5

Functional Protein Interactions in Steroid Receptor-Chaperone Complexes

Thomas Ratajczak[1,2,*], Rudi K. Allan[1,2],
Carmel Cluning[1,2] and Bryan K. Ward[1,2]
[1]*Laboratory for Molecular Endocrinology, Western Australian Institute for Medical Research and the UWA Centre for Medical Research, The University of Western Australia, Nedlands WA,*
[2]*Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Hospital Avenue, Nedlands WA, Australia*

1. Introduction

Heat shock protein 90 (Hsp90) is unique in that it chaperones a select group of client proteins and assists their folding in preparation for key regulatory roles in cellular signalling. Steroid receptors are among the most extensively studied Hsp90 chaperone substrates and belong to the large nuclear receptor superfamily of hormone-activated transcription factors that respond to hormonal cues through conformational changes induced by hormone binding within the ligand-binding domain (LBD). In an ATP-dependent assembly process, high affinity hormone binding is achieved through the direct interaction of the steroid receptor LBD with Hsp90 and specific Hsp90-associated chaperones. After synthesis, steroid receptors enter the Hsp90 chaperoning pathway by initial assembly with Hsp40, followed by incorporation of Hsp70 and Hip. The binding of Hop and Hsp90 then generates an intermediate receptor complex which is further modified by the release of Hsp70 and Hop, allowing a transition of the receptor to hormone-binding competency. Recruitment of p23 leads to formation of mature receptor complexes capable of binding hormone with high affinity and characterized by the additional presence of one of the immunophilin cochaperones, FKBP51, FKBP52, CyP40 and PP5. This dynamic assembly of receptors to a hormone-activatable state, together with a selective functionality of receptors associated with specific Hsp90-immunophilin complexes provides mechanisms through which Hsp90 and the immunophilin cochaperones may regulate hormone-induced signalling events. This may occur directly by enhancing hormone binding as has been observed for AR, GR and PR associated with Hsp90-FKBP52 complexes or indirectly by facilitating nuclear import of receptor as seen

* Corresponding Author

subsequent to the hormone-induced exchange of FKBP51 by FKBP52 in GR-Hsp90 complexes. For more in depth summaries related to the mechanism and functional consequences of steroid receptor assembly with the Hsp90 chaperone machine, readers are referred to recent reviews (Echeverria & Picard, 2010; Picard, 2006; Pratt & Toft, 2003; Ratajczak *et al*., 2003; Riggs *et al*., 2004; Smith & Toft, 2008).

It is understood that ligand binding induces conformational changes within the steroid receptor LBD, facilitating release of Hsp90 and its cochaperones and exposing elements required for homodimerization, nuclear translocation and DNA binding. The mechanisms through which Hsp90 chaperone machinery regulates the physiological response to steroid hormones mediated by steroid receptors remain unclear. In early work, multiple approaches that included deletion analyses, peptide competition studies and use of the *in vitro* receptor-Hsp90 heterocomplex assembly system present in rabbit reticulocyte lysate were aimed at defining the regions within steroid receptors and Hsp90 responsible for interaction (Pratt & Toft, 1997). These revealed that the GR LBD was essential for formation of apo-GR-Hsp90 heterocomplexes and defined a ~100-amino acid minimal segment (human GR residues 550-653) required for high-affinity Hsp90-binding. The region contains the so-called signature sequence (human GR residues 577-596) that is conserved among steroid receptors, and may contribute to the stability of receptor-Hsp90 interaction. Despite the identification of this core Hsp90 interaction domain, other results suggested a role for nearly all of the LBD in GR association with Hsp90. Similar studies with PR and ERα also concluded that several regions throughout the LBD participate in the assembly of receptor-Hsp90 complexes, although for ERα the much less stable association of the LBD with Hsp90 requires a short upstream sequence (human ERα residues 251-71), located at the C-terminal end of the DNA-binding domain, to confer increased stability. Since Hsp90 has not been shown to bind directly to this upstream sequence, it has been proposed that the region may alternatively serve as a contact site for Hsp90 cochaperones (e.g. FKBP52) (Pratt & Toft, 1997).

Studies by the Toft laboratory, with mutants of chicken Hsp90α translated *in vitro* in reticulocyte lysate, have shown that the PR-Hsp90 interaction can tolerate deletion of the first 380-residues within the 728-amino acid chicken Hsp90α sequence to produce a hormone-activatable receptor. By contrast, selected regions (amino acids 381-441 and 601-677) in the C-terminal half of chicken Hsp90α were shown to be particularly important for PR-Hsp90 binding, with their deletion also interfering with receptor hormone responsiveness (Sullivan & Toft, 1993). An alternate approach by Baulieu and coworkers, in which human GR was coexpressed in baculovirus-infected insect cells with wild type or mutant chicken Hsp90α containing selective internal deletions (ΔA: 221-290; ΔB: 530-581; ΔZ: 392-419), revealed a loss of GR-Hsp90 interaction upon deletion of region A within the N-terminal domain, whereas deletions of regions B and Z afforded aggregated receptor-Hsp90 complexes in which receptor was unable to bind hormone (Cadepond *et al*., 1993). An extension of these studies by the same laboratory, to chicken ERα and human MR, also concluded that deletion of the A domain in chicken Hsp90α negates interaction with both receptors (Binart *et al*., 1995). None of the deletions affected ERα hormone-binding capacity, but MR failed to bind aldosterone with removal of region B. Although these investigations led to conflicting conclusions in relation to the role of the Hsp90 N-terminal domain in receptor interaction, it is appreciated that the introduced modifications may have caused

structural perturbations leading to a disruption of Hsp90 functions elsewhere in the protein, possibly hampering valid interpretation of the results (Pratt & Toft, 1997).

Recent developments have led to the crystallographic analysis of steroid receptors, as well as Hsp90 and several of its cochaperones. At the same time, the use of the yeast two-hybrid system has revealed novel interactions between specific steroid receptors and selected cochaperones involved in the Hsp90 chaperoning pathway. Additionally, further insight is now available into the mechanism(s) that underlie the potentiation of AR, GR and PR by FKBP52. This review provides a summary of this recent progress with a focus on steroid receptor, Hsp90 and cochaperone contact domains that mediate interactions important for steroid receptor function.

2. Hsp90-steroid receptor interactions

2.1 GR LBD sub-regions required for assembly of apo-GR-Hsp90 complexes; GR structure

Further endeavours to identify sequences within the GR LBD critical for Hsp90 recognition were undertaken jointly by the Simons and Pratt laboratories. In initial studies using COS-7 cell-expressed receptor chimeras comprising glutathione S-transferase (GST) fused to the N-terminal end of an intact rat GR LBD and testing for recovered Hsp90, they found that a 7-residue amino-terminal truncation of the LBD eliminated both Hsp90 and steroid binding (Xu *et al.*, 1998). This allowed them to determine the 7-amino acid sequence, TPTLVSL (equivalent to amino acids 547-553 in rat GR and residues 529-535 in human GR, see Fig. 4), to be essential for the GR-Hsp90 interaction. Alignment of this sequence with the corresponding region in other steroid receptors revealed a conserved hydrophobic domain contained within helix 1 of the receptor LBD structure. It was proposed that the sequence defined a structure important for the unfolding of the hormone binding pocket, permitting steroid access and resulting in the exposure of a hydrophobic contact domain for stable Hsp90 interaction (Xu *et al.*, 1998). Extending the 7-amino acid sequence to include Leu554 in rat GR (Leu536 in human GR), gave the sequence TPTLVSLL and led to the recognition of the LXXLL protein-protein interaction motif within helix 1 (Giannoukos *et al.*, 1999). Such motifs have previously been shown to mediate interactions between transcriptional coactivators and members of the steroid/nuclear receptor super family (Ratajczak, 2001). Mutation of the first two leucine residues within the motif (L550S/L553S in rat GR) caused an increased rate of steroid dissociation, resulting in a dramatic loss of transcriptional activity. From a predicted GR structure, the GR LBD was seen as a "hinged pocket" with helices 1-6 comprising one side of the steroid-binding domain. In this model, the LXXLL motif within helix 1 was proposed to function as a hydrophobic clasp, helping to close one end of the steroid binding pocket by forming intramolecular contacts with residues in helices 8 and 9 on the opposite arm of the pocket, as well as residues in helix 3 and the intervening loop between helices 3 and 4 (Giannoukos *et al.*, 1999). The LXXLL motif was proposed then to play a key role in stabilizing GR LBD tertiary structure and would, as a consequence, make important contributions to steroid binding activity.

A mutational study of specific rat GR LBD residues within the previously defined minimal high affinity binding segment for Hsp90 revealed that alanine substitution of the conserved

Pro643 (analogous to human GR Pro625) profoundly reduced both the stability of the GR-Hsp90 heterocomplex, as well as transcriptional activity, despite retaining almost normal hormone-binding affinity (Caamano *et al*., 1998). The negative effect on transcriptional function was related to a defect in nuclear translocation for the mutated receptor. Together the results strengthened the case for the requirement of Hsp90 as a critical component of steroid receptor signalling and identified an essential role for proline residue 643, located within an exposed hydrophobic loop between helices 5 and 6 in the receptor, in maintaining the apo-GR-Hsp90 interaction.

The x-ray structure of the human GR LBD, liganded to dexamethasone, resembles those for AR and PR, bound to their respective agonists and confirmed a helical sandwich arrangement for the steroid binding pocket (Bledsoe *et al*., 2002). Pro625 was shown to be a key residue of a novel receptor dimerization interface involving reciprocal hydrophobic interactions between the helix 5-6 loop residues, Pro625 and Ile628 from each LBD and a hydrophobic bond network between the LBDs involving residues within the helix 1-3 loops (see Fig. 4). Since Pro625 is also central to the stability of GR-Hsp90 heterocomplexes, the finding suggested an overlap between the interface for receptor dimerization and an important contact domain for Hsp90. Indeed, this may form part of the mechanism that allows the Hsp90 chaperone complex to restrict transactivation of receptor in the absence of hormone (Picard, 2006). In comparison to GR, studies have revealed that ERα is less reliant on Hsp90 regulatory control over its hormone-dependent function (Picard *et al*., 1990), allowing the ERα LBD to mediate dimerization in the absence of hormone *in vivo* (Aumais *et al*., 1997). ERα homodimer formation in the LBD is mediated through helix 10, thus differing in configuration to that of GR (Bledsoe *et al*., 2002). It is of interest that for ERα, substitution of a valine residue for Gly400, also within the helix 5-6 loop of the ERα LBD, induces a conformational change that destabilizes the receptor LBD, promoting a stronger, more stable association with Hsp90, similar to that for GR and rendering receptor transactivation more hormone-dependent (Aumais *et al*., 1997).

2.2 Hsp90 structure; Amphipathic helices 1 and 2 in the Hsp90 C-terminal domain with potential for GR-binding

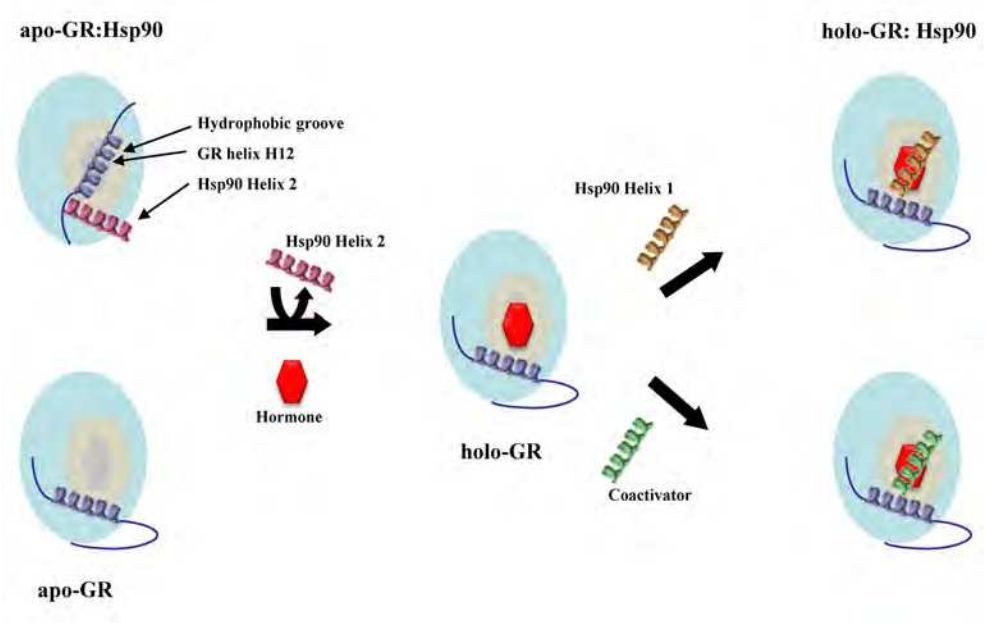
The x-ray crystal structure of the C-terminal dimerization domain of htpG, the *Escherichia coli* Hsp90, was recently solved by Agard and coworkers, revealing a dimerization motif defined by a four-helix bundle interface derived from the interaction of helices 4 and 5 of one monomer with equivalent helices from a second monomer (Harris *et al*., 2004). The structure also identified helix 2, a flexible, solvent exposed amphipathic helix, as a potential chaperone substrate-binding site. Hydrophobic residues within helix 2 are strongly conserved in Hsp90 homologues across species, suggesting an important underlying function. This was supported by other studies in which deletion of a region encompassing the corresponding helix 2 sequence in yeast Hsp82 impaired viability (Louvion *et al*., 1996), while the point mutation, A587T, which defines the start of the helix, compromised the ability of Hsp82 to promote GR activity and caused a general reduction in Hsp90 function (Nathan &Lindquist, 1995). Core hydrophobic residues within the helix 2 sequence were observed to share sequence similarity with helix 12 of steroid receptors, leading to a proposal that Hsp90 helix 2 acts as a receptor helix 12 mimic in apo-receptor-Hsp90

complexes, occupying the normal activation function 2 (AF2) position of helix 12 following hormone binding (Jackson *et al.*, 2004). Structural elucidation of full-length yeast Hsp90 (Ali *et al.*, 2006) allowed the recognition of helix 1, also consisting of a solvent-exposed, hydrophobic surface within the Hsp90 C-terminal domain, as a possible contact site for protein-protein interactions (Fang *et al.*, 2006). The highly conserved hydrophobic sequence of this helix closely matches the LXXLL recognition motif of the Steroid Receptor Coactivator/p160 family of coactivators that modulate receptor transcriptional activity by interacting with the AF2 agonist-induced hydrophobic groove of nuclear receptors (Ratajczak, 2001).

2.3 Flexible positioning of receptor LBD helix 12; Hsp90 helix 2 induces apo-GR helix 12 to adopt the GR-RU486 antagonist conformation

Recent studies by Darimont and coworkers have confirmed that Hsp90 helix 2 stabilizes unliganded GR by engaging apo-GR at the position normally occupied by receptor helix 12 in response to hormonal activation and forcing the flexible helix 12 to bind to the hydrophobic groove, at the same time preventing receptor interaction with coactivators (Fang *et al.*, 2006). The resulting structure corresponds to the native conformation of unliganded GR, with an orientation of helix 12 similar to that in antagonist (RU486)-bound GR (Fang *et al.*, 2006; Kauppi *et al.*, 2003). On agonist binding, hormone-induced conformational changes within the LBD of holo-GR promote the replacement of Hsp90 helix 2 by receptor helix 12, causing loss of Hsp90 chaperone machinery and establishing the AF2 contact domain for coactivator interaction. Alternatively, the new structure might facilitate Hsp90 helix 1 binding to the hydrophobic groove. Since Hsp90 helices 1 and 2 are proximally located at the Hsp90 C-terminus, this exchange of receptor-Hsp90 interactions, which is partly determined by the dynamics of receptor helix 12, may likely be achieved within the one receptor-Hsp90 complex (Fig. 1).

The hormone-induced progression from apo- to holo-GR-Hsp90 complexes, through changes in the mode of receptor-Hsp90 interaction resulting from altered receptor LBD conformation, provides a suitable model for visualising the transition between inactive and active receptor that may also involve the participation of Hsp90 cochaperones such as FKBP51 and FKBP52. Although FKBP51 is the preferred cochaperone in mature GR-Hsp90 complexes (Barent *et al.*, 1998; Nair *et al.*, 1997), FKBP52 has been shown to promote increased GR hormone binding affinity and to potentiate the transcriptional activity of the receptor (Riggs *et al.*, 2003). It is possible that the observed hormone-induced interchange of FKBP51 by FKBP52 in GR-Hsp90 complexes, resulting in the favoured nuclear translocation of receptor complexes (Davies *et al.*, 2002), might be initiated by a change in GR LBD conformation elicited by the transfer of receptor interaction from Hsp90 helix 2 to helix 1, both helices being close to the common TPR binding site for immunophilin cochaperones in the C-terminal region of Hsp90. Unique steroid receptor LBD conformations then might be an important determinant of receptor preferences for specific immunophilin cochaperones within receptor-Hsp90 complexes (e.g. FKBP51 in GR, PR and MR complexes (Barent *et al.*, 1998; Nair *et al.*, 1997); PP5, the major cochaperone in GR complexes (Silverstein *et al.*, 1997) and CyP40, the prevalent immunophilin in ER complexes (Ratajczak *et al.*, 1990)), allowing these cochaperones to potentially modulate receptor function (Ratajczak *et al.*, 2003; Smith & Toft, 2008).



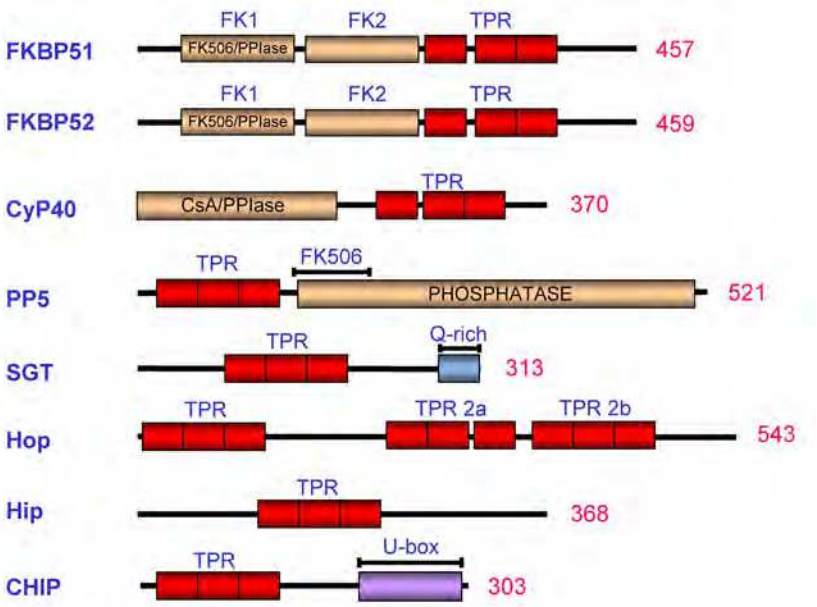
Hsp90 helix 2 binds apo-GR at the position normally occupied by GR helix H12, forcing H12 to dock within the hydrophobic groove, thus stabilizing the unliganded hormone-binding pocket. With hormone binding, GR H12 replaces Hsp90 helix 2 providing contacts for AF2-interacting coactivators or for Hsp90 helix 1.

Fig. 1. Hsp90 interactions with apo-GR and holo-GR.

3. Hsp90/Hsp70-cochaperone interactions

3.1 TPR cochaperones

Folding of newly synthesized peptides to functionally mature proteins, such as steroid receptors, is actively regulated by Hsp70 and Hsp90 with their cochaperones in what is known as the Hsp70/Hsp90-based chaperone machinery (Pratt & Toft, 2003). Cochaperones can regulate the nucleotide status, and thus function, of Hsp70 and Hsp90, and deliver non-native proteins to their respective polypeptide-binding domains for folding. Those cochaperones that regulate Hsp70 include Hsp40, Hsc70-interacting protein (Hip), Hsp-organizing protein (Hop) and small glutamine-rich TPR protein (SGT), while Hsp90 is regulated by cochaperones that include Hop, p23, PP5, CyP40, FKBP51 and FKBP52. C-terminal of Hsp70-interacting protein (CHIP) is another cochaperone that regulates both Hsp70 and Hsp90. Fig. 2 shows the domain architecture of the immunophilin and other TPR cochaperones with an established role in Hsp70 and/or Hsp90 chaperone function.



TPR domains are depicted in red whilst other specialized functional domains are highlighted in other various colours and labelled accordingly. Abbreviations: FKBP, FK506-binding protein; PPIase, peptidylprolyl isomerase; TPR, tetratricopeptide repeat; CyP40, cyclophilin 40; CsA, cyclosporin A; PP5, protein phosphatase 5; SGT, small glutamine-rich TPR protein; Hop, Hsp-organizing protein; Hip, Hsc70-interacting protein; CHIP, C-terminal of Hsp70-interacting protein.

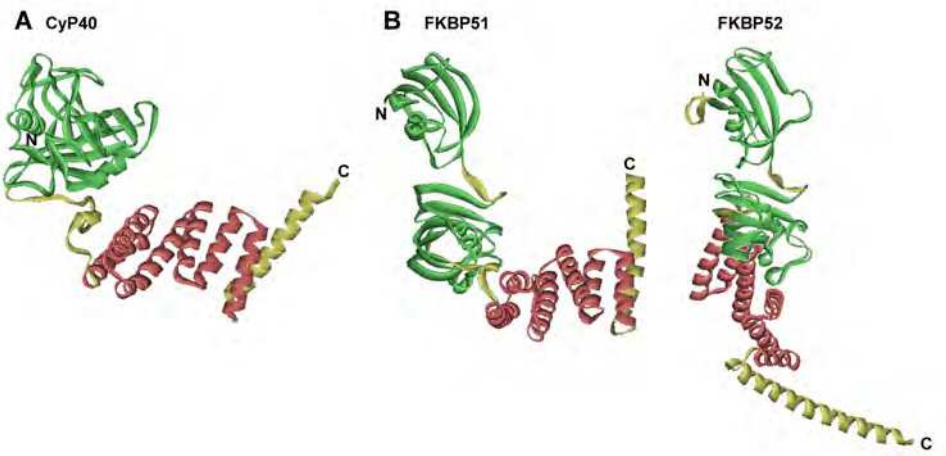
Fig. 2. Schematic presentation of the domain structures of TPR-containing proteins associated with the Hsp70/Hsp90 chaperone machinery.

Since the crystallization of the PP5 TPR domain, the structures of several other steroid receptor-associated TPR-containing proteins have been solved. There are now full-length structures available for bovine CyP40, human FKBP52, PP5 and Hop, human and squirrel monkey FKBP51, and mouse CHIP, as well as the structure of the human SGT TPR domain. It is known that TPR domains in these proteins can mediate interactions with Hsp70 and/or Hsp90 (Angeletti *et al.*, 2002; Smith, 2004), but in addition to their Hsp-recognition domains, each also possesses other localized functional domains important for their own conformation and/or the regulation of associated proteins.

3.1.1 CyP40, FKBP51 and FKBP52

CyP40 and the two FKBPs have a similar structural arrangement, each possessing an N-terminal binding site for the immunosuppressants cyclosporin A or FK506, respectively, and a C-terminal TPR domain (Sinars *et al.*, 2003; Taylor *et al.*, 2001; Wu *et al.*, 2004). The cyclophilin domain of CyP40 is similar to other single-domain cyclophilins (Kallen *et al.*, 1998). In FKBP51 and FKBP52, FK506 binds to the first of two FKBP domains, termed FK1, while the second domain, called FK2, lacks drug-binding activity. Bound immunosuppressants inhibit the peptidylprolyl isomerase (PPIase) activity of the

cyclophilin and FK1 domains, which may be important for target protein regulation by direct or indirect association. Fig. 3 provides a structural comparison between CyP40, FKBP51 and FKBP52 immunophilin cochaperones.



A, CyP40 and **B,** FKBP51, FKBP52. The CsA-binding domain (CyP40) and FK regions (FKBP51 and FKBP52) are shown in green. Core TPR domains for CyP40, FKBP51 and FKBP52 are depicted in red, with the final extended helices, at the C-terminal ends of each protein, shown in yellow.

Fig. 3. Ribbon representations of molecular structures of TPR-containing proteins.

3.1.2 PP5

PP5 is a phosphatase that dephosphorylates serine and threonine residues on target proteins (Barford, 1996; Cohen, 1997). Crystallisation of the full-length phosphatase in the absence of ligands or binding partners revealed the structural organization of the autoinhibited form of PP5 (Yang *et al.*, 2005). The TPR domain in PP5 is oriented to the N-terminus and is linked to a C-terminal phosphatase catalytic domain followed by a short C-terminal subdomain. In this inactive conformation, the TPR domain engages with the catalytic domain in such a way as to restrict target protein access to the enzymatic site, and this structure is stabilized by the C-terminal subdomain. Suppression of catalytic activity can be abolished by an allosteric conformational change that disrupts the TPR-catalytic domain interface, and this can be induced upon binding of polyunsaturated fatty acids or Hsp90 to the TPR domain (Chen & Cohen, 1997; Ramsey & Chinkers, 2002; Skinner *et al.*, 1997).

3.1.3 Hop

Hop plays a dual role in mature steroid receptor complex assembly by recruiting Hsp90 to preformed Hsp70-receptor complexes and inhibiting the ATPase of Hsp90 for client loading onto the chaperone for subsequent folding (Chen *et al.*, 1996b; Chen & Smith, 1998; Dittmar *et al.*, 1996; Kosano *et al.*, 1998; Prodromou *et al.*, 1999; Siligardi *et al.*, 2004). Hop has an N-terminal TPR domain (TPR1) followed by an aspartic acid/proline (DP)-rich region, and two more adjacent TPR domains (TPR2a and TPR2b) followed by a second DP-rich region.

3.1.4 Hip

Hip functions as a transient component of native steroid receptor complexes and enters the assembly cycle once Hsp70 ATPase activity has been stimulated by Hsp40 (Frydman & Höhfeld, 1997; Höhfeld *et al.*, 1995). Hip acts to stabilize the ADP-bound state of Hsp70 that is necessary for high affinity interaction with unfolded substrates (Frydman &Höhfeld, 1997; Höhfeld *et al.*, 1995). Structurally, Hip consists of an N-terminal oligomerization domain that is important for the functional maturation of GR in yeast (Nelson *et al.*, 2004), a central TPR domain and an adjacent highly charged region which are both required for Hsp70 binding (Prapapanich *et al.*, 1996b) and a C-terminal DP-rich domain that helps direct the intermediate stage recruitment of Hop-Hsp90 during assembly of steroid receptor complexes (Prapapanich *et al.*, 1998).

3.1.5 CHIP

The cochaperones described above are involved in maintaining an activatable conformation of Hsp70/Hsp90-dependent "clients", but TPR proteins also function to mediate the degradation of misfolded proteins, indicating a role in quality control (Cyr *et al.*, 2002). Selection of proteins for degradation is mediated by E3 ubiquitin ligases, and CHIP is a member of this enzymatic class (Jiang *et al.*, 2001; Murata *et al.*, 2001). CHIP has an N-terminal TPR domain and a C-terminal U-box domain that mediates its ligase activity, which promotes ubiquitylation of target substrates prior to their degradation by the proteasome.

3.1.6 SGT

Human SGT binds to viral protein U (Vpu) and Group specific Antigen, 2 proteins associated with human immunodeficiency virus-1, and the rat homologue was identified as an interactor of the non-structural protein NS-1 of the parvovirus H-1. The central TPR domain in SGT is flanked by an N-terminal dimerization domain and a C-terminal glutamine-rich domain involved in association with type 1 glucose transporter (Callahan *et al.*, 1998; Cziepluch *et al.*, 1998; Liou & Wang, 2005).

3.2 Regulation of Hsp70 and Hsp90 ATPases by TPR cochaperones

Both Hsp70 and Hsp90 require ATP for their functional association with substrates (Pratt & Toft, 2003). In the case of a steroid receptor, Hip binding to the N-terminal ATPase domain of Hsp70, possibly through a unique TPR binding site located within this region (see below), stabilizes the Hsp70-receptor complex (Frydman & Höhfeld, 1997; Höhfeld *et al.*, 1995) in a step that may be important for recognition by Hop and loading of the receptor onto Hsp90 for further processing. Hop contains three distinct TPR domains (TPR1, TPR2a, TPR2b) (Fig. 2), with TPR1 and TPR2a providing anchor points for the C-terminal EEVD peptides of Hsp70 and Hsp90, respectively. These specific interactions, coupled with domain-domain interactions, also involving its TPR domains, allow Hop to play a key role in coordinating the actions of Hsp70 and Hsp90 (Carrigan *et al.*, 2006; Chen *et al.*, 1996b; Chen & Smith, 1998; Odunuga *et al.*, 2003; Prodromou *et al.*, 1999; Ramsey *et al.*, 2009; Scheufler *et al.*, 2000). While the TPR acceptor site for Hop in the C-terminal region of Hsp90 serves to anchor the

cochaperone, studies have shown that Sti1, the yeast homologue of Hop, markedly inhibits the ATPase activity of yeast Hsp90 through secondary interactions that block the ATP-binding pocket in the Hsp90 N-terminal domain (Prodromou *et al.*, 1999). By directly competing with Sti1 for binding to Hsp90, the CyP40 yeast homologue Cpr6 can negate the Sti1-mediated blockade of Hsp90 ATPase activity following TPR protein exchange (Prodromou *et al.*, 1999). In contrast, in vitro studies with human Hop determined that the cochaperone had no influence on the weak basal ATPase activity of human Hsp90, but significantly inhibited the increased rate of ATP hydrolysis by Hsp90 in response to interaction with the ligand binding domain of GR, an established Hsp90 client protein (McLaughlin *et al.*, 2002). On the other hand, FKBP52, which like CyP40 binds competitively with Hop to the C-terminal TPR interaction site of Hsp90, was shown to enhance Hsp90 ATPase activity stimulated by GR (McLaughlin *et al.*, 2002). This control over ATP utilization is important for the functional activity of newly synthesized substrates, but ATPase regulation is also required for the degradation of improperly folded substrates. CHIP can bind Hsp70 and inhibit Hsp40-stimulated Hsp70 ATPase activity, and has been reported to deplete cellular GR levels (Ballinger *et al.*, 1999; Connell *et al.*, 2001). Therefore, CHIP can be regarded as a degradatory cochaperone of Hsp70 and Hsp90. SGT negatively regulates Hsp70 such that the chaperone has a reduced ability to refold denatured luciferase (Angeletti *et al.*, 2002).

3.3 Determinants of Hsp70 and Hsp90 interaction with TPR cochaperones

Deletion studies were the first to demonstrate that TPR domains mediated binding to Hsp90 (Barent *et al.*, 1998; Chen *et al.*, 1996a; Radanyi *et al.*, 1994; Ratajczak & Carrello, 1996). Determination of the TPR domain structure of PP5 revealed that the packing of adjacent TPR units generated an exposed groove capable of accepting a target protein peptide (Das *et al.*, 1998). Although TPR motifs are highly degenerate, they display a consistent pattern of key residues important for structural integrity. The two α-helical sub-domains in each TPR motif are arranged such that the groove is mainly composed of residues from the A helix of each repeat, while B helix residues are buried to form the structural backbone of the superhelix, and this groove forms a critical Hsp recognition surface.

In a PP5 mutagenesis study, Russell and coworkers carefully selected A helix residues with side-chains extended into the groove and identified four basic residues important for PP5-Hsp90 interaction (Russell *et al.*, 1999). These amino acids are highly conserved in other Hsp90-binding TPR proteins, and mutation of aligned residues in CyP40 confirmed their importance in Hsp90 recognition (Ward *et al.*, 2002). The key recognition sequence for the TPR domain in these proteins is the EEVD peptide located at the extreme C-terminus of Hsp90 (Carrello *et al.*, 1999; Chen *et al.*, 1998; Young *et al.*, 1998), which is conserved in Hsp70. Crystallization of individual Hop TPR domains with Hsp70 and Hsp90 N-terminally extended EEVD peptides has defined the mechanism of TPR domain-peptide interaction (Scheufler *et al.*, 2000). The TPR1 domain of Hop binds to Hsp70, while the TPR2a domain mediates Hsp90 recognition (Chen *et al.*, 1996b; Lassle *et al.*, 1997). The groove in each TPR domain accommodates their respective peptide in an extended conformation where the ultimate aspartate residue is tightly held by electrostatic interactions with TPR residue side-chains in a two-carboxylate clamp. Additional EEVD contacts involve hydrogen-bonding, while amino acids upstream of the EEVD enhance the affinity of the peptides for TPR

domains and mediate specificity of Hsp70 and Hsp90 to TPR1 and TPR2a, respectively. Notably, Hop TPR2a provides an example of where an additional sequence within the TPR domain doesn't disrupt the overall structure. TPR2a contains an insertion between units 2 and 3 that extends the helices by a single turn but does not impact Hsp90 peptide recognition (Scheufler *et al.*, 2000).

The Hsp90 dimerization domain, located in the C-terminal region upstream of the MEEVD peptide, contributes to TPR cochaperone recognition (Chen *et al.*, 1998) and contains the putative binding site for novobiocin, a coumarin-based Hsp90 inhibitor (Marcu *et al.*, 2000). *In vitro* studies demonstrated that novobiocin had a differential effect on Hsp90-immunophilin cochaperone interaction, suggesting that the TPR cochaperones modulate Hsp90 function through distinct contacts within the Hsp90 C-terminal domain (Allan *et al.*, 2006).

Although EEVD interactions with the TPR domain groove are critical for Hsp binding, regions outside of the TPR domains are also important in mediating recognition. TPR domains are typically followed by a seventh α-helix that packs against and extends beyond the TPR domain and has been shown to be involved in binding Hsp90 in addition to the TPR domain. FKBP51 and FKBP52 have different affinities for Hsp90 and are assembled differentially with specific receptor complexes, and these differences map in part to sequences C-terminal of their respective TPR domains (Barent *et al.*, 1998; Cheung-Flynn *et al.*, 2003; Pirkl & Buchner, 2001). The charge-Y motif was identified and found to be essential for FKBP-Hsp90 interaction, which was also confirmed for CyP40, but sequences further downstream in FKBP51 and FKBP52 differentially regulated Hsp90 binding (Allan *et al.*, 2006; Cheung-Flynn *et al.*, 2003; Ratajczak & Carrello, 1996). The acidic linker flanking the N-terminus of the CyP40 TPR domain was also shown to be important for efficient interaction (Mok *et al.*, 2006; Ratajczak & Carrello, 1996). Although an interaction partner for Hop TPR2b has yet to be identified, mutations in TPR2b reduced Hop interaction with both Hsp70 and Hsp90, while mutations in the C-terminal DP-rich region inhibited Hop binding to Hsp70 (Chen & Smith, 1998; Nelson *et al.*, 2003).

3.4 Alternative modes of Hsp70 and Hsp90 recognition by TPR cochaperones

Like Hop, CHIP binds to both Hsp70 and Hsp90 (Ballinger *et al.*, 1999; Connell *et al.*, 2001), but CHIP interacts with either of these major chaperones through a single TPR domain. Recent elucidation of the binding of Hsp90 C-terminal peptide (NH_2-DDTSRMEEVD) with the CHIP TPR domain has revealed that the peptide sequence is not accommodated in an extended conformation as for Hop, but turns at the methionine residue and becomes buried within a hydrophobic pocket (Zhang *et al.*, 2005). This pocket can accommodate either the methionine or isoleucine that lies immediately upstream of the EEVD sequence in Hsp90 and Hsp70, respectively, and the peptide is twisted, negating the role of upstream residues in conferring the same specificity seen in binding Hop TPR domains. SGT also recognizes Hsp70 and Hsp90 via its single TPR domain, but possibly through a different mechanism to that described for CHIP as SGT lacks the residues that form the hydrophobic pocket which allows the respective C-terminal peptides in the chaperones to twist (Dutta & Tan, 2008).

Hydrophobic pockets themselves may also be important structural features within TPR domains that confer Hsp specificity, as the crystal structure of Hop TPR2a with the non-cognate Hsp70 peptide shows the hydrophobic pocket to be less accommodating for the Ile

(-5) residue in the extended Hsp70 peptide than Met (-5) in the extended Hsp90 peptide, with the notable feature of a lack of bending by the Hsp70 peptide, such as with CHIP, to perhaps enhance affinity for TPR2A (Kajander *et al.*, 2009).

General cell UNC-45 (GCUNC-45), a member of the UNC-45/Cro1/She4p (UCS) protein family, is a TPR protein that regulates PR chaperoning by Hsp90 by preventing activation of Hsp90 ATPase activity (Chadli *et al.*, 2006). Hsp90-binding experiments in the presence of Hop revealed a novel GCUNC-45 TPR recognition site in the N-terminal domain of Hsp90, which also bound FKBP52 (Chadli *et al.*, 2008a). Further analysis defined a non-contiguous EEVD-like motif, centered in and around the Hsp90 N-terminal ATP-binding pocket, arranged in a structural conformation that can recognize TPR domains. Nucleotide binding negatively regulates the interaction. These authors also alluded to CyP40 binding to the N-terminal interaction motif, although Onuoha and coworkers have recently confirmed CyP40 interaction only with the C-terminal domain of Hsp90 (Onuoha *et al.*, 2008). GCUNC-45 is the first cochaperone to display a preferential association with Hsp90β over the Hsp90α isoform, resulting in functional Hsp90β-GCUNC-45 interactions that more efficiently block progression of PR chaperoning than seen with Hsp90α-GCUNC-45 complexes (Chadli *et al.*, 2008b). An EEVD-like motif interaction with a TPR domain has also been described for androgen receptor recognition by SGT, where binding is mediated by the first 2 TPR motifs of the SGT TPR domain and the hinge region located between the DNA-binding and ligand-binding domains in the receptor (Buchanan *et al.*, 2007).

Hip has similarly been reported to bind the Hsp70 N-terminal ATPase domain via its TPR domain (Höhfeld *et al.*, 1995). Through this interaction, Hip, originally identified in progesterone receptor complex assembly (Prapapanich *et al.*, 1996a; Smith, 1993), can stabilize substrate-Hsp70 binding and competitively counteract the destabilizing effects of the non-TPR cochaperone BAG1 (Bimston *et al.*, 1998; Gebauer *et al.*, 1997; Höhfeld & Jentsch, 1997; Takayama *et al.*, 1997). The Hip-Hsp70 interaction also allows for the simultaneous association of Hip with Hsp70-Hop complexes (Gebauer *et al.*, 1997; Prapapanich *et al.*, 1996a). By analogy with the mode of GCUNC-45 interaction with Hsp90, there is the possibility that Hip targets a similar TPR recognition site in the N-terminal region of Hsp70. However, Hip is unique among the steroid receptor-associated TPR proteins in terms of Hsp recognition in that it binds Hsp70 independently of EEVD interactions (Höhfeld *et al.*, 1995), and that efficient binding may be due to a greater requirement for additional Hsp-interaction determinants, such as the adjacent highly charged region and a C-terminal DP-repeat domain (Prapapanich *et al.*, 1998). It is possible the mechanism of Hsp70 recognition by Hip is not unique, but may be utilized by some of the steroid-receptor TPR cochaperones to interact with binding partners in distinct cellular pathways. Dutta and Tan (2008) reported the SGT TPR domain is sufficient to bind Vpu and identified the sequence ^{31}KILRQ^{35} in Vpu as being important for this interaction.

4. p23 and Cdc37 interaction with Hsp90

p23 is an essential component involved in stabilizing mature steroid receptor-Hsp90 complexes and binds to the ATP-bound conformation of a Hsp90 dimer characterised by high affinity for client proteins (Ali *et al.*, 2006; Felts & Toft, 2003; McLaughlin *et al.*, 2006;

Richter *et al.*, 2004). Conformational changes that accompany ATP binding promote dimeric interaction between the N-terminal domains of the Hsp90 C-terminal dimer to form distinct binding surfaces for separate p23 molecules, thus further underpinning the ATP-bound conformation (Ali *et al.*, 2006; Karagöz *et al.*, 2010). In a recent model proposed for the Hsp90 cochaperone cycle, entry of an immunophilin cochaperone into an existing client protein-Hsp90-Sti1/Hop-Hsp70 complex forms an intermediate complex important for cycle progression. Conversion of Hsp90 to the closed conformation on ATP and subsequent p23 binding then favours the release of Sti1/Hop (Li *et al.*, 2011).

Cdc37 serves as an adaptor predominantly facilitating protein kinase interaction with Hsp90, although additional client proteins, including steroid receptors have been identified (MacLean & Picard, 2003). Similar to Hop, Cdc37 arrests the Hsp90 ATPase cycle and functions as an "early" cochaperone for the recruitment of protein kinase clients to the Hsp90 machinery. Hsp90 binding maps to the Cdc37 C-terminal region, while kinase interaction occurs via the N-terminal domain (Roe *et al.*, 2004). Hsp90 ATPase activity is coupled to an opening and closing of a molecular clamp generated by the constitutive C-terminal Hsp90 dimer at one end in combination with the ATP-dependent association of the N-terminal domains at the other (Prodromou *et al.*, 2000). A structural view of the Hsp90-Cdc37 complex shows Cdc37 located as a dimer between the N-terminal domains of the clamp, thus preventing their interaction (Roe *et al.*, 2004). With cycle progression, loss of one Cdc37 monomer leads to the formation of a stable $(Hsp90)_2$-Cdc37-kinase complex (Vaughan *et al.*, 2006; Vaughan *et al.*, 2008).

5. Receptor-cochaperone interactions

5.1 Cortisol resistance in New World primates; The key role of FKBP51; Structures of FKBP51 and FKBP52

Analysis of glucocorticoid resistance in New World primates, such as squirrel monkey, has demonstrated that the high circulating cortisol levels result from elevated expression and greatly increased incorporation of FKBP51 into GR-Hsp90 complexes, causing a significant decrease in GR hormone binding affinity (Denny *et al.*, 2000; Reynolds *et al.*, 1999; Scammell *et al.*, 2001). FKBP51 then appears to have a major role in stabilizing an inactive receptor conformation. The FK506 drug-binding pocket of FKBP51 is inaccessible to FK506 in low affinity hormone-binding GR heterocomplexes. However, incubation of receptor cytosols from squirrel monkey lymphocytes with FK506 prevented assembly of FKBP51 with GR-Hsp90 complexes, correlating with a sharp increase in receptor hormone binding and affinity. On the other hand, recognition of FK506 by FKBP52 appeared unaffected by whether the immunophilin exists as a component of mature, high affinity hormone-binding GR complexes or not (Denny *et al.*, 2000; Tai *et al.*, 1992). Furthermore, the immunosuppressant blocks FKBP52-mediated potentiation of GR activity (Riggs *et al.*, 2003). The inhibitory influence of FKBP51 on GR activity requires both FK domains, as well as Hsp90 binding, but is not reliant on FKBP51 PPIase activity (Denny *et al.*, 2005). FK506 may likely serve to sterically hinder receptor LBD interactions with the FK1 domain of FKBP51 and FKBP52 essential for inhibitory and activation effects on receptor, respectively. This differential action of FK506 may arise from distinct domain orientations that have been

defined from recent structures of the two immunophilins (Sinars *et al.*, 2003; Wu *et al.*, 2004). Unique interactions between receptor and the FKBP51 and FKBP52 cochaperones have been further highlighted by results showing that deletion of the Asp195, His196, Asp197 insertion within the FK2 domain of FKBP51 compromised assembly of the immunophilin into PR complexes, whereas removal of the corresponding FK2 insertion loop from FKBP52 had no affect on receptor association (Sinars *et al.*, 2003). This raises the possibility that direct interaction of FK2 in FKBP51 with PR might favour the preferred association of FKBP51 over FKBP52 with this receptor.

5.2 Cortisol resistance in the guinea-pig; Do guinea pig GR LBD changes favour FKBP51 binding over FKBP52?

In contrast to the New World primates, the cause of glucocorticoid resistance in the guinea pig, a New World hystricomorph, has been delineated to an unstructured loop between helix 1 and helix 3 of the guinea pig GR LBD. Five amino acid substitutions in this region differentiate guinea pig GR from the human receptor, with at least four contributing to the low binding affinity phenotype (Fuller *et al.*, 2004). It has been predicted that these crucial residues (Ile538, His539, Ser540, Thr545 and Ser546) lying on the surface of the guinea pig GR LBD, disrupt a contact domain for FKBP52, favouring increased association with FKBP51 and conformational changes that compromise high affinity cortisol binding. Using a yeast-based assay (Riggs *et al.*, 2003) with rat GR substituted in the helix 1 to helix 3 loop with the guinea pig GR-specific residues, we have recently confirmed that FKBP52 can efficiently potentiate the transcriptional activity of the mutated GR, thus discounting a central role of this region in receptor-FKBP52 interaction [Cluning C and Ratajczak T, unpublished observations].

5.3 FKBP52 potentiation of AR, GR and PR

Direct interaction studies between bacterially expressed FKBP52 and GST-tagged, wild type human GR and C-terminal truncation mutants of the receptor purified from Sf9 cell extracts, identified a 35-amino acid region (hGR 465-500), between the DNA-binding domain and the LBD, to be sufficient for FKBP52 binding, with optimal interaction requiring involvement of the LBD (Silverstein *et al.*, 1999). However, recent demonstration of FKBP52 potentiation of GR activity in association with increased receptor hormone binding affinity has definitively localized the FKBP52 effect to the GR LBD (hGR 521-777) and at the same time pointed to a requirement of FKBP52 PPIase activity residing in the FK1 domain (Riggs *et al.*, 2003). Studies with FKBP52 knockout mouse strains have extended the critical physiological role of FKBP52 to cellular responses controlled by both AR (Cheung-Flynn *et al.*, 2005) and PR (Tranguch *et al.*, 2005; Yang *et al.*, 2006), while similar influences of this immunophilin cochaperone on ERα (Riggs *et al.*, 2003) and MR (Gallo *et al.*, 2007) activity have not been observed, despite the assembly of FKBP52 with Hsp90 complexes containing these receptors.

5.4 Molecular basis of FKBP52 action; Potential interaction of FKBP52 with the BF3 regulatory site

An initial understanding that FKBP52 potentiation of AR, GR and PR activity was dependent on the FK1-mediated PPIase function of the immunophilin, prompted speculation that FKBP52

might target a key proline likely to be conserved among these receptors and that this critical residue would be located on the surface of the LBD, accessible to the cochaperone and in a position where it might influence the shape of the ligand binding pocket (Cheung-Flynn *et al.*, 2005). Although several such candidate prolines exist in the intervening loops between receptor LBD helices, a more extensive mutational analysis of the FK1 catalytic site has excluded a role for the FKBP52 PPIase activity in receptor potentiation (Riggs *et al.*, 2007). Rather, recent evidence has identified a loop overhanging the FK1 catalytic pocket in FKBP52 that is responsible for the functional difference between FKBP52 and FKBP51 relating to AR (and GR/PR) potentiation (Riggs *et al.*, 2007). It is proposed that a critical proline within this loop (human FKBP52 Pro119) allows specific contact with a region of the AR LBD (a structural feature that is also common to GR and PR), thus helping to stabilize an LBD conformation favourable for high affinity hormone binding and leading to efficient transcriptional activation (Riggs *et al.*, 2007). It is speculated that a leucine substitution within the corresponding FK1 sequence of FKBP51 alters the loop conformation sufficiently to disrupt this functionally important contact. The possibility exists that in the hormone-induced transition from inactive to active states of AR-Hsp90 complexes associated with FKBP51 and FKBP52, respectively, Hsp90 orients FKBP52 to achieve unique interactions with the receptor LBD, allowing Hsp90 to facilitate optimal hormone binding and to further fine-tune the hormonal response.

Prior to investigations establishing a noncatalytic involvement of the FKBP52 PPIase domain in the modulation of receptor function, an early attempt to identify the putative proline substrate for FKBP52 isomerase activity within the AR LBD utilized AR-P723S, a proline mutant associated with androgen insensitivity syndrome (Cheung-Flynn *et al.*, 2005). Although predicted to display basal activity, coupled with a lack of response to hormone in the presence of FKBP52, this mutant was characterized by subnormal activity in the absence of FKBP52, showing full restoration to wild type receptor activity levels with the cochaperone on exposure to hormone (Cheung-Flynn *et al.*, 2005). Such a favoured response reflects a greater dependence of the AR-Pro723S mutant on FKBP52 for normal activity. Pro723 lies within the signature sequence conserved among all steroid receptors (Brelivet *et al.*, 2004), close to a region directly involved in ligand binding and is situated in a solvent exposed loop between helices 3 and 4, which combine together with the mobile helix 12 to form the AF2 coactivator binding pocket (He *et al.*, 2004; Matias *et al.*, 2000b). For AR, AF2 initially has a preferred interaction with the AR N-terminal domain, resulting in an intramolecular fold that precedes receptor dimerization and appears critical for AR function (He *et al.*, 2001; He *et al.*, 2004; Schaufele *et al.*, 2005). Pro723 also forms part of the recently identified BF-3 surface that has the ability to allosterically alter the AF2 binding pocket of AR (Estébanez-Perpiñá *et al.*, 2007) (Fig. 4). BF-3 residues altered through natural mutations linked to androgen insensitivity and those associated with prostate cancer, either diminish or enhance AR AF2 activity, respectively, underlining the importance of the BF-3 surface for AR function (Estébanez-Perpiñá *et al.*, 2007). FKBP52 rescue of AR-Pro723S activity might signify FKBP52 influence over some part of the BF-3 allosteric regulatory site leading to conformational changes that allow full recovery of AR activity. Indeed, Cox and coworkers have recently identified small-molecule inhibitors of FKBP52-enhanced AR function in prostate cancer cells that target a region of the AR LBD overlapping the BF3 surface (De Leon *et al.*, 2011) (Fig. 4). Multiple residues that contribute to the FKBP52 sensitivity of AR, some of which form part of the binding site for MJC13, the lead compound, have been

identified (De Leon *et al.*, 2011) (Fig. 4). Since MJC13 helps to maintain an intact AR-Hsp90-FKBP52 complex at low hormone concentrations, it is possible that the inhibitor interferes with a critical next step - a hormone-induced, FKBP52-dependent transitory change in AR conformation necessary for nuclear translocation. Sequence comparisons have revealed some conservation of BF-3 residues within the LBDs for AR, GR, MR and PR, suggesting the presence of BF-3-like regulatory domains in each receptor (Estébanez-Perpiñá *et al.*, 2007) (Fig. 4). A very limited conservation of these residues is apparent in ERα, suggesting the formation of a BF-3 type surface that is unique to this receptor (Estébanez-Perpiñá *et al.*, 2007) (Fig. 4). Both ERα and MR behave differently to AR, GR and PR, through their inability to respond to FKBP52. Certain structural differences within their LBDs distinguish these two receptors from the other members of this subfamily (De Leon *et al.*, 2011) (Fig. 4). Since FKBP52 also regulates GR and PR activity, most likely through specific BF3 surfaces, there is the potential for the development of FKBP52-specific inhibitors targeting GR and PR function to treat a range of steroid hormone-based diseases (Moore *et al.*, 2010). The BF-3 pocket is a potential target for second-site modulators that can allosterically block agonist-activated AR function to inhibit prostate cancer cell growth (Joseph *et al.*, 2009).

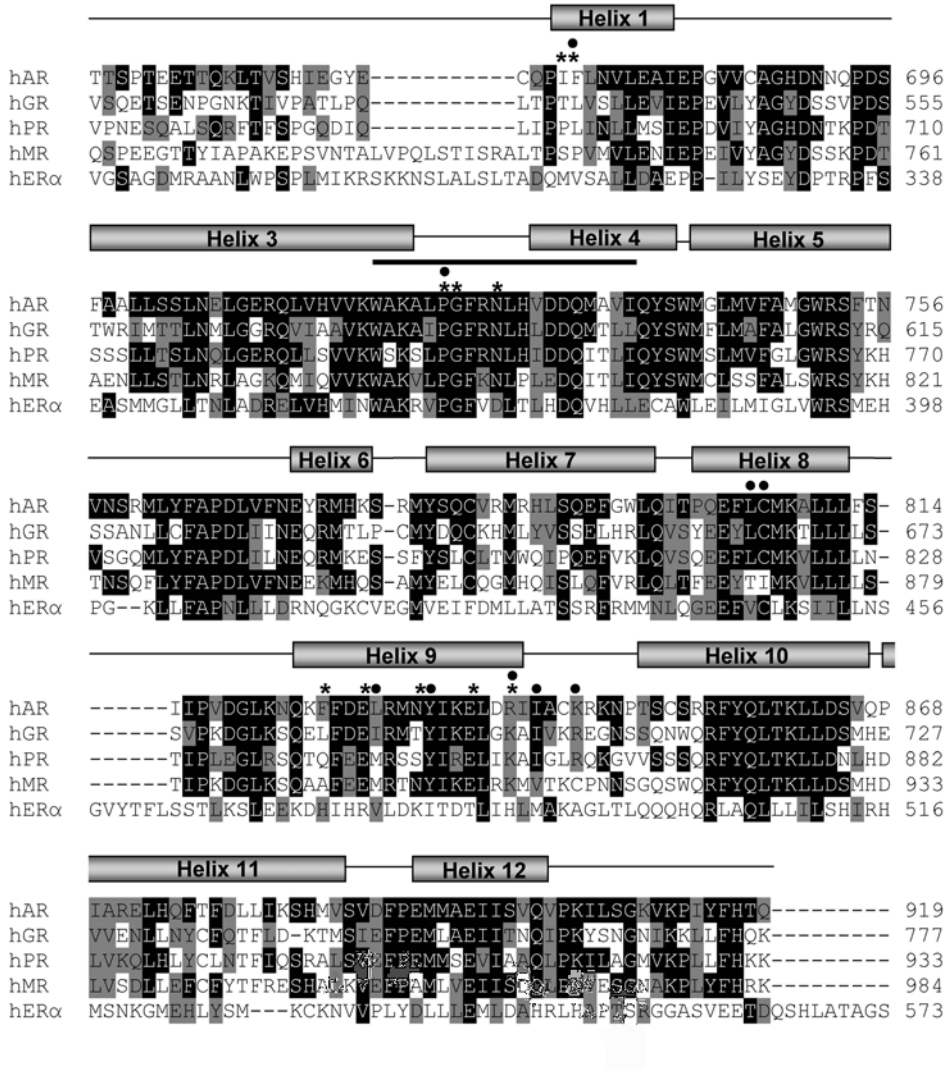
5.4.1 FKBP51 is an androgen-regulated gene that promotes assembly of mature AR-Hsp90 complexes

FKBP51 is recognised as a highly sensitive AR-regulated gene that functions as an important component of a feed-forward mechanism linked to the partial reactivation of AR-signalling pathways in the absence of androgens, leading to the outgrowth of androgen-independent tumours (Amler *et al.*, 2000; Febbo *et al.*, 2005; Magee *et al.*, 2006; Mousses *et al.*, 2001; Tomlins *et al.*, 2007). Sanchez and coworkers have confirmed a significantly increased expression of FKBP51, but not that of FKBP52, in most prostate cancer tissues and in androgen-dependent and androgen-independent cell lines (Periyasamy *et al.*, 2010), suggesting that FKBP51 might have a critical role in prostate cancer growth and progression. FKBP51 overexpression was found to increase the AR transcriptional response by facilitating hormone-binding competence through the assembly of the AR LBD with mature FKBP51-Hsp90-p23 complexes (Ni *et al.*, 2010), resulting in higher levels of androgen-liganded receptor and providing a pathway for AR-dependent signalling and growth in a low-androgen environment. The ability of FKBP51 to enhance AR transcription and chaperone complex assembly appears to be dependent on FKBP51 PPIase activity mediated by the FK1 domain and requires Hsp90 binding through its TPR domain (Ni *et al.*, 2010).

6. Receptor LBD contacts with other Hsp90 cochaperones

6.1 PP5; GCUNC-45; SGT

The domain structure of the Hsp90 cochaperone, PP5, a serine/threonine protein phosphatase (Chen *et al.*, 1994; Chinkers, 1994), is characterised by a C-terminal phosphatase catalytic domain and an N-terminal TPR domain that competes with FKBP51, FKBP52 and CyP40 for the TPR binding site at the Hsp90 C-terminus during assembly into mature steroid receptor-Hsp90 complexes (Banerjee *et al.*, 2008; Chen *et al.*, 1996a; Hinds Jr & Sanchez, 2008). Through its TPR domain, PP5 has also been shown to bind directly to ERα and ERβ, an interaction that targets the LBDs of these receptors, but does not require the C-terminal region incorporating



NCBI accession numbers for receptor sequences are: AR – NP000035, ERα – NP000116, GR – NP001018087, MR – NP000892, PR – NP000917. The ERα sequence has 595 amino acids and is shown terminated at residue 573. LBD helices are based on the structure of AR liganded to R1881 (Matias *et al.*, 2000a) (PDB ID 1E3G). The nuclear receptor signature sequence is indicated (thick black line). Residues that map to the BF-3 allosteric regulatory site defined for AR are highlighted with an asterisk (*). Multiple residues that contribute to the FKBP52 sensitivity of AR and form the putative binding site for MJC13 (De Leon *et al.*, 2011) are highlighted with a black circle (•). Identical residues are shown white against black; conserved residues (black on grey) are based on the following scheme: (P, G), (M, C), (Y, W, F, H), (L, V, I, A), (K, R), (E, Q, N, D) and (S, T).

Fig. 4. Multiple sequence alignment of human steroid receptor LBDs.

the helix 11-12 loop and helix 12 central to AF2 function (Ikeda *et al.*, 2004). PP5 was found to function as a negative regulator of ERα transcription *in vivo* by inhibiting epidermal growth factor (EGF)-dependent phosphorylation of Ser118 in the receptor N-terminal domain. Although demonstration of a direct PP5-ERα interaction was consistent with a non-involvement of Hsp90, a role for this major molecular chaperone in the *in vivo* effects of PP5 on ERα function cannot be discounted. Similar observations have been reported for GR with evidence suggesting that PP5-dependent modulation of receptor N-terminal phosphorylation within the GR-Hsp90 apo-receptor complex is mediated through contacts between the phosphatase and receptor LBD (Wang *et al.*, 2007).

A yeast two-hybrid screen, using bait encompassing both the hinge region and LBD of human PR, liganded with the mixed antagonist RU486, identified GCUNC-45 as a PR-binding protein (Chadli *et al.*, 2006). Presence of two LXXLL motifs (similar to NR boxes of known transcriptional coregulatory proteins) within the interacting clone, corresponding to the C-terminal end of GCUNC-45, suggested a mode of interaction similar to that for receptor recognition of transcription coactivators (Ratajczak, 2001), although this remains to be confirmed. Both FKBP52 and CyP40 compete with GCUNC-45 for the N-terminal TPR site, with nucleotides causing a reduction in Hsp90 binding affinity for these cochaperones in this region and favouring their interaction with the Hsp90 C-terminus during progression of receptor to a hormone-binding state (Chadli *et al.*, 2008b). GCUNC-45 therefore, appears to have a role upstream of FKBP52 and CyP40, at an intermediate stage of the receptor activation pathway.

The Hsp70/Hsp90 cochaperone, SGT, has been shown to interact through its TPR domain with the hinge region of human AR, which contains a peptide sequence structurally resembling the EEVD binding site for TPR proteins at the extreme C-terminus of Hsp70 and Hsp90 (Buchanan *et al.*, 2007). It has been proposed that, as a component of AR-Hsp90 complexes, SGT regulates the ligand sensitivity of AR signalling by limiting receptor trafficking to the nucleus at low hormone concentrations and maintaining the receptor within the cytoplasm of the cell.

6.2 p23; Cdc37

Disruption of the p23 gene in mice has revealed that although p23 is not essential for overall perinatal development its absolute requirement for perinatal survival is linked to impaired GR function arising most likely from instability of GR-Hsp90 complexes in the absence of p23 (Grad *et al.*, 2006; Picard, 2006). These findings suggest that GR might be a key molecular target for p23. Overexpression experiments with p23 in tissue culture cells have revealed both positive and negative influences on GR function (Freeman *et al.*, 2000; Wochnik *et al.*, 2004), as well as differential effects on other steroid receptors - increasing PR activity, while decreasing the activities of AR, ERα and MR (Freeman *et al.*, 2000). In yeast, p23 has been shown to be a positive regulator of ERα transcriptional activation, being most effective at low ERα levels and hormone concentrations, consistent with the proposed role for p23 as a component of mature ERα-Hsp90 complexes (Knoblauch & Garabedian, 1999). Ectopic expression of p23 in MCF-7 breast cancer cells increased both hormone-dependent and hormone-independent ERα transcriptional activity (Knoblauch & Garabedian, 1999).

Thus, while the major impact of p23 on ERα is likely to be through an Hsp90-dependent effect on estradiol binding, p23 overexpression may also influence receptor activity independent of ligand binding and may participate in the disassembly of receptors at cognate response elements (Freeman *et al.*, 2000; Freeman & Yamamoto, 2001; Freeman & Yamamoto, 2002). It is of interest that although p23 increases AR transcriptional activity in a variety of mammalian cell lines, partly by increasing ligand binding competence of the receptor, Hsp90 inhibitors could not abolish the AR coactivation potential of p23, consistent with an Hsp90-independent role of p23 in AR function (Querol Cano L and Bevan CL, unpublished observations).

Genetic studies in yeast have revealed that Cdc37 plays a role in AR hormone-dependent transactivation through functional interactions with the AR LBD, although the hormone-binding properties of the receptor appear to be unaffected (Fliss *et al.*, 1997). The association with Cdc37 is specific to AR since it does not occur with closely related nuclear receptors such as GR (Rao *et al.*, 2001). Depletion of Cdc37 using RNA interference caused growth arrest in both AR-positive and AR-negative prostate cancer cells, and in the former led to a loss of AR transcriptional activity with a concomitant decrease in androgen-dependent gene expression (Gray *et al.*, 2007). The targeting of Cdc37 in prostate cancer causes growth inhibition that correlates with decreased signalling through multiple pathways - the extracellular signal-regulated kinase (ERK) and Akt kinase cascades, as well as reduced AR-dependent signalling (Gray *et al.*, 2008).

7. Conclusions

We have arrived at a better understanding of the molecular mechanisms that allow the Hsp90 chaperone to modulate steroid receptor function through direct contact with receptor LBDs. Critical to this regulation is the ability of Hsp90 to coordinate and bring to receptor-Hsp90 complexes a selection of cochaperones whose specialized influences target receptor LBDs and combine, at various stages of the receptor activation pathway, to alter receptor hormone-binding status, cellular location and transcriptional activity. A number of these cochaperones may impact on steroid receptor function independently of Hsp90. Substantial gaps still remain, however in our knowledge of how the interplay between Hsp90 and its cochaperones affects receptor function. For example, while it is known the CyP40 yeast homologue, Cpr6, regulates Hsp90 ATPase activity during receptor assembly (Prodromou *et al.*, 1999) and studies of a second yeast homologue, Cpr7, have provided some insight into the role of this immunophilin in Hsp90-dependent signalling by steroid receptors (Duina *et al.*, 1996; Duina *et al.*, 1998), a coherent mechanism at the molecular level has yet to be defined. From the structural similarity between CyP40 and FKBP52, both being characterized by N-terminal PPIase and C-terminal TPR domains, it is tempting to draw parallels for their mechanism of action. Within steroid receptor-Hsp90 complexes it is possible that, as for FKBP52, the CyP40 PPIase domain forms productive interactions with the receptor LBD, serving to modulate receptor conformation and function. This may be of relevance for the function of ERα, purification of which led to the isolation of CyP40 in ERα-Hsp90 complexes (Ratajczak *et al.*, 1993) and for the regulation of AR in prostate cancer where CyP40 appears to be overexpressed (Periyasamy *et al.*, 2010).

Hsp90 is required for the proper function of several key regulatory proteins including multiple tyrosine and serine/threonine kinases and steroid receptors, many of which are involved in promoting malignancy (Calderwood *et al*., 2006; Pearl, 2005; Whitesell & Lindquist, 2005). The aim of targeting and pharmacological manipulation of the Hsp90 chaperoning system has led to the ongoing development and clinical evaluation of novel Hsp90 and chaperone inhibitors for potential application in therapies against selected malignancies (Donnelly *et al.*, 2010; Kim *et al.*, 2009), syndromes arising from dysfunctional protein folding and neurodegenerative diseases (Jinwal *et al.*, 2010). With growing understanding of the novel mechanisms through which Hsp90 cochaperones modulate the function of specific clients, strategies are now evolving for the targeting of chaperone-client interactions in a wide range of human diseases (De Leon *et al*., 2011; Gray *et al*., 2008).

8. Abbreviations

Hsp, heat shock protein; TPR, tetratricopeptide repeat; PPIase, prolylpeptidyl isomerase; FKBP, FK506-binding protein; CyP40, cyclophilin 40; PP5, serine/threonine protein phosphatase type 5; GCUNC-45, general cell UNC-45; αSGT, small glutamine-rich tetratricopeptide repeat containing protein α; AR, androgen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; LBD, ligand-binding domain; AF2, activation function 2; GST, glutathione S-transferase.

9. Acknowledgments

The authors wish to acknowledge support from the National Health & Medical Research Council of Australia, the National Breast Cancer Foundation and the Sir Charles Gairdner Hospital Research Fund. The authors also thank colleagues for permitting citation of their data prior to publication.

10. References

Ali M. M. U., Roe S. M., Vaughan C. K., Meyer P., Panaretou B., Piper P. W., Prodromou C. & Pearl L. H. (2006). Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. *Nature*, Vol.440, No.7087, pp. 1013-1017.

Allan R. K., Mok D., Ward B. K. & Ratajczak T. (2006). Modulation of chaperone function and cochaperone interaction by novobiocin in the C-terminal domain of Hsp90: evidence that coumarin antibiotics disrupt Hsp90 dimerization. *J Biol Chem*, Vol.281, No.11, pp. 7161-7171.

Amler L. C., Agus D. B., LeDuc C., Sapinoso M. L., Fox W. D., Kern S., Lee D., Wang V., Leysens M., Higgins B., Martin J., Gerald W., Dracopoli N., Cordon-Cardo C., Scher H. I. & Hampton G. M. (2000). Dysregulated expression of androgen-responsive and nonresponsive genes in the androgen-independent prostate cancer xenograft model CWR22-R. *Cancer Res*, Vol.60, No.21, pp. 6134-6141.

Angeletti P. C., Walker D. & Panganiban A. T. (2002). Small glutamine-rich protein/viral protein U-binding protein is a novel cochaperone that affects heat shock protein 70 activity. *Cell Stress Chaperones*, Vol.7, No.3, pp. 258-268

Aumais J. P., Lee H. S., Lin R. & White J. H. (1997). Selective interaction of Hsp90 with an estrogen receptor ligand-binding domain containing a point mutation. *J Biol Chem*, Vol.272, No.18, pp. 12229-12235.

Ballinger C. A., Connell P., Wu Y., Hu Z., Thompson L. J., Yin L.-Y. & Patterson C. (1999). Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol*, Vol.19, No.6, pp. 4535-4545.

Banerjee A., Periyasamy S., Wolf I. M., Hinds T. D., Yong W., Shou W. & Sanchez E. R. (2008). Control of glucocorticoid and progesterone receptor subcellular localization by the ligand-binding domain is mediated by distinct interactions with tetratricopeptide repeat proteins. *Biochemistry*, Vol.47, No.39, pp. 10471-10480.

Barent R. L., Nair S. C., Carr D. C., Ruan Y., Rimerman R. A., Fulton J., Zhang Y. & Smith D. F. (1998). Analysis of FKBP51/FKBP52 chimeras and mutants for Hsp90 binding and association with progesterone receptor complexes. *Mol Endocrinol*, Vol.12, No.3, pp. 342-354.

Barford D. (1996). Molecular mechanisms of the protein serine/threonine phosphatases. *Trends Biochem Sci*, Vol.21, No.11, pp. 407-412.

Bimston D., Song J., Winchester D., Takayama S., Reed J. C. & Morimoto R. I. (1998). BAG-1, a negative regulator of Hsp70 chaperone activity, uncouples nucleotide hydrolysis from substrate release. *EMBO J*, Vol.17, No.23, pp. 6871-6878.

Binart N., Lombès M. & Baulieu E. E. (1995). Distinct functions of the 90 kDa heat-shock protein (hsp90) in oestrogen and mineralocorticosteroid receptor activity: effects of hsp90 deletion mutants. *Biochem J*, Vol.311, 797-804.

Bledsoe R. K., Montana V. G., Stanley T. B., Delves C. J., Apolito C. J., McKee D. D., Consler T. G., Parks D. J., Stewart E. L., Willson T. M., Lambert M. H., Moore J. T., Pearce K. H. & Xu H. E. (2002). Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell*, Vol.110, 93-105.

Brelivet Y., Kammerer S., Rochel N., Poch O. & Moras D. (2004). Signature of the oligomeric behaviour of nuclear receptors at the sequence and structural level *EMBO Rep*, Vol.5, No.4, pp. 423-429.

Buchanan G., Ricciardelli C., Harris J. M., Prescott J., Yu Z. C.-L., Jia L., Butler L. M., Marshall V. R., Scher H. I., Gerald W. L., Coetzee G. A. & Tilley W. D. (2007). Control of androgen receptor signaling in prostate cancer by the cochaperone small glutamine rich tetratricopeptide repeat containing protein α. *Cancer Res*, Vol.67, No.20, pp. 10087-10096.

Caamano C. A., Morano M. I., Dalman F. C., Pratt W. B. & Akil H. (1998). A conserved proline in the Hsp90 binding region of the glucocorticoid receptor is required for Hsp90 heterocomplex stabilization and receptor signaling. *J Biol Chem*, Vol.273, No.32, pp. 20473-20480.

Cadepond F., Binart N., Chambraud B., Jibard N., Schweizer-Groyer G., Segard-Maurel I. & Baulieu E. E. (1993). Interaction of glucocorticosteroid receptor and wild-type or mutated 90-kDa heat shock protein coexpressed in baculovirus-infected Sf9 cells. *PNAS*, Vol.90, No.22, pp. 10434-10438.

Calderwood S. K., Khaleque M. A., Sawyer D. B. & Ciocca D. R. (2006). Heat shock proteins in cancer: chaperones of tumorigenesis. *Trends Biochem Sci*, Vol.31, No.3, pp. 164-172.

Callahan M. A., Handley M. A., Lee Y.-H., Talbot K. J., Harper J. W. & Panganiban A. T. (1998). Functional interaction of human immunodeficiency virus type 1 Vpu and Gag with a novel member of the tetratricopeptide repeat protein family. *J Virol*, Vol.72, No.6, pp. 5189-5197.

Carrello A., Ingley E., Minchin R. F., Tsai S. & Ratajczak T. (1999). The common tetratricopeptide repeat acceptor site for steroid receptor-associated immunophilins and Hop is located in the dimerization domain of Hsp90. *J Biol Chem*, Vol.274, No.5, pp. 2682-2689.

Carrigan P. E., Sikkink L. A., Smith D. F. & Ramirez-Alvarado M. (2006). Domain:domain interactions within Hop, the Hsp70/Hsp90 organizing protein, are required for protein stability and structure. *Protein Sci*, Vol.15, No.3, pp. 522-532.

Chadli A., Bruinsma E. S., Stensgard B. & Toft D. (2008a). Analysis of Hsp90 cochaperone interactions reveals a novel mechanism for TPR protein recognition. *Biochemistry*, Vol.47, No.9, pp. 2850-2857.

Chadli A., Felts S. J. & Toft D. O. (2008b). GCUNC-45 is the first Hsp90 co-chaperone to show α/β isoform specificity *J Biol Chem*, Vol.283, No.15, pp. 9509-9512.

Chadli A., Graham J. D., Abel M. G., Jackson T. A., Gordon D. F., Wood W. M., Felts S. J., Horwitz K. B. & Toft D. (2006). GCUNC-45 is a novel regulator for the progesterone receptor/Hsp90 chaperoning pathway. *Mol Cell Biol*, Vol.26, No.5, pp. 1722-1730.

Chen M.-S., Silverstein A. M., Pratt W. B. & Chinkers M. (1996a). The tetratricopeptide repeat domain of protein phosphatase 5 mediates binding to glucocorticoid receptor heterocomplexes and acts as a dominant negative mutant. *J Biol Chem*, Vol.271, No.50, pp. 32315-32320.

Chen M. X. & Cohen P. T. W. (1997). Activation of protein phosphatase 5 by limited proteolysis or the binding of polyunsaturated fatty acids to the TPR domain. *FEBS Lett*, Vol.400, No.1, pp. 136-140.

Chen M. X., McPartlin A. E., Brown L., Chen Y. H., Barker H. M. & Cohen P. T. W. (1994). A novel human protein serine/ threonine phosphatase, which possesses four tetretricopeptide repeat motifs and localizes to the nucleus. *EMBO J*, Vol.13, No.18, pp. 4278-4290.

Chen S., Prapapanich V., Rimerman R. A., Honore B. & Smith D. F. (1996b). Interactions of p60, a mediator of progesterone receptor assembly, with heat shock proteins Hsp90 and Hsp70. *Mol Endocrinol*, Vol.10, No.6, pp. 682-693.

Chen S. & Smith D. F. (1998). Hop as an adaptor in the heat shock protein 70 (Hsp70) and Hsp90 chaperone machinery. *J Biol Chem*, Vol.273, No.52, pp. 35194-35200.

Chen S., Sullivan W. P., Toft D. O. & Smith D. F. (1998). Differential interactions of p23 and the TPR-containing proteins Hop, Cyp40, FKBP52 and FKBP51 with Hsp90 mutants. *Cell Stress Chaperones*, Vol.3, No.2, pp. 118-129.

Cheung-Flynn J., Prapapanich V., Cox M. B., Riggs D. L., Suarez-Quian C. & Smith D. F. (2005). Physiological role for the cochaperone FKBP52 in androgen receptor signaling. *Mol Endocrinol*, Vol.19, No.6, pp. 1654-1666.

Cheung-Flynn J., Roberts P. J., Riggs D. L. & Smith D. F. (2003). C-terminal sequences outside the tetratricopeptide repeat domain of FKBP51 and FKBP52 cause differential binding to Hsp90. *J Biol Chem*, Vol.278, No.19, pp. 17388-17394.

Chinkers M. (1994). Targeting of a distinctive protein-serine phosphatase to the protein kinase-like domain of the atrial natriuretic peptide receptor. *PNAS*, Vol.91, No.23, pp. 11075-11079.

Cohen P. T. W. (1997). Novel protein serine/threonine phosphatases: variety is the spice of life. *Trends Biochem Sci*, Vol.22, No.7, pp. 245-251.

Connell P., Ballinger C. A., Jiang J., Wu Y., Thompson L. J., Hohfeld J. & Patterson C. (2001). The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat Cell Biol*, Vol.3, No.1, pp. 93-96.

Cyr D. M., Höhfeld J. & Patterson C. (2002). Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. *Trends Biochem Sci*, Vol.27, No.7, pp. 368-375.

Cziepluch C., Kordes E., Poirey R., Grewenig A., Rommelaere J. & Jauniaux J.-C. (1998). Identification of a novel cellular TPR-containing protein, SGT, that interacts with the nonstructural protein NS1 of parvovirus H-1. *J Virol*, Vol.72, No.5, pp. 4149-4156.

Das A. K., Cohen P. T. W. & Barford D. (1998). The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. *EMBO J*, Vol.17, No.5, pp. 1192-1199.

Davies T. H., Ning Y.-M. & Sanchez E. R. (2002). A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. *J Biol Chem*, Vol.277, No.7, pp. 4597-4600.

De Leon J. T., Iwai A., Feau C., Garcia Y., Balsiger H. A., Storer C. L., Suro R. M., Garza K. M., Lee S., Sang Kim Y., Chen Y., Ning Y.-M., Riggs D. L., Fletterick R. J., Guy R. K., Trepel J. B., Neckers L. M. & Cox M. B. (2011). Targeting the regulation of androgen receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells. *PNAS*, Vol.108, No.29, pp. 11878-11883.

Denny W. B., Prapapanich V., Smith D. F. & Scammell J. G. (2005). Structure-function analysis of Squirrel Monkey FK506-binding protein 51, a potent inhibitor of glucocorticoid receptor activity. *Endocrinology*, Vol.146, No.7, pp. 3194-3201.

Denny W. B., Valentine D. L., Reynolds P. D., Smith D. F. & Scammell J. G. (2000). Squirrel monkey immunophilin FKBP51 is a potent inhibitor of glucocorticoid receptor binding. *Endocrinology*, Vol.141, No.11, pp. 4107-4113.

Dittmar K. D., Hutchison K. A., Owens-Grillo J. K. & Pratt W. B. (1996). Reconstitution of the steroid receptor·Hsp90 heterocomplex assembly system of rabbit reticulocyte lysate. *J Biol Chem*, Vol.271, No.22, pp. 12833-12839.

Donnelly A. C., Zhao H., Reddy Kusuma B. & Blagg B. S. J. (2010). Cytotoxic sugar analogues of an optimized novobiocin scaffold. *Med Chem Commun*, Vol.1, No.2, pp. 165-170.

Duina A. A., Chang H.-C. J., Marsh J. A., Lindquist S. & Gaber R. F. (1996). A cyclophilin function on Hsp90-dependent signal transduction. *Science*, Vol.274, 1713-1715.

Duina A. A., Marsh J. A., Kurtz R. B., Chang H.-C. J., Lindquist S. & Gaber R. F. (1998). The peptidyl-prolyl isomerase domain of the CyP-40 cyclophilin homolog Cpr7 is not

required to support growth or glucocorticoid receptor activity in *Saccharomyces cerevisiae*. *J Biol Chem*, Vol.273, No.18, pp. 10819-10822.

Dutta S. & Tan Y.-J. (2008). Structural and functional characterization of human SGT and its interaction with Vpu of the human immunodeficiency virus type 1. *Biochemistry*, Vol.47, No.38, pp. 10123-10131.

Echeverria P. C. & Picard D. (2010). Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility. *Biochim Biophys Acta - Mol Cell Res*, Vol.1803, No.6, pp. 641-649.

Estébanez-Perpiñá E., Arnold L. A., Nguyen P., Rodrigues E. D., Mar E., Bateman R., Pallai P., Shokat K. M., Baxter J. D., Guy R. K., Webb P. & Fletterick R. J. (2007). A surface on the androgen receptor that allosterically regulates coactivator binding. *PNAS*, Vol.104, No.41, pp. 16074-16079.

Fang L., Ricketson D., Getubig L. & Darimont B. (2006). Unliganded and hormone-bound glucocorticoid receptors interact with distinct hydrophobic sites in the Hsp90 C-terminal domain. *PNAS*, Vol.103, No.49, pp. 18487-18492.

Febbo P. G., Lowenberg M., Thorner A. R., Brown M., Loda M. & Golub T. R. (2005). Androgen mediated regulation and functional implications of FKBP51 expression in prostate cancer. *J Urol*, Vol.173, No.5, pp. 1772-1777.

Felts S. J. & Toft D. O. (2003). p23, a simple protein with complex activities. *Cell Stress Chaperones*, Vol.8, No.2, pp. 108-113.

Fliss A. E., Fang Y., Boschelli F. & Caplan A. J. (1997). Differential *in vivo* regulation of steroid hormone receptor activation by Cdc37p. *Mol Biol Cell*, Vol.8, No.12, pp. 2501-2509.

Freeman B. C., Felts S. J., Toft D. O. & Yamamoto K. R. (2000). The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies. *Genes Dev*, Vol.14, 422-434.

Freeman B. C. & Yamamoto K. R. (2001). Continuous recycling: a mechanism for modulatory signal transduction. *Trends Biochem Sci*, Vol.26, No.5, pp. 285-290.

Freeman B. C. & Yamamoto K. R. (2002). Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science*, Vol.296, No.5576, pp. 2232-2235.

Frydman J. & Höhfeld J. (1997). Chaperones get in touch: the Hip-Hop connection. *Trends Biochem Sci*, Vol.22, No.3, pp. 87-92.

Fuller P. J., Smith B. J. & Rogerson F. M. (2004). Cortisol resistance in the New World revisited. *Trends Endocrinol Metab*, Vol.15, No.7, pp. 296-299.

Gallo L. I., Ghini A. A., Piwien Pilipuk G. & Galigniana M. D. (2007). Differential recruitment of tetratricopeptide repeat domain immunophilins to the mineralocorticoid receptor influences both heat-shock protein 90-dependent retrotransport and hormone-dependent transcriptional activity. *Biochemistry*, Vol.46, No.49, pp. 14044-14057.

Gebauer M., Zeiner M. & Gehring U. (1997). Proteins interacting with the molecular chaperone Hsp70/Hsc70: physical associations and effects on refolding activity. *FEBS Lett*, Vol.417, No.1, pp. 109-113.

Giannoukos G., Silverstein A. M., Pratt W. B. & Simons Jr S. S. (1999). The seven amino acids (547-553) of rat glucocorticoid receptor required for steroid and Hsp90 binding

contain a functionally independent LXXLL motif that is critical for steroid binding. *J Biol Chem*, Vol.274, No.51, pp. 36527-36536.

Grad I., McKee T. A., Ludwig S. M., Hoyle G. W., Ruiz P., Wurst W., Floss T., Miller C. A., III & Picard D. (2006). The Hsp90 cochaperone p23 is essential for perinatal survival. *Mol Cell Biol*, Vol.26, No.23, pp. 8976-8983.

Gray P. J., Prince T., Cheng J., Stevenson M. A. & Calderwood S. K. (2008). Targeting the oncogene and kinome chaperone CDC37. *Nat Rev Cancer*, Vol.8, No.7, pp. 491-495.

Gray P. J., Stevenson M. A. & Calderwood S. K. (2007). Targeting Cdc37 inhibits multiple signaling pathways and induces growth arrest in prostate cancer cells. *Cancer Res*, Vol.67, No.24, pp. 11942-11950.

Harris S. F., Shiau A. K. & Agard D. A. (2004). The crystal structure of the carboxy-terminal dimerization domain of htpG, the *Escherichia coli* Hsp90, reveals a potential substrate binding site. *Structure*, Vol.12, No.6, pp. 1087-1097.

He B., Bowen N. T., Minges J. T. & Wilson E. M. (2001). Androgen-induced NH_2- and COOH-terminal interaction inhibits p160 coactivator recruitment by activation function 2. *J Biol Chem*, Vol.276, No.45, pp. 42293-42301.

He B., Gampe Jr R. T., Kole A. J., Hnat A. T., Stanley T. B., An G., Stewart E. L., Kalman R. I., Minges J. T. & Wilson E. M. (2004). Structural basis for androgen receptor interdomain and coactivator interactions suggests a transition in nuclear receptor activation function dominance. *Mol Cell*, Vol.16, No.3, pp. 425-438.

Hinds Jr T. D. & Sánchez E. R. (2008). Protein phosphatase 5. *Int J Biochem Cell Biol*, Vol.40, No.11, pp. 2358-2362.

Höhfeld J. & Jentsch S. (1997). GrpE-like regulation of the Hsc70 chaperone by the anti-apoptotic protein BAG-1. *EMBO J*, Vol.16, No.20, pp. 6209-6216.

Höhfeld J., Minami Y. & Hartl F.-U. (1995). Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. *Cell*, Vol.83, No.4, pp. 589-598.

Ikeda K., Ogawa S., Tsukui T., Horie-Inoue K., Ouchi Y., Kato S., Muramatsu M. & Inoue S. (2004). Protein phosphatase 5 is a negative regulator of estrogen receptor-mediated transcription. *Mol Endocrinol*, Vol.18, No.5, pp. 1131-1143.

Jackson S. E., Queitsch C. & Toft D. (2004). Hsp90: from structure to phenotype. *Nat Struct Mol Biol*, Vol.11, No.12, pp. 1152-1155.

Jiang J., Ballinger C. A., Wu Y., Dai Q., Cyr D. M., Höhfeld J. & Patterson C. (2001). CHIP is a U-box-dependent E3 ubiquitin ligase. Identification of Hsc70 as a target for ubiquitylation. *J Biol Chem*, Vol.276, 42938-42944.

Jinwal U. K., Koren J., Borysov S. I., Schmid A. B., Abisambra J. F., Blair L. J., Johnson A. G., Jones J. R., Shults C. L., O'Leary J. C., Jin Y., Buchner J., Cox M. B. & Dickey C. A. (2010). The Hsp90 cochaperone, FKBP51, increases Tau stability and polymerizes microtubules. *J Neurosci*, Vol.30, No.2, pp. 591-599.

Joseph J. D., Wittmann B. M., Dwyer M. A., Cui H., Dye D. A., McDonnell D. P. & Norris J. D. (2009). Inhibition of prostate cancer cell growth by second-site androgen receptor antagonists. *PNAS*, Vol.106, No.29, pp. 12178-12183.

Kajander T., Sachs J. N., Goldman A. & Regan L. (2009). Electrostatic interactions of Hsp-organizing protein tetratricopeptide domains with Hsp70 and Hsp90: computational analysis and protein engineering. *J Biol Chem*, Vol.284, 25364-25374.

Kallen J., Mikol V., Taylor P. & D.Walkinshaw M. (1998). X-ray structures and analysis of 11 cyclosporin derivatives complexed with cyclophilin A. *J Mol Biol*, Vol.283, No.2, pp. 435-449.

Karagöz G. E., Duarte A. M. S., Ippel H., Uetrecht C., Sinnige T., van Rosmalen M., Hausmann J., Heck A. J. R., Boelens R. & Rüdiger S. G. D. (2010). N-terminal domain of human Hsp90 triggers binding to the cochaperone p23. *PNAS*, Vol.108, No.2, pp. 580-585.

Kauppi B., Jakob C., Farnegardh M., Yang J., Ahola H., Alarcon M., Calles K., Engstrom O., Harlan J., Muchmore S., Ramqvist A.-K., Thorell S., Ohman L., Greer J., Gustafsson J.-A., Carlstedt-Duke J. & Carlquist M. (2003). The three-dimensional structures of antagonistic and agonistic forms of the glucocorticoid receptor ligand-binding domain: RU-486 induces a transconformation that leads to active antagonism. *J Biol Chem*, Vol.278, No.25, pp. 22748-22754.

Kim Y. S., Alarcon S. V., Lee S., Lee M. J., Giaccone G., Neckers L. & Trepel J. B. (2009). Update on Hsp90 inhibitors in clinical trial. *Curr Top Med Chem*, Vol.9, No.15, pp. 1479-1492.

Knoblauch R. & Garabedian M. J. (1999). Role for Hsp90-associated cochaperone p23 in estrogen receptor signal transduction. *Mol Cell Biol*, Vol.19, No.5, pp. 3748-3759.

Kosano H., Stensgard B., Charlesworth M. C., McMahon N. & Toft D. (1998). The assembly of progesterone receptor-Hsp90 complexes using purified proteins. *J Biol Chem*, Vol.273, No.49, pp. 32973-32979.

Lassle M., Blatch G. L., Kundra V., Takatori T. & Zetter B. R. (1997). Stress-inducible, murine protein mSTI1. Characterization of binding domains for heat shock proteins and in vitro phosphorylation by different kinases. *J Biol Chem*, Vol.272, No.3, pp. 1876-1884.

Li J., Richter K. & Buchner J. (2011). Mixed Hsp90-cochaperone complexes are important for the progression of the reaction cycle. *Nat Struct Mol Biol*, Vol.18, No.1, pp. 61-66.

Liou S.-T. & Wang C. (2005). Small glutamine-rich tetratricopeptide repeat-containing protein is composed of three structural units with distinct functions. *Arch Biochem Biophys*, Vol.435, No.2, pp. 253-263.

Louvion J.-F., Warth R. & Picard D. (1996). Two eukaryote-specific regions of Hsp82 are dispensable for its viability and signal transduction functions in yeast. *PNAS*, Vol.93, No.24, pp. 13937-13942.

MacLean M. & Picard D. (2003). Cdc37 goes beyond Hsp90 and kinases. *Cell Stress Chaperones*, Vol.8, No.2, pp. 114-119.

Magee J., Chang L., Stormo G. & Milbrandt J. (2006). Direct, androgen receptor-mediated regulation of the FKBP5 gene via a distal enhancer element. *Endocrinology*, Vol.147, No.1, pp. 590-598.

Marcu M. G., Chadli A., Bouhouche I., Catelli M. & Neckers L. M. (2000). The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. *J Biol Chem*, Vol.275, No.47, pp. 37181-37186.

Matias P. M., Donner P., Coelho R., Thomaz M., Peixoto C., Macedo S., Otto N., Joschko S., Scholz P., Wegg A., Bäsler S., Schäfer M., Egner U. & Carrondo M. A. (2000a). Structural evidence for ligand specificity in the binding domain of the human

androgen receptor: implications for pathogenic gene mutations. *J. Biol. Chem.*, Vol.275, No.34, pp. 26164-26171.

Matias P. M., Donner P., Coelho R., Thomaz M., Peixoto C., Macedo S., Otto N., Joschko S., Scholz P., Wegg A., Bäsler S., Schäfer M., Egner U. & Carrondo M. A. (2000b). Structural evidence for ligand specificity in the binding domain of the human androgen receptor: implications for pathogenic gene mutations. *J Biol Chem*, Vol.275, No.34, pp. 26164-26171.

McLaughlin S. H., Smith H. W. & Jackson S. E. (2002). Stimulation of the weak ATPase activity of human Hsp90 by a client protein. *J Mol Biol*, Vol.315, No.4, pp. 787-798.

McLaughlin S. H., Sobott F., Yao Z.-p., Zhang W., Nielsen P. R., Grossmann J. G. n., Laue E. D., Robinson C. V. & Jackson S. E. (2006). The co-chaperone p23 arrests the Hsp90 ATPase cycle to trap client proteins. *J Mol Biol*, Vol.356, No.3, pp. 746-758.

Mok D., Allan R. K., Carrello A., Wangoo K., Walkinshaw M. D. & Ratajczak T. (2006). The chaperone function of cyclophilin 40 maps to a cleft between the prolyl isomerase and tetratricopeptide repeat domains. *FEBS Lett*, Vol.580, No.11, pp. 2761-2768.

Moore T. W., Mayne C. G. & Katzenellenbogen J. A. (2010). Minireview: Not picking pockets: nuclear receptor alternate-site modulators (NRAMs). *Mol Endocrinol*, Vol.24, No.4, pp. 683-695.

Mousses S., Wagner U., Chen Y., Kim J. W., Bubendorf L., Bittner M., Pretlow T., Elkahloun A. G., Trepel J. B., Kallioniemi O.-P. & (2001). Failure of hormone therapy in prostate cancer involves systematic restoration of androgen responsive genes and activation of rapamycin sensitive signaling. *Oncogene*, Vol.20, No.46, pp. 6718-6723.

Murata S., Minami Y., Minami M., Chiba T. & Tanaka K. (2001). CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep*, Vol.2, No.12, pp. 1133–1138.

Nair S. C., A.Rimerman R., Toran E. J., Chen S., Prapapanich V., Butts R. N. & Smith D. F. (1997). Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. *Mol Cell Biol*, Vol.17, No.2, pp. 594-603.

Nathan D. F. & Lindquist S. (1995). Mutational analysis of Hsp90 function: interactions with a steroid receptor and a protein kinase. *Mol Cell Biol*, Vol.15, No.7, pp. 3917-3925.

Nelson G. M., Huffman H. & Smith D. F. (2003). Comparison of the carboxy-terminal DP-repeat region in the co-chaperones Hop and Hip. *Cell Stress Chaperones*, Vol.8, No.2, pp. 125-133.

Nelson G. M., Prapapanich V., Carrigan P. E., Roberts P. J., Riggs D. L. & Smith D. F. (2004). The heat shock protein 70 cochaperone Hip enhances functional maturation of glucocorticoid receptor. *Mol Endocrinol*, Vol.18, No.7, pp. 1620-1630.

Ni L., Yang C.-S., Gioeli D., Frierson H., Toft D. O. & Paschal B. M. (2010). FKBP51 promotes assembly of the Hsp90 chaperone complex and regulates androgen receptor signaling in prostate cancer cells. *Mol Cell Biol*, Vol.30, No.5, pp. 1243-1253.

Odunuga O. O., Hornby J. A., Bies C., Zimmermann R., Pugh D. J. & Blatch G. L. (2003). Tetratricopeptide repeat motif-mediated Hsc70-mSTI1 interaction. *J Biol Chem*, Vol.278, No.9, pp. 6896-6904.

Onuoha S. C., Coulstock E. T., Grossmann J. G. & Jackson S. E. (2008). Structural studies on the co-chaperone Hop and its complexes with Hsp90. *J Mol Biol*, Vol.379, No.4, pp. 732-744.

Pearl L. H. (2005). Hsp90 and Cdc37 - a chaperone cancer conspiracy. *Curr Opin Genet Dev*, Vol.15, No.1, pp. 55-61.

Periyasamy S., Hinds T., Jr., Shemshedini L., Shou W. & Sanchez E. R. (2010). FKBP51 and Cyp40 are positive regulators of androgen-dependent prostate cancer cell growth and the targets of FK506 and cyclosporin A. *Oncogene*, Vol.29, No.11, pp. 1691-1701.

Picard D. (2006). Chaperoning steroid hormone action. *Trends Endocrinol Metab*, Vol.17, No.6, pp. 229-236.

Picard D., Khursheed B., Garabedian M. J., Fortin M. G., Lindquist S. & Yamamoto K. R. (1990). Reduced levels of Hsp90 compromise steroid receptor action *in vivo*. *Nature*, Vol.348, 166-168.

Pirkl F. & Buchner J. (2001). Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and CyP40. *J Mol Biol*, Vol.308, No.4, pp. 795-806.

Prapapanich V., Chen S., Nair S. C., Rimerman R. A. & Smith D. F. (1996a). Molecular cloning of human p48, a transient component of progesterone receptor complexes and an Hsp70-binding protein. *Mol Endocrinol*, Vol.10, No.4, pp. 420-431.

Prapapanich V., Chen S. & Smith D. F. (1998). Mutation of Hip's carboxy-terminal region inhibits a transitional stage of progesterone receptor assembly. *Mol Cell Biol*, Vol.18, No.2, pp. 944-952.

Prapapanich V., Chen S., Toran E. J., Rimerman R. A. & Smith D. F. (1996b). Mutational analysis of the hsp70-interacting protein Hip. *Mol Cell Biol*, Vol.16, No.11, pp. 6200-6207.

Pratt W. B. & Toft D. O. (1997). Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev*, Vol.18, No.3, pp. 306-360.

Pratt W. B. & Toft D. O. (2003). Regulation of signaling protein function and trafficking by the Hsp90/Hsp70-based chaperone machinery. *Exp Biol Med*, Vol.228, No.2, pp. 111-133.

Prodromou C., Panaretou B., Chohan S., Siligardi G., O'Brien R., Ladbury J. E., Roe S. M., Piper P. W. & Pearl L. H. (2000). The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. *EMBO J*, Vol.19, No.16, pp. 4383-4392.

Prodromou C., Siligardi G., O'Brien R., Woolfson D. N., Regan L., Panaretou B., Ladbury J. E., Piper P. W. & Pearl L. H. (1999). Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. *EMBO J*, Vol.18, No.3, pp. 754-762.

Radanyi C., Chambraud B. & Baulieu E. E. (1994). The ability of the immunophilin FKBP59-HBI to interact with the 90-kDa heat shock protein is encoded by its tetratricopeptide repeat domain. *PNAS*, Vol.91, 11197-11201.

Ramsey A. J. & Chinkers M. (2002). Identification of potential physiological activators of protein phosphatase 5. *Biochemistry*, Vol.41, No.17, pp. 5625-5632.

Ramsey A. J., Russell L. C. & Chinkers M. (2009). C-terminal sequences of Hsp70 and Hsp90 as non-specific anchors for tetratricopeptide repeat (TPR) proteins. *Biochem J*, Vol.423, No.3, pp. 411-419.

Rao J., Lee P., Benzeno S., Cardozo C., Albertus J., Robins D. M. & Caplan A. J. (2001). Functional interaction of human Cdc37 with the androgen receptor but not with the glucocorticoid receptor. *J Biol Chem*, Vol.276, No.8, pp. 5814-5820.

Ratajczak T. (2001). Protein coregulators that mediate estrogen receptor function. *Reprod Fertil Dev*, Vol.13, 221-229.

Ratajczak T. & Carrello A. (1996). Cyclophilin 40 (CyP-40), mapping of its Hsp90 binding domain and evidence that FKBP52 competes with CyP-40 for Hsp90 binding. *J Biol Chem*, Vol.271, No.6, pp. 2961-2965.

Ratajczak T., Carrello A., Mark P. J., Warner B. J., Simpson R. J., Moritz R. L. & House A. K. (1993). The cyclophilin component of the unactivated estrogen receptor contains a tetratricopeptide repeat domain and shares identity with p59 (FKBP59). *J Biol Chem*, Vol.268, No.18, pp. 13187-13192.

Ratajczak T., Hlaing J., Brockway M. J. & Hahnel R. (1990). Isolation of untransformed bovine estrogen receptor without molybdate stabilization. *J Steroid Biochem*, Vol.35, No.5, pp. 543-553.

Ratajczak T., Ward B. K. & Minchin R. F. (2003). Immunophilin chaperones in steroid receptor signalling. *Curr Top Med Chem*, Vol.3, No.12, pp. 1348-1357.

Reynolds P. D., Ruan Y., Smith D. F. & Scammell J. G. (1999). Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51. *J Clin Endocrinol Metab*, Vol.84, No.2, pp. 663-669.

Richter K., Walter S. & Buchner J. (2004). The co-chaperone Sba1 connects the ATPase reaction of Hsp90 to the progression of the chaperone cycle. *J Mol Biol*, Vol.342, No.5, pp. 1403-1413.

Riggs D. L., Cox M. B., Cheung-Flynn J., Prapapanich V., Carrigan P. E. & Smith D. F. (2004). Functional specificity of co-chaperone interactions with Hsp90 client proteins. *Crit Rev Biochem Mol Biol*, Vol.39, 279-295.

Riggs D. L., Cox M. B., Tardif H. L., Hessling M., Buchner J. & Smith D. F. (2007). Noncatalytic role of the FKBP52 peptidyl-prolyl isomerase domain in the regulation of steroid hormone signaling. *Mol Cell Biol*, Vol.27, No.24, pp. 8658-8669.

Riggs D. L., Roberts P. J., Chirillo S. C., Cheung-Flynn J., Prapapanich V., Ratajczak T., Gaber R., Picard D. & Smith D. F. (2003). The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling *in vivo*. *EMBO J*, Vol.22, No.5, pp. 1158-1167.

Roe S. M., Ali M. M. U., Meyer P., Vaughan C. K., Panaretou B., Piper P. W., Prodromou C. & Pearl L. H. (2004). The mechanism of Hsp90 regulation by the protein kinase-specific cochaperone p50cdc37. *Cell*, Vol.116, No.1, pp. 87-98.

Russell L. C., Whitt S. R., Chen M.-S. & Chinkers M. (1999). Identification of conserved residues required for the binding of a tetratricopeptide repeat domain to heat shock protein 90. *J Biol Chem*, Vol.274, No.29, pp. 20060-20063.

Scammell J. G., Denny W. B., Valentine D. L. & Smith D. F. (2001). Overexpression of the FK506-binding immunophilin FKBP51 is the common cause of glucocorticoid

resistance in three New World primates. *Gen Comp Endocrinol*, Vol.124, No.2, pp. 152-165.

Schaufele F., Carbonell X., Guerbadot M., Borngraeber S., Chapman M. S., Ma A. A. K., Miner J. N. & Diamond M. I. (2005). The structural basis of androgen receptor activation: intramolecular and intermolecular amino–carboxy interactions. *PNAS*, Vol.102, No.28, pp. 9802-9807.

Scheufler C., Brinker A., Bourenkov G., Pegoraro S., Moroder L., Bartunik H., Hartl F. U. & Moarefi I. (2000). Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell*, Vol.101, No.2, pp. 199-210.

Siligardi G., Hu B., Panaretou B., Piper P. W., Pearl L. H. & Prodromou C. (2004). Co-chaperone regulation of conformational switching in the Hsp90 ATPase cycle. *J Biol Chem*, Vol.279, No.50, pp. 51989-51998.

Silverstein A. M., Galigniana M. D., Chen M.-S., Owens-Grillo J. K., Chinkers M. & Pratt W. B. (1997). Protein phosphatase 5 is a major component of glucocorticoid receptor·Hsp90 complexes with properties of an FK506-binding immunophilin. *J Biol Chem*, Vol.272, No.26, pp. 16224-16230.

Silverstein A. M., Galigniana M. D., Kanelakis K. C., Radanyi C., Renoir J.-M. & Pratt W. B. (1999). Different regions of the immunophilin FKBP52 determine its association with the glucocorticoid receptor, Hsp90, and cytoplasmic dynein. *J Biol Chem*, Vol.274, No.52, pp. 36980-36986.

Sinars C. R., Cheung-Flynn J., Rimerman R. A., Scammell J. G., Smith D. F. & Clardy J. (2003). Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes. *PNAS*, Vol.100, No.3, pp. 868-873.

Skinner J., Sinclair C., Romeo C., Armstrong D., Charbonneau H. & Rossie S. (1997). Purification of a fatty acid-stimulated protein-serine/threonine phosphatase from bovine brain and its identification as a homolog of protein phosphatase 5. *J Biol Chem*, Vol.272, No.36, pp. 22464-22471.

Smith D. F. (1993). Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes. *Mol Endocrinol*, Vol.7, No.11, pp. 1418-1429.

Smith D. F. (2004). Tetratricopeptide repeat cochaperones in steroid receptor complexes. *Cell Stress Chaperones*, Vol.9, No.2, pp. 109-121.

Smith D. F. & Toft D. O. (2008). Minireview. The intersection of steroid receptors with molecular chaperones: observations and questions. *Mol Endocrinol*, Vol.22, No.10, pp. 2229-2240.

Sullivan W. P. & Toft D. O. (1993). Mutational analysis of hsp90 binding to the progesterone receptor. *J Biol Chem*, Vol.268, No.27, pp. 20373-20379.

Tai P. K., Albers M. W., Chang H., Faber L. E. & Schreiber S. L. (1992). Association of a 59-kilodalton immunophilin with the glucocorticoid receptor complex. *Science*, Vol.256, No.5061, pp. 1315-1318.

Takayama S., Bimston D. N., Matsuzawa S.-i., Freeman B. C., Aime-Sempe C., Xie Z., Morimoto R. I. & Reed J. C. (1997). BAG-1 modulates the chaperone activity of Hsp70/Hsc70. *EMBO J*, Vol.16, No.16, pp. 4887-4896.

Taylor P., Dornan J., Carrello A., Minchin R. F., Ratajczak T. & Walkinshaw M. D. (2001). Two structures of cyclophilin 40: folding and fidelity in the TPR domains. *Structure*, Vol.9, No.5, pp. 431-438.

Tomlins S. A., Mehra R., Rhodes D. R., Cao X., Wang L., Dhanasekaran S. M., Kalyana-Sundaram S., Wei J. T., Rubin M. A., Pienta K. J., Shah R. B. & Chinnaiyan A. M. (2007). Integrative molecular concept modeling of prostate cancer progression. *Nat Genet*, Vol.39, No.1, pp. 41-51.

Tranguch S., Cheung-Flynn J., Daikoku T., Prapapanich V., Cox M. B., Xie H., Wang H., Das S. K., Smith D. F. & Dey S. K. (2005). Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation. *PNAS*, Vol.102, No.40, pp. 14326-14331.

Vaughan C. K., Gohlke U., Sobott F., Good V. M., Ali M. M. U., Prodromou C., Robinson C. V., Saibil H. R. & Pearl L. H. (2006). Structure of an Hsp90-Cdc37-Cdk4 complex. *Mol Cell*, Vol.23, No.5, pp. 697-707.

Vaughan C. K., Mollapour M., Smith J. R., Truman A., Hu B., Good V. M., Panaretou B., Neckers L., Clarke P. A., Workman P., Piper P. W., Prodromou C. & Pearl L. H. (2008). Hsp90-dependent activation of protein kinases is regulated by chaperone-targeted dephosphorylation of Cdc37. *Mol Cell*, Vol.31, No.6, pp. 886-895.

Wang Z., Chen W., Kono E., Dang T. & Garabedian M. J. (2007). Modulation of glucocorticoid receptor phosphorylation and transcriptional activity by a C-terminal-associated protein phosphatase. *Mol Endocrinol*, Vol.21, No.3, pp. 625-634.

Ward B. K., Allan R. K., Mok D., Temple S. E., Taylor P., Dornan J., Mark P. J., Shaw D. J., Kumar P., Walkinshaw M. D. & Ratajczak T. (2002). A structure-based mutational analysis of cyclophilin 40 identifies key residues in the core tetratricopeptide repeat domain that mediate binding to Hsp90. *J Biol Chem*, Vol.277, No.43, pp. 40799-40809.

Whitesell L. & Lindquist S. L. (2005). Hsp90 and the chaperoning of cancer. *Nat Rev Cancer*, Vol.5, No.10, pp. 761-772.

Wochnik G. M., Young J. C., Schmidt U., Holsboer F., Hartl F. U. & Rein T. (2004). Inhibition of GR-mediated transcription by p23 requires interaction with Hsp90. *FEBS Lett*, Vol.560, 35-38.

Wu B., Li P., Liu Y., Lou Z., Ding Y., Shu C., Ye S., Bartlam M., Shen B. & Rao Z. (2004). 3D structure of human FK506-binding protein 52: implications for the assembly of the glucocorticoid receptor/Hsp90/immunophilin heterocomplex. *PNAS*, Vol.101, No.22, pp. 8348-8353.

Xu M., Dittmar K. D., Giannoukos G., Pratt W. B. & Simons Jr S. S. (1998). Binding of hsp90 to the glucocorticoid receptor requires a specific 7-amino acid sequence at the amino terminus if the hormone-binding domain. *J Biol Chem*, Vol.273, No.22, pp. 13918-13924.

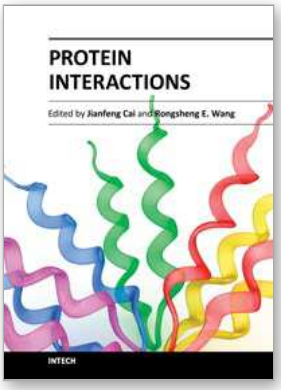
Yang J., Roe S. M., Cliff M. J., Williams M. A., Ladbury J. E., Cohen P. T. W. & Barford D. (2005). Molecular basis for TPR domain-mediated regulation of protein phosphatase 5. *EMBO J*, Vol.24, No.1, pp. 1-10.

Yang Z., Wolf I. M., Chen H., Periyasamy S., Chen Z., Yong W., Shi S., Zhao W., Xu J., Srivastava A., Sanchez E. R. & Shou W. (2006). FK506-binding protein 52 is

essential to uterine reproductive physiology controlled by the progesterone receptor A isoform. *Mol Endocrinol*, Vol.20, No.11, pp. 2682-2694.

Young E. T., Saario J., Kacherovsky N., Chao A., Sloan J. S. & Dombek K. M. (1998). Characterization of a p53-related activation domain in Adr1p that is sufficient for ADR1-dependent gene expression. *J Biol Chem*, Vol.273, No.48, pp. 32080-32087.

Zhang M., Windheim M., Roe S. M., Peggie M., Cohen P., Prodromou C. & Pearl L. H. (2005). Chaperoned ubiquitylation--crystal structures of the CHIP U Box E3 ubiquitin ligase and a CHIP-Ubc13-Uev1a complex. *Mol Cell*, Vol.20, No.4, pp. 525-538.



Protein Interactions

Edited by Dr. Jianfeng Cai

ISBN 978-953-51-0244-1
Hard cover, 464 pages
Publisher InTech
Published online 16, March, 2012
Published in print edition March, 2012

Protein interactions, which include interactions between proteins and other biomolecules, are essential to all aspects of biological processes, such as cell growth, differentiation, and apoptosis. Therefore, investigation and modulation of protein interactions are of significance as it not only reveals the mechanism governing cellular activity, but also leads to potential agents for the treatment of various diseases. The objective of this book is to highlight some of the latest approaches in the study of protein interactions, including modulation of protein interactions, development of analytical techniques, etc. Collectively they demonstrate the importance and the possibility for the further investigation and modulation of protein interactions as technology is evolving.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821