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Functional Specificity of Co-Chaperone Interactions with Hsp90 Client Proteins

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A wide array of proteins in signal transduction pathways depend on Hsp90 and other chaperone components for functional maturation, regulation, and stability. Among these Hsp90 client proteins are steroid receptors, members from other classes of transcription factors, and representatives of both serine/threonine and tyrosine kinase families. Typically, dynamic complexes form on the client protein, and these consist of Hsp90- plus bound co-chaperones that often have enzymatic activities. In addition to its direct influence on client folding, Hsp90 locally concentrates co-chaperone activity within the client complex, and dynamic exchange of co-chaperones on Hsp90 facilitates sampling of co-chaperone activities that may, or may not, act on the client protein. We are just beginning to understand the nature of biochemical and molecular interactions between co-chaperone and Hsp90-bound client. This review focuses on the differential effects of Hsp90 co-chaperones toward client protein function and on the specificity that allows co-chaperones to discriminate between even closely related clients.

Keywords Hsp90, immunophilin, co-chaperone, Cdc37, Xap2, FKBP52, FKBP51, CyP40, PP5

INTRODUCTION

The 90 kDa heat shock protein (Hsp90) is a highly abundant molecular chaperone that comprises approximately 1% to 2% of total cellular protein in the absence of stress (Welch & Feramisco, 1982). Two cytosolic isoforms are differentially expressed and are both required for viability in all eukaryotes. Early studies showed that Hsp90 is upregulated under stress conditions (Finkelstein & Strausberg, 1983); however, the classification of Hsp90 as a true molecular chaperone remained in question due to its extremely slow rate of ATP hydrolysis *in vitro* (Panaretou *et al.*, 1998). The ATPase activity observed was presumed to be the result of contaminating kinases (Shi *et al.*, 1994) and Hsp70 (Schneider *et al.*, 1996), which notoriously co-purifies with Hsp90. The crystal structure of the N-terminal nucleotide binding domain (Prodromou *et al.*, 1997) and the discovery of Hsp90 inhibitors (e.g., geldanamycin) that bind this site (Stebbins *et al.*, 1997) helped to resolve the debate and showed that Hsp90 is a molecular chaperone that requires ATP binding and hydrolysis for function (Panaretou *et al.*, 1998). Hsp90 associates as a dimer with a set of highly diverse client proteins; typically, this association occurs at latter stages of the client folding process and often follows client interactions with Hsp70 and other chaperones. Early work on Hsp90 clients focused on steroid hormone receptors and kinases, but during the last decade the diversity of recognized Hsp90 clients has greatly expanded (see the website maintained by Dr. Didier Picard for an up-to-date listing: http://www.picard.ch/DP/downloads/Hsp90interactors.pdf).

Interactions between chaperone and steroid receptors have been extensively studied and provide a useful model for Hsp90 clients in general. Steroid receptors depend on ordered assembly using chaperone and co-chaperone proteins to reach functionally mature conformations that are competent for hormone binding. *In vitro* assembly assays for progesterone receptor (PR) and glucocorticoid receptor (GR) chaperone complexes using either rabbit reticulocyte lysate or purified proteins showed that Hsp40, Hsp 70, Hsp organizing protein (Hop), Hsp90, and the Hsp90-binding co-chaperone p23 are both necessary and sufficient to restore efficient hormone binding (Dittmar *et al.*, 1996; Hutchison *et al.*, 1994; Kosano *et al.*, 1998; Pratt

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& Toft, 1997; Scherrer *et al.*, 1990; Smith *et al.*, 1990b; Smith *et al.*, 1995). The steroid receptor assembly pathway has been thoroughly reviewed (Pratt & Toft, 1997; Pratt & Toft, 2003) and is briefly described here. Hsp40 binding to the receptor hormone binding domain initiates the assembly pathway (Hernandez *et al.*, 2002); in turn, Hsp70 is recruited to the nascent complex. Dependent upon ATP hydrolysis by Hsp70, formation of an intermediate complex occurs, in which Hop and the Hsp70 interacting protein (Hip) associate with the receptor-Hsp70 complex. Hop concomitantly binds Hsp70 and Hsp90, thus acting as a bridge factor to link the receptor-Hsp70 complex to Hsp90. In ways that are as yet not well understood, Hsp90 becomes directly bound to the receptor, Hsp70 and its co-chaperones dissociate, and Hsp90 co-chaperones are recruited in their place. Exchange of the receptor to the Hsp90 complex results in a final folding event that enables the receptor to bind hormonal ligand.

Investigators have used co-immunoprecipitation approaches to identify Hsp90 client proteins. Also, due to the high conservation of Hsp90 function, yeast genetic models have proven valuable for identifying and characterizing Hsp90 clients. Finally, the identification of specific Hsp90 inhibitors such as geldanamycin (Whitesell *et al.*, 1994) and radicicol (Schulte *et al.*, 1998; Sharma *et al.*, 1998) has greatly facilitated identification of novel clients and increased our understanding of the molecular and cellular roles of Hsp90. These small molecules bind to the Hsp90 N-terminal nucleotide binding site and effectively disrupt the ATPase cycle that drives Hsp90 function and client protein maturation (reviewed in Prodromou & Pearl, 2003).

As Hsp90/client complexes have been compared, it is clear that Hsp90 co-chaperone frequencies vary among client complexes. While some co-chaperones are found with diverse Hsp90 clients, others are highly specific for particular clients. This review focuses on two classes of Hsp90 co-chaperone that have distinctive client repertoires. First, we discuss selected examples of co-chaperones that share a conserved tetratricopeptide repeat (TPR) domain that targets a common binding site on Hsp90 (TPR co-chaperones were recently reviewed in Smith, 2004). Two of these are members of the FK506 binding family of immunophilins, FKBP51 and FKBP52, which are favored in steroid receptor complexes and which have antagonistic actions toward some steroid receptors. Another is Xap2 (hepatitis virus protein X-associated protein 2), which associates with Hsp90 and is found almost exclusively in aryl hydrocarbon receptor (AhR) complexes. In contrast to the TPR-containing co-chaperones, Cdc37 lacks a TPR domain and is most commonly found in Hsp90 complexes with kinase clients (a listing of Cdc37 interacting proteins is also maintained by Picard: http://www.picard.ch/DP/downloads/Cdc37interactors.pdf).

We have not attempted to review all Hsp90 co-chaperones and acknowledge that, among the co-chaperones we neglect, are several important and distinctive proteins. The more general co-chaperones p23 and Hop have recently been reviewed (Felts & Toft, 2003; Smith, 2004), and additional Hsp90-binding TPR co-chaperones with unique properties, such as yeast Cns1 (Dolinski *et al.*, 1998; Marsh *et al.*, 1998) or metazoan UNC-45 (reviewed in Gonczy, 2004; Hutagalung *et al.*, 2002), also play critical roles in client protein maturation, regulation, or degradation. We also do not address subcellular localization of co-chaperones, which can be an important factor in the range of cellular activities, but this topic was recently covered in an excellent review (Young *et al.*, 2003). In narrowing our scope to a few examples of functional specificity in Hsp90 co-chaperones, we apologize to those whose relevant work we have neglected.

STEROID RECEPTOR-ASSOCIATED IMMUNOPHILINS

Discovery of Steroid Receptor-Associated Immunophilins and Their Client Range

Among the Hsp90-binding co-chaperones in steroid receptor complexes are large members of the FK506 and cyclosporin binding families of immunophilins (FKBP and CyP, respectively), which compete for binding a common site on Hsp90, and each of which is a peptidylprolyl isomerase (PPIase) (Galat, 2003). The commonly observed receptor-associated immunophilins are FKBP52, FKBP51, and CyP40. In addition, the protein phosphatase PP5 (Chinkers, 1994), which binds FK506 with low affinity and shares some sequence homology with the FKBP PPIase domain (Silverstein *et al.*, 1997), competes with other immunophilins for binding Hsp90 and assembling with steroid receptor complexes. Thus, we now recognize that mature, Hsp90-bound steroid receptors exist as a collection of complexes that are individually distinguished by the immunophilin component.

FKBP52 (also termed p59, Hsp56, p50, HBI, FKBP59, or FKBP4) was first identified in steroid receptor complexes by Faber and colleagues (Tai *et al.*, 1986) and was later recognized as a novel member of the FKBP family after molecular cloning of the cDNA (Tai *et al.*, 1992). FKBP51 (also termed p54, FKBP54, or FKBP5) was first observed in immunoaffinity-purified chicken PR complexes (Smith *et al.*, 1990a) and was shown to share approximately 70% amino acid sequence similarity with FKBP52 (Baughman *et al.*, 1995; Nair *et al.*, 1997). CyP40 was first found in estrogen receptor (ER) complexes (Ratajczak *et al.*, 1993; Ratajczak *et al.*, 1990), and PP5 was first shown to assemble with GR complexes (Chen *et al.*, 1996).

Although immunophilins are typically characterized as participants of steroid-receptor-Hsp90 complex assembly, their association with Hsp90 clients is not limited to steroid receptors. They can also be found in complexes with heat shock transcription factor 1 (HSF), AhR, the Src-related kinase Fes (Nair *et al.*, 1995), with other cytoplasmic kinases(Hartson *et al.*, 2000; Scroggins *et al.*, 2003), or with mutant p53 (Galigniana *et al.*, 2004). These interactions are largely Hsp90-dependent and probably reflect piggybacking of immunophilin into the Hsp90-client complex. On the other hand, the Hsp90-binding immunophilins have also been shown to have stable, Hsp90-independent interactions with other proteins. The PPIase domain of FKBP52 binds a protein component in dynein complexes (Galigniana *et al.*, 2002), and this interaction may relate to intracellular transport of receptor complexes, as discussed below. Yeast two-hybrid screens detected FKBP52 directly binding to interferon regulatory factor 4 (Mamane *et al.*, 2000), copper transport protein Atox1 (Sanokawa-Akakura *et al.*, 2004), or transient receptor protein channels for Ca^{2+} transport (Sinkins *et al.*, 2004). FKBP52 also interacts with protein FAP48, which may play a role in T-cell activation (Chambraud *et al.*, 1996; Krummrei *et al.*, 2003). Surprisingly, FKBP52 can directly bind adeno-associated virus DNA and regulate replication of the viral genome (Qing *et al.*, 2001; Qing *et al.*, 1998). The TPR-containing phosphatase PP5 can directly bind the atrial natriuretic peptide receptor (Chinkers, 1994) or the apoptosis signal-regulating kinase 1 (Morita *et al.*, 2001). Currently, direct interacting partners other than Hsp90 have not been described for CyP40 or FKBP51, but such partners probably exist.

Structural Features of Immunophilins and Their Roles in Client Selectivity

Three-dimensional crystal or NMR structures have been solved for full-length CyP40 (Taylor *et al.*, 2001) and FKBP51 (Sinars *et al.*, 2003) and for domains of FKBP52 (Craescu *et al.*, 1996; Li *et al.*, 2003; Wu *et al.*, 2004) and PP5 (Das *et al.*, 1998). Each shares a similar TPR domain that forms the Hsp90-binding site and targets co-chaperone to the C-terminal region of Hsp90, which contains the highly conserved MEEVD sequence that terminates Hsp90. Co-crystal structures with an MEEVD peptide bound to TPR domains of the Hsp90 co-chaperone Hop (Scheufler *et al.*, 2000) or FKBP52 (Wu *et al.*, 2004) reveal the basis for MEEVD interactions with the TPR groove. Mutations in the C-terminal region of Hsp90, however, differentially affect binding of co-chaperones (Chen *et al.*, 1998), and sequences outside the core TPR domain of co-chaperones can distinctively impact Hsp90 binding (Barent *et al.*, 1998; Cheung-Flynn *et al.*, 2003).

Each of the receptor-associated immunophilins has functional domains conjoined either upstream or downstream from the common TPR domain (Figure 1). CyP40 has a single PPIase domain N-terminal to the TPR. The protein phosphatase domain of PP5 lies downstream of the TPR. Both FKBP51 and FKBP52 have two N-terminal domains, both of which are derived from an ancestral PPIase that is similar to other FKBP family members but structurally distinct from the CyP40 PPIase domain. The first domain of the pair (FK1) retains PPIase activity and is the site for FK506 binding; the second domain (FK2) does not have PPIase or drug-binding activity, despite its structural similarity to FK1 (Nair *et al.*, 1997; Sinars *et al.*, 2003). The enzymatic domains of these co-chaperones effectively accessorize Hsp90 with activities that can act upon receptor or other client proteins bound by Hsp90.

Dynamic Sampling and Preferential Associations

The interactions between chaperones and steroid receptor complexes are dynamic. Under conditions *in vitro* that promote maximal functional maturation of receptor complexes, Hsp90 dissociates from the receptor after approximately 5 minutes (Smith, 1993), timing that correlates with the slow ATPase cycle of Hsp90 (Grenert *et al.*, 1999; Panaretou *et al.*, 1998; Prodromou & Pearl, 2003; Richter & Buchner, 2001; Wegele *et al.*, 2003). Immunophilin cycling on the mature PR complex is more rapid than release of Hsp90; immunophilin residence times ranged from 0.5 to 1.5 minutes in one study (Nair *et al.*, 1997). We can draw two conclusions from these kinetics. First, the mature Hsp90-receptor complex is continuously sampling its environment for available co-chaperones. Second, because immunophilins differ in the duration each resides in a receptor complex, interactions between a particular immunophilin and other components in the receptor complex seem unique. Some of the differential interaction probably relates to distinct affinities of immunophilins for Hsp90 (Pirkl & Buchner, 2001), but the patterns of interaction also differ depending on the particular steroid receptor component. In comparing GR, PR, and ER complexes (Barent *et al.*, 1998; Silverstein *et al.*, 1997), PP5 and FKBP51 are more abundant in GR complexes, whereas FKBP51, but not PP5, is preferred in PR complexes. CyP40 is most abundant in ER complexes. FKBP51 association with PR complexes is uniquely sensitive to hormone binding by receptor (Smith *et al.*, 1993; Smith *et al.*, 1990a), and hormone binding stimulates a rapid exchange of FKBP51 for FKBP52 in GR complexes (Davies *et al.*, 2002). Thus, the immunophilin component that piggybacks on Hsp90 into steroid receptor complexes is capable of sensing which receptor is present in the complex and of assessing the hormone-binding state of that receptor. It is reasonable to propose that immunophilins can form direct contacts with

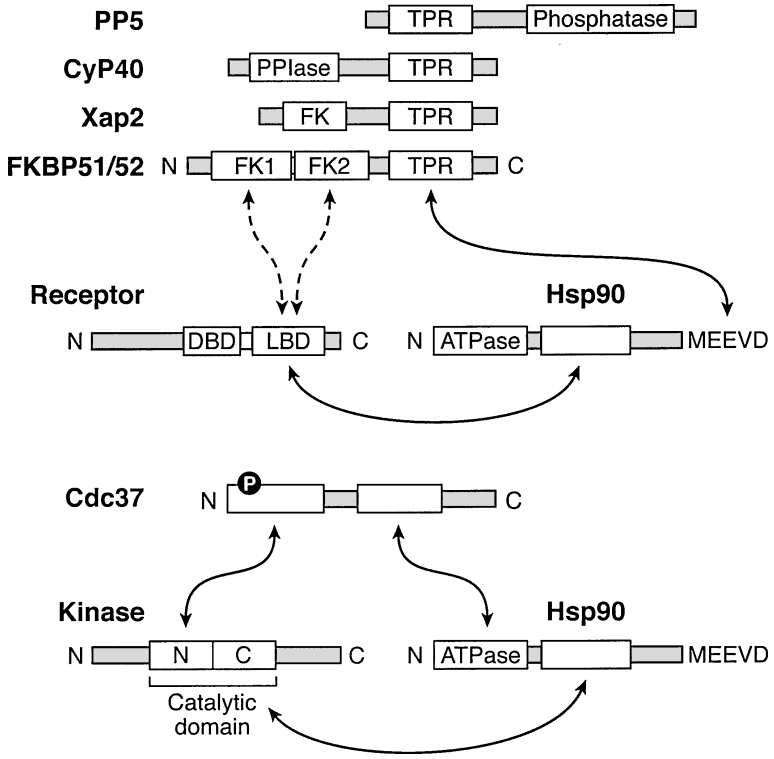

FIG. 1. Schematic representations of the Hsp90 co-chaperones and their interactions with clients. **Top.** Structural comparison of the TPR-containing proteins and their interactions with the receptor client and Hsp90. The TPR domains compete for binding to the MEEVD motif and other features in the C-terminal region of Hsp90. Unique co-chaperone domains upstream or downstream of the TPR domain may direct interactions with receptor clients. The middle domain and other regions of Hsp90 bind client protein, in this case the ligand binding domain (LBD) of steroid receptor, and bring co-chaperone into close proximity with the client. A productive interaction can then occur between co-chaperone and client; as illustrated here, FK domain interactions with the receptor LBD or potentially with the DNA binding domain (DBD) or other receptor region. **Bottom.** Cdc37 structure and interactions with kinase client and Hsp90. The highly conserved N-terminal domain contains the site of phosphorylation by casein kinase II ("P"). This domain interacts primarily with the N-lobe of the kinase catalytic domain. The middle portion of Cdc37 interacts with the N-terminal ATPase domain of Hsp90. The kinase catalytic domain is recognized by the middle domain of Hsp90. Note that the TPR proteins and Cdc37 do not share docking sites on Hsp90, suggesting that they can bind the same Hsp90 complex.

receptor through non-TPR domains; ternary complexes of Hsp90, receptor, and immunophilin could facilitate interaction, and selective interactions between immunophilin and receptor could contribute to different patterns of association when comparing complexes on different steroid receptors. Consistent with this concept, direct binding of FKBP52 to GR has been reported (Silverstein *et al.*, 1999), but GR binding was not observed with N-terminal or C-terminal fragments of FKBP52, suggesting that different domains contribute to binding. FKBP51 associates preferentially in PR complexes, and site-directed mutation of unique residues in FK2 abrogated preferential association with PR complexes, without altering Hsp90 binding or affecting the basic ability of FKBP51 to assemble into receptor complexes (Sinars *et al.*, 2003). Further study is needed to more clearly define sites of interaction between components in receptor complexes.

Functions of Immunophilins in Receptor Complexes

In the last several years, the Pratt lab has provided evidence that supports a potential role for immunophilins in nuclear translocation of Hsp90 clients such as GR and p53 (Pratt *et al.*, 2004). According to their findings, FKBP52, CyP40, or PP5 in the Hsp90/receptor complex can concomitantly bind a dynein-dynactin motor complex on cytoskeleton (Galigniana *et al.*, 2001, 2002, 2004). FKBP51 purportedly lacks the ability to bind dynein complexes. Because hormone binding can induce switching of FKBP51 for FKBP52 in GR complexes (Davies *et al.*, 2002), one could imagine a corresponding shift of GR to dynein complexes that facilitates nuclear import. There are appealing aspects to this model, and some experimental evidence supports immunophilin-mediated transport of GR complexes. On the other hand, this model faces a conceptual hurdle when

one considers the dynamics of immunophilins in receptor complexes; the observed compositions of various steroid receptor complexes; the apparent redundancy of FKBP52, CyP40, and PP5 in directing receptor complexes to dynein; and the distinctive subcellular localization patterns for different receptors in the absence of hormone. Much remains to be learned of the mechanisms that facilitate nuclear transport of steroid receptors and the potential roles for immunophilins in these processes.

Accumulating evidence suggests that immunophilins can selectively modulate hormone-binding affinity of some steroid receptors. Studies by Scammell and colleagues provided the first indication that cortisol resistance in New World primates is attributable to FKBP51 (Denny *et al.*, 2000; Reynolds *et al.*, 1999; Scammell *et al.*, 2001). They have shown that FKBP51 can reduce GR hormone-binding affinity by fivefold or greater. In squirrel monkey tissues, FKBP51 is constitutively expressed at elevated levels compared to those in human tissues, and the squirrel monkey FKBP51 is more potent in reducing GR hormone-binding affinity. FKBP51 has also been shown to inhibit PR activity in transfected vertebrate cells (Hubler *et al.*, 2003).

In a yeast model that measures the impact of human immunophilins on steroid receptor activity, FKBP51 does not directly inhibit GR function (Riggs *et al.*, 2003); on the other hand, FKBP52 was found to elevate GR hormone-binding affinity. Co-expression of FKBP51 with FKBP52 returned GR to a lower affinity state; this reversal could not be attributed to competitive displacement of FKBP52 from Hsp90 complexes, given that PP5, which preferentially assembles with GR similar to FKBP51, was unable to lower GR affinity when co-expressed with FKBP52. Therefore, despite the relatedness of FKBP51 and FKBP52, these co-chaperones affect the sensitivity of GR to hormone in an opposing manner. The mechanism by which FKBP52 up-shifts GR hormone-binding affinity was investigated in the yeast model (Riggs *et al.*, 2003). Potentiation was absent in a TPR point mutation that disrupts Hsp90 binding, emphasizing the importance of Hsp90's organizing role in receptor complexes. Additionally, FKBP52-mediated potentiation was blocked either by FK506, which inhibits PPIase activity, or by point mutation of active site residues in the PPIase domain. FKBP52 PPIase was also required for functional changes in two Hsp90-independent targets of FKBP52, IRF-4 (Mamane *et al.*, 2000; Sharma *et al.*, 2000) and Atox1 (Sanokawa-Akakura *et al.*, 2004). Because FKBP52 does not enhance ER function, chimeric receptors with swapped functional domains were used to localize potentiation to the ligand binding domain of GR (Riggs *et al.*, 2003). These observations support a role for FKBP52 PPIase (FK1) in GR potentiation and suggest the presence of a relevant proline substrate in the GR ligand binding domain. Whether FKBP51 reverses potentiation by targeting the same putative site or by some other mechanism has not yet been determined.

In the yeast model, our laboratory has recently observed that human ER and mineralocorticoid receptor (MR) are unaffected by either FKBP and that both GR and androgen receptor (AR) are similarly potentiated in the presence of FKBP52 (unpublished observations). Thus, as with varied retention times of immunophilins in receptor complexes (discussed above), varied functional consequences are related to immunophilin association with different steroid receptors. To address the physiological importance of receptor-associated immunophilins, our laboratory has recently generated mouse gene knockout models for FKBP51 and FKBP52 (manuscripts in preparation). Both sexes of FKBP52 null mice are infertile. Males display abnormal virilization and dysgenesis of secondary sexual organs consistent with androgen insensitivity. Female mice appear to have a maternal defect that prevents successful pregnancy, a phenotype that may relate to progesterone insensitivity in the uterus. FKBP51 mutant mice have no overt defects and are reproductively normal; this observation confirms that FKBP52 and FKBP51 have nonredundant functions and suggests that mice compensate for loss of FKBP51 but cannot for loss of FKBP52.

The functional significance of CyP40 or PP5 participation in steroid receptor complexes is not well understood. Two yeast orthologs exist for CyP40 (Duina *et al.*, 1996a; Duina *et al.*, 1996b); deletion of one of these, Cpr7, impairs GR function but also causes a general growth defect. GR function is not restored by expression of human CyP40 in Cpr7-deficient yeast, so it is unclear whether Cpr7 accurately represents CyP40 function in vertebrate GR complexes. Published reports have stated that PP5 can either positively or negatively regulate GR function (Chen *et al.*, 1996; Silverstein *et al.*, 1997), and cellular studies showed direct binding of PP5 to ER, resulting in inhibition of ER phosphorylation and transcriptional activation (Ikeda *et al.*, 2004). Apart from steroid receptors, PP5 was found to down-regulate the heme-regulated eIF2α kinase, HRI, in an Hsp90-dependent manner (Shao *et al.*, 2002). Our laboratory has not observed a significant effect of PP5 on any functional readout of human steroid receptors in the yeast model, but that finding does not exclude the possibility that PP5 serves important cellular functions in native contexts.

Regulation of Immunophilin Expression and Activity

Because receptor-associated immunophilins can differentially regulate steroid receptor activity, the level and balance of immunophilin activities in cells probably influence cellular responses to steroid hormones. Several observations suggest that steroid hormones and other factors

actively regulate immunophilin gene expression, which in turn can alter cellular sensitivity to hormones. FKBP51 gene expression is directly upregulated by glucocorticoids (Baughman *et al.*, 1995; Yoshida *et al.*, 2002), progesterone (Hubler *et al.*, 2003; Kester *et al.*, 1997), or androgens (Zhu *et al.*, 2001); consequently, increased levels of FKBP51 could down-modulate GR, PR, or AR activity in target tissues. CyP40 and FKBP52 gene expressions are induced by estrogens in an MCF-7 breast cancer cell line (Kumar *et al.*, 2001), with implications for ER or PR function in mammary cells. Likewise, estrogen-stimulated PP5 expression in MCF-7 cells may contribute to estrogen-independent growth of these cells (Urban *et al.*, 2001). FKBP52 expression can be induced by heat shock (Sanchez, 1990), which has implications for integrating cellular stress responses involving both HSF and GR pathways (Jones *et al.*, 2004). Post-translational modification of immunophilins might also influence steroid signaling. For example, FKBP52 is a substrate for casein kinase II *in vitro*, and the resulting phosphorylation inhibited FKBP52 binding to Hsp90 (Miyata *et al.*, 1997). Tyrosine phosphorylation of FKBP52 affects its ability to bind adeno-associated virus DNA (Qing *et al.*, 2003), but it is unknown whether tyrosine phosphorylation influences FKBP52 assembly and function in steroid receptor complexes. Long-chain fatty acids bind the TPR domain of PP5 and de-repress phosphatase activity (Ramsey & Chinkers, 2002; Sinclair *et al.*, 1999; Skinner *et al.*, 1997); given the structural similarity of the PP5 and immunophilin TPR domains, it is reasonable to speculate that fatty acids might similarly regulate aspects of immunophilin function. These various mechanisms for regulation of expression and activity of receptor-associated co-chaperones imply evolutionary adaptations that enhance physiological control of steroid-dependent pathways.

XAP2

Discovery of Xap2 and the Identification of Xap2-Regulated Hsp90 Client Proteins

Chen and Perdew, using protein cross-linking and co-immunoprecipitation approaches, identified an unknown 43 kDa protein that associated with the AhR-Hsp90 complex (Chen & Perdew, 1994). Three steroid hormone receptor-associated immunophilins were chosen as possible candidates for the unknown 43 kDa protein; however, antibodies directed against these immunophilins did not detect the 43 kDa protein on Western blots. Two years later, a 36 kDa protein was identified in a yeast two-hybrid screen for proteins that associate with the hepatitis B virus protein X from a human peripheral lymphocyte cDNA library (Kuzhandaivelu *et al.*, 1996). The interaction between the 36 kDa protein and protein X was further confirmed by co-precipitation *in vitro*. Thus, the unknown 36 kDa protein was named the hepatitis B virus (HBV) protein X-associated protein 2 (Xap2). Subsequently, Xap2 overexpression was shown to inhibit transactivation mediated by protein X. During the two years following the identification and cloning of Xap2, three independent laboratories showed an interaction between Xap2 and the AhR-Hsp90 complex. Two of these laboratories identified an unknown protein in yeast two-hybrid screens for AhR-interacting proteins (Carver & Bradfield, 1997; Ma & Whitlock, 1997); this protein was named AhR interacting protein (AIP) (Ma & Whitlock, 1997) and AhR associated protein 9 (ARA9) (Carver & Bradfield, 1997). AIP, ARA9, and Xap2 are now known to be the same protein. Finally, using reverse genetic approaches, Meyer and coworkers showed that the unknown 43 kDa protein found in association with the AhR-Hsp90 complex four years earlier was Xap2 (Meyer *et al.*, 1998). A functional role for Xap2/AhR interaction was confirmed by showing that Xap2 overexpression influences AhR-mediated expression of a reporter gene in both yeast and mammalian cells (Ma & Whitlock, 1997; Meyer *et al.*, 1998; Miller, 2002).

Co-immunoprecipitation using *in vitro* transcribed and translated proteins showed that Xap2 specifically associated with AhR, but did not interact with GR (Carver *et al.*, 1998). In this same set of experiments, Xap2's affinity for AhR was approximately threefold higher than that of FKBP52. Xap2 enhanced AhR protein levels in COS-1 cells, but neither FKBP52 nor PP5 affected AhR protein levels (Meyer *et al.*, 2000). Thus, Xap2 has generally been considered an AhR-specific co-factor. Recently, however, Xap2 and Hsp90 were co-immunoprecipitated with the peroxisome proliferator-activated receptor α (Sumanasekera *et al.*, 2003). Coexpression of Xap2 and PPARα in COS-1 cells resulted in reduced PPARα-mediated expression of a luciferase reporter gene. Thus, PPAR α is the second Hsp90 client protein and the first nuclear receptor known to functionally interact with Xap2.

The client specificity of Xap2 is clearly restricted, given that no interaction with GR has been observed. Xap2 can also discriminate among members of the basic helix-loop-helix (bHLH)/Per-Arnt-Sim (PAS) family of transcription factors, of which AhR is a member. First, Xap2 does not interact with Arnt, the heterodimeric partner for AhR in active transcription complexes, but Arnt is also not a client for Hsp90 (Nair *et al.*, 1995). Another bHLH/PAS protein, hypoxia inducible factor-1α (HIF-1α), is an Hsp90 client (Minet *et al.*, 1999) and shares many structural and regulatory similarities with AhR. Reporter gene activation by AhR was reduced by antisense depletion of Xap2, but reporter activation by an HIF-1α chimera containing the bHLH domain from AhR was unaffected (Lees *et al.*, 2003), suggesting that Xap2 does not similarly interact with HIF-1α.

Xap2 may also have roles in Hsp90-independent processes. As mentioned above, Xap2 functionally interacts with HBV protein X (Kuzhandaivelu *et al.*, 1996). In addition, Xap2 is also known to interact with the Epstein-Barr virus protein EBNA-3 (Kashuba *et al.*, 2000) and the cyclic AMP-specific phosphodiesterase PDE4A5 (Bolger *et al.*, 2003). The biological and functional significance of these Hsp90-independent interactions remains unknown.

Structural and Functional Relationships of Xap2

Specificity of Xap2 for Hsp90 clients must result from unique structural features of Xap2 and client protein. The N-terminus of Xap2 shares regions of strong sequence homology with FKBP12 and the FK domains of FKBP52 and FKBP51 (Carver & Bradfield, 1997; Ma & Whitlock, 1997; Meyer *et al.*, 1998). Unlike FKBP12 and FKBP52, however, Xap2 does not bind FK506 (Carver *et al.*, 1998), and the presence of PPIase activity in the Xap2 FK domain has not been determined. Also similar to FKBP52 and other Hsp90 co-chaperones, Xap2 contains a related TPR domain (Carver & Bradfield, 1997) that binds the same Hsp90 acceptor site (Bell & Poland, 2000). FKBP52 can assemble with Hsp90-AhR complexes (Nair *et al.*, 1995), albeit weakly and non-functionally (Carver *et al.*, 1998).

Xap2 associates with a C-terminal portion (aa 380 to 419) of the AhR PAS domain, which includes binding sites for ligand and Hsp90 (Meyer & Perdew, 1999). Because this interaction is ATP-dependent and sensitive to geldanamycin (Bell & Poland, 2000), Hsp90 probably facilitates binding of Xap2 to AhR. Xap2 binding to Hsp90 may not be required, however, for the association of Xap2 with AhR, given that a TPR point mutant (K266A) abolishes binding to Hsp90, but not to AhR (Bell & Poland, 2000). Thus, Xap2 binding to AhR is dependent on Hsp90 in the sense that Hsp90 is required for the receptor to reach the mature conformation that can bind Xap2.

Interestingly, Xap2 effects on murine AhR (mAhR) and human AhR (hAhR) are functionally distinct (Ramadoss *et al.*, 2004). Xap2 was capable of facilitating nucleocytoplasmic shuttling of mAhR-YFP in COS-1 cells, whereas Xap2 had no effect on hAhR-YFP. Furthermore, Xap2 inhibited hAhR-mediated expression of a reporter gene, while it enhanced mAhR-mediated gene expression. Amino acid sequences of mAhR and hAhR differ in the C-terminal region involved in Xap2 interaction.

Domain mapping of Xap2 showed that the C-terminal half containing the TPR domain is sufficient for binding both AhR and Hsp90, whereas the N-terminal half containing the FK domain is not required (Carver *et al.*, 1998). Although the C-terminal half of Xap2 alone is capable of interacting with AhR, this interaction is non-functional, as assessed by the ability of Xap2 to influence the subcellular localization of AhR (Kazlauskas *et al.*, 2002). Thus, the FK domain is functionally important for changes in client protein activity.

Functional Consequences of Xap2 Association with AhR

AhR complexes assembled in reticulocyte lysate that lacks Xap2 assemble with Hsp90 and are competent for binding ligand; additionally, after ligand binding Hsp90 dissociates, AhR dimerizes with Arnt, and the heterodimer binds AhR response elements on DNA (Meyer *et al.*, 1998). Thus, Xap2 is not required for several aspects of AhR function. On the other hand, Xap2 is required for AhR to function at full capacity. Xap2 expression in human cells enhanced ligand-induced expression of a luciferase reporter (Meyer *et al.*, 1998) or of CYP1A1, an endogenous AhR-regulated gene (Ma & Whitlock, 1997). Xap2 was also capable of influencing AhR-mediated expression of a reporter gene in a yeast model system (Carver *et al.*, 1998; Miller, 2002). It is clear that Xap2 has a role in cellular AhR function, yet the mechanism of action is unresolved. Several laboratories showed that Xap2 enhances the stability of the receptor (LaPres *et al.*, 2000; Meyer & Perdew, 1999; Meyer *et al.*, 2000), and this increased stability results from Xap2's ability to protect AhR from ubiquitination and proteasomal degradation (Kazlauskas *et al.*, 2000; Lees *et al.*, 2003). This finding suggests that, in the presence of Xap2, more receptors would be available to bind ligand. It is unclear, however, whether the increase in receptor levels also results in an increase in mature receptors that are competent to bind ligand with high affinity. Xap2 has also been shown to affect nucleocytoplasmic shuttling of AhR (Berg & Pongratz, 2002; Kazlauskas *et al.*, 2000; Kazlauskas *et al.*, 2001; Petrulis *et al.*, 2000). mAhR is predominately localized to the nuclei of cells. In studies conducted by Petrulis *et al.*, expression of Xap2 resulted in the redistribution of mAhR-YFP to the cytoplasm in COS-1 cells (Petrulis *et al.*, 2003). Thus, Xap2 seems to act as a cytoplasmic retention factor. The use of an antibody directed against a portion of the mAhR nuclear localization signal (NLS) was able to bind to mAhR-Xap2 complexes. Thus, Xap2 does not block the NLS on mAhR. In contrast to the results from other laboratories, Xap2 repressed mAhR mediated expression of a reporter gene in these studies. Interestingly, Xap2 was able to inhibit the binding of AhR to importin β *in vitro* (Petrulis *et al.*, 2003), which suggests that Xap2 can alter the conformation of the bipartite NLS on mAhR. Whether any of the proposed mechanisms by which Xap2 regulates AhR would have positive or negative consequences for the receptor signaling pathway is unclear and seems to depend on the levels of Xap2 present in the cell. The positive

effects on AhR signaling observed when Xap2 is expressed at high levels are likely due to the stabilization of the AhR-Hsp90 complex in the cytoplasm resulting in enhanced receptor stability as a secondary consequence. Interestingly, Xap2 negatively influences p23 binding to the AhR-Hsp90 complex, but not to Hsp90 alone, when the pool size of Xap2 is only increased two- to threefold (personal communication, Gary Perdew, Pennsylvania State University). Given p23's role in stabilizing the AhR-Hsp90 complex, this finding can explain the negative effects of Xap2 on AhR signaling that have been observed in some studies. In conclusion, Xap2 displays functional specificity for Hsp90 client proteins that are distinct from those regulated by other TPR-containing proteins such as FKBP52. Furthermore, the mechanism by which Xap2 regulates AhR seems to be functionally distinct from that of FKBP52 in steroid receptor complexes.

Cdc37

Discovery of Cdc37 and Identification of Kinase Clients

Studies of the Rous sarcoma virus transforming protein (v-Src) in vertebrate cells identified a high molecular weight complex of v-Src with a 50 kDa protein (referred to as p50 or pp50) and a 90 kDa protein that was identified as Hsp90 (Brugge *et al.*, 1981; Oppermann *et al.*, 1981). A functional link between Hsp90 and v-Src became apparent through the work of Lindquist and coworkers, who showed that, in yeast, Hsp90 is required for v-Src stability and activity (Kimura *et al.*, 1997; Nathan & Lindquist, 1995; Xu & Lindquist, 1993). The more general role of Hsp90 in kinase complexes became apparent when HRI was found in functional association with Hsp90 (Matts & Hurst, 1989; Rose *et al.*, 1987).

Molecular cloning of a p50 cDNA (Stepanova *et al.*, 1996) showed that it is the metazoan ortholog of yeast Cdc37 (recently reviewed in MacLean & Picard, 2003). Yeast Cdc37 was first identified in genetic screens for temperature-sensitive mutations causing G_1 cell cycle arrest (Reed, 1980). Unlike the TPR-containing co-chaperones described above, the chaperone Cdc37 is essential for growth in *S. cerevisiae* (Gerber *et al.*, 1995) and *C. elegans* (Kamath *et al.*, 2003). It is now known that an important target of Cdc37 is Cdc28, a cyclin-dependant kinase that regulates entry into the cell cycle. In the *cdc37-1* mutant, Cdc28 is unable to complex with cyclins, impairing its activity and stability at restrictive temperatures (Gerber *et al.*, 1995).

The functional relationship between kinases, Cdc37, and Hsp90 was further supported by the identification of both Cdc37 and Hsp90 in a genetic screen for dominant mutations impairing sevenless receptor tyrosine kinase in *Drosophila* (Cutforth & Rubin, 1994). Numerous studies have since shown that Cdc37 and Hsp90 collaborate to stabilize and activate a large number of protein kinases that mediate diverse processes such as cell cycle regulation and signaling.

Interactions Governing the Assembly of Cdc37/Hsp90/Kinase Complexes

Studies in yeast suggest that the co-chaperone Hop plays an important role in recruiting Cdc37 to the kinase-containing Hsp90 complex. Physical interaction between the yeast Hop ortholog Sti1 and Cdc37 has been observed in yeast extracts and with purified proteins (Abbas-Terki *et al.*, 2002). The biological significance of this interaction has been shown genetically. Combinations of *cdc37* and *sti1* mutations are synthetically lethal (Abbas-Terki *et al.*, 2002), and strains lacking Sti1 are defective in maturation of the Ste11 kinase, a defect that can be suppressed by overexpression of Cdc37 (Lee *et al.*, 2004).

After Cdc37 is in the Hsp90/client complex, a major function is to bind both Hsp90 and kinase and to stabilize kinase clients (reviewed in Hunter & Poon, 1997). The central region of Cdc37 contains the Hsp90 binding domain (Scholz *et al.*, 2001; Shao *et al.*, 2003). Co-crystallization of this domain of human Cdc37 and the N-terminal nucleotide binding domain of yeast Hsp90 reveals that Cdc37 locks the lid of the nucleotide binding pocket of Hsp90 in an open position, thus suppressing its ATPase activity (Roe *et al.*, 2004). This interruption of the Hsp90 ATPase cycle results in Hsp90 being maintained in a conformation responsive for client loading (Siligardi *et al.*, 2002). Kinase client interactions with metazoan Cdc37 have been localized to the highly conserved N-terminal domain of Cdc37 (Grammatikakis *et al.*, 1999; Shao *et al.*, 2003), which contains a consensus casein kinase II phosphorylation site, implicating it in the regulation of Cdc37 activity, as discussed below. Despite only about 20% overall amino acid identity between metazoan and yeast Cdc37 (mostly confined to the first 40 amino acids), they are functionally similar (Bandhakavi *et al.*, 2003; Lee *et al.*, 2002).

How does Cdc37 selectively recognize kinase clients that have diverse structures and functions? Although it has long been known that Cdc37/Hsp90 recognizes the catalytic domain of many kinases, the details of these interactions are only beginning to emerge. Although the C-lobe of the catalytic domain is essential for salt resistant client/Hsp90/Cdc37 tertiary complexes, the N-lobe alone is sufficient to bind Hsp90 and Cdc37 (Hartson *et al.*, 1998). The structure of the N-lobe is conserved among kinases and consists of a five-stranded β-sheet. In the case of Cdk4 and Raf1, point mutations in the Gly-X-Gly-X-X-Gly sequence (which overlaps the first two β-stands) abolish Cdc37 binding (Zhao *et al.*, 2004). This motif is

found in several nucleotide binding proteins and serves to anchor the non-transferable phosphates of ATP (Taylor & Radzio-Andzelm, 1994). Whether these mutations remove Cdc37 contacts, or simply alter the β-sheet structure, remains to be determined. Studies on the Lck kinase also confirm the importance of this structure, although weak Cdc37/Hsp90 interactions are observed even when the first three β-strands are deleted (Prince & Matts, 2004). Taken together, these results suggest that Cdc37 recognizes the β-strands of the N-lobe (with possibly some additional specific contacts) and may function by packing the strands properly into the β-sheet structure.

In addition to stabilizing the Hsp90-client complex and regulating the Hsp90 ATPase activity, Cdc37 has an intrinsic chaperone activity that mimics Hsp90. *In vitro* Cdc37 stabilizes purified unfolded β-galactosidase in an activation competent state but does not refold it and also stabilizes the inherently unstable casein kinase II (Kimura *et al.*, 1997). This chaperone activity raises the question of whether Cdc37 can act independently of Hsp90. A truncated yeast Cdc37 that is unable to bind Hsp90 can replace the essential functions of the full-length protein, although only when overexpressed (Lee *et al.*, 2002). Likewise overexpression of a similar form of the metazoan Cdc37 partially stabilizes a temperature-sensitive mutant kinase Hck (Scholz *et al.*, 2001). These results suggest that *in vivo* Cdc37 may be able to function independently of the Hsp90 complex, although rather inefficiently. It remains to be determined whether this type of Hsp90 independent activity is physiologically significant or simply represents residual activity normally present in the Cdc37-Hsp90 collaboration.

Cdc37 Client Range and Activation

With few exceptions, the Cdc37 clients described thus far are kinases. Initial experiments with GR failed to support a role for Cdc37 in steroid hormone signaling. Cdc37 was not observed in steroid receptor- or AhR-Hsp90 complexes assembled in rabbit reticulocyte lysates, although it was readily detectable in parallel assemblies of kinase-Hsp90 complexes (Nair *et al.*, 1996). Yeast strains having reduced levels of Hsp90 are defective in both v-Src activation and steroid signaling, yet although overexpression of yeast Cdc37 suppresses the defect in v-Src maturation, GR remains inactive (Kimura *et al.*, 1997). Consistent with these results, GR function was normal in a strain expressing mutant Cdc37; surprisingly, however, AR function was defective in this yeast strain (Fliss *et al.*, 1997). Physical interaction has been observed between Cdc37 and the AR ligand binding domain (but not GR) in a geldanamycin and hormone-sensitive manner (Rao *et al.*, 2001). It is not clear what factors distinguish recognition of these two closely related steroid receptors by Cdc37, and the physiological significance of Cdc37 interaction with AR has not been determined. The second example of a non-kinase client is the duck hepatitis B virus reverse transcriptase (Wang *et al.*, 2002). Cdc37 interacts with this transcriptase, possibly recognizing structural motifs similar to the Raf kinase. A mutant Cdc37 that is unable to bind Hsp90 inhibits reverse transcription *in vitro* and viral replication and RNA packaging in cell culture. Apparently, this virus has co-opted the Cdc37/Hsp90 chaperone complex for the maturation of this rather unusual protein. Despite these exceptions, the vast majority of Cdc37 clients identified over the past 20 years are kinases.

Are all kinases clients? Some kinases have been presumed to not be clients, based on the lack of physical interactions as determined by co-immunoprecipitation, yeast two-hybrid analysis, or other binding assays. A range of Cdc37-client interactions from weak/transient to strong/long-lived might be expected. Additionally, it has been established that kinases are first recognized by Cdc37/Hsp90 at different stages of synthesis. Although HRI interacts with Cdc37 co-translationally (Shao *et al.*, 2003), Lck does not (Scroggins *et al.*, 2003). This finding may reflect differences in the accessibility of the binding site in the nascent versus mature clients. Thus, the ways in which Cdc37 recognizes kinases appear to differ significantly both physically and temporally, and some of the approaches used to capture this interaction may not be sufficiently sensitive. For example, although v-Src was one of the first Cdc37/Hsp90 clients identified because of its relatively strong interactions with the chaperone complex, the normal cellular homolog, c-Src, was not proven to be Hsp90-dependent until a more sensitive yeast assay was performed almost 20 years later (Xu *et al.*, 1999). Apparently, v-Src has a greater reliance on chaperones due to numerous mutations in the C-terminal region.

In some cases, experiments measuring different aspects of Hsp90 functional interaction may yield apparently contradictory results. The Cdc28 kinase was shown to be a Cdc37 client using genetic and biochemical approaches. In a yeast strain containing the temperature-sensitive *cdc37-1* mutant allele, the interaction between Cdc28 kinase and cyclins is defective, the levels of Cdc28 are reduced, and the thermosensitivity of this strain is suppressed by overexpression of Cdc28 (Gerber *et al.*, 1995; Mort-Bontemps-Soret *et al.*, 2002). Nonetheless, no evidence supports a direct physical interaction between full-length proteins (Mort-Bontemps-Soret *et al.*, 2002). In part, this may be due to Cdc37 interacting with nascent Cdc28 *in vivo* (Farrell & Morgan, 2000). Thus, as a broader range of functional assays for Hsp90 dependence are employed, the list of kinase clients will likely expand.

How does Cdc37/Hsp90 activate kinases, and is this maturation regulated? The presumption is that Cdc37/

Hsp90 recognizes and properly folds the kinase to activate the catalytic domain of the client, although the molecular details of this transformation are unknown. It is interesting to note the similarities and differences between kinases and the steroid hormone receptor clients (Figure 2). In either case, the partially folded kinase domain or ligand binding domain (Immature Client in Figure 2) is recognized by the Hsp90 complex, and the target domain is remodeled (Mature Client in Figure 2) to expose a cleft that interacts with ATP or hormone. The fact that the kinases uniquely contain Cdc37 enables differential regulation of this broad class of Hsp90 client. Indeed, the proper function of both yeast and metazoan Cdc37 has been shown to require phosphorylation at an absolutely conserved N-terminal serine residue, possibly by casein kinase II (Bandhakavi *et al.*, 2003; Miyata & Nishida, 2004; Shao *et al.*, 2003). Given that casein kinase II is also a Cdc37 client, this could create a positive feedback loop in which phosphorylated Cdc37 activates casein kinase II, which in turn keeps Cdc37 phosphorylated (Bandhakavi *et al.*, 2003). Presumably, this positive feedback is then interrupted by a phosphatase removing this essential modification. Interestingly, the immunophilin-like protein phosphatase PP5 is found in an HRI-Cdc37-Hsp90 complex and negatively regulates the maturation of the client (Shao *et al.*, 2003). As in AhR and steroid receptor complexes, the TPR-containing co-chaperone may regulate events subsequent to client refolding and maturation (Modulated Clients). These results suggest that Hsp90 serves not only as a collaborator of Cdc37 in protein folding, but also as a scaffold for the recruitment of regulatory proteins.

DISCUSSION

Co-chaperones can directly regulate Hsp90 ATPase activity or collaborate with Hsp90 to promote client protein folding, functional maturation, and stability. In addition, Hsp90-bound co-chaperones can also use inherent activities to act directly on client proteins and modulate client protein activity in a client-specific manner. Examples of specificity we discussed include the actions of Xap2 toward AhR but not steroid receptors, FKBP52-mediated potentiation of GR but not ER, and Cdc37-dependent maturation of kinases. In many cases, co-chaperone actions require concomitant binding of client and co-chaperone to Hsp90. One reason for concomitant binding may be that Hsp90 induces a client conformation necessary for co-chaperone recognition. A second, non-mutually exclusive reason could be that Hsp90 locally concentrates and/or orients co-chaperone to facilitate interaction with a client. This concentrating mechanism could afford broader co-chaperone specificity by mitigating the cost of lowered affinity for any particular client. From an evolutionary perspective, local concentration through Hsp90 would provide an adaptive opportunity for a relatively small number of co-chaperones to act on a broad range of clients. Moreover, dynamic exchange of co-chaperones on client-associated Hsp90 provides an efficient mechanism for client to sample various co-chaperone activities. In this sampling scenario, specificity arises from chance compatibility in the stochastic pairing of a co-chaperone and client brought together in complex with Hsp90. The requisite biochemical interaction that occurs in a productive client/co-chaperone pairing has not been defined but can be postulated. If co-chaperone recognizes an appropriate feature on the client protein (e.g., a suitably positioned PPIase substrate or phospho-peptide substrate for PP5), productive interactions can occur. Otherwise, one co-chaperone is passively replaced by another on the Hsp90-client complex.

The physiological relevance of specific co-chaperone–mediated changes in client protein function is emerging through animal models. FKBP52 knockout mice have striking reproductive phenotypes that relate to defective steroid hormone signaling. As new gene disruption models are generated for other Hsp90 co-chaperones, we can anticipate phenotypes that underscore non-redundant roles for each co-chaperone. By careful analysis of these phenotypes, one should be able to identify specific client proteins that depend on co-chaperone function and assess whether the relevant clients have a co-dependence on Hsp90.

Small molecule inhibitors/activators of co-chaperone function could be highly useful research reagents and potentially useful clinical agents. As members of the FK506 binding or cyclosporin binding families of immunophilins, FKBP52, FKBP51, and Cyp40 are known drug targets, but no one has yet identified a ligand specific for CyP40 and not for other cyclophilins, or one that adequately discriminates between FKBP51, FKBP52, and other FKBP family members. PP5 phosphatase activity can be stimulated by certain fatty acids, and there is the prospect that highly specific physiological or pharmacological ligands for PP5 can be identified. Either random screening or rational design approaches could be applied to generating specific co-chaperone ligands.

If, as appears likely, co-chaperones have discreet functional roles in physiological processes, then it could be efficacious to target individual co-chaperones for therapeutic ends. Given that FKBP52 is important for AR function, a specific inhibitor of FKBP52 might be a useful adjunct in the treatment of androgen-dependent prostate cancer. A specific inhibitor of FKBP51, which can desensitize GR or PR, might enhance glucocorticoid potency in anti-inflammatory therapies or promote full-term pregnancies in females with progesterone insufficiency. The flip-side of the physiological coin is that

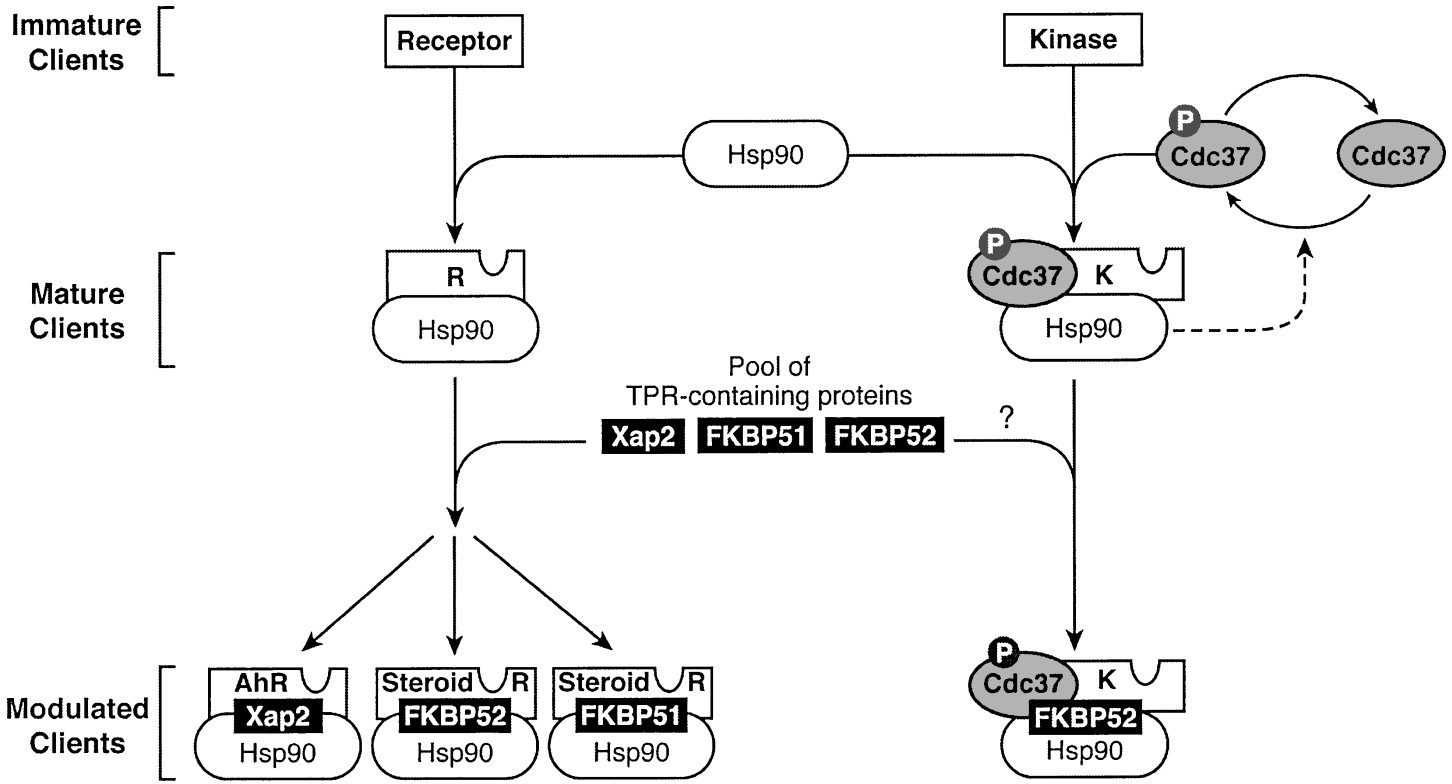

FIG. 2. Client maturation by the Hsp90 chaperone and modulation of activity by co-chaperones. Immature Client (receptor or kinase) is remodeled by association with Hsp90, and in the case of kinases, also Cdc37 to generate the Mature Client conformation. Cdc37 is itself phosphorylated to enhance co-chaperone activity and maturation of kinase clients. In contrast to Cdc37, the TPR-containing co-chaperones (Xap2, FKBP51, and FKBP52, as well as PP5, CyP40 and others) are not essential for maturation of the client, but instead can selectively modulate client function. For simplicity, multiple chaperones and additional co-chaperones that participate in assembly of Hsp90/client complexes are not shown.

co-chaperones could be targets for environmental toxins. For example, because FKBP52 is important for mouse reproductive development and success, one could imagine an endocrine disruptor that acts by blocking FKBP52 and thereby altering reproductive development and fitness.

There are many opportunities for exploration in the field of co-chaperones. More co-chaperones and clients are yet to be discovered, and molecular mechanisms involving even known client complexes are poorly understood. The regulation of co-chaperone function has barely been addressed and promises to be a productive area for study. Structural studies have been very helpful, but more must be learned about how co-chaperones interface with Hsp90 and with client proteins and greater utilization can be made of available structures. Finally, physiological roles for most co-chaperones have yet to be determined in vertebrate models; nonetheless, there are attractive clinical prospects for compounds that inhibit or enhance co-chaperone function.

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