antibody. There is no immunodetectable Hop in the supernatant fraction, which is our purified Hsp40-free, Hop-free Hsp70.

Human p23 (37) was purified from 10 ml of bacterial lysate by chromatography on DE52 as described (15) followed by hydroxylapatite chromatography. Fractions containing p23 were identified by immunoblotting, pooled, concentrated by Amicon filtration to ~1.5 ml, dialyzed against HKD buffer, aliquoted, and stored at −70 °C. The bacterial expression of YDJ-1 has been described previously (38, 39) as has the expression of human Hop (12). For purification of YDJ-1, bacterial sonicates were cleared by centrifugation, and YDJ-1 was purified by sequential chromatography on DE52 and hydroxylapatite. The YDJ-1 containing fractions identified by immunoblotting were pooled, concentrated by Amicon filtration, dialyzed against HKD buffer, flash frozen, and stored at −70 °C. Purification of human Hop was carried out in a similar manner by sequential chromatography on DE52 and hydroxylapatite.

**RESULTS**

Elimination of Hop from Reticulocyte Lysate—It was shown previously that immunodepletion of Hop from reticulocyte lysate markedly reduced but did not eliminate its progesterone receptor-Hsp90 heterocomplex assembly activity (34). Similar immunodepletion of a DE52 fraction of reticulocyte lysate reduced but did not eliminate the ability of a system reconstituted from lysate subfractions to convert the GR to the steroid binding form (12). In the experiment of Fig. 1A, we have eliminated all immune detectible Hop from reticulocyte lysate by immunoadsorbing it twice with the DS14F5 antibody against Hop. Although this immune depleted lysate was less active at activating steroid binding activity of the GR, it was still half as effective as Hop-containing lysate extracted with nonimmune antibody (Fig. 1B). It could be argued that trace amounts of Hop that are not immunodetectible are responsible for the GR activation that occurs in the immunodepleted lysate. Thus, we tested the requirement for Hop in a purified, five-protein system that is very effective at GR-Hsp90 heterocomplex assembly.

Generation of GR Steroid Binding Activity without Hop—In the experiment of Fig. 2, stripped GR (lane 2) was incubated with the complete five-protein system (Hsp90, Hsp70, Hop, YDJ-1, and p23) (lane 3) or with all components of the system except one protein. At the end of the incubation, the immune pellets were washed and steroid binding activity was assayed. Elimination of Hsp90 (lane 4) or Hsp70 (lane 5) or the heterocomplex stabilizers p23 and molybdate (lane 8) reduced receptor activation to levels near that of the stripped receptor. Elimination of Hop (lane 6) reduced activation, but activation was still nearly 50% of that achieved by the complete system.

In our previous reconstitution studies we have utilized GR immunoadsorbed from L cell cytosol where we reactivate 75–100% of the steroid binding activity with reticulocyte lysate (35). In this work, for the first time, we are reactivating mouse GR overexpressed in Sf9 cells, and we are immunoadsorbing much more GR protein. As shown in Fig. 2, the five-protein system actually generates more steroid binding activity (lane 3) than that of the unstripped receptor bound to insect Hsp90 (lane 1). Because there is so much GR protein in the immune pellets from Sf9 lysate, we can run lanes with standards of albumin or Hsp90 on the same gel and assay the amount of receptor by scanning the Coomassie Blue-stained proteins. Thus, from the specific activity of the bound [3H]triamcinolone acetonide, we can calculate that with reticulocyte lysate we reactivate ~15% of the GR in the immune pellet and with the five-protein system we reactivate ~12% of the receptors. The use of the Sf9 lysate saves us a tremendous amount of effort
Hop Increases the Rate of GR Activation—In the experiment of Fig. 4, GR was incubated with purified Hsp90, Hsp70, YDJ-1, and p23 without Hop (open circles) or with 0.12 μg (open triangles) or 1.2 μg (solid squares) of purified Hop. Hop clearly increases the rate at which steroid binding is generated. In the absence of Hop, receptor activation continues throughout the extended incubation time to approach the maximum level obtained with Hop.

Geldanamycin Blocks Heterocomplex Assembly in the Absence of Hop—Geldanamycin is a benzoquinone ansamycin antibiotic that binds to the nucleotide-binding site of Hsp90 and specifically blocks Hsp90 function (21, 22, 40). When steroid receptor-Hsp90 heterocomplexes are assembled in reticulocyte lysate in the presence of geldanamycin, they cannot bind steroid, despite the fact that Hsp90 and Hsp70 are present in a heterocomplex with the receptor (32). In contrast to receptor heterocomplexes formed in lysate in the absence of geldanamycin, heterocomplexes formed in the presence of geldanamycin contain Hop and do not contain p23 (32). The effect of geldanamycin on GR-Hsp90 heterocomplex assembly by the purified system is shown in Fig. 5. When the stripped GR was incubated with the purified five-protein system (lane 2), the receptor was assembled into a heterocomplex containing Hsp90, Hsp70, Hop, YDJ-1, and p23, and the GR was converted to the steroid binding state. In the presence of geldanamycin (lane 3), however, the amount of Hop is increased, p23 is absent, and the resulting heterocomplex does not bind steroid.

In the absence of Hop (lane 4), the GR is still assembled into a heterocomplex with Hsp90 and converted to the steroid binding state, and addition of geldanamycin (lane 5) blocks the generation of steroid-binding sites. However, in contrast to the results obtained with both reticulocyte lysate and the purified five-protein system, GR incubated with geldanamycin in this Hop-free system is associated with little or no Hsp90. It should be noted that the incubation time in this experiment has been extended to 45 min to permit more receptor activation in the absence of Hop, as was shown in Fig. 4. The data of Fig. 5 show that the Hop-containing intermediate assembly complex that is trapped by geldanamycin treatment of reticulocyte lysate is not an obligatory stage in the formation of functional (i.e. steroid binding) GR-Hsp90 heterocomplexes.
**YDJ-1/Hsp40 Is Not Required for GR Activation**—In the 2 years since we originally examined the role of Hsp40 (provided then as now in the form of the yeast homolog YDJ-1) in GR-Hsp90 heterocomplex assembly and receptor activation to the steroid binding state (15), we have optimized both our protein purification procedures and the relative concentrations of the five proteins in the minimal system such that we achieve more extensive assembly and activation. In our previous work with the less active and less purified system, we could easily demonstrate that YDJ-1/Hsp40 potentiated GR-Hsp90 heterocomplex assembly, but we always saw some activity in our Hsp40-free system, and we could not determine unequivocally whether or not Hsp40 was essential for assembly (15). As shown in lane 7 of Fig. 2, there is some GR activation when YDJ-1 is omitted from the purified assembly system. The experiment of Fig. 6 was performed to determine whether GR activation would occur under the most rigorous conditions of elimination of Hsp40. In this experiment, GR was incubated with a purified Hsp40-free chaperone system (Hsp90, Hsp70, Hop, and p23). Receptor was activated to the steroid binding state in the complete absence of Hsp40 (open circles), and activation was increased by YDJ-1 (closed circles). The inset shows that the purified Hsp70 used in this experiment (lane 1) is free of Hsp40 and compares it with Hsp70 purified by our usual two-step procedure (lane 2), which contains trace amounts of Hsp40. We conclude that, like Hop, Hsp40 potentiates but is not required for the Hsp90-dependent folding change in the GR LBD that results in steroid binding activity.

**GR Activation with Only Hsp70 and Hsp90**—In reticulocyte lysate, receptor-Hsp90 heterocomplexes are dynamically cycling in that they are simultaneously undergoing assembly and disassembly (31). Using the purified system, we have shown that heterocomplex disassembly is inhibited by p23 and molybdate (14). As seen in Fig. 2 (lane 8), incubation of the GR with a purified chaperone system without p23 does not yield stable GR-Hsp90 heterocomplexes that can be assayed by washing the immune pellet and incubating them with \(^{3}H\)steroid, as we have done in the experiments of Figs. 1–6 (14). However, when \(^{3}H\)triamcinolone acetonide is present during the incubation at 30 °C with a chaperone mixture lacking p23, the steroid can bind to some of the GR-Hsp90 complexes as soon as they are formed and before they disassemble. Thus, the steroid binding can be used as an indirect assay of heterocomplex assembly (14, 41).

In the experiment of Fig. 7A, we have incubated the GR with purified Hsp90 and Hsp40-free Hsp70 and Hsp90 in the presence of \(^{3}H\)triamcinolone acetonide. It can be seen that Hsp70 and Hsp90 yield only trace activation when present alone, but substantial steroid binding activity is generated by the two proteins together, and this activation is ATP-dependent. When heterocomplex disassembly is retarded by the presence of p23 and molybdate, more steroid binding activity can be detected in this Hop-free and Hsp40-free assembly system. We have noted that preincubation of the immunoadsorbed GR with an ATP-regenerating system, followed by washing of the immune pellet, eliminates even trace activation upon subsequent incubation of the GR with Hsp90. This preincubation procedure also reduces the maximum extent of receptor reactivation by either the five-protein system or reticulocyte lysate by about one-half. As shown in Fig. 7B, under this condition where neither Hsp70 nor Hsp90 alone provide any activation, the two chaperones together yield some ATP-dependent activation. Again, when heterocomplex disassembly is retarded by the
Hop (Fig. 5) provides useful insight into the assembly process. The proximity of the two essential chaperones to each other. GR and Hsp70 work together to make an efficient folding machinery. It is not known whether Hop acts as an adaptor in that sense simply by bringing the two proteins together (42). We predict that direct interaction of the two proteins on the receptor is required as the folding change is brought about. Hop may act as an adaptor in that sense simply by bringing the two essential chaperones into proximity to or contact with each other to make an efficient folding machinery. It is not known whether Hop directly affects Hsp90 or Hsp70 functions during GR-Hsp90 heterocomplex assembly or during the folding change that is brought about in the LBD by the Hsp90-based chaperone system.

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