Chapter 2
Functions of the Hsp90-Binding FKBP Immunophilins

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Abstract Hsp90 functionally interacts with a broad array of client proteins, but in every case examined Hsp90 is accompanied by one or more co-chaperones. One class of co-chaperone contains a tetratricopeptide repeat domain that targets the co-chaperone to the C-terminal region of Hsp90. Within this class are Hsp90-binding peptidylprolyl isomerases, most of which belong to the FK506-binding protein (FKBP) family. Despite the common association of FKBP co-chaperones with Hsp90, it is now clear that the client protein influences, and is influenced by, the particular FKBP bound to Hsp90. Examples include Xap2 in aryl hydrocarbon receptor complexes and FKBP52 in steroid receptor complexes. In this chapter, we discuss the known functional roles played by FKBP co-chaperones and, where possible, relate distinctive functions to structural differences between FKBP members.

Keywords Immunophilin · FKBP · Hsp90 · Steroid hormone receptor

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Introduction

Immunophilins are a large, functionally diverse group of proteins that are defined by their ability to bind immunosuppressive ligands. The immunophilins minimally contain a peptidyl-prolyl cis-trans isomerase (PPIase; also termed rotamase) domain to which the immunosuppressive drugs bind. Early investigations into the PPIase enzymatic activity led to the belief that the immunosuppressive drugs elicited their effects by inhibiting the PPIase activity. However, some compounds binding the PPIase active site efficiently inhibit PPIase activity without inducing immunosuppression, so PPIase activity is not critical for immune responses. It is now known that effector domains on the immunosuppressive drugs project from the PPIase pocket. This allows the immunophilin-drug complex to bind tightly to and inhibit calcineurin or target of rapamycin, signal transduction proteins required for immune responses (see Hamilton and Steiner 1998 for a detailed review on the mechanisms by which immunophilins and their ligands suppress immune responses).

Since the initial identification of the immunophilin proteins, multiple family members have been identified in all major branches of life. Some immunophilins are small proteins containing only a single PPIase domain while others are large multidomain proteins that contain one or more PPIase domains, as well as additional functional domains. The immunophilins are divided into two groups based on their ability to bind different immunosuppressive ligands: the FK506 binding proteins (FKBP), which also bind rapamycin, and the cyclosporin-A binding proteins or cyclophilins (CyP). The PPIase domains of FKBP and cyclophilins are structurally distinct and likely evolved independently. On the other hand, some members of either the FKBP or cyclophilin families contain a structurally similar tetratricopeptide repeat (TPR) domain that targets binding to heat shock protein 90 (Hsp90).

Hsp90 is an abundant molecular chaperone that interacts with a broad array of protein clients that regulate numerous important cellular pathways. Among the known Hsp90 clients are transcription factors (e.g., steroid hormone receptors, heat shock transcription factor 1, aryl hydrocarbon receptor), both serine/threonine and tyrosine kinases (e.g., Raf and Src-related kinases), and key regulatory enzymes (e.g., nitric oxide synthase and telomerase). A compilation of known Hsp90 clients maintained by Didier Picard at Univ. of Geneva can be accessed at: http://www.picard.ch/downloads/Hsp90interactors.pdf.

In concert with other chaperone proteins, Hsp90 facilitates client folding and proteolytic stability but can also promote client degradation. In the case of steroid receptors, Hsp90 and its associated co-chaperones also regulate receptor activity. Hsp90 binding to steroid receptors must be preceded by transient receptor interactions with Hsp40, Hsp70, and associated co-chaperones. Hsp90, which is recruited as a dimer in the latter stages of complex assembly, binds directly to the receptor ligand binding domain and stabilizes a receptor conformation that is competent for hormone binding. Proteins that are associated with Hsp90 in the functionally mature

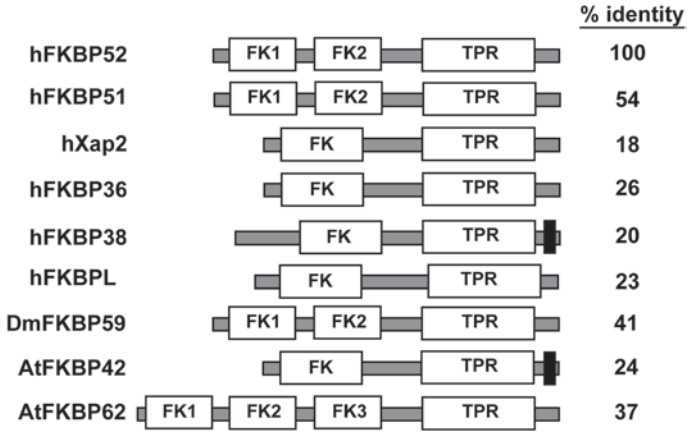

Fig. 2.1 Domain organization of representative Hsp90-binding TPR-containing FKBPs from vertebrate, insect, and plant sources were selected for comparison of domain organizations. The proteins are human FKBP52 (acc. # NP_002005), human FKBP51 (acc. # Q13451), human FKBPL (acc. # NP_071393.2), human Xap2 (acc. # O00170), human FKBP36 (acc. # NP_003593), human FKBP38 (acc. # NP_036313.3), *Drosophila melanogaster* FKBP59 (acc. # AAF18387), *Arabadopsis thaliana* FKBP42 (acc. # CAC00654), and *Arabadopsis thaliana* FKBP62 (acc. # AAB82062). The percent amino acid identity of each compared to human FKBP52 was determined from ClustalW2 alignments (http://www.ebi.ac.uk/clustalw). Each protein shown has at least one FKBP12-like domain (FK), which in some cases has peptidylprolyl isomerase activity and is the binding site for the immunosuppressant drug FK506, and one tetratricopeptide repeat domain (TPR), which is typically an Hsp90 binding site. The black box in the C-terminus of AtFKBP42 is a transmembrane domain used for anchoring the protein to the plasma and vacuolar membranes

receptor complex are p23, a co-chaperone that stabilizes Hsp90 binding to receptor, and any one of several TPR co-chaperones, including the immunophilin/PPIases FKBP52 (also termed p59, Hsp56, p50, HBI, FKBP59, and FKBP4), FKBP51 (also termed p54, FKBP54, and FKBP5), and CyP40, or the protein phosphatase PP5. As discussed below, receptor activity can vary depending on the particular TPR co-chaperone in mature receptor heterocomplexes.

The domain organization for several TPR co-chaperones is compared in Fig. 2.1. These co-chaperones compete for a common binding site in the C-terminal region of Hsp90 that includes the highly conserved -MEEVD sequence that terminates Hsp90. Co-crystallographic structures have shown how an MEEVD pentapeptide associates with the TPR binding pocket (Scheufler et al. 2000; Wu et al. 2004). Although the TPR domains for each of these co-chaperones are structurally similar and interact in a similar manner with Hsp90, the client protein bound by Hsp90 can influence the rank order of co-chaperone recruitment to Hsp90-client complexes (reviewed in Riggs et al. 2004). For instance, PP5 and FKBP51 are preferred components in glucocorticoid receptor (GR) complexes, FKBP51 is preferred in progesterone receptor (PR) complexes, and CyP40 is relatively enhanced in estrogen receptor (ER) complexes (Silverstein et al. 1997; Barent et al. 1998). On the other

hand, another TPR-containing FKBP, the hepatitis B virus protein X associated protein 2 (Xap2; also termed AIP, ARA9, and FKBP37) shows little interaction with steroid receptors but is strongly associated with the aryl hydrocarbon receptor-Hsp90 complex (Ma and Whitlock 1997; Meyer et al. 1998). The distinctive patterns of preference for co-chaperone association in client complexes is one line of evidence that the co-chaperones bound to Hsp90 can also interact with the Hsp90-bound client.

In addition to FKBP52, FKBP51, and XAP2, several other FKBP family members contain TPR domains that are known or likely to bind Hsp90. FKBP36 is structurally similar to XAP2 but is required for male fertility and homologous chromosome pairing in meiosis (Crackower et al. 2003). FKBP38 is a unique family member that is anchored to the mitochondrial and endoplasmic reticulum membranes, and is involved in a variety of processes including protein folding and trafficking, apoptosis, neural tube formation, cystic fibrosis transmembrane conductance regulator (CFTR) trafficking, and viral replication (reviewed in Edlich and Lucke 2011). FK506-binding protein like (FKBPL) protein is a divergent member of the FKBP family that can associate and functionally regulate steroid hormone receptors, has antiangiogenic properties, has a role in the DNA damage response, and controls tumor growth (reviewed in Robson and James 2012). *Drosophila melanogaster* express a TPR-containing immunophilin (DmFKBP59) that has high similarity to FKBP52/51 in vertebrates (Goel et al. 2001; Zaffran 2000). Plants have several FKBP genes that encode TPR domains; for example, in *Arabidopsis thaliana* there are 4 such genes: AtFKBP42, AtFKBP62, AtFKBP65 and AtFKBP72 (Romano et al. 2005; He et al. 2004). Although prokaryotic and Archaeal genomes also contain FKBP family members (Maruyama et al. 2004), none of these genes encode a TPR domain.

Structure/Function Relationships of Steroid Receptor-Associated FKBPs

X-ray crystallographic structures have been resolved for full-length FKBP51 and for overlapping fragments of FKBP52 (Fig. 2.2). FKBP51 and FKBP52 share greater than 60% amino acid sequence similarity, and individual domains do not differ markedly between FKBP51 and FKBP52. Both share a similar TPR domain composed of three tandem repeats of the degenerate 34-amino acid motif, which is a typical characteristic of TPR proteins (Blatch and Lassle 1999). Each repeat adopts a helix-turn-helix conformation and adjacent units stack in parallel to form a saddle-shaped domain with a concave binding pocket for Hsp90. In addition to the TPR domain, both FKBP51 and FKBP52 have two N-terminal domains, each of which is structurally similar to FKBP12. FK506-binding and PPIase activities reside in the most N-terminal domain (FK1), which has a pocket and active site residues similar to FKBP12. Due to several amino acid differences, the second domain (FK2) lacks drug binding and PPIase activity (Sinars et al. 2003).

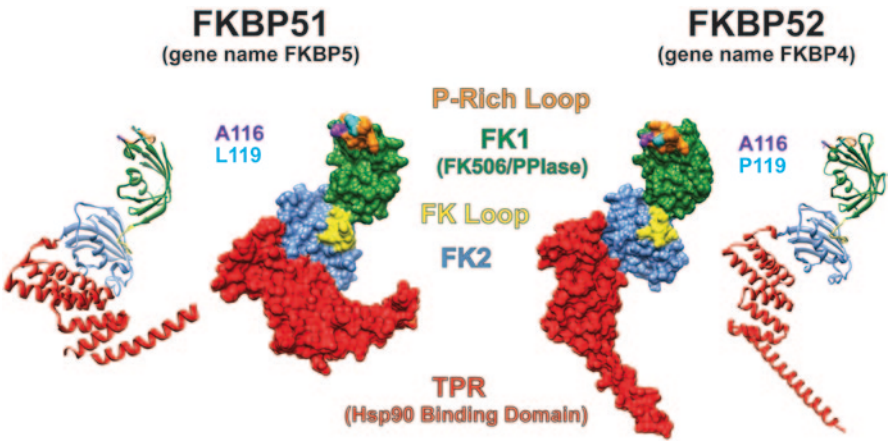

Fig. 2.2 Structural and functional characteristics of FKBP51 and FKBP52. Both ribbon and molecular surface depictions of the X-ray crystallographic structures for human FKBP51 (A; protein data bank number 1KT0) and a composite of two partial structures for human FKBP52 (B; protein data bank numbers 1Q1C and 1P5Q) are shown. In either protein the two FKBP12-like domains (FK1 and FK2, *green* and *blue* respectively) are indicated, the first of which has FK506 binding and PPIase activities. PPIase activity is not required for receptor regulation. The proline-rich loop (*orange*) that overhangs the PPIase catalytic pocket is critical for FKBP52 function and is responsible for the functional difference between FKBP51 and FKBP52. Two functionally critical residues (A116 and L119 in FKBP51 and A116 and P119 in FKBP52) within this loop are highlighted. The FK1 domain, the proline-rich loop in particular, is hypothesized to serve as an interaction surface within the Hsp90-receptor heterocomplex. A loop structure containing a CKII phosphorylation site in the hinge region between FK1 and FK2 is pointed out (*yellow*). The C-terminal TPR domain (*red*) consists of three helix-loop-helix motifs that form the Hsp90 binding pocket. Structures of the individual domains are highly similar between the two proteins, but the angle between FK2 and TPR domains of FKBP51 is more acute and probably more constrained than in FKBP52. The FKBP51 and FKBP52 structure models shown were constructed using UCSF Chimera version 1.5

The most striking difference in crystal structures relates to apparent domain:domain orientations. The FKBP52 structure shown in Fig. 2.2 is a composite model derived from merging the separate FK1-FK2 and FK2-TPR structures. The composite model suggests that the FKBP52 TPR domain is aligned in a more linear fashion with the FK domains rather than in the kinked conformation seen with FKBP51 (Fig. 2.2). In fact, the static orientations shown in crystal structures are likely more dynamic in solution, but the different crystal orientations are perhaps telling. Amino acid side chains unique to FKBP51 form a salt bridge between FK2 and TPR that would stabilize the domain:domain interaction in FKBP51 relative to FKBP52, which lacks this salt bridge. The apparently more malleable structure of FKBP52 might allow interactions within the receptor heterocomplex that are strained in FKBP51.

Significant progress has been made in understanding functionally important domains and residues on FKBP52 that contribute to the distinct ability to regulate steroid hormone receptor activity. Random mutagenesis studies in *S. cerevisiae*

demonstrated that two point mutations (A116V and L119P) in the FKBP51 FK1 domain, which does not potentiate steroid hormone receptor activity under normal conditions, confer full receptor potentiating ability to FKBP51, similar to that of FKBP52 (Riggs et al. 2007). This suggests that FKBP51 and FKBP52 functionally diverged at some point in evolution by only a few residues. A recent study suggests that there are differences in conformational dynamics between FKBP51 and FKBP52 within the proline-rich loop (Mustafi et al. 2014). 15N NMR relaxation measurements demonstrated that only the proline-rich loop in FKBP51 displays significantly larger line broadening, which is completely suppressed in the presence of the L11P mutation. These data suggest not only that differences in the proline-rich loop confer distinct functions to FKBP51 and FKBP52, but also that the proline-rich loop is functionally important for FKBP52 regulation of receptor activity. The current hypothesis holds that the FKBP52 proline-rich loop serves as an interaction surface, and the interaction partner is likely the receptor hormone binding domain (Sivils et al. 2011; De Leon et al. 2011).

Recent evidence by Bracher et al. demonstrate that the FK1-FK2 domains portray a flexible hinge that may account for regulatory differences between FKBP51 and FKBP52 (Bracher et al. 2013). It is hypothesized that the FK2 domain of FKBP52 contains an activation mechanism based on the calmodulin-binding motif at the C-terminus, yet this region is unable to bind FK506 and rapamycin, and lacks PPIase activity (Chambraud et al. 1993; Pirkl and Buchner 2001; Rouviere et al. 1997).

FKBP51 and FKBP52 also differ in the hinge region connecting FK1 and FK2 domains (FK loop). The FK loop of FKBP52 contains a -TEEED- sequence that has been identified as an *in vitro* substrate for casein kinase II; the corresponding sequence in FKBP51, -FED-, lacks the threonine phosphorylation site. Phosphorylation of FKBP52 is potentially important since the phospho-protein is reported to lose Hsp90 binding (Miyata et al. 1997). This difference was further tested using comparative analysis of FKBP51 and FKBP52 FK linker sequences (Cox et al. 2007). While the phosphomimetic mutation T143E had no effect on FKBP52 binding to Hsp90 in this study, the mutation did abrogate FKBP52 regulation of receptor activity. It is predicted that phosphorylation of residue T143 in the FKBP52 FK linker reorients the entire FK1 conformation, thereby eliminating FK1 interactions with the receptor hormone binding domain.

Cellular and Physiological Functions of Hsp90-Associated FKBPs

FKBP52

FKBP52 is expressed in most vertebrate tissues and cell lines, although its expression can be up-regulated by heat stress (Sanchez 1990), by estrogen in MCF-7 breast

cancer cells (Kumar et al. 2001), and by the homeobox transcription factor HoxA-10 in the peri-implantation mouse uterus (Daikoku et al. 2005). FKBP52 associates with steroid receptor complexes in an Hsp90-dependent manner, but FKBP52 is not required in a defined cell-free assembly system for receptor to reach the mature conformation that is competent for hormone binding (Dittmar et al. 1996; Kosano et al. 1998). Nonetheless, FKBP52 in cells potentiates hormone-dependent reporter gene activation by GR (Riggs et al. 2003), AR (Cheung-Flynn et al. 2005), and PR (Tranguch et al. 2005). Potentiation of hormone signaling can be related to an increase in receptor affinity for hormone (Riggs et al. 2003; Davies et al. 2005), but there may be additional mechanisms by which FKBP52 enhances receptor activity.

In concordance with hormone binding affinity changes, domain-swapping experiments between GR and ER, which is not potentiated by FKBP52, demonstrated that FKBP52 potentiation is localized to the ligand binding domain of GR (Riggs et al. 2003). FKBP52-dependent potentiation of receptor activity is abrogated in point mutants that are defective for Hsp90 binding, and potentiation is blocked by the PPIase inhibitor FK506 (Riggs et al. 2003; Cheung-Flynn et al. 2005). One model to explain these findings is that Hsp90 recruits FKBP52 to the receptor heterocomplex such that the FK1 PPIase can effectively catalyze isomerization of one or more proline substrates in the receptor ligand binding domain. However, studies have shown that point mutations within the FKBP52 PPIase pocket that eliminate PPIase activity have no effect on FKBP52 potentiation of receptor activity (Riggs et al. 2007). Thus, FK506-mediated inhibition of FKBP52 function likely occurs through the inhibition of FK1 interactions as opposed to inhibition of PPIase enzymatic activity. As discussed above, the FKBP52 FK1 domain as a whole is functionally important and the proline-rich loop that overhangs the PPIase pocket could serve as a functionally important interaction surface that contacts the receptor hormone binding domain within the receptor-chaperone heterocomplex. A structure-based screen for small molecules targeting an alternative surface of the androgen receptor hormone binding domain identified a series of fenamic acid molecules that allosterically affect coactivator binding at the activation function 2 (AF2) site through interaction with a surface cleft termed binding function 3 (BF3) (Estebanez-Perpina et al. 2007). Steroid hormone receptor structural comparisons identified this region to be a highly conserved regulatory surface that could serve as a therapeutic target for hormone-dependent diseases (Buzon et al. 2012). Interestingly, mutations within the AR BF3 surface (F673P, P723S, and C806Y) result in increased dependence on FKBP52 for function. In addition, a drug termed MJC13 that specifically inhibits FKBP52-regulated AR activity is predicted to target the BF3 surface (De Leon et al. 2011). Thus, the BF3 surface is a putative FKBP52 interaction and/or regulatory surface, and FKBP52 interaction with the receptor BF3 surface could allosterically affect receptor interactions at the AF2 site. In addition to the AR BF3 surface, recent studies suggest that the Helix 1–3 (H1-H3) loop in the GR LBD is an important site of FKBP regulation. Glucocorticoid insensitivity in guinea pig has been linked to sequence differences in the H1-H3 loop and substitution of the guinea pig H1-H3 loop into rat GR resulted in increased FKBP51-mediated repression of receptor activity. It is hypothesized that changes in the H1-H3 loop result in

changes within the GR-Hsp90 heterocomplex that favor FKBP51 repression over FKBP52 potentiation (Cluning et al. 2013).

FKBP52 has been shown by *in vitro* studies to have a chaperone activity that is independent of Hsp90 binding or PPIase (Bose et al. 1996; Pirkl and Buchner 2001). Like Hsp90 and numerous other chaperone components, FKBP52 can hold misfolded proteins in a non-aggregated state that is amenable to refolding. The possibility that chaperone holding activity displayed by FKBP52 plays some role in altering receptor activity cannot be dismissed, but this appears unlikely since holding activity is highly redundant among chaperone components. Furthermore, holding activity, unlike FKBP52-dependent potentiation of receptor activity, is neither PPIase- nor Hsp90-dependent. Unfortunately, no one has identified an FKBP52 mutation that disrupts holding activity in a discrete manner.

In an effort to extend biochemical and cellular data to the physiological level FKBP52 gene knockout (52KO) mice were generated, independently, by two groups (Cheung-Flynn et al. 2005; Yong et al. 2007). The mutant mice have striking reproductive phenotypes that can be attributed, at least in part, to loss of steroid receptor activity. Male 52KO mice are infertile and display abnormal virilization with persistent nipples, ambiguous external genitalia, and dysgenic seminal vesicles and prostate (Cheung-Flynn et al. 2005; Yong et al. 2007). These developmental defects are consistent with androgen insensitivity in these tissues. Testicular morphology, descent, histology, and spermatogenesis are normal and androgen production and release from testes is unimpaired; these developmental features are not highly androgen-dependent. On the other hand, sperm isolated from the epididymis have abnormal tail morphology and reduced motility suggestive of a defect in sperm maturation within the epididymis, a process that is androgen-dependent. Cellular studies confirm that FKBP52 is required for full AR function, which provides a rational explanation for androgen insensitivity in tissues of 52KO males.

52KO females have no gross morphological abnormalities, yet are completely infertile (Tranguch et al. 2005). Oocyte formation and release are not markedly impaired, and oocytes are competent for *in vitro* and *in vivo* fertilization. Infertility is due, at least in part, to a maternal failure of embryonic implantation and uterine decidualization. During the early stages of pregnancy, the 52KO uterus does not display the usual molecular or physiological markers for implantation. These events are largely dependent on progesterone actions, and both molecular and cellular studies confirm that FKBP52 is required for full PR activity. Additionally, FKBP52 is related to the etiology of endometriosis given that 52KO mice display increased endometrial lesions, inflammation, cell proliferation, and angiogenesis, and FKBP52 protein levels are reduced in human endometrial tissues (Hirota et al. 2008).

FKBP52 is critical for reproductive development and success in both male and female mice and its role can be traced to support of AR and PR function. Although GR-related phenotypes are not readily apparent, cellular and biochemical studies suggest that 52KO animals should display phenotypes related to reduced GR activity. Given that abnormal Mendelian ratios are not observed for heterozygous crosses,

the 52KO phenotype does include partial embryonic lethality. This combined with the reproductive defects leads to difficulty in obtaining sufficient numbers of 52KO animals for experiments. Thus, heterozygous fkbp52-deficient mice (52+/–) were generated to determine the *in vivo* roles for FKBP52 in GR-mediated physiology. 52+/– mice displayed phenotypes associated with reduced GR signaling including increased susceptibility to high-fat diet-induced hepatic steatosis, hyperglycemia, hyperinsulinemia, and behavioral alterations under basal and chronic stress conditions (Wadekar et al. 2004; Warrier et al. 2010).

Although FKBP52 does not alter ER function in cellular studies and 52KO mice show no signs of estrogen insensitivity, FKBP52 expression is upregulated by estrogens and FKBP52 is over-expressed in breast tumors (Ward et al. 1999). In addition, the FKBP52 gene is methylated in ER-negative, but not in ER-positive breast cancer cells (Ostrow et al. 2009). Thus, a few studies have identified FKBP52 as a potential regulator of at least ER expression in breast cancer.

Despite the fact that FKBP52 was initially discovered in the immune system, it is ubiquitously expressed and particularly abundant in the central nervous system. Thus, it is not surprising that FKBP52 is involved in neurodegenerative tauopathies including Alzheimer's (AD) and Pick's disease, fronto-temporal dementia and Parkinsonism linked to chromosome 17 (FTDP), and progressive supranuclear palsy (Haelens et al. 2007; Hernandez and Avila 2007). The defining neuropathological characteristic of tauopathies is the aberrant aggregation of insoluble hyperphosphorylated microtubule-associated protein (MAP) tau within the neurons, which is termed neurofibrillary tangles (NFTs) and is also referred to as paired helical filaments (PHF) (Cao and Konsolaki 2011). Recent studies have shown FKBP52's direct interaction with tau, particularly with its hyperphosphorylated form, has antagonistic effects on tubulin polymerization and microtubule assembly (Chambraud et al. 2007; Chambraud et al. 2010). In addition, FKBP52 was recently shown to induce Tau-P301L oligimerization and assembly into filaments (Giustiniani et al. 2014). More importantly, knockdown of FKBP52 was shown to restore axonal outgrowth and branching caused by Tau-P301L expression, thereby validating FKBP52 as an attractive therapeutic target in tauopathies. FKBP52 is known to be involved in subcellular rearrangement. Studies by Quintá et al. demonstrated that the overexpression of FKBP52 can induce neuronal differentiation and neurite outgrowth (Quintá et al. 2010).

Recent reports have shown that copper (Cu) contributes to the neuropathology of AD by interacting with copper binding domains of amyloid precursor proteins (APPs) and beta-amyloid (Aβ) peptides causing the formation of amyloid plaques and disrupting metal ion homeostasis (Barnham and Bush 2008; Drago et al. 2008; Kong et al. 2007). FKBP52 is involved in the regulation of cellular Cu homeostasis by interacting directly with the copper transport protein Atox1 (Sanokawa-Akakura et al. 2004), which is part of the Cu efflux machinery in neurons. In addition, both genetic and cellular data in Drosophila suggest a novel role for FKBP52 in the regulation of intracellular Cu homeostasis via binding to APP, thus, modulating the toxicity level of Aβ peptides (Sanokawa-Akakura et al. 2010).

S100A proteins belong to the EF-hand type calcium (Ca^{2+}) sensing protein family that are linked to regulation of various intracellular processes and are often expressed in a cell- and tissue-specific fashion (Santamaria-Kisiel et al. 2006; Wright et al. 2009). Based on biochemical evidence, it has been demonstrated that S100A1 and S100A6 interact with FKBP52 by competing with Hsp90 for the TPR domain in a Ca^{2+}-dependent manner (Shimamoto et al. 2010). Cellular data has linked S100A1s involvement in the neuronal cell dysfunction/death that occurs in AD by reducing APP expression and stabilizing the intracellular Ca^{2+} homeostasis (Zimmer et al. 2005). It seems that the function of FKBP52 can be regulated by Ca^{2+} homeostasis within the cell leading to effects on the phosphorylation of tau and pathology in AD. Interestingly, a Drosophila orthologue of FKBP52 termed FKBP59 was found to interact with the Ca^{2+} channel protein TRPL in photoreceptor cells and to influence Ca^{2+} influx (Goel et al. 2001). Subsequent studies revealed that FKBP52 similarly interacts with a subset of rat transient receptor potential channel (TRPC) proteins that form Ca^{2+} channels in the mammalian brain (Sinkins et al. 2004). The C-terminus of FKBP52 contains a predicted calmodulin binding domain, which enables the protein to bind to calmodulin-Sepharose in a Ca^{2+}-dependent manner, the biological function of which is still unknown (Silverstein et al. 1999).

Apart from the well-established roles of FKBP52 in steroid hormone receptor function, FKBP52, as with other Hsp90 co-chaperones, has been identified in a variety of client-Hsp90 heterocomplexes such as those containing kinases, aryl hydrocarbon receptor, and heat shock transcription factor; however, many of these interactions might reflect passive, transient association of the protein with Hsp90 and have no functional impact on client activity. FKBP52 is also linked to various Hsp90-independent interactions. Aside from the aforementioned Hsp90-independent interactors, FKBP52 has been found to interact directly with the interferon regulatory factor 4 (Mamane et al. 2000), which regulates gene expression in B and T lymphocytes, forms a complex with tyrosine kinase receptor RET51, which is involved in the development and maintenance of the nervous system (Fusco et al. 2010) and FKBP associated protein 48 (Chambraud et al. 1996), which influences proliferation of Jurkat T cells (Krummrei et al. 2003). Each of these interactions was found to be disrupted by FK506 and to target the FKBP52 PPIase domain to specific proline sites in each partner protein. Phenotypes potentially related to these interactions have not yet been assessed in 52KO mice. Not only does FKBP52 interact with proteins, but also FKBP52 is capable of directly binding adeno-associated virus DNA and regulating replication of the viral genome (Qing et al. 2001; Zhong et al. 2004). The relevant DNA binding site in FKBP52 has not been identified.

FKBP51

FKBP51/p54/FKBP54 was originally identified as a component of chicken PR complexes (Smith et al. 1990; Smith et al. 1993a; Smith et al. 1993b) and is now

known to assemble as an Hsp90 co-chaperone with all steroid receptors and other Hsp90-client complexes. FKBP51 is functionally similar in some ways to FKBP52; both have similar PPIase activity in the presence of model peptide substrates, both hold misfolded proteins in a folding competent state, and they compete for binding a common site on Hsp90 (Nair et al. 1997; Pirkl et al. 2001). As noted above, the overall structural similarity of these FKBPs is consistent with these shared functional properties, yet their distinct effects on steroid receptor activity belie these similarities. In addition to the aforementioned structural differences between FKBP51 and FKBP52, another distinction is that the FKBP51 gene is highly inducible by glucocorticoids, androgens, and progesterone (Baughman et al. 1995; Kester et al. 1997; Zhu et al. 2001; Yoshida et al. 2002; Vermeer et al. 2003; Hubler et al. 2003; Febbo et al. 2005).

FKBP51 acts as an inhibitor of GR, PR, and MR function excluding AR. The first indication of its inhibitory role came from studies by Scammell and colleagues of glucocorticoid resistance in New World primates (Reynolds et al. 1999; Denny et al. 2000). In squirrel monkeys, GR has a relatively low affinity for hormone yet the cloned monkey GR has an affinity similar to human GR *in vitro*. This observation led to a search for cellular factors in monkey cells that reduced GR binding affinity. A key factor identified was FKBP51, which is constitutively overexpressed in squirrel monkey cells as well as cells of other New World primates, all of which display some degree of glucocorticoid resistance. Human FKBP51 was also found to inhibit GR function but not to the degree of squirrel monkey FKBP51, which differs in amino acid sequence from its human counterpart at 15 of 457 amino acids. These differences are scattered fair evenly along the sequence, and mapping studies have shown that amino acid changes in several domains contribute to the more potent inhibitory actions of squirrel monkey FKBP51 (Denny et al. 2005). Crystal structures for both human and squirrel monkey FKBP51 have been solved (Sinars et al. 2003); although functionally relevant structural changes are not yet apparent, comparison of these structures should ultimately help to understand why inhibitory potencies differ. The function of FKBP51 is dichotomous with respect to regulation of the steroid hormone receptors. *In vitro* experiments have shown that overexpression of human FKBP51 reduces glucocorticoid binding affinity and nuclear translocation of GR which forms an ultra-short negative feedback loop for receptor activity (Wochnik et al. 2005). This model is in agreement with the aforementioned data from squirrel monkeys that have a general resistance to glucocorticoids even though they express GR that has the full potential to bind cortisol with high affinity. Another interesting possibility by which FKBP51 decreases overall GR signaling is by promoting nuclear translocation of the transcriptionally inactive β isoform of GR (Zhang et al. 2008). Interestingly, FKBP51 has an opposing effect on AR; it increases the receptor signaling in prostate cancer cells. Using both recombinant protein- and cell-based assays, Ni *et al*. demonstrated that FKBP51 stimulates chaperone complex association with AR, which further enhances AR ligand binding and androgen-dependent transcription and cell growth, resulting in an ultra-short positive feedback loop (Ni et al. 2010).

In a yeast model for studying functional interactions between steroid receptors and human FKBPs, FKBP51 does not inhibit the activity of GR; however, FKBP51 can effectively reverse the potentiation of GR activity conferred by FKBP52 (Riggs et al. 2003). Therefore, FKBP51 acts as an antagonist of FKBP52. FKBP51 has also been shown to inhibit PR function (Hubler et al. 2003), presumably through a similar inhibition of FKBP52-mediated potentiation. The mechanism by which FKBP51 antagonizes FKBP52's ability to enhance steroid receptor function is not understood. Other Hsp90-binding TPR proteins do not block FKBP52 actions, so it does not appear that competitive displacement of FKBP52 from receptor complexes by FKBP51 can fully account for antagonism. On the other hand, FKBP51 is known to preferentially associate with PR and GR complexes (Nair et al. 1997; Barent et al. 1998). Domain swapping studies indicate that the FK1 PPIase domain partially contributes to antagonism but sequences in the FK2 and TPR domain also play a role (Riggs et al. 2003; Denny et al. 2005).

Given that FKBP51 gene expression is inducible by some steroid hormones and FKBP51 can both activate and inhibit receptor function, one can reasonably speculate that FKBP51 serves as a cellular modulator of hormone responsiveness. In cells unexposed to hormone, FKBP52 actions would predominate and promote a robust response to hormone. As a consequence, FKBP51 levels would rise and partially desensitize cells to a secondary hormone exposure in most systems excluding AR-mediated prostate cancer cells. These effects can be demonstrated in cellular models, but the physiological importance of this mechanism must be established with animal models. Toward this goal, FKBP51 gene knockout (51KO) mice were generated. Homozygous mutant animals are grossly normal and reproductively viable, so FKBP51 does not appear to be critical in the same physiological processes as FKBP52. Nonetheless, modulatory actions of FKBP51 are relevant but subject to compensatory physiological mechanisms. Interestingly, double knockout of both FKBP51 and FKBP52 genes is embryonic lethal in mice, suggesting either that FKBP51 and FKBP52 have a critical, mutually redundant function or that FKBP51 and FKBP52 function in a common developmental pathway that requires the distinct actions of both immunophilins.

The hypothalamic-pituitary-adrenal (HPA) axis controls stress response and is associated with susceptibility to depression as well as antidepressant efficacy (Touma et al. 2011; O'Leary et al. 2011). The HPA axis is regulated via negative feedback of GR activity and FKBP51. GR resistance is conferred by the overexpression of FKBP51, which is associated with an impaired negative feedback mechanism (Denny et al. 2005). Polymorphisms in the FKBP5 gene are associated with an increased susceptibility for depression, an increased response to antidepressants, and an increased risk of posttraumatic stress disorder in response to adverse early life events (Binder et al. 2008; Binder et al. 2004). In addition, genotype-directed environment-induced gene programming through FKBP5 gene methylation was recently shown to mediate gene-childhood trauma interactions (Klengel et al. 2013). Recent studies have shown that FKBP51 is a modulator of the cortisol-HPA axis response to chronic stress and related psychiatric disorders (Hartmann et al. 2012; O'Leary et al. 2011; Tatro et al. 2009; Touma et al. 2011). Indeed, 51KO mice

displayed diminished physiological and neuroendocrine response to the adverse effects of chronic stress with fast recovery from acute stress episodes. The null mice also showed reduced adrenal gland weight and lower levels of basal corticosterone suggesting an enhanced sensitivity of GR due to the loss of FKBP51.

As aforementioned, aggregation of MAP tau into neurofibrillary tangles in neurons is the hallmark of tauopathies. *In vitro* studies demonstrated that PPIase activity of FKBP51 regulates and balances the phosphorylation state of tau for microtubule stabilization (Jinwal et al. 2010; Koren et al. 2011). Interestingly, knockdown of FKBP51 dramatically reduced tau levels while inhibiting its PPIase activity led to increased stability and accumulation of phosphorylated tau (Jinwal et al. 2010). In addition, overexpression of FKBP51 prevented tau clearance and produced oligomeric tau in the brain, facilitating its neurotoxicity (Blair et al. 2013; Jinwal et al. 2010). Studies by Blair et al. demonstrated that upregulation of FKBP51 expression is attributed to a decrease in FKBP5 methylation in which the process appears to be inversely proportional over time (Blair et al. 2013). This provides an explanation for the detection of increased FKBP51 protein levels in aged murine brains, and the manifestation of depression and cognitive deficits in AD patients.

Aside from its role in steroid receptor function, FKBP51 has been identified in a wide array of Hsp90-independent complexes. Biochemical and cellular studies have demonstrated that FKBP51 inhibits apoptosis in irradiated melanoma cells (Romano et al. 2010), promotes dephosphorylation of Akt and downregulation of the Akt pathway (Pei et al. 2009), and is associated with polymorphisms in *fkbp5* as seen in affective and anxiety disorders (Binder 2009). Furthermore, FKBP51 has been shown to regulate NFκB pathways. FKBP51 was identified (Bouwmeester et al. 2004) by a proteomic approach in complex with IKKα, one of the serine/threonine kinases that stimulates phosphorylation and degradation of the NFκB inhibitor IκB. Knockdown of FKBP51 expression was shown to inhibit IKKα activation and thereby block TNFα-induced activation of NFκB, which confirmed the functional significance of FKBP51 in IKKα complexes. Perhaps related to FKBP51-dependent regulation of NFκB pathways, overexpression of FKBP51 has been correlated (Giraudier et al. 2002) with idiopathic myelofibrosis, a rare clonal stem cell disorder. Experimental overexpression of FKBP51 was subsequently shown to stimulate NFκB activity and, as a consequence, to increase secretion of pro-fibrotic TGF-β1 (Komura et al. 2005). IKKα had previously been shown to be an Hsp90 client (Broemer et al. 2004), so it is possible that, analogous to steroid receptor complexes, FKBP51 assembles with IKKα as a heterocomplex with Hsp90. Whether FKBP51 Hsp90 binding or PPIase is required for regulation of IKKα has not been determined.

Cytoplasmic Transport

There is strong evidence that Hsp90-binding immunophilins play a key role in the subcellular relocalization of some transcription factors, the pioneer studies having

been performed with steroid receptors. In the absence of ligand, some members of the steroid-receptor family such as GR or MR reside primarily in the cytoplasm, whereas others such as ER or PR are mostly nuclear in a constitutive manner even in the absence of hormone. Regardless of their primary localization, receptors are constantly shuttling in a highly dynamic manner between the nucleus and the cytoplasm (Elbi et al. 2004; Galigniana et al. 2010a; Madan and DeFranco 1993). Therefore, the final localization of a given receptor under a certain biological condition is the resultant of the proper displacement of that dynamic equilibrium between both cellular compartments. Accordingly, the presence of hormone favors the import driven mechanism that results in the nuclear concentration of GR. Although some molecules can escape to the cytoplasm, they are transported back to the nucleus and vice versa, the opposite situation is also true when receptors are primarily cytoplasmic. In summary, the degree of cytoplasmic or nuclear localization reflects both the rate of nuclear import and the rate of nuclear export in a given moment (Galigniana et al. 2010a).

It has always been assumed that simple diffusion is the driving force for steroid hormone receptor movement. The classic model for receptor trafficking was posited several years ago (Dahmer et al. 1984) and supported the heuristic notion that the receptor-chaperone heterocomplex is dissociated immediately after steroid binding (a process usually referred to as 'transformation'). Therefore, transformation was originally thought to be a key cytoplasmic requirement to favor the release of the receptor from the cytoplasmic anchoring sites and to permit its consequent nuclear translocation. Today, the experimental evidence shows that rather than an early event in the molecular mechanism of activation of steroid receptors, transformation is a nuclear process (Galigniana et al. 2010a; Grossmann et al. 2012; Presman et al. 2014).

The original finding that some TPR-domain proteins such as FKBP52, CyP40 and the PPIase-like protein phosphatase PP5 are able to interact with the motor protein dynein (Galigniana et al. 2002), led to the idea that they may be involved in the retrotransport of the receptors. It was demonstrated that dynein is also present in the native GR/Hsp90/FKBP52 heterocomplex (Galigniana et al. 2001), and that such association is FKBP52-dependent via the peptidyl-prolyl isomerase domain of the immunophilin (Galigniana et al. 2001; Galigniana et al. 2010b). The enzymatic activity, however, is related neither to the protein-protein interaction nor to the molecular mechanism of transport. The disruption of such complex or the lack of expression of FKBP52 impairs (but not totally abolishes) the cytoplasmic transport of GR to the nucleus (Galigniana et al. 2001; Galigniana et al. 2010b; Tatro et al. 2009), such that the half-life for nuclear translocation is increased one order of magnitude under this abnormal situation (from 5 min to 40–50 min for GR). This means that the cytoplasmic retention of the receptor when the transportosome is inactivated is indeed a temporal event. Thus, it can be predicted that incubation times with steroid longer than 40–60 min will show the receptor in the nucleus anyway. A retrotransport delay may have physiological consequences when the biological response should be fast, for example, in stressing situations, and can be envisaged when travelling distances are long, such as in axons. In this case, GR

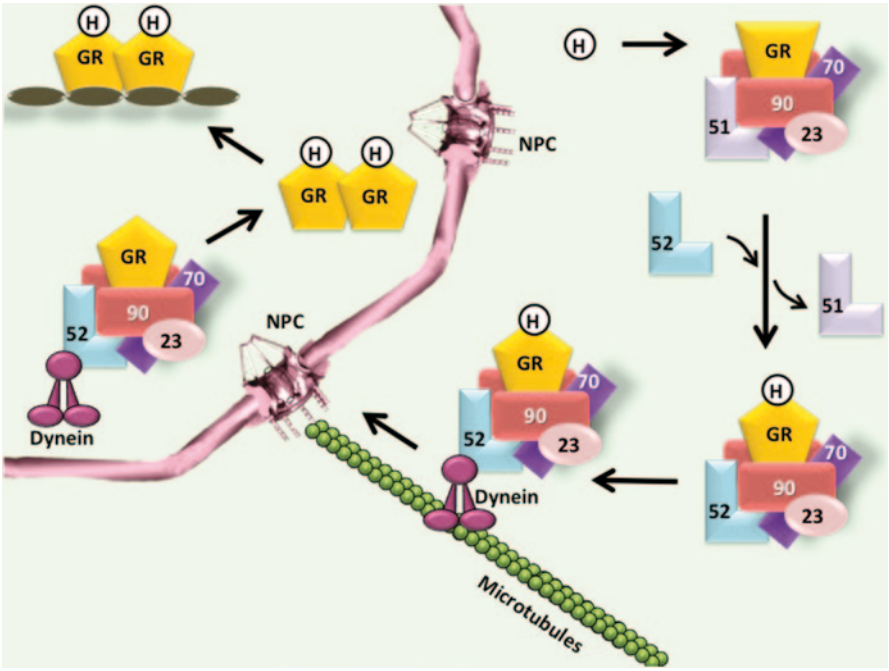

Fig. 2.3 Model of glucocorticoid receptor activation. In the absence of hormone (H), the GR exists in the cytoplasm associated with the Hsp90-based heterocomplex formed by a dimer of Hsp90, and one molecule of Hsp70, p23 and FKBP51. Upon steroid binding, FKBP51 is replaced by FKBP52, an immunophilin able to recruit the dynein/dynactin motor complex. The whole GR heterocomplex is retrotransported on microtubules tracks and translocates through the nuclear pore complex (NPC) to the nucleoplasm still associated to the heterocomplex. Transformation (i.e., Hsp90-complex dissociation) occurs in the nuclear compartment followed by receptor dimerization. The receptor is targeted to the promoter binding-sites to trigger the proper biological response and the heterocomplex is recycled

does not reach the nucleus because it is targeted to proteosomal degradation along its pathway (Galigniana et al. 2004a).

On the other hand, FKBP51, the highly homologous partner that shows low affinity for dynein motors (Wochnik et al. 2005; Galigniana et al. 2010b), acts as a competitive inhibitor of FKBP52. Therefore, it is not surprising that upon ligand binding FKBP51 is released from steroid receptor complexes and replaced by FKBP52 (Davies et al. 2002), which in turn recruits the dynein/dynactin motor complex (Fig. 2.3). In line with this fact, it has been proposed that the FKBP52/FKBP51 expression ratio may be one of the key regulatory factors for the nuclear retention of steroid receptors (Galigniana et al. 2010b; Tatro et al. 2009; Gallo et al. 2007).

It is possible that other TPR-domain immunophilins that are also able to interact with dynein, such as CyP40 and PP5, may replace FKBP52 in the transport machinery, although this has not been demonstrated. Nonetheless, recent evidence showed

that the Hsp90-binding immunophilin FKBPL/WISp39 also favors GR retrotransport in a similar fashion as FKBP52 (McKeen et al. 2008).

Importantly, the active Hsp90-, FKBP52-dependent mechanism for cytoplasmic transport first described for GR has also been found for other factors such as MR (Galigniana et al. 2010b), AR (Thomas et al. 2006), ecdysone receptor (Vafopoulou and Steel 2012), p53 (Galigniana et al. 2004b), RAC3 (Colo et al. 2008), and adeno-associated virus-2 (AAV) (Zhao et al. 2006). This immunophilin-dependent model for soluble protein trafficking implies that the proteins of the heterocomplex should remain associated to the client cargo during the passage through the nuclear pore complex. In line with this speculation, it was demonstrated that the whole Hsp90-FKBP52 heterocomplex cross-linked to corticosteroid receptors (Galigniana et al. 2010b; Echeverria et al. 2009) is able to translocate intact in a hormone-dependent manner through the nuclear pore of digitonin-permeabilized cells, suggesting that steroid-receptor transformation and its subsequent dimerization must be a nuclear event. This was recently confirmed by using different methodologies (Galigniana et al. 2010b; Grossmann et al. 2012; Presman et al. 2014; Presman et al. 2010).

Studies of reconstitution of the Hsp90-FKBP52 heterocomplex with purified proteins or reticulocyte lysate as a source of chaperones (Echeverria et al. 2009), demonstrated that the interaction of GR with structures of the nuclear pore such as nucleoporins (NUPs) is strengthened when both factors, GR and NUPs, are chaperoned. On the other hand, the discovery that NUPs are Hsp90- and FKBP52-interacting proteins also suggests a potential regulatory role of these chaperones for the nuclear import process. In this regard, it has always been very difficult to explain how single factors such as importins could shield the multitude of different protein-, RNA- and DNA-binding domains in transport cargoes that are import substrates. It could be speculated that these chaperones associated to importins, NUPs, and the cargo itself may act as a cooperative system to prevent aggregation of cargoes when a hydrophobic domain is exposed during the translocation step. This may justify why there is a more efficient interaction between NUPs and GR when both proteins are associated to the Hsp90-FKBP52 complex compared to both ‘naked’ proteins (Echeverria et al. 2009).

The association of FKBP52 and PP5 with Nup62 seems to be Hsp90-dependent, as was suggested by the almost-complete dissociation of these immunophilins from Nup62 in the presence of the Hsp90-disrupting agent radicicol (Echeverria et al. 2009). However, indirect immunofluorescence assays performed with intact cells treated with radicicol still show the presence of the immunophilins in the perinuclear ring, suggesting that they may also bind in an Hsp90-independent manner to other perinuclear structures. Nonetheless, competition experiments with the TPR domain overexpressed in intact cells showed that the perinuclear signal of FKBP52 was totally abolished, indicating that most, if not all, types of association of the immunophilin with any structure of the nuclear envelope require the TPR domain.

Xap2

Apart from the highly characterized steroid hormone receptor-associated FKBPs, several other TPR-containing FKBPs are present in higher vertebrates. As mentioned in earlier sections of this chapter, Xap2 is a TPR-containing immunophilin that is found almost extensively in AhR complexes. As the name implies, Xap2 also functionally interacts with the hepatitis B virus protein X (Kuzhandaivelu et al. 1996). Recently, Xap2 was shown to exert an inhibitory effect on both GR and ERα, but not ERβ activity, and may inhibit AR and PR as well (Cai et al. 2011; Laenger et al. 2009; Schulke et al. 2010). In addition, Xap2 is known to have functional interactions with peroxisome proliferator activated receptor α (PPARα) (Sumanasekera et al. 2003) and thyroid hormone receptor β, however, these interactions have not been extensively characterized. AhR is a ligand-dependent transcription factor that mediates the physiological response to specific environmental contaminants termed polycyclic aromatic hydrocarbons, the most notorious of which is 2,3,7,8-tetrachlorodibenzo-p-dioxin. Similar to steroid receptors, AhR requires assembly with Hsp90 and p23 to achieve a mature ligand-binding conformation (reviewed in Petrulis and Perdew 2002), although the AhR ligand binding domain is unrelated to steroid receptor ligand binding domains. AhR complexes also contain an FKBP component, but in this case it is Xap2 rather FKBP52 or FKBP51.

As with FKBP51 and FKBP52, Xap2 has a C-terminal TPR domain that is known to facilitate binding to the MEEVD motif on Hsp90 (Carver et al. 1998) (Fig. 2.1). In addition Xap2 contains one N-terminal FK domain that lacks drug binding and also likely lacks PPIase activity. Although the FK domain is not required for Hsp90 binding, it is required for an interaction with the AhR-Hsp90 complex that functionally influences receptor activity (Carver et al. 1998; Kazlauskas et al. 2002). In a cell-free assembly system that lacks Xap2, AhR is capable of assembling with Hsp90 and binding ligand, and upon ligand binding AhR is capable of binding AhR response elements on DNA (Meyer et al. 1998). Again, similar to FKBP52 or FKBP51 in steroid receptor complexes, Xap2 is not required for basal maturation of AhR activity, but in both yeast and mammalian systems, Xap2 can modulate AhR-mediated reporter gene expression (Miller 2002; Ma and Whitlock 1997; Meyer et al. 1998; Carver et al. 1998). By titrating the relative level of Xap2 protein in cells, AhR activity can be enhanced or decreased. For example, when Xap2 is expressed at a level 2- to 3-fold higher than normal, binding of p23 in the AhR-Hsp90 complex is reduced (Hollingshead et al. 2004). Displacement of p23 by high levels of Xap2 would destabilize binding of Hsp90 to AhR and reduce the proportion of AhR in functionally mature complexes. Conversely, there is also evidence that at elevated Xap2 levels, AhR is protected from ubiquitination and proteosomal degradation which would increase total AhR levels (Lees et al. 2003; LaPres et al. 2000; Meyer et al. 2000; Meyer and Perdew 1999; Kazlauskas et al. 2000). Finally, several studies suggest that Xap2 facilitates nucleocytoplasmic shuttling of AhR following ligand binding (Berg and Pongratz 2002; Petrulis et al. 2000; Kazlauskas et al. 2000; Kazlauskas et al. 2001; Petrulis et al. 2003).

The physiological relevance of Xap2 interactions with AhR complexes has not been examined in a whole animal model, but Xap2 could potentially influence any of several physiological and pathological pathways mediated by AhR. Mice that are homozygous for a disrupted AhR gene have many physiological and developmental defects; among these are immune system impairment, hepatic fibrosis, cardiac hypertrophy, impaired insulin regulation, and defects in ovarian and vascular development (Fernandez-Salguero et al. 1995; Lahvis et al. 2005; Thackaberry et al. 2003; Benedict et al. 2000). In addition, many of the toxic and teratogenic effects produced by AhR ligands require an intact AhR signaling pathway (Mimura and Fujii-Kuriyama 2003; Fernandez-Salguero et al. 1996). For example, dioxin induced defects in prostate development are absent in AhR knockout mice (Lin et al. 2002). In a conditional Xap2 hepatic knockout mouse model, AhR and Cyp1b1 levels were significantly reduced, however Cyp1a1 and Cyp1a2 were induced to levels seen in wild type mice in response to dioxin challenge (Nukaya et al. 2010). Development of a mouse strain lacking Xap2 would aid in determining the role Xap2 plays in these processes and might validate Xap2 as a potential target for therapeutic intervention. In addition to the above functional interactions, Xap2 has several other interacting partners including, but not limited to, PDE4A5 and 2A3, HSC70, TIF-2, TRβ1, RET, and TOMM20; thereby modulating a host of physiological functions (Reviewed in Trivellin and Korbonits 2011).

FKBP36

FKBP36 (gene name *FKBP6* in humans) is another TPR-containing FKBP that is structurally similar to Xap2, yet functionally distinct. FKBP36 has a single N-terminal FK domain and a C-terminal TPR domain. *In vitro* studies show that FKBP36 binds Hsp90 and can assemble with steroid receptor complexes (unpublished observation), but there is currently no evidence that FKBP36 alters receptor activity. FKBP36 mRNA is broadly expressed in vertebrate tissues with an exceptionally high level observed in the testis; male *FKBP6* knockout mice lack sperm and FKBP36 was shown to be a critical component in meiotic synaptonemal complexes (Crackower et al. 2003). FKBP36 interacts with and inhibits GAPDH activity and expression (Jarczowski et al. 2009). FKBP36 forms a complex with Hsp90 and GAPDH and this complex may regulate GAPDH activity in a manner akin to FKBP/Hsp90/ steroid receptor complexes (Jarczowski et al. 2009). FKBP36 can exert an effect on GAPDH in an Hsp90 independent manner by either directly inhibiting NAD^+ binding to GAPDH or by decreasing GAPDH expression (Jarczowski et al. 2009). Patients with Williams syndrome, which is characterized by congenital cardiovascular defects, dysmorphic facial features, mental retardation, growth defects, azoospermia, and hypercalcemia, are typically haploinsufficient for *FKBP6* (Meng et al. 1998); however, the contribution of

FKBP6 deletion in this syndrome is not clear since several contiguous genes on chromosome 11, including genes for elastin and LIM-Kinase 1, are also deleted in these patients and clearly contribute to some phenotypic aspects.

FKBP38

FKBP38 (gene name *FKBP8*) contains a glutamate-rich domain, FK domain, three TPR domains, and a calmodulin-binding motif. FKBP38 is ubiquitously expressed in all tissues, with high expression in neuronal tissues. Among the FKBP family, FKBP38 is novel in several respects, including a unique C-terminal transmembrane anchor domain, used to localize FKBP38 to both the mitochondrial and ER membranes. Although FKBP38 contains a PPIase domain, PPIase activity is regulated. The structure of the PPIase domain is similar to the prototypical family member, FKBP12; however, there are important differences in the three-dimensional structure of the loop and the binding pocket of the active site (Maestre-Martinez et al. 2006; Kay 1996). The loss of several aromatic residues in the active site leads to lower PPIase activity, even upon activation, and low affinity for FK506 (Maestre-Martinez et al. 2006; Edlich et al. 2006). FKBP38 PPIase activation is dependent on the calmodulin-binding domain and calmodulin/Ca^{2+} binding stimulates PPIase activity (Edlich et al. 2005; Edlich et al. 2007b; Maestre-Martinez et al. 2010).

FKBP38 participates in a number of cellular processes involving protein folding and trafficking, apoptosis, neural tube formation, CFTR trafficking, and viral replication (Edlich and Lucke 2011; Banasavadi-Siddegowda et al. 2011). FKBP38 interacts with the anti-apoptotic proteins Bcl-2 in regulating apoptosis and appears to have both pro- and anti-apoptotic activity that is likely tissue specific (Shirane and Nakayama 2004). In general, FKBP38 anti-apoptotic activity appears to regulate apoptosis by transporting Bcl-2 to the mitochondrial membrane stabilizing Bcl-2 and inhibiting apoptosis (Shirane and Nakayama 2004). Two mechanisms on how FKBP38 protects Bcl-2 from degradation have been explored. One involves the interaction between FKBP38 and a caspase cleavage site located within Bcl-2 (Choi et al. 2010). When FKBP38 is associated with Bcl-2 access to the caspase cleavage site may be blocked, preventing caspase-mediated Bcl-2 degradation (Choi et al. 2010). The second mechanism is through an interaction between the S4 subunit of the 19S proteasome complex, thereby regulating proteasome activity. However, in neuroblastoma cells the active FKBP38/calmodulin/Ca^{2+} complex has a pro-apoptotic affect by interfering with the ability of Bcl-2 to interact with and block pro-apoptotic proteins (Edlich et al. 2005). In this case, an interaction between Hsp90 and the FKB38/calmodulin/Ca^{2+} complex interferes with FKP38 pro-apoptotic activity, which could impede apoptosis (Edlich et al. 2007a).

FKBP38 is also implicated in the regulation of mTOR signaling through an interaction with Rheb (Rosner et al. 2003). mTOR regulates a wide range of cellular processes, including cell cycle and cell growth, in response to various conditions,

including fluctuations in nutrient and energy levels, and growth factors (Yang and Guan 2007). The FKBP12/rapamycin complex interacts with and inhibits mTOR activity (Brown et al. 1994). However, FKBP38 interacts with and antagonizes mTOR in a rapamycin-independent manner (Bai et al. 2007). Overexpression of FKBP38 decreases the induction of mTOR-regulated genes, and siRNA-induced reduction of FKBP38 increased mTOR activity (Bai et al. 2007). Rheb disrupts the mTOR/FKBP38 complex by binding to FKBP38 in a nutrient-dependent manner leading to an induction of mTOR-responsive genes (Bai et al. 2007).

FKBP38 is also involved in neural tube formation as the loss of FKBP38 leads to gross abnormalities during embryonic formation of the nervous system (Wong et al. 2008). It has been speculated that this is due to deregulation of the Sonic hedgehog (SHH) pathway during neural tube formation, where FKBP38 is a SHH antagonist, and the loss of FKBP38 function leads to over activity of SHH during development resulting in neuronal malformation (Cho et al. 2008).

In addition to the regulatory role in response to nutritional conditions, FKBP38 is also involved in the cellular response to hypoxia. Hypoxia-inducible transcription factors (HIFs) are involved in the cellular response to low oxygen levels, and, under normal conditions, are quickly degraded by prolyl-4-hydroxylase (PDH) enzymes (Wenger et al. 2005). FKBP38 interacts with PHD2 at the endoplasmic reticulum and mitochondrial membranes, and regulates PDH2 activity through proteasomal degradation, thereby regulating HIF stability and downstream gene expression in response to hypoxic conditions (Barth et al. 2009).

FKBP38 is involved in CFTR synthesis and folding by negatively regulating CFTR synthesis and positively regulating folding (Banasavadi-Siddegowda et al. 2011). Knockdown of FKBP38 increased CFTR production, but reduced post-translational modification, resulting in a lower expression of functional CFTR (Banasavadi-Siddegowda et al. 2011). Interestingly, FKBP38 PPIase activity is required for the regulation of CFTR folding.

Finally, FKBP38 is required for replication of the hepatitis C virus (HCV). In HCV infection the viral nonstructural protein 5A (NS5A) has been shown to form a complex with FKBP38 and Hsp90 at the mitochondrial and endoplasmic reticulum membranes (Wang et al. 2006). Either knockdown of FKBP38 with siRNA or inhibition of Hsp90 activity with geldanamycin results in decreased HCV RNA replication (Okada et al. 2004).

FKBPL

FKBPL shares the same general structure as other members of the FKBP family, including a TPR domain that facilitates Hsp90 binding and a PPIase domain, which lacks catalytic activity (Robson et al. 1999; Sunnotel et al. 2010). FKBPL was initially discovered while screening for genes that were protective against ionizing radiation (Robson et al. 1997; Robson et al. 1999). FKBPL is most closely related

to the larger FKBP52 (26% identity) (Robson and James 2012). However, the PPIase domain only shares 17% identity with the FKBP52 PPIase region (Robson and James 2012). The FKBPL TPR domain shares 33% amino acid identity with FKBP52 and has the ability to interact with Hsp90 stabilizing steroid hormone receptor conformations as well as stabilizing newly synthesized p21 preventing its degradation (Robson and James 2012; Jascur et al. 2005). There is conflicting data on FKBPL and its role in conferring radiation resistance. Jascur *et al.* originally showed that, in response to high-dose radiation, the FKBPL/Hsp90/p21 complex stabilized p21 leading to G2 cell cycle arrest, which conferred a pro-survival effect. However, more recent data has demonstrated that there is a down-regulation of p21 in response to radiation exposure and decreased p21 was involved in pro-survival after radiation exposer (Chu et al. 2004; Robson et al. 1999; Robson et al. 2000). In addition to radiation resistance, FKBPL plays a significant role in tumor progression (Robson et al. 1997; Robson et al. 1999; Robson et al. 2000; Jascur et al. 2005). In tumor cells, FKBPL appears to participate in not only growth of the tumor, but also in the sensitivity of the tumor to various chemotherapeutic agents (Bublik et al. 2010). For example, high levels of GSTE-1 interact with the FKBPL/Hsp90/p21 complex, which leads to p21 stabilization leading to resistance to the chemotherapeutic agent Taxane (Bublik et al. 2010). Although the exact radio- and chemo-protective role of FKBPL needs to be elucidated, the data clearly show that FKBPL is an important factor in cell-cycle progression, cell survival, and tumor progression.

Like other Hsp90-associated FKBP proteins, FKBPL also forms complexes with various steroid hormone receptors (reviewed in Erlejman et al. 2014). FKBPL and Hsp90 appear to stabilize AR, ER, and GR/Hsp90 complexes (Sunnotel et al. 2010; McKeen et al. 2008; McKeen et al. 2010). Similar to FKBP52, FKBPL affects the AR-dependent expression of prostate-specific antigen (Sunnotel et al. 2010). Sunnotel *et al.* demonstrated that two populations of azoospermic males had alterations in their FKBPL gene, which may alter FKBPL interaction with AR and contribute to infertility in the two populations. FKBPL was also shown to colocalize with the GR/Hsp90 complex (McKeen et al. 2008). Dexamethasone treatment resulted in the colocalization of FKBPL and GR in the nucleus and the up-regulation of GR-response genes in a prostate cancer cell line (McKeen et al. 2008). Translocation of the FKBPL/GR complex appears to be mediated by an interaction with dynamitin motor proteins, similar to the mechanism described for FKBP52 (McKeen et al. 2008).

FKBPL expression is regulated by estrogen and FKBPL functionally interacts with the ER/Hsp90 complex (McKeen et al. 2010). In addition, FKBPL expression correlates with breast cancer tumor growth as FKBPL and ER expression are inversely related; increased FKBPL levels lead to decreased ER expression (McKeen et al. 2010; Abukhdeir et al. 2008). Overexpression of FKBPL is associated with increased survival of untreated breast cancer patients and sensitizes cancer cells to the anti-proliferative effect of both tamoxifen and fulvestrant, which promotes increased recurrence-free survival (McKeen et al. 2011;

Han et al. 2006). Interestingly, overexpression of related FKBP proteins in tumors is associated with a poor treatment outcome and prognosis (Romano et al. 2010; Solassol et al. 2011). Conversely, increased levels of FKBPL correlate to a more positive response to treatment and a more favorable prognosis (McKeen et al. 2010; McKeen et al. 2011; Han et al. 2006). FKBPL stability is regulated by RBCK1, and as with FKBPL, RBCK1 is up-regulated by estrogen and can interact with the FKBPL/ER/Hsp90 complex (Donley et al. 2013). Increased expression of both FKBPL and RBCK1 appear to correlate with increased survival; however, elevated RBCK1 levels reduce the efficacy of tamoxifen (Donley et al. 2013). The interactions leading to tumor survival and progression still need to be explored further.

Finally, FKBPL possesses anti-angiogenic properties (Yakkundi et al. 2013). In a mouse xenograft tumor model overexpression of FKBPL resulted in decreased tumor growth and tumor necrosis (Crabb et al. 2009). The anti-angiogenic effects of FKBPL are mediated through the N-terminal portion of the protein comprised of amino acids 34–58, termed peptide AD-01, which is currently being explored as a novel anti-angiogenic drug (Valentine et al. 2011; Yakkundi et al. 2013).

Plant FKBPs

Hsp90-binding TPR immunophilins have been identified in all eukaryotes examined. A few examples of plant TPR-containing FKBPs are shown in Fig. 2.1. The TPR domain of each FKBP is very similar in amino acid sequence to that of vertebrate proteins; these are presumed to bind Hsp90, but that has not been determined in all cases. The plant and insect FKBPs contain one or more PPIase-related domain and can contain other functional domains. For example, AtFKBP42 contains a C-terminal transmembrane domain that localizes the protein to the inner plasma membrane and the vacuolar membrane (Kamphausen et al. 2002; Geisler et al. 2003; Geisler et al. 2004).

There is ample evidence to suggest that the plant and insect FKBPs are physiologically important. Mutations in AtFKBP42 cause the severe developmental phenotypes termed twisted dwarf 1 (TWD) (Geisler et al. 2003) and ultracurvata (UCU2) (Perez-Perez et al. 2004). The mechanism by which these phenotypes occur likely involves impairment of membrane transport of the growth hormone auxin, as AtFKBP42 is known to interact with several ATP-binding cassette transporters on the plasma and vacuolar membranes (Geisler et al. 2004; Geisler et al. 2003; Liu et al. 2001). Mutations in AtFKBP72 result in a class of mutants termed pasticcino or pas mutants, which are characterized by a wide variety of developmental defects (Vittorioso et al. 1998). Two Hsp90-binding TPR FKBPs in wheat, wFKBP72 and the heat shock-inducible wFKBP77, have been shown in transgenic plants to distinctively influence developmental patterns (Kurek et al. 2002).

Summary

In addressing the physiological importance of PPIases, Heitman and colleagues (Dolinski et al. 1997) generated an *S. cerevisiae* strain that lacked all 12 PPIase genes in the FKBP and cyclophilin families; the pluri-mutant strain displayed some growth abnormalities but was viable, thus demonstrating that these genes collectively are non-essential in yeast. Nonetheless, it has become increasingly clear that the Hsp90-binding FKBP immunophilins, through interactions with steroid receptors, kinases, and other cellular factors, play important physiological and pathological roles in mammals. Significant progress has been made on the elucidation of these roles and the definition of underlying molecular mechanisms. The identification of specific inhibitors will likely quicken in the coming few years and lead to therapeutic targeting of individual Hsp90-associated FKBP immunophilins for the treatment of a variety of human diseases.

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References

Abukhdeir AM, Vitolo MI, Argani P, De Marzo AM, Karakas B, Konishi H, Gustin JP, Lauring J, Garay JP, Pendleton C, Konishi Y, Blair BG, Brenner K, Garrett-Mayer E, Carraway H, Bachman KE, Park BH (2008) Tamoxifen-stimulated growth of breast cancer due to p21 loss. Proc Natl Acad Sci U S A 105(1):288–293

Bai X, Ma D, Liu A, Shen X, Wang QJ, Liu Y, Jiang Y (2007) Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. Science 318(5852):977–980

Banasavadi-Siddegowda YK, Mai J, Fan Y, Bhattacharya S, Giovannucci DR, Sanchez ER, Fischer G, Wang X (2011) FKBP38 peptidylprolyl isomerase promotes the folding of cystic fibrosis transmembrane conductance regulator in the endoplasmic reticulum. J Biol Chem 286(50):43071–43080

Barent RL, Nair SC, Carr DC, Ruan Y, Rimerman RA, Fulton J, Zhang Y, Smith DF (1998) Analysis of FKBP51/FKBP52 chimeras and mutants for Hsp90 binding and association with progesterone receptor complexes. Mol Endocrinol 12(3):342–354

Barnham KJ, Bush AI (2008) Metals in Alzheimer's and Parkinson's diseases. Curr Opin Chem Biol 12(2):222–228. doi:10.1016/j.cbpa.2008.02.019

Barth S, Edlich F, Berchner-Pfannschmidt U, Gneuss S, Jahreis G, Hasgall PA, Fandrey J, Wenger RH, Camenisch G (2009) Hypoxia-inducible factor prolyl-4-hydroxylase PHD2 protein abundance depends on integral membrane anchoring of FKBP38. J Biol Chem 284(34):23046–23058

Baughman G, Wiederrecht GJ, Campbell NF, Martin MM, Bourgeois S (1995) FKBP51, a novel T-cell-specific immunophilin capable of calcineurin inhibition. Mol Cell Biol 15(8):4395–4402

Benedict JC, Lin TM, Loeffler IK, Peterson RE, Flaws JA (2000) Physiological role of the aryl hydrocarbon receptor in mouse ovary development. Toxicol Sci 56(2):382–388

Berg P, Pongratz I (2002) Two parallel pathways mediate cytoplasmic localization of the dioxin (aryl hydrocarbon) receptor. J Biol Chem 277(35):32310–32319

Binder EB (2009) The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology 34(Suppl 1):S186–195. doi:10.1016/j.psyneuen.2009.05.021

Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Kunzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Kohnlein O, Dabitz H, Bruckl T, Muller N, Pfister H, Lieb R, Mueller JC, Lohmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B (2004) Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nat Genet 36(12):1319–1325

Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA 299(11):1291–1305

Blair LJ, Nordhues BA, Hill SE, Scaglione KM, O'Leary JC 3rd, Fontaine SN, Breydo L, Zhang B, Li P, Wang L, Cotman C, Paulson HL, Muschol M, Uversky VN, Klengel T, Binder EB, Kayed R, Golde TE, Berchtold N, Dickey CA (2013) Accelerated neurodegeneration through chaperone-mediated oligomerization of tau. J Clin Invest 123(10):4158–4169. doi:10.1172/JCI69003

Blatch GL, Lassle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21(11):932–939

Bose S, Weikl T, Bugl H, Buchner J (1996) Chaperone function of Hsp90-associated proteins. Science 274(5293):1715–1717

Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, Cruciat C, Eberhard D, Gagneur J, Ghidelli S, Hopf C, Huhse B, Mangano R, Michon AM, Schirle M, Schlegl J, Schwab M, Stein MA, Bauer A, Casari G, Drewes G, Gavin AC, Jackson DB, Joberty G, Neubauer G, Rick J, Kuster B, Superti-Furga G (2004) A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. Nat Cell Biol 6(2):97–105

Bracher A, Kozany C, Hahle A, Wild P, Zacharias M, Hausch F (2013) Crystal structures of the free and ligand-bound FK1-FK2 domain segment of FKBP52 reveal a flexible inter-domain hinge. J Mol Biol 425(22):4134–4144

Broemer M, Krappmann D, Scheidereit C (2004) Requirement of Hsp90 activity for IkappaB kinase (IKK) biosynthesis and for constitutive and inducible IKK and NF-kappaB activation. Oncogene 23(31):5378–5386

Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature 369(6483):756–758

Bublik DR, Scolz M, Triolo G, Monte M, Schneider C (2010) Human GTSE-1 regulates p21(CIP1/WAF1) stability conferring resistance to paclitaxel treatment. J Biol Chem 285(8):5274–5281

Buzon V, Carbo LR, Estruch SB, Fletterick RJ, Estebanez-Perpina E (2012) A conserved surface on the ligand binding domain of nuclear receptors for allosteric control. Mol Cell Endocrinol 348(2):394–402

Cai W, Kramarova TV, Berg P, Korbonits M, Pongratz I (2011) The immunophilin-like protein XAP2 is a negative regulator of estrogen signaling through interaction with estrogen receptor alpha. PLoS One 6(10):e25201

Cao W, Konsolaki M (2011) FKBP immunophilins and Alzheimer's disease: a chaperoned affair. J Biosci 36(3):493–498

Carver LA, LaPres JJ, Jain S, Dunham EE, Bradfield CA (1998) Characterization of the Ah receptor-associated protein, ARA9. J Biol Chem 273(50):33580–33587

Chambraud B, Rouviere-Fourmy N, Radanyi C, Hsiao K, Peattie DA, Livingston DJ, Baulieu EE (1993) Overexpression of p59-HBI (FKBP59), full length and domains, and characterization of PPlase activity. Biochem Biophys Res Commun 196(1):160–166

Chambraud B, Radanyi C, Camonis JH, Shazand K, Rajkowski K, Baulieu EE (1996) FAP48, a new protein that forms specific complexes with both immunophilins FKBP59 and FKBP12. Prevention by the immunosuppressant drugs FK506 and rapamycin. J Biol Chem 271(51):32923–32929

Chambraud B, Belabes H, Fontaine-Lenoir V, Fellous A, Baulieu EE (2007) The immunophilin FKBP52 specifically binds to tubulin and prevents microtubule formation. FASEB J 21(11):2787–2797. doi:10.1096/fj.06-7667com

Chambraud B, Sardin E, Giustiniani J, Dounane O, Schumacher M, Goedert M, Baulieu EE (2010) A role for FKBP52 in Tau protein function. Proc Natl Acad Sci U S A 107(6):2658–2663

Cheung-Flynn J, Prapapanich V, Cox MB, Riggs DL, Suarez-Quian C, Smith DF (2005) Physiological role for the cochaperone FKBP52 in androgen receptor signaling. Mol Endocrinol 19(6):1654–1666

Cho A, Ko HW, Eggenschwiler JT (2008) FKBP8 cell-autonomously controls neural tube patterning through a Gli2- and Kif3a-dependent mechanism. Dev Biol 321(1):27–39

Choi BH, Feng L, Yoon HS (2010) FKBP38 protects Bcl-2 from caspase-dependent degradation. J Biol Chem 285(13):9770–9779

Chu K, Teele N, Dewey MW, Albright N, Dewey WC (2004) Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3sigma and CDKN1A (p21) knockout cell lines. Radiat Res 162(3):270–286

Cluning C, Ward BK, Rea SL, Arulpragasam A, Fuller PJ, Ratajczak T (2013) The helix 1–3 loop in the glucocorticoid receptor LBD is a regulatory element for FKBP cochaperones. Mol Endocrinol 27(7):1020–1035

Colo GP, Rubio MF, Nojek IM, Werbajh SE, Echeverria PC, Alvarado CV, Nahmod VE, Galigniana MD, Costas MA (2008) The p160 nuclear receptor co-activator RAC3 exerts an anti-apoptotic role through a cytoplasmatic action. Oncogene 27(17):2430–2444

Cox MB, Riggs DL, Hessling M, Schumacher F, Buchner J, Smith DF (2007) FK506-binding protein 52 phosphorylation: a potential mechanism for regulating steroid hormone receptor activity. Mol Endocrinol 21(12):2956–2967

Crabb SJ, Patsios D, Sauerbrei E, Ellis PM, Arnold A, Goss G, Leighl NB, Shepherd FA, Powers J, Seymour L, Laurie SA (2009) Tumor cavitation: impact on objective response evaluation in trials of angiogenesis inhibitors in non-small-cell lung cancer. J Clin Oncol 27(3):404–410

Crackower MA, Kolas NK, Noguchi J, Sarao R, Kikuchi K, Kaneko H, Kobayashi E, Kawai Y, Kozieradzki I, Landers R, Mo R, Hui CC, Nieves E, Cohen PE, Osborne LR, Wada T, Kunieda T, Moens PB, Penninger JM (2003) Essential role of Fkbp6 in male fertility and homologous chromosome pairing in meiosis. Science 300(5623):1291–1295

Dahmer MK, Housley PR, Pratt WB (1984) Effects of molybdate and endogenous inhibitors on steroid-receptor inactivation, transformation, and translocation. Annu Rev Physiol 46:67–81.

Daikoku T, Tranguch S, Friedman DB, Das SK, Smith DF, Dey SK (2005) Proteomic analysis identifies immunophilin FK506 binding protein 4 (FKBP52) as a downstream target of Hoxa10 in the periimplantation mouse uterus. Mol Endocrinol 19(3):683–697

Davies TH, Ning YM, Sanchez ER (2002) A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem 277(7):4597–4600

Davies TH, Ning YM, Sanchez ER (2005) Differential control of glucocorticoid receptor hormone-binding function by tetratricopeptide repeat (TPR) proteins and the immunosuppressive ligand FK506. Biochemistry 44(6):2030–2038

De Leon JT, Iwai A, Feau C, Garcia Y, Balsiger HA, Storer CL, Suro RM, Garza KM, Lee S, Kim YS, Chen Y, Ning YM, Riggs DL, Fletterick RJ, Guy RK, Trepel JB, Neckers LM, Cox MB (2011) Targeting the regulation of androgen receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells. Proc Natl Acad Sci U S A 108(29):11878–11883. doi:10.1073/pnas.1105160108

Denny WB, Valentine DL, Reynolds PD, Smith DF, Scammell JG (2000) Squirrel monkey immunophilin FKBP51 is a potent inhibitor of glucocorticoid receptor binding. Endocrinology 141(11):4107–4113

Denny WB, Prapapanich V, Smith DF, Scammell JG (2005) Structure-function analysis of squirrel monkey FK506-binding protein 51, a potent inhibitor of glucocorticoid receptor activity. Endocrinology 146(7):3194–3201

Dittmar KD, Hutchison KA, Owens-Grillo JK, Pratt WB (1996) Reconstitution of the steroid receptor hsp90 heterocomplex assembly system of rabbit reticulocyte lysate. J Biol Chem 271(22):12833–12839

Dolinski K, Muir S, Cardenas M, Heitman J (1997) All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 94(24):13093–13098

Donley C, McClelland K, McKeen HD, Nelson L, Yakkundi A, Jithesh PV, Burrows J, McClements L, Valentine A, Prise KM, McCarthy HO, Robson T (2013) Identification of RBCK1 as a novel regulator of FKBPL: implications for tumor growth and response to tamoxifen. Oncogene 5(10):306

Drago D, Bolognin S, Zatta P (2008) Role of metal ions in the abeta oligomerization in Alzheimer's disease and in other neurological disorders. Curr Alzheimer Res 5(6):500–507

Echeverria PC, Mazaira G, Erlejman A, Gomez-Sanchez C, Piwien Pilipuk G, Galigniana MD (2009) Nuclear import of the glucocorticoid receptor-hsp90 complex through the nuclear pore complex is mediated by its interaction with Nup62 and importin beta. Mol Cell Biol 29(17):4788–4797

Edlich F, Lucke C (2011) From cell death to viral replication: the diverse functions of the membrane-associated FKBP38. Curr Opin Pharmacol 11(4):348–353

Edlich F, Weiwad M, Erdmann F, Fanghanel J, Jarczowski F, Rahfeld JU, Fischer G (2005) Bcl-2 regulator FKBP38 is activated by Ca2+/calmodulin. EMBO J 24(14):2688–2699

Edlich F, Weiwad M, Wildemann D, Jarczowski F, Kilka S, Moutty MC, Jahreis G, Lucke C, Schmidt W, Striggow F, Fischer G (2006) The specific FKBP38 inhibitor N-(N′,N′-dimethylcarboxamidomethyl)cycloheximide has potent neuroprotective and neurotrophic properties in brain ischemia. J Biol Chem 281(21):14961–14970

Edlich F, Erdmann F, Jarczowski F, Moutty MC, Weiwad M, Fischer G (2007a) The Bcl-2 regulator FKBP38-calmodulin-Ca2+ is inhibited by Hsp90. J Biol Chem 282(21):15341–15348

Edlich F, Maestre-Martinez M, Jarczowski F, Weiwad M, Moutty MC, Malesevic M, Jahreis G, Fischer G, Lucke C (2007b) A novel calmodulin-Ca2+ target recognition activates the Bcl-2 regulator FKBP38. J Biol Chem 282(50):36496–36504

Elbi C, Walker DA, Romero G, Sullivan WP, Toft DO, Hager GL, DeFranco DB (2004) Molecular chaperones function as steroid receptor nuclear mobility factors. Proc Natl Acad Sci U S A 101(9):2876–2881

Erlejman AG, Lagadari M, Toneatto J, Piwien-Pilipuk G, Galigniana MD (2014) Regulatory role of the 90-kDa-heat-shock protein (Hsp90) and associated factors on gene expression. Biochim Biophys Acta 1839(2):71–87

Estebanez-Perpina E, Arnold LA, Nguyen P, Rodrigues ED, Mar E, Bateman R, Pallai P, Shokat KM, Baxter JD, Guy RK, Webb P, Fletterick RJ (2007) A surface on the androgen receptor that allosterically regulates coactivator binding. Proc Natl Acad Sci U S A 104(41):16074–16079

Febbo PG, Lowenberg M, Thorner AR, Brown M, Loda M, Golub TR (2005) Androgen mediated regulation and functional implications of fkbp51 expression in prostate cancer. J Urol 173(5):1772–1777

Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science 268(5211):722–726

Fernandez-Salguero PM, Hilbert DM, Rudikoff S, Ward JM, Gonzalez FJ (1996) Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. Toxicol Appl Pharmacol 140(1):173–179

Fusco D, Vargiolu M, Vidone M, Mariani E, Pennisi LF, Bonora E, Capellari S, Dirnberger D, Baumeister R, Martinelli P, Romeo G (2010) The RET51/FKBP52 complex and its involvement in Parkinson disease. Hum Mol Genet 19(14):2804–2816. doi:10.1093/hmg/ddq181

Galigniana MD, Radanyi C, Renoir JM, Housley PR, Pratt WB (2001) Evidence that the peptidylprolyl isomerase domain of the hsp90-binding immunophilin FKBP52 is involved in both dynein interaction and glucocorticoid receptor movement to the nucleus. J Biol Chem 276(18):14884–14889

Galigniana MD, Harrell JM, Murphy PJ, Chinkers M, Radanyi C, Renoir JM, Zhang M, Pratt WB (2002) Binding of hsp90-associated immunophilins to cytoplasmic dynein: direct binding and *in vivo* evidence that the peptidylprolyl isomerase domain is a dynein interaction domain. Biochemistry 41(46):13602–13610

Galigniana MD, Harrell JM, Housley PR, Patterson C, Fisher SK, Pratt WB (2004a) Retrograde transport of the glucocorticoid receptor in neurites requires dynamic assembly of complexes with the protein chaperone hsp90 and is linked to the CHIP component of the machinery for proteasomal degradation. Brain Res Mol Brain Res 123(1–2):27–36

Galigniana MD, Harrell JM, O'Hagen HM, Ljungman M, Pratt WB (2004b) Hsp90-binding immunophilins link p53 to dynein during p53 transport to the nucleus. J Biol Chem 279(21):22483–22489

Galigniana MD, Echeverria PC, Erlejman AG, Piwien-Pilipuk G (2010a) Role of molecular chaperones and TPR-domain proteins in the cytoplasmic transport of steroid receptors and their passage through the nuclear pore. Nucleus 1(4):299–308

Galigniana MD, Erlejman AG, Monte M, Gomez-Sanchez C, Piwien-Pilipuk G (2010b) The hsp90-FKBP52 complex links the mineralocorticoid receptor to motor proteins and persists bound to the receptor in early nuclear events. Mol Cell Biol 30(5):1285–1298

Gallo LI, Ghini AA, Pilipuk GP, Galigniana MD (2007) Differential recruitment of tetratricorpeptide repeat domain immunophilins to the mineralocorticoid receptor influences both heat-shock protein 90-dependent retrotransport and hormone-dependent transcriptional activity. Biochemistry 46(49):14044–14057

Geisler M, Kolukisaoglu HU, Bouchard R, Billion K, Berger J, Saal B, Frangne N, Koncz-Kalman Z, Koncz C, Dudler R, Blakeslee JJ, Murphy AS, Martinoia E, Schulz B (2003) TWISTED DWARF1, a unique plasma membrane-anchored immunophilin-like protein, interacts with Arabidopsis multidrug resistance-like transporters AtPGP1 and AtPGP19. Mol Biol Cell 14(10):4238–4249

Geisler M, Girin M, Brandt S, Vincenzetti V, Plaza S, Paris N, Kobae Y, Maeshima M, Billion K, Kolukisaoglu UH, Schulz B, Martinoia E (2004) Arabidopsis immunophilin-like TWD1 functionally interacts with vacuolar ABC transporters. Mol Biol Cell 15(7):3393–3405

Giraudier S, Chagraoui H, Komura E, Barnache S, Blanchet B, LeCouedic JP, Smith DF, Larbret F, Taksin AL, Moreau-Gachelin F, Casadevall N, Tulliez M, Hulin A, Debili N, Vainchenker W (2002) Overexpression of FKBP51 in idiopathic myelofibrosis regulates the growth factor independence of megakaryocyte progenitors. Blood 100(8):2932–2940

Giustiniani J, Chambraud B, Sardin E, Dounane O, Guillemeau K, Nakatani H, Paquet D, Kamah A, Landrieu I, Lippens G, Baulieu EE, Tawk M (2014) Immunophilin FKBP52 induces Tau-P301L filamentous assembly *in vitro* and modulates its activity in a model of tauopathy. Proc Natl Acad Sci U S A 111(12):4584–4589

Goel M, Garcia R, Estacion M, Schilling WP (2001) Regulation of drosophila trpl channels by immunophilin fkbp59. J Biol Chem 276(42):38762–38773

Grossmann C, Ruhs S, Langenbruch L, Mildenberger S, Stratz N, Schumann K, Gekle M (2012) Nuclear shuttling precedes dimerization in mineralocorticoid receptor signaling. Chem Biol 19(6):742–751

Haelens A, Tanner T, Denayer S, Callewaert L, Claessens F (2007) The hinge region regulates DNA binding, nuclear translocation, and transactivation of the androgen receptor. Cancer Res 67(9):4514–4523. doi:10.1158/0008-5472.CAN-06-1701

Hamilton GS, Steiner JP (1998) Immunophilins: beyond immunosuppression. J Med Chem 41(26):5119–5143

Han W, Han MR, Kang JJ, Bae JY, Lee JH, Bae YJ, Lee JE, Shin HJ, Hwang KT, Hwang SE, Kim SW, Noh DY (2006) Genomic alterations identified by array comparative genomic hybridization as prognostic markers in tamoxifen-treated estrogen receptor-positive breast cancer. BMC Cancer 6(92):92

Hartmann J, Wagner KV, Liebl C, Scharf SH, Wang XD, Wolf M, Hausch F, Rein T, Schmidt U, Touma C, Cheung-Flynn J, Cox MB, Smith DF, Holsboer F, Muller MB, Schmidt MV (2012) The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress. Neuropharmacology 62(1):332–339. doi:10.1016/j.neuropharm.2011.07.041

He Z, Li L, Luan S (2004) Immunophilins and parvulins. Superfamily of peptidyl prolyl isomerases in Arabidopsis. Plant Physiol 134(4):1248–1267

Hernandez F, Avila J (2007) Tauopathies. Cell Mol Life Sci 64(17):2219–2233. doi:10.1007/s00018-007-7220-x

Hirota Y, Tranguch S, Daikoku T, Hasegawa A, Osuga Y, Taketani Y, Dey SK (2008) Deficiency of immunophilin FKBP52 promotes endometriosis. Am J Pathol 173(6):1747–1757. doi:10.2353/ajpath.2008.080527

Hollingshead BD, Petrulis JR, Perdew GH (2004) The aryl hydrocarbon (Ah) receptor transcriptional regulator hepatitis B virus X-associated protein 2 antagonizes p23 binding to Ah receptor-Hsp90 complexes and is dispensable for receptor function. J Biol Chem 279(44):45652–45661

Hubler TR, Denny WB, Valentine DL, Cheung-Flynn J, Smith DF, Scammell JG (2003) The FK506-binding immunophilin FKBP51 is transcriptionally regulated by progestin and attenuates progestin responsiveness. Endocrinology 144(6):2380–2387

Jarczowski F, Jahreis G, Erdmann F, Schierhorn A, Fischer G, Edlich F (2009) FKBP36 is an inherent multifunctional glyceraldehyde-3-phosphate dehydrogenase inhibitor. J Biol Chem 284(2):766–773

Jascur T, Brickner H, Salles-Passador I, Barbier V, El Khissiin A, Smith B, Fotedar R, Fotedar A (2005) Regulation of p21(WAF1/CIP1) stability by WISp39, a Hsp90 binding TPR protein. Mol Cell 17(2):237–249

Jinwal UK, Koren J III, Borysov SI, Schmid AB, Abisambra JF, Blair LJ, Johnson AG, Jones JR, Shults CL, O'Leary JC III, Jin Y, Buchner J, Cox MB, Dickey CA (2010) The Hsp90 cochaperone, FKBP51, increases Tau stability and polymerizes microtubules. J Neurosci 30(2):591–599

Kamphausen T, Fanghanel J, Neumann D, Schulz B, Rahfeld JU (2002) Characterization of Arabidopsis thaliana AtFKBP42 that is membrane-bound and interacts with Hsp90. Plant J 32(3):263–276

Kay JE (1996) Structure-function relationships in the FK506-binding protein (FKBP) family of peptidylprolyl cis-trans isomerases. Biochem J 314:361–385

Kazlauskas A, Poellinger L, Pongratz I (2000) The immunophilin-like protein XAP2 regulates ubiquitination and subcellular localization of the dioxin receptor. J Biol Chem 275(52):41317–41324

Kazlauskas A, Sundstrom S, Poellinger L, Pongratz I (2001) The hsp90 chaperone complex regulates intracellular localization of the dioxin receptor. Mol Cell Biol 21(7):2594–2607

Kazlauskas A, Poellinger L, Pongratz I (2002) Two distinct regions of the immunophilin-like protein XAP2 regulate dioxin receptor function and interaction with hsp90. J Biol Chem 277(14):11795–11801

Kester HA, van der Leede BM, van der Saag PT, van der Burg B (1997) Novel progesterone target genes identified by an improved differential display technique suggest that progestin-induced growth inhibition of breast cancer cells coincides with enhancement of differentiation. J Biol Chem 272(26):16637–16643

Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB (2013) Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci 16(1):33–41

Komura E, Tonetti C, Penard-Lacronique V, Chagraoui H, Lacout C, Lecouedic JP, Rameau P, Debili N, Vainchenker W, Giraudier S (2005) Role for the nuclear factor kappaB pathway in

transforming growth factor-beta1 production in idiopathic myelofibrosis: possible relationship with FK506 binding protein 51 overexpression. Cancer Res 65(8):3281–3289
Kong GK, Adams JJ, Cappai R, Parker MW (2007) Structure of Alzheimer's disease amyloid precursor protein copper-binding domain at atomic resolution. Acta Crystallogr Sect F Struct Biol Cryst Commun 63(Pt 10):819–824. doi:10.1107/S1744309107041139
Koren J 3rd, Jinwal UK, Davey Z, Kiray J, Arulselvam K, Dickey CA (2011) Bending tau into shape: the emerging role of peptidyl-prolyl isomerases in tauopathies. Mol Neurobiol 44(1):65–70. doi:10.1007/s12035-011-8182-4
Kosano H, Stensgard B, Charlesworth MC, McMahon N, Toft D (1998) The assembly of progesterone receptor-hsp90 complexes using purified proteins. J Biol Chem 273(49):32973–32979
Krummrei U, Baulieu EE, Chambraud B (2003) The FKBP-associated protein FAP48 is an antiproliferative molecule and a player in T cell activation that increases IL2 synthesis. Proc Natl Acad Sci U S A 100(5):2444–2449
Kumar P, Mark PJ, Ward BK, Minchin RF, Ratajczak T (2001) Estradiol-regulated expression of the immunophilins cyclophilin 40 and FKBP52 in MCF-7 breast cancer cells. Biochem Biophys Res Commun 284(1):219–225
Kurek I, Stoger E, Dulberger R, Christou P, Breiman A (2002) Overexpression of the wheat FK506-binding protein 73 (FKBP73) and the heat-induced wheat FKBP77 in transgenic wheat reveals different functions of the two isoforms. Transgenic Res 11(4):373–379
Kuzhandaivelu N, Cong YS, Inouye C, Yang WM, Seto E (1996) XAP2, a novel hepatitis B virus X-associated protein that inhibits X transactivation. Nucleic Acids Res 24(23):4741–4750
Laenger A, Lang-Rollin I, Kozany C, Zschocke J, Zimmermann N, Ruegg J, Holsboer F, Hausch F, Rein T (2009) XAP2 inhibits glucocorticoid receptor activity in mammalian cells. FEBS Lett 583(9):1493–1498
Lahvis GP, Pyzalski RW, Glover E, Pitot HC, McElwee MK, Bradfield CA (2005) The aryl hydrocarbon receptor is required for developmental closure of the ductus venosus in the neonatal mouse. Mol Pharmacol 67(3):714–720
LaPres JJ, Glover E, Dunham EE, Bunger MK, Bradfield CA (2000) ARA9 modifies agonist signaling through an increase in cytosolic aryl hydrocarbon receptor. J Biol Chem 275(9):6153–6159
Lees MJ, Peet DJ, Whitelaw ML (2003) Defining the role for XAP2 in stabilization of the dioxin receptor. J Biol Chem 278(38):35878–35888
Lin TM, Ko K, Moore RW, Simanainen U, Oberley TD, Peterson RE (2002) Effects of aryl hydrocarbon receptor null mutation and in utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on prostate and seminal vesicle development in C57BL/6 mice. Toxicol Sci 68(2):479–487
Liu G, Sanchez-Fernandez R, Li ZS, Rea PA (2001) Enhanced multispecificity of arabidopsis vacuolar multidrug resistance-associated protein-type ATP-binding cassette transporter, AtMRP2. J Biol Chem 276(12):8648–8656
Ma Q, Whitlock JP Jr (1997) A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Biol Chem 272(14):8878–8884
Madan AP, DeFranco DB (1993) Bidirectional transport of glucocorticoid receptors across the nuclear envelope. Proc Natl Acad Sci U S A 90(8):3588–3592
Maestre-Martinez M, Edlich F, Jarczowski F, Weiwad M, Fischer G, Lucke C (2006) Solution structure of the FK506-binding domain of human FKBP38. J Biomol NMR 34(3):197–202
Maestre-Martinez M, Haupt K, Edlich F, Jahreis G, Jarczowski F, Erdmann F, Fischer G, Lucke C (2010) New structural aspects of FKBP38 activation. Biol Chem 391(10):1157–1167
Mamane Y, Sharma S, Petropoulos L, Lin R, Hiscott J (2000) Posttranslational regulation of IRF-4 activity by the immunophilin FKBP52. Immunity 12(2):129–140
Maruyama T, Suzuki R, Furutani M (2004) Archaeal peptidyl prolyl cis-trans isomerases (PPIases) update 2004. Front Biosci 9:1680–1720
McKeen HD, McAlpine K, Valentine A, Quinn DJ, McClelland K, Byrne C, O'Rourke M, Young S, Scott CJ, McCarthy HO, Hirst DG, Robson T (2008) A novel FK506-like binding protein

interacts with the glucocorticoid receptor and regulates steroid receptor signaling. Endocrinology 149(11):5724–5734
McKeen HD, Byrne C, Jithesh PV, Donley C, Valentine A, Yakkundi A, O'Rourke M, Swanton C, McCarthy HO, Hirst DG, Robson T (2010) FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. Cancer Res 70(3):1090–1100
McKeen HD, Brennan DJ, Hegarty S, Lanigan F, Jirstrom K, Byrne C, Yakkundi A, McCarthy HO, Gallagher WM, Robson T (2011) The emerging role of FK506-binding proteins as cancer biomarkers: a focus on FKBPL. Biochem Soc Trans 39(2):663–668
Meng X, Lu X, Morris CA, Keating MT (1998) A novel human gene FKBP6 is deleted in Williams syndrome. Genomics 52(2):130–137
Meyer BK, Perdew GH (1999) Characterization of the AhR-hsp90-XAP2 core complex and the role of the immunophilin-related protein XAP2 in AhR stabilization. Biochemistry 38(28):8907–8917
Meyer BK, Pray-Grant MG, Vanden Heuvel JP, Perdew GH (1998) Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity. Mol Cell Biol 18(2):978–988
Meyer BK, Petrulis JR, Perdew GH (2000) Aryl hydrocarbon (Ah) receptor levels are selectively modulated by hsp90-associated immunophilin homolog XAP2. Cell Stress Chaperones 5(3):243–254
Miller CA (2002) Two tetratricopeptide repeat proteins facilitate human aryl hydrocarbon receptor signalling in yeast. Cell Signal 14(7):615–623
Mimura J, Fujii-Kuriyama Y (2003) Functional role of AhR in the expression of toxic effects by TCDD. Biochim Biophys Acta 1619(3):263–268
Miyata Y, Chambraud B, Radanyi C, Leclerc J, Lebeau MC, Renoir JM, Shirai R, Catelli MG, Yahara I, Baulieu EE (1997) Phosphorylation of the immunosuppressant FK506-binding protein FKBP52 by casein kinase II: regulation of HSP90-binding activity of FKBP52. Proc Natl Acad Sci U S A 94(26):14500–14505
Mustafi SM, LeMaster DM, Hernandez G (2014) Differential conformational dynamics in the closely homologous FK506-binding domains of FKBP51 and FKBP52. Biochem J 461(1):115–123
Nair SC, Rimerman RA, Toran EJ, Chen S, Prapapanich V, Butts RN, Smith DF (1997) Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. Mol Cell Biol 17(2):594–603
Ni L, Yang CS, Gioeli D, Frierson H, Toft DO, Paschal BM (2010) FKBP51 promotes assembly of the Hsp90 chaperone complex and regulates androgen receptor signaling in prostate cancer cells. Mol Cell Biol 30(5):1243–1253
Nukaya M, Lin BC, Glover E, Moran SM, Kennedy GD, Bradfield CA (2010) The aryl hydrocarbon receptor-interacting protein (AIP) is required for dioxin-induced hepatotoxicity but not for the induction of the Cyp1a1 and Cyp1a2 genes. J Biol Chem 285(46):35599–35605
Okada M, Hatakeyama T, Itoh H, Tokuta N, Tokumitsu H, Kobayashi R (2004) S100A1 is a novel molecular chaperone and a member of the Hsp70/Hsp90 multichaperone complex. J Biol Chem 279(6):4221–4233. doi:10.1074/jbc.M309014200
O'Leary JC 3rd, Dharia S, Blair LJ, Brady S, Johnson AG, Peters M, Cheung-Flynn J, Cox MB, de Erausquin G, Weeber EJ, Jinwal UK, Dickey CA (2011) A new anti-depressive strategy for the elderly: ablation of FKBP5/FKBP51. PLoS One 6(9):e24840. doi:10.1371/journal.pone.0024840
Ostrow KL, Park HL, Hoque MO, Kim MS, Liu J, Argani P, Westra W, Van Criekinge W, Sidransky D (2009) Pharmacologic unmasking of epigenetically silenced genes in breast cancer. Clin Cancer Res 15(4):1184–1191
Pei H, Li L, Fridley BL, Jenkins GD, Kalari KR, Lingle W, Petersen G, Lou Z, Wang L (2009) FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt. Cancer Cell 16(3):259–266

Perez-Perez JM, Ponce MR, Micol JL (2004) The ULTRACURVATA2 gene of Arabidopsis encodes an FK506-binding protein involved in auxin and brassinosteroid signaling. Plant Physiol 134(1):101–117

Petrulis JR, Perdew GH (2002) The role of chaperone proteins in the aryl hydrocarbon receptor core complex. Chem Biol Interact 141(1–2):25–40

Petrulis JR, Hord NG, Perdew GH (2000) Subcellular localization of the aryl hydrocarbon receptor is modulated by the immunophilin homolog hepatitis B virus X-associated protein 2. J Biol Chem 275(48):37448–37453

Petrulis JR, Kusnadi A, Ramadoss P, Hollingshead B, Perdew GH (2003) The hsp90 Co-chaperone XAP2 alters importin beta recognition of the bipartite nuclear localization signal of the Ah receptor and represses transcriptional activity. J Biol Chem 278(4):2677–2685

Pirkl F, Buchner J (2001) Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40. J Mol Biol 308(4):795–806

Pirkl F, Fischer E, Modrow S, Buchner J (2001) Localization of the chaperone domain of FKBP52. J Biol Chem 276(40):37034–37041

Presman DM, Alvarez LD, Levi V, Eduardo S, Digman MA, Marti MA, Veleiro AS, Burton G, Pecci A (2010) Insights on glucocorticoid receptor activity modulation through the binding of rigid steroids. PLoS One 5(10):e13279

Presman DM, Ogara MF, Stortz M, Alvarez LD, Pooley JR, Schiltz RL, Grontved L, Johnson TA, Mittelstadt PR, Ashwell JD, Ganesan S, Burton G, Levi V, Hager GL, Pecci A (2014) Live cell imaging unveils multiple domain requirements for *in vivo* dimerization of the glucocorticoid receptor. PLoS Biol 12(3):e1001813

Qing K, Hansen J, Weigel-Kelley KA, Tan M, Zhou S, Srivastava A (2001) Adeno-associated virus type 2-mediated gene transfer: role of cellular FKBP52 protein in transgene expression. J Virol 75(19):8968–8976

Quintá HR, Maschi D, Gomez-Sanchez C, Piwien Pilipuk G, Galigniana MD (2010) Subcellular rearrangement of hsp90-binding immunophilins accompanies neuronal differentiation and neurite outgrowth. J Neurochem 115:716–734

Reynolds PD, Ruan Y, Smith DF, Scammell JG (1999) Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51. J Clin Endocrinol Metab 84(2):663–669

Riggs DL, Roberts PJ, Chirillo SC, Cheung-Flynn J, Prapapanich V, Ratajczak T, Gaber R, Picard D, Smith DF (2003) The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling *in vivo*. EMBO J 22(5):1158–1167

Riggs DL, Cox MB, Cheung-Flynn J, Prapapanich V, Carrigan PE, Smith DF (2004) Functional specificity of co-chaperone interactions with Hsp90 client proteins. Crit Rev Biochem Mol Biol 39:279–295

Riggs DL, Cox MB, Tardif HL, Hessling M, Buchner J, Smith DF (2007) Noncatalytic role of the FKBP52 peptidyl-prolyl isomerase domain in the regulation of steroid hormone signaling. Mol Cell Biol 27(24):8658–8669

Robson T, James IF (2012) The therapeutic and diagnostic potential of FKBPL; a novel anticancer protein. Drug Discov Today 17(11–12):544–548

Robson TA, Lohrer H, Bailie JR, Hirst DG, Joiner MC, Arrand JE (1997) Gene regulation by low-dose ionizing radiation in a normal human lung epithelial cell line. Biochem Soc Trans 25(1):335–342

Robson T, Joiner MC, Wilson GD, McCullough W, Price ME, Logan I, Jones H, McKeown SR, Hirst DG (1999) A novel human stress response-related gene with a potential role in induced radioresistance. Radiat Res 152(5):451–461

Robson T, Price ME, Moore ML, Joiner MC, McKelvey-Martin VJ, McKeown SR, Hirst DG (2000) Increased repair and cell survival in cells treated with DIR1 antisense oligonucleotides: implications for induced radioresistance. Int J Radiat Biol 76(5):617–623

Romano P, Gray J, Horton P, Luan S (2005) Plant immunophilins: functional versatility beyond protein maturation. New Phytol 166(3):753–769

Romano S, D'Angelillo A, Pacelli R, Staibano S, De LE, Bisogni R, Eskelinen EL, Mascolo M, Cali G, Arra C, Romano MF (2010) Role of FK506-binding protein 51 in the control of apoptosis of irradiated melanoma cells. Cell Death Differ 17(1):145–157

Rosner M, Hofer K, Kubista M, Hengstschlager M (2003) Cell size regulation by the human TSC tumor suppressor proteins depends on PI3K and FKBP38. Oncogene 22(31):4786–4798

Rouviere N, Vincent M, Craescu CT, Gallay J (1997) Immunosuppressor binding to the immunophilin FKBP59 affects the local structural dynamics of a surface beta-strand: time-resolved fluorescence study. Biochemistry 36(24):7339–7352

Sanchez ER (1990) Hsp56: a novel heat shock protein associated with untransformed steroid receptor complexes. J Biol Chem 265(36):22067–22070

Sanokawa-Akakura R, Dai H, Akakura S, Weinstein D, Fajardo JE, Lang SE, Wadsworth S, Siekierka J, Birge RB (2004) A novel role for the immunophilin FKBP52 in copper transport. J Biol Chem 279(27):27845–27848

Sanokawa-Akakura R, Cao W, Allan K, Patel K, Ganesh A, Heiman G, Burke R, Kemp FW, Bogden JD, Camakaris J, Birge RB, Konsolaki M (2010) Control of Alzheimer's amyloid beta toxicity by the high molecular weight immunophilin FKBP52 and copper homeostasis in Drosophila. PLoS One 5(1):e8626. doi:10.1371/journal.pone.0008626

Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS (2006) Calcium-dependent and -independent interactions of the S100 protein family. Biochem J 396(2):201–214. doi:10.1042/BJ20060195

Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. Cell 101(2):199–210

Schulke JP, Wochnik GM, Lang-Rollin I, Gassen NC, Knapp RT, Berning B, Yassouridis A, Rein T (2010) Differential impact of tetratricopeptide repeat proteins on the steroid hormone receptors. PLoS One 5(7):e11717. doi:10.1371/journal.pone.0011717

Shimamoto S, Kubota Y, Tokumitsu H, Kobayashi R (2010) S100 proteins regulate the interaction of Hsp90 with Cyclophilin 40 and FKBP52 through their tetratricopeptide repeats. FEBS Lett 584(6):1119–1125

Shirane M, Nakayama KI (2004) Immunophilin FKBP38, an inherent inhibitor of calcineurin, targets Bcl-2 to mitochondria and inhibits apoptosis. Nippon Rinsho 62(2):405–412

Silverstein AM, Galigniana MD, Chen MS, Owens-Grillo JK, Chinkers M, Pratt WB (1997) Protein phosphatase 5 is a major component of glucocorticoid receptor hsp90 complexes with properties of an FK506-binding immunophilin. J Biol Chem 272(26):16224–16230

Silverstein AM, Galigniana MD, Kanelakis KC, Radanyi C, Renoir JM, Pratt WB (1999) Different regions of the immunophilin FKBP52 determine its association with the glucocorticoid receptor, hsp90, and cytoplasmic dynein. J Biol Chem 274(52):36980–36986 [In Process Citation]

Sinars CR, Cheung-Flynn J, Rimerman RA, Scammell JG, Smith DF, Clardy J (2003) Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes. Proc Natl Acad Sci U S A 100(3):868–873

Sinkins WG, Goel M, Estacion M, Schilling WP (2004) Association of immunophilins with mammalian TRPC channels. J Biol Chem 279(33):34521–34529

Sivils JC, Storer CL, Galigniana MD, Cox MB (2011) Regulation of steroid hormone receptor function by the 52-kDa FK506-binding protein (FKBP52). Curr Opin Pharmacol 19:19

Smith DF, Faber LE, Toft DO (1990) Purification of unactivated progesterone receptor and identification of novel receptor-associated proteins. J Biol Chem 265(7):3996–4003

Smith DF, Albers MW, Schreiber SL, Leach KL, Deibel MR Jr (1993a) FKBP54, a novel FK506-binding protein in avian progesterone receptor complexes and HeLa extracts. J Biol Chem 268(32):24270–24273

Smith DF, Baggenstoss BA, Marion TN, Rimerman RA (1993b) Two FKBP-related proteins are associated with progesterone receptor complexes. J Biol Chem 268(24):18365–18371

Solassol J, Mange A, Maudelonde T (2011) FKBP family proteins as promising new biomarkers for cancer. Curr Opin Pharmacol 11(4):320–325

Sumanasekera WK, Tien ES, Turpey R, Vanden Heuvel JP, Perdew GH (2003) Evidence that peroxisome proliferator-activated receptor alpha is complexed with the 90-kDa heat shock protein and the hepatitis virus B X-associated protein 2. J Biol Chem 278(7):4467–4473

Sunnotel O, Hiripi L, Lagan K, McDaid JR, De Leon JM, Miyagawa Y, Crowe H, Kaluskar S, Ward M, Scullion C, Campbell A, Downes CS, Hirst D, Barton D, Mocanu E, Tsujimura A, Cox MB, Robson T, Walsh CP (2010) Alterations in the steroid hormone receptor co-chaperone FKBPL are associated with male infertility: a case-control study. Reprod Biol Endocrinol 8(22):22

Tatro ET, Everall IP, Kaul M, Achim CL (2009) Modulation of glucocorticoid receptor nuclear translocation in neurons by immunophilins FKBP51 and FKBP52: implications for major depressive disorder. Brain Res 1286:1–12

Thackaberry EA, Bedrick EJ, Goens MB, Danielson L, Lund AK, Gabaldon D, Smith SM, Walker MK (2003) Insulin regulation in AhR-null mice: embryonic cardiac enlargement, neonatal macrosomia, and altered insulin regulation and response in pregnant and aging AhR-null females. Toxicol Sci 76(2):407–417

Thomas M, Harrell JM, Morishima Y, Peng HM, Pratt WB, Lieberman AP (2006) Pharmacologic and genetic inhibition of hsp90-dependent trafficking reduces aggregation and promotes degradation of the expanded glutamine androgen receptor without stress protein induction. Hum Mol Genet 15(11):1876–1883

Touma C, Gassen NC, Herrmann L, Cheung-Flynn J, Bull DR, Ionescu IA, Heinzmann JM, Knapman A, Siebertz A, Depping AM, Hartmann J, Hausch F, Schmidt MV, Holsboer F, Ising M, Cox MB, Schmidt U, Rein T (2011) FK506 binding protein 5 shapes stress responsiveness: modulation of neuroendocrine reactivity and coping behavior. Biol Psychiatry 70(10):928–936. doi:10.1016/j.biopsych.2011.07.023

Tranguch S, Cheung-Flynn J, Daikoku T, Prapapanich V, Cox MB, Xie H, Wang H, Das SK, Smith DF, Dey SK (2005) Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation. Proc Natl Acad Sci U S A 102(40):14326–14331

Trivellin G, Korbonits M (2011) AIP and its interacting partners. J Endocrinol 210(2):137–155

Vafopoulou X, Steel CG (2012) Cytoplasmic travels of the ecdysteroid receptor in target cells: pathways for both genomic and non-genomic actions. Front Endocrinol (Lausanne) 3(43):43

Valentine A, O'Rourke M, Yakkundi A, Worthington J, Hookham M, Bicknell R, McCarthy HO, McClelland K, McCallum L, Dyer H, McKeen H, Waugh DJ, Roberts J, McGregor J, Cotton G, James I, Harrison T, Hirst DG, Robson T (2011) FKBPL and peptide derivatives: novel biological agents that inhibit angiogenesis by a CD44-dependent mechanism. Clin Cancer Res 17(5):1044–1056

Vermeer H, Hendriks-Stegeman BI, van der Burg B, van Buul-Offers SC, Jansen M (2003) Glucocorticoid-induced increase in lymphocytic FKBP51 messenger ribonucleic acid expression: a potential marker for glucocorticoid sensitivity, potency, and bioavailability. J Clin Endocrinol Metab 88(1):277–284

Vittorioso P, Cowling R, Faure JD, Caboche M, Bellini C (1998) Mutation in the Arabidopsis PASTICCINO1 gene, which encodes a new FK506- binding protein-like protein, has a dramatic effect on plant development. Mol Cell Biol 18(5):3034–3043

Wadekar SA, Li D, Sanchez ER (2004) Agonist-activated glucocorticoid receptor inhibits binding of heat shock factor 1 to the heat shock protein 70 promoter *in vivo*. Mol Endocrinol 18(3):500–508

Wang J, Tong W, Zhang X, Chen L, Yi Z, Pan T, Hu Y, Xiang L, Yuan Z (2006) Hepatitis C virus non-structural protein NS5A interacts with FKBP38 and inhibits apoptosis in Huh7 hepatoma cells. FEBS Lett 580(18):4392–4400

Ward BK, Mark PJ, Ingram DM, Minchin RF, Ratajczak T (1999) Expression of the estrogen receptor-associated immunophilins, cyclophilin 40 and FKBP52, in breast cancer. Breast Cancer Res Treat 58(3):267–280

Warrier M, Hinds TD Jr, Ledford KJ, Cash HA, Patel PR, Bowman TA, Stechschulte LA, Yong W, Shou W, Najjar SM, Sanchez ER (2010) Susceptibility to diet-induced hepatic steatosis and glucocorticoid resistance in FK506-binding protein 52-deficient mice. Endocrinology 151(7):3225–3236

Wenger RH, Stiehl DP, Camenisch G (2005) Integration of oxygen signaling at the consensus HRE. Sci STKE 2005(306):re12

Wochnik GM, Ruegg J, Abel GA, Schmidt U, Holsboer F, Rein T (2005) FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. J Biol Chem 280(6):4609–4616

Wong RL, Wlodarczyk BJ, Min KS, Scott ML, Kartiko S, Yu W, Merriweather MY, Vogel P, Zambrowicz BP, Finnell RH (2008) Mouse Fkbp8 activity is required to inhibit cell death and establish dorso-ventral patterning in the posterior neural tube. Hum Mol Genet 17(4):587–601

Wright NT, Cannon BR, Zimmer DB, Weber DJ (2009) S100A1: structure, function, and therapeutic potential. Curr Chem Biol 3(2):138–145

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. Proc Natl Acad Sci U S A 101(22):8348–8353

Yakkundi A, McCallum L, O'Kane A, Dyer H, Worthington J, McKeen HD, McClements L, Elliott C, McCarthy HO, Hirst DG, Robson T (2013) The anti-migratory effects of FKBPL and its peptide derivative, AD-01: regulation of CD44 and the cytoskeletal pathway. PLoS One 8(2):e55075

Yang Q, Guan KL (2007) Expanding mTOR signaling. Cell Res 17(8):666–681

Yong W, Yang Z, Periyasamy S, Chen H, Yucel S, Li W, Lin LY, Wolf IM, Cohn MJ, Baskin LS, Sanchez ER, Shou W (2007) Essential role for co-chaperone Fkbp52 but not Fkbp51 in androgen receptor-mediated signaling and physiology. J Biol Chem 282(7):5026–5036

Yoshida NL, Miyashita T, U M, Yamada M, Reed JC, Sugita Y, Oshida T (2002) Analysis of gene expression patterns during glucocorticoid-induced apoptosis using oligonucleotide arrays. Biochem Biophys Res Commun 293(4):1254–1261

Zaffran S (2000) Molecular cloning and embryonic expression of dFKBP59, a novel Drosophila FK506-binding protein. Gene 246(1–2):103–109

Zhang X, Clark AF, Yorio T (2008) FK506-binding protein 51 regulates nuclear transport of the glucocorticoid receptor beta and glucocorticoid responsiveness. Invest Ophthalmol Vis Sci 49(3):1037–1047. doi:10.1167/iovs.07-1279

Zhao W, Zhong L, Wu J, Chen L, Qing K, Weigel-Kelley KA, Larsen SH, Shou W, Warrington KH Jr, Srivastava A (2006) Role of cellular FKBP52 protein in intracellular trafficking of recombinant adeno-associated virus 2 vectors. Virology 353(2):283–293

Zhong L, Qing K, Si Y, Chen L, Tan M, Srivastava A (2004) Heat-shock treatment-mediated increase in transduction by recombinant adeno-associated virus 2 vectors is independent of the cellular heat-shock protein 90. J Biol Chem 279(13):12714–12723

Zhu W, Zhang JS, Young CY (2001) Silymarin inhibits function of the androgen receptor by reducing nuclear localization of the receptor in the human prostate cancer cell line LNCaP. Carcinogenesis 22(9):1399–1403

Zimmer DB, Chaplin J, Baldwin A, Rast M (2005) S100-mediated signal transduction in the nervous system and neurological diseases. Cell Mol Biol (Noisy-le-grand) 51(2):201–214