FKBP52 is a high molecular mass immunophilin possessing peptidylprolyl isomerase (PPase) activity that is inhibited by the immunosuppressant drug FK506. FKBP52 is a component of steroid receptor-hsp90 heterocomplexes, and it binds to hsp90 via a region containing three tetratricopeptide repeats (TPRs). Here we demonstrate by cross-linking of the purified proteins that there is one binding site for FKBP52 in the C-terminus of hsp90. This accounts for the common heterotrameric structure of native receptor heterocomplexes being one molecule of receptor, 2 molecules of hsp90, and 1 molecule of a TPR domain protein. Immunoadsorption of FKBP52 from reticulocyte lysate also yields co-immunoadsorption of cytoplasmic dynein, and we show that co-immunoadsorption of dynein is competed by a fragment of FKBP52 containing its PPase domain, but not by a TPR domain fragment that blocks FKBP52 binding to hsp90. Using purified proteins, we also show that FKBP52 binds directly to the hsp90-free glucocorticoid receptor.

Because neither the PPase fragment nor the TPR fragment affects the binding of FKBP52 to the glucocorticoid receptor under conditions in which they block FKBP52 binding to dynein or hsp90, respectively, different regions of FKBP52 must determine its association with these three proteins.

More than a dozen transcription factors and a dozen protein kinases are known to exist in heterocomplexes with the abundant, ubiquitous, and essential protein chaperone hsp90 (12 for review, see Refs. 1 and 2). In addition to hsp90, native steroid receptor heterocomplexes contain one of several proteins that possess tetratricopeptide repeats (TPRs), which are degenerative sequences of 34 amino acids involved in protein-protein interactions (3). The TPR proteins in steroid receptor-hsp90 heterocomplexes include high molecular mass immunophilins such as FKBP52 (4–7), FKBP51 (8–10), and CyP-40 (11–13) as well as the protein serine/threonine phosphatase PP5 (14, 15). The TPR domains of these proteins are required for their binding to hsp90 (14, 16–19), and the TPR proteins and protein fragments containing only the TPR domains compete with each other for binding (12, 15, 18, 20) to a TPR domain acceptor site (20) that is located in the C-terminal 12-kDa domain of hsp90 (21). The immunophilins have been shown to exist in independent receptor-hsp90FKBP52 and receptor-hsp90-CyP-40 heterocomplexes (12, 13), and inasmuch as the TPR proteins compete for the binding of each other, these complexes must be very dynamic in the sense that a single receptor-hsp90 heterocomplex could be associated sequentially with several different TPR proteins over a short time.

The aryl hydrocarbon receptor (AHR) is recovered from the cytosol in AHR-hsp90 heterocomplexes that contain a 37-kDa FKBP homolog with three TPRs that has been called ARA9, AIP, or XAP2 (22–24). ARA9/AIP/XAP2 is specifically associated with AHR-hsp90 heterocomplexes and not glucocorticoid receptor (GR)-hsp90 heterocomplexes, whereas the reverse is the case for FKBP52 (25). Thus, there are differences in heterocomplex composition between AHR-hsp90 heterocomplexes and steroid receptor-hsp90 heterocomplexes, and there may well be differences in the relative amount of one immunophilin versus another recovered in hsp90 heterocomplexes with different steroid receptors (19).

In contrast to the nuclear receptors, the dominant protein recovered in protein kinase-hsp90 heterocomplexes is a 50-kDa phosphoprotein originally identified as a component of the p60co-s-hsp90 heterocomplex (for review, see Refs. 26 and 27). This 50-kDa protein has been cloned and identified as the vertebrate homolog of the yeast cell cycle control protein Cdc37 (28–30), and it is called p50cdc37. Genetic evidence suggests that this protein is required for Src function (31) and for signaling via the sevenless receptor, a protein-tyrosine kinase of Drosophila (32). p50cdc37 does not contain TPRs, and it binds to a site on hsp90 that is different from the TPR acceptor site, but the two sites must be topologically adjacent to each other because TPR proteins compete for the binding of p50cdc37 to hsp90 and p50cdc37 is not recovered in the same hsp90 heterocomplexes as the TPR proteins (33). Both the cyclin-dependent protein kinase Cdk4 and the serine/threonine kinase v-Raf exist in heterocomplexes containing hsp90 and p50cdc37, and p50cdc37 has been shown to bind directly to both Cdk4 (29) and Raf (33) as well as to hsp90. The combination of exclusive binding of p50cdc37 versus a TPR domain protein to hsp90 plus direct binding of p50cdc37 to the kinase appears to account for...
selection of the dominant hsp90-p50<sup>cdc7</sup>-hsp90<sup>cdc7</sup> composition that is observed with a variety of protein kinase heterocomplexes immunoadsorbed from the cytosol (33).

Although native steroid receptor-hsp90 heterocomplexes contain one of the TPR domain proteins, they do not contain p50<sup>cdc7</sup> (34, 35); however, no direct binding of TPR protein to a steroid receptor has been detected that would account for selection of an immunophilin versus p50<sup>cdc7</sup> as a partner in the heterocomplex. Cross-linking studies of steroid receptor-hsp90 heterocomplexes have revealed a common heterotetrameric structure containing 1 molecule of receptor, 2 molecules of hsp90, and 1 molecule of immunophilin (36–38). However, it is not known whether there are two TPR acceptor sites/hsp90 dimer and one is blocked when the receptor is bound or whether there is one TPR acceptor site/hsp90 dimer irrespective of the presence of the chaperoned protein. In this paper, we examine three protein-protein interactions of the abundant immunophilin FKBP52. By cross-linking of complexes formed with purified proteins, we show that there is one FKBP52-binding site/dimer of hsp90. We also demonstrate direct binding of FKBP52 to the GR and provide evidence for a contact site residing between amino acids 465 and 500 of the human GR (hGR). We have previously reported that cytoplasmic FKBP52 is localized to microtubules (39, 40) and that immunoadsorption of FKBP52 from the cytosol is accompanied by co-immunoadsorption of cytoplasmic dynein (39). Here we show that co-immunoadsorption of dynein is blocked by a bacterially expressed fragment of FKBP52 that contains its peptidylprolyl isomerase (PPIase) domain, suggesting that this conserved region of FKBP may be involved directly or indirectly in the association with dynein.

The role of the immunophilins and p50<sup>cdc7</sup>-hsp90 in the actions of nuclear receptors and signaling protein kinases is unknown. The high molecular mass TPR domain immunophilins were discovered because they were components of steroid receptor-hsp90 heterocomplexes, and it was originally thought that their PPIase activity might be required for the proper folding of the receptors and assembly of steroid-hsp90 heterocomplexes. However, it became clear that this is not their role (see Ref. 1 for review). Another proposal is that the immunophilins and p50<sup>cdc7</sup> serve to target the movement of the receptors and protein kinases in the appropriate anterograde or retrograde direction to their sites of action in the nucleus and at the plasma membrane (20, 41). The ability of FKBP52 to interact directly with the GR and either directly or indirectly with the microtubule-associated motor protein dynein is consistent with the immunophilin performing such a role in targeting of receptor movement.

**EXPERIMENTAL PROCEDURES**

**Materials**

Untreated rabbit reticulocyte lysate was from Green Hectares (Oregon, WI). 125<sup>i</sup>-Conjugated goat anti-mouse and anti-rabbit IgGs were from NEN Life Science Products. Horseradish peroxidase-conjugated goat anti-mouse IgG, monoclonal nonimmune IgG and IgM, preimmune rabbit serum, monoclonal anti-glutathione S-transferase (GST) clone GST-2 ascites, glutathione cross-linked agarose, purified glutathione S-transferase, mouse monoclonal IgM (clone 70.1) against the intermediate chain of dynein, and purified recombinant FKBP12 were from Sigma. Actigel-ALD was from Stergene Bioseparations Inc. (San Gabriel, CA), and Complete-Mini protease inhibitor mixture was from Roche Molecular Biochemicals. Horseradish peroxidase-conjugated goat anti-rabbit IgG was from Pierce, and FK506 was from Alexis Biochemicals (San Diego, CA). Anti-cyclophilin 40 antibody (COOH-terminal peptide) and 3G3 anti-hsp90 monoclonal IgM were from Affinity Bioreagents (Golden, CO), and anti-FLAG M2 monoclonal IgG was from Kodak Scientific Imaging Systems. The UPJ56 antisera against FKBP52 (42) was a gift from Dr. Karen Leach (The Upjohn Co.), and the EC1 anti-FKBP52 monoclonal antibody was a gift from Dr. Lee Faber (Medical College of Ohio, Toledo, OH). The rabbit antisera against hsp70 and hsp90 (43) was provided by Dr. Ettore Appella (NCI, National Institutes of Health). Rabbit antisera against P5, FLAG-P5, and the FLAG-tagged TPR domain of rat P5, prepared as described previously (14), were kindly provided by Dr. Michael Chinkers (University of South Alabama, Mobile, AL). FLAG-P5<sup>cdc7</sup>-hsp90<sup>cdc7</sup> monoclonal serum to p50<sup>cdc7</sup>, prepared as described previously (33), were a kind gift from Dr. Nicholas Grammatikakis (Tufts University School of Medicine, Boston, MA). The DS145F monoclonal antibody against Hsp (hsp organizer protein) (44) was kindly provided by Dr. David Smith (University of Nebraska, Omaha, NE). The baculoviruses expressing GST-cdc37<sup>pp56</sup>-transferase, mouse monoclonal IgM (clone 70.1) against the intermediate chain of dynein, and purified recombinant FKBP12 were from Drs. Ganesan Srinivasan and E. Brad Thompson (University of Texas Medical Branch, Galveston, TX). The pGEX-2T plasmids encoding GST-rabbit FKBP52 and the GST-rabbit FKBP52 PPIase domain (amino acids 19–262) were prepared as described previously (46). The pGEXAT plasmids encoding GST-rabbit FKBP52 domain I plus hinge (amino acids 1–15), GST-FKBP52 core domain I (amino acids 51–158), and GST-FKBP52 domain II (amino acids 149–267) were prepared as described previously (47).

**Methods**

**Cell Culture and Cytosol Preparation**—Sf9 cells were harvested, washed, resuspended in 1 volume of HE buffer (10 mM Hepes, pH 7.4, and 1 mM EDTA) with 1 tablet of Complete-Mini protease inhibitor mixture/3 ml of buffer, and ruptured by Dounce homogenization. Homogenates were centrifuged for 15 min at 12,000 × g.

**Immunoadsorption**—For immunoadsorption of immunophilins, GST-P5, or FLAG-p50<sup>cdc7</sup>-hsp90<sup>cdc7</sup>, aliquots (150 µl) of rabbit reticulocyte lysate were immunoadsorbed for 2 h at 4 °C to 10 µl of protein A-agarose prebound with the UPJ56 antisera against FKBP52 (2%), anti-CyP-40 (2%), or 6 µg of anti-FLAG M2 monoclonal antibody. Immune pellets were washed three times by suspension in 1 ml of TEG buffer (10 mM Tris, pH 7.6, 50 mM NaCl, 4 mM EDTA, 10% (w/v) glycerol) with 20 mM sodium molybdate and centrifugation prior to gel electrophoresis.

For immunoadsorption of hsp90 and its associated proteins from rabbit reticulocyte lysate, lysate was diluted 10-fold with HE buffer (HE buffer + 10% glycerol), and 100-µl aliquots were immunoadsorbed to 15-µl pellets of Actigel-ALD precomplexed with either nonimmune IgM or 3G3 anti-hsp90 monoclonal IgM. Immune pellets were rotated with the diluted lysate for 2 h at 4 °C and then washed three times by suspension in 1 ml of HE buffer prior to protein resolution by SDS-polyacrylamide gel electrophoresis and Western blotting.

**Western Blotting**—Immunoblots were probed with 0.1% hsp70/hsp90 antisera against hsp90; 0.1% UPJ56 antisera or 1 µg/ml EC1 monoclonal IgG against FKBP52; 0.1% P5 antisera; 0.1% antisera against p50<sup>cdc7</sup>; 0.1% anti-cyclophilin 40 antibody against CyP-40; 0.1% DS145F against Hsp; 0.1% anti-GST ascertes against GST-GR, GST-465<sup>z</sup>, and GST-500<sup>z</sup>; or 0.05% anti-dynein intermediate chain antibody against cytoplasmic dynein. The immunoblots were then incubated a second time with the appropriate 125<sup>i</sup>-conjugated counterantibody to visualize immunoreactive bands.

**Expression of GST Fusions**—pGEX-2T plasmids expressing GST-rabbit FKBP52 or GST-FKBP52 Pro<sup>1</sup>–Ser<sup>242</sup> fragment were used to transform Escherichia coli strain BL21(DE3). pGEXAT plasmids expressing GST-FKBP52 Met<sup>1</sup>–Gly<sup>184</sup>, GST-FKBP52 Gly<sup>182</sup>–Lys<sup>138</sup>, or GST-FKBP52 Gly<sup>148</sup>–Leu<sup>267</sup> were used to transform E. coli strain UT5600. Purification of the fusion proteins was performed by binding GST-FKBP52 to GSH-agarose and incubation at 4 °C with thrombin, which cleaves at a site between the GST domain and FKBP52.

**Binding of Purified FKBP52 to GST-IGR**—Sf9 cells were infected with baculovirus encoding GST-IGR, GST-465<sup>z</sup>, or GST-500<sup>z</sup> at a multiplicity of infection of 3; and after 48 h, cytosol was prepared. Twenty-five µl of Sf9 cytosol or 15 µg of GST (as a control) were immobilized on 15 µl of glutathione cross-linked to agarose, and the mixtures were rotated for 2 h at 4 °C. The glutathione pellets were washed three times by suspension in 1 ml of phosphate-buffered saline and then two times with TEG buffer. The pellets were then suspended in TEG buffer containing 10 µg/ml of protein A-agarose prebound with the UPJ56 antisera against FKBP52 (2%), anti-CyP-40 (2%), or 6 µg of anti-FLAG M2 monoclonal antibody and incubated at 30 °C for 2 h to dissociate the GR from the hsp90. After the incubation, the pellets were washed twice with 1 ml of radioimmunoprecipitation assay buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) and two times with TEG buffer. The washed pellets were incubated with 25 µl of purified bacterially expressed FKBP52 or the PPIase fragment of FKBP52 and adjusted to a final volume of 50 µl with 10 mM Hepes, pH 7.6, 150 mM NaCl, 100 mM KCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS before loading onto SDS–polyacrylamide gel electrophoresis and Western blotting.
RESULTS

Binding of FKBP52 to hsp90—As summarized above, the immunophilins bind via TPR domains to a TPR acceptor site in the C-terminal domain of hsp90, and the stoichiometry of steroid receptor heterocomplexes is 1 molecule of receptor, 2 molecules of hsp90, and 1 molecule of immunophilin. To resolve whether there is one TPR acceptor site/hsp90 dimer or whether there is one acceptor site on each monomer with one being blocked when the receptor is bound to an hsp90 dimer, we performed the cross-linking experiment shown in Fig. 1. In this experiment, purified hsp90 and purified FKBP52 were mixed together and cross-linked with glutaraldehyde. Fig. 1 shows the two proteins after they were resolved by SDS gel electrophoresis and Western blotting. Lanes 1–3 were probed with an antiserum that recognizes both hsp70 and hsp90, and lanes 5–7 were probed with the UPJ56 antiserum against FKBP52. Lane 4 was split, and each half was probed with the respective antiserum. As shown in lane 2, when hsp90 alone was treated with glutaraldehyde, the majority of the protein was recovered as a 180-kDa hsp90 dimer. In contrast, little if any FKBP52 was cross-linked as a homodimer (lane 6). When the two proteins were mixed together, glutaraldehyde treatment yielded a slow migrating form that reacted with both the antibody against hsp90 (lane 3) and the antibody against FKBP52 (lane 5). This band was formed at the expense of some hsp90-hsp90 dimer, and it migrated as expected for an hsp90-hsp90-FKBP52 heterotrimer. This direct evidence strongly supports a model in which there is one TPR acceptor site/hsp90 dimer.

hsp90 is a very abundant protein in the cytosol, and immunoadsorption of immunophilins yields co-adsorption of immunophilins:hsp90 heterocomplexes (20). The relative amount of a TPR protein that is associated with hsp90 should depend on both its affinity for the TPR acceptor site on the hsp90 dimer and its abundance in the cytosol relative to other TPR proteins. The experiment of Fig. 2 was done to determine the relative amounts of several TPR proteins that are bound to hsp90 in reticulocyte lysate. hsp90 was immunoadsorbed from a small volume of rabbit reticulocyte lysate with the 3G3 monoclonal antibody IgM, and the amount of protein remaining in the lysate after adsorption with nonimmune IgM (lane 1) or the 3G3 antibody (lane 2) is shown, as is the amount of protein recovered in the nonimmune (lane 3) or 3G3 immune (lane 4) pellets. Under these conditions, we immunoadsorbed essentially all of the hsp90 protein and co-immunoadsorbed all of the Hop protein. Hop is required for assembly of receptor:hsp90 heterocomplexes, and it contains six tetratricopeptide repeats (see Ref. 1 for review). PP5 contains four TPRs in a TPR domain that binds very tightly to hsp90 (15, 33), and most of the PP5 in reticulocyte lysate is bound to hsp90. Only a portion of the immunophilins FKBP52 and CyP-40, both of which have three TPRs, is bound to hsp90 (Fig. 2). Of these two immunophilins, CyP-40 is known to be more weakly bound in GR:hsp90 heterocomplexes (12), and less is associated with the washed hsp90 immune pellet (Fig. 2). Although p50cdc37 does not have TPRs, its binding site is close to the TPR acceptor site on hsp90 (33), and only a portion of the p50cdc37 in reticulocyte lysate is bound to hsp90.

Binding of FKBP52 to the GR—Fig. 3A shows that FKBP52 also binds directly to the GR. In this experiment, GST-GR was expressed in Sf9 cells or GST alone was immobilized on glutathione-agarose and stripped of associated insect hsp90 prior to incubation with purified FKBP52. A small amount of insect hsp90 is visible in lane 2 of the original autoradiogram that was not present in the stripped samples of GST-GR in lanes 4 and 6. As shown in lane 6, FKBP52 bound to GST-GR, but it did not...
bind to GST alone (lane 5). GST-GR does not bind the purified TPR domain fragment of PP5, and the PP5 TPR domain does not compete for the binding of FKB52 to GST-GR (data not shown) under conditions in which it blocks FKB52 binding to hsp90 (33). As shown in Fig. 3B, GST-GR did not bind the purified PPIase fragment of FKB52 (lane 6), and the FKB52 PPIase domain did not compete for the binding of FKB52 to GST-GR (lane 8) under conditions in which we will show that it blocks co-immunoadsorption of dynein with FKB52 (see Fig. 8).

In the experiment of Fig. 4, GST-GR that was immobilized on glutathione-agarose and stripped of insect hsp90 was mixed with purified FKB52, and the mixture was cross-linked with glutaraldehyde. The amount of full-length GST-GR was reduced when FKB52 and glutaraldehyde were present (cf. lanes 2 and 3), and a slow migrating band (lane 4). This band migrated at an apparent molecular mass predicted for a cross-linked GST-500* fragment (see Fig. 8).

Further localization of potential binding site(s) for FKB52 within this region, we assayed the binding of purified FKB52 in two mutants of the hGR, GST-500* and GST-465*. As diagrammed in Fig. 5, GST-500* is a carboxyl-terminal truncation of the GR lacking the hormone-binding domain and the portion of the hinge region carboxyl-terminal to NL1 (nuclear localization signal 1 (50)) at amino acids 491–498 of the hGR (51), whereas GST-465* lacks half of the carboxyl-terminal zinc finger and NL1 as well. As shown in the Western blot of Fig. 5, FKB52 binds to GST-500* (lane 3), although the binding was reduced compared with GST-GR (lane 2); and there is no binding of FKB52 to GST-465* (lane 4).

In their early study of cross-linking of the untransformed GR heteromer, Gehring and co-workers (49) identified a small peak of cross-linked product that was the size of the receptor plus an ~50-kDa polypeptide. This cross-linked product was also obtained on cross-linking of the nt1 (increased nuclear transfer) mutant GR (49). The nt1 mutant is composed of the DNA-binding, hinge, and hormone-binding domains of the GR, and the 50-kDa component was later identified as FKB52 (36). To further localize a potential binding site(s) for FKB52 within this region, we assayed the binding of purified FKB52 in two mutants of the hGR, GST-500* and GST-465*. As diagrammed in Fig. 5, GST-500* is a carboxyl-terminal truncation of the GR lacking the hormone-binding domain and the portion of the hinge region carboxyl-terminal to NL1 (nuclear localization signal 1 (50)) at amino acids 491–498 of the hGR (51), whereas GST-465* lacks half of the carboxyl-terminal zinc finger and NL1 as well. As shown in the Western blot of Fig. 5, FKB52 bound to GST-500* (lane 3), although the binding was reduced compared with GST-GR (lane 2); and there is no binding of FKB52 to GST-465* (lane 4).

Binding of FKB52 to Dynein—we have reported previously that immunoadsorption of FKB52 from Chinese hamster ovary cell or chicken brain cytosol with UPJ56 is accompanied by co-immunoadsorption of both intermediate and heavy chains of cytoplasmic dynein; UPJ56 does not itself recognize dynein; and the presence of dynein in UPJ56 immune pellets is specific for the presence of FKB52 (39). As shown in Fig. 6, immune adsorption of FKB52 from rabbit reticulocyte lysate was accompanied by co-adsorption of dynein, again suggesting that dynein is bound, either directly or via other proteins, to cytosolic FKB52. Immune adsorption of CyP-40 usually (but not always) yielded co-adsorption of trace amounts of dynein (Fig. 6), suggesting a possible weak complex containing the two proteins. Immune adsorption of either PP5 or p50-protein was not accompanied by co-adsorption of dynein.

To determine the domain of FKB52 involved in the interaction with dynein, lysate from bacteria expressing the GST-FKB52 Pro19–Ser302 fragment or lysate from control bacteria expressing GST was mixed with reticulocyte lysate, and rabbit FKB52 was immunoadsorbed. The FKB52 fragment encompasses the N-terminal domain with high PPIase homology, and it possesses PPIase activity (47). As shown in Fig. 7A, immunoadsorption of FKB52 yielded co-immunoadsorption of both hsp90 and dynein (cf. lanes 1 and 2), and the binding of dynein (but not hsp90) was competed by the PPIase fragment (lanes 4.
and 6), but not by FKBP12 (lane 7).

In Fig. 7B, either control Sf9 lysate or lysate from Sf9 cells expressing the TPR domain of PP5 was added to reticulocyte lysate prior to immunoadsorption of FKBP52. Immunoadsorption of FKBP52 from reticulocyte alone yielded co-adsorption of dynein and rabbit hsp90 (lane 2). In the mixture of reticulocyte lysate and control Sf9 lysate, dynein was still co-adsorbed, and co-adsorbed hsp90 was largely the more rapidly migrating in- nect hsp90 (lane 3), which had exchanged with rabbit hsp90. The presence of the PP5 TPR domain in the lysate mixture did not affect co-adsorption of dynein under conditions in which FKBP52 binding to hsp90 was blocked (lane 4). As shown in Fig. 7C, dynein was co-adsorbed with FKBP52, regardless of whether or not its PP1ase domain was bound by FK506.

The FKBP52 Pro19–Ser262 fragment used to compete for dynein co-adsorption in Fig. 7A contains FKBP52 domain I, which shares 49% sequence identity with FKBP12, followed by a short hinge connector segment and domain II, which possesses much less homology to FKBP12 (52). The experiment of Fig. 8A was performed to determine what portion of the PP1ase fragment was sufficient for competition for dynein co-adsorption. Lysates from bacteria expressing GST-FKBP52 Met6–

and Gly148 (lane 5), which encompasses domain I and the hinge, and GST-FKBP52 Gly32–Lys138 (lane 6), which encompasses the core of domain I without the hinge, competed for dynein co-adsorption with FKBP52. In contrast, bacterial lysate expressing a comparable amount of GST-FKBP52 Gly618–Leu262 (lane 7), which encompasses domain II, yielded much less competition. Thus, domain I alone is sufficient for competition for dynein binding to FKBP52.

To rule out any contribution of GST in the fusion proteins, the entire PP1ase fragment (amino acids 19–262) and domain I plus the hinge (amino acids 6–148) were separated from GST by thrombin cleavage and added as the purified fragments to reticulocyte lysate. As shown in Fig. 8 (B and C, lanes 4), both fragments competed for dynein co-adsorption with FKBP52.

**DISCUSSION**

The cross-linking data in Fig. 1 support a model in which 1 molecule of FKBP52 is bound per dimer of hsp90. In the event that heterotrimerers containing 2 molecules of hsp90 and 2 molecules of FKBP52 were formed, they should be readily resolved from the heterotrimer on our gels. Because no slower migrating bands were detected, we conclude that there is one TPR acceptor site/dimer. hsp90 forms homodimers with very
**Protein Interactions with FKBP52**

AHR-hsp90 heterocomplexes (22–25), binds directly to the AHR as well as to hsp90 (57). It is likely that the native GR-hsp90 heterocomplexes, which contain FKBP52 and no p50c production, are selected because FKBP52 binds to the GR as shown in Figs. 3–5. The region of FKBP52 that determines its binding to the GR is not known, but the lack of competition for binding by either the P5 TPR domain or the FKBP52 PPIase domain suggests that neither region is involved. The cross-linking data of Fig. 4 suggest that FKBP52 binds directly to the GR, and the mutant GR data of Fig. 5 suggest that the 35-amin acid segment between amino acids 465 and 500 of the hGR is sufficient for FKBP52 binding. However, GST-500 binds less FKBP52 than GST-GR (Fig. 5), suggesting that the hormone-binding domain also contributes to FKBP52 binding. Segment 465–500 of the GR that is sufficient for FKBP52 contains NL1, and a second nuclear localization signal is located within the hormone-binding domain (50). One possible way to explain the observations of Fig. 5 is that FKBP52 binds to both NL1 and NL2; thus, binding is reduced from the wild-type GR in GST-500 and eliminated in GST-465.

Although the PPIase domain fragment of FKBP52 does not compete for FKBP52 binding to the GR (Fig. 3B) or for FKBP52 binding to hsp90 (Fig. 7A), it does compete for the co-adsorption of cytoplasmic dynein with FKBP52 (Fig. 7A), suggesting that this region of the immunophilin is responsible for either direct binding to dynein or dynein binding via another protein. The immunophilin CyP-40 may also engage in a similar interaction (Fig. 6), but the interaction is so weak that we cannot be sure of it. Although the PPIase domain appears to be involved in the dynein co-adsorption, PPIase activity is not required. This is inferred from the fact that the PPIase inhibitor FK506 does not affect the interaction (Fig. 7C). Although the PPIase domain (domain I of FKBP52 competes for the co-adsorption of dynein with FKBP52 (Fig. 8A), FKBP12 does not compete (Fig. 7A). The lack of an effect of FK506 or FKBP12 on the co-adsorption of dynein with FKBP52 is not unique. Chambraud et al. (58) used PPIase domain I of FKBP52 as bait in a yeast two-hybrid screen to identify a peroxisomal enzyme as a potential FKBP-associated protein. In cell-free experiments, it was shown that the enzyme did not bind FKBP12, and its interaction with FKBP52 was not affected by FK506. Also, it should be noted that FK506-bound FKBP52 or its PPIase domain does not inhibit calcineurin activity in vitro (59), whereas FK506-bound FKBP12 binds to and inhibits the calcineurin phosphatase (60). Thus, there seem to be clear differences in the protein-protein interactions of the FKBP52 PPIase domain and its homolog, FKBP12.

To eventually define the functions of the high molecular mass components of receptor-hsp90 heterocomplexes and the function of the p50c protein kinase-hsp90 heterocomplexes, it is important to define the proteins with which these components interact and the domains responsible for those interactions. We suggest from this and previous work that FKBP52 has at least four regions determining protein interactions. In the domain structure of FKBP52 suggested by the sequence of Callebaut et al. (52), domain I has the highest homology (49%) to FKBP12. This domain expressed alone has PPIase activity (47), and a proteolytic fragment comprising domain I binds FK506 (61). The competition data of Fig. 8 suggest that this region of FKBP52 accounts for co-adsorption of dynein. Domain III contains three TPRs, and both deletion of this TPR domain (16, 19) and competition with TPR domain fragments (12, 15, 20, 33) block FKBP52 binding to hsp90. The C terminus of FKBP52 contains a predicted calmodulin-binding site (52), and FKBP52 binds calmodulin-Sepharose in a calcium-dependent manner (62). Additionally, we show here...
that FKBP52 binds directly to the GR. The precise region of FKBP52 that binds the GR is not yet known, but it appears to be separate from the regions involved in binding to dynen or hsp90 because neither the FKBP52 PPIase fragment nor the hsp90 FKBP52 interacts with the GR. The precise region of 18. Ratajczak, T., and Carrello, A. (1996) J. Biol. Chem.

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