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Molecular Chaperone Activity and Biological Regulatory Actions of the TPR-Domain Immunophilins FKBP51 and FKBP52

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Abstract: Immunophilins comprise a family of intracellular proteins with peptidyl-prolyl-(*cis/trans*)-isomerase activity. These foldases are abundant, ubiquitous, and able to bind immunosuppressant drugs, from which the term immunophilin derives. Family members are found in abundance in virtually all organisms and subcellular compartments, and their amino acid sequences are conserved phylogenetically. Immunophilins possess the ability to function as molecular chaperones favoring the proper folding and biological regulation of their biological actions. Their ability to interact via their TPR domains with the 90-kDa heat-shock protein, and through this chaperone, with several signalling cascade factors is of particular importance. Among the family members, the highly homologous proteins FKBP51 and FKBP52 were first characterized due to their ability to interact with steroid hormone receptors. Since then, much progress has been made in understanding the mechanisms by which they regulate receptor signaling and the resulting roles they play not only in endocrine processes, but also in cell architecture, neurodifferentiation, and tumor progression. In this article we review the most relevant features of these two immunophilins and their potential as pharmacologic targets.

Keywords: FK506, FKBP51, FKBP52, Hsp70, Hsp90, Immunophilin, Peptidylprolyl isomerase, TPR.

INTRODUCTION

Immunophilins (IMMs) comprise a large family of intracellular proteins characterized by the ability to interconvert Xaa-Pro bonds in a *cis*↔*trans* reversible manner (Fig. **1**). Such peptidyl-prolyl-(*cis/trans*)-isomerase (PPIase) activity, also named rotamase, is present in most of the members of the family and is inhibited by binding of immunosuppressant drugs such as cyclosporine A or FK506. Some of these proteins possess a PPIase domain and are able to bind the inhibitory drug, but they show no intrinsic enzymatic activity. Therefore, the signature domain of the family is the presence of the PPIase domain rather than its isomerase enzymatic activity or the ability to bind the immunosuppressant ligands.

IMMs are classified into two large subfamilies according to their ability to recognize specific immunosuppressant drugs [1]. They are named cyclophilins (or CyPs) when they bind cyclosporine A (brand names are Gengraf, Neoral, and Sandimmune), a cyclic non-ribosomal peptide of 11 amino acids that contains a single D-amino acid, which is rarely encountered in nature (see structure in Fig. **1**). A second subfamily is named FK506-binding proteins (or FKBPs) because they bind the cyclic macrolide FK506, also called tacrolimus or fujimycin (trade names Prograf, Advagraf, and Protopic). Many members of this FKBP subfamily also bind rapamycin, also named sirolimus (brand name Rapamune). A third subfamily of IMMs comprises parvulins, which conserve the homology in the PPIase domain, but they do not bind immunosuppressive drugs (see [2, 3] for recent updates related to this subfamily).

Only the low molecular weight immunophilins FKBP12 (gene name *fkbp1A*,) and CyPA (gene name *pp1A*) are related to the immunosuppressive effect when the drug•IMM complex inhibits the Ser/Thr-phosphatase activity of PP2B/calcineurin. This prevents the activation by dephosphorylation of the transcription factor NFAT (nuclear factor of activated T cells) and its subsequent nuclear translocation, which avoids the production of interleukins and interferon-γ (see [4] for a recent review). On the other hand, FKBP12-rapamycin complexes inhibit the large (*M*r 289,000) multidomain Ser/Thr kinase belonging to the PI3K family, mTOR kinase, preventing interleukin-2 signaling and cell cycle progression from G1 to S [5]. Beyond CD8(+) and regulatory T-cell control, mTOR protein is involved in critical biological functions of T helper cells or dendritic cells [6], and mTOR inhibitors are currently being tested for cancer treatment (see [7] for a recent review).

In contrast to the smallest members of the family, high molecular weight IMMs have a more complex architecture and are not related to immunosupression. The archetype of this subfamily is the 52-kDa FK506-binding protein, FKBP52 (gene name *fkbp4*). Like its highly homologous

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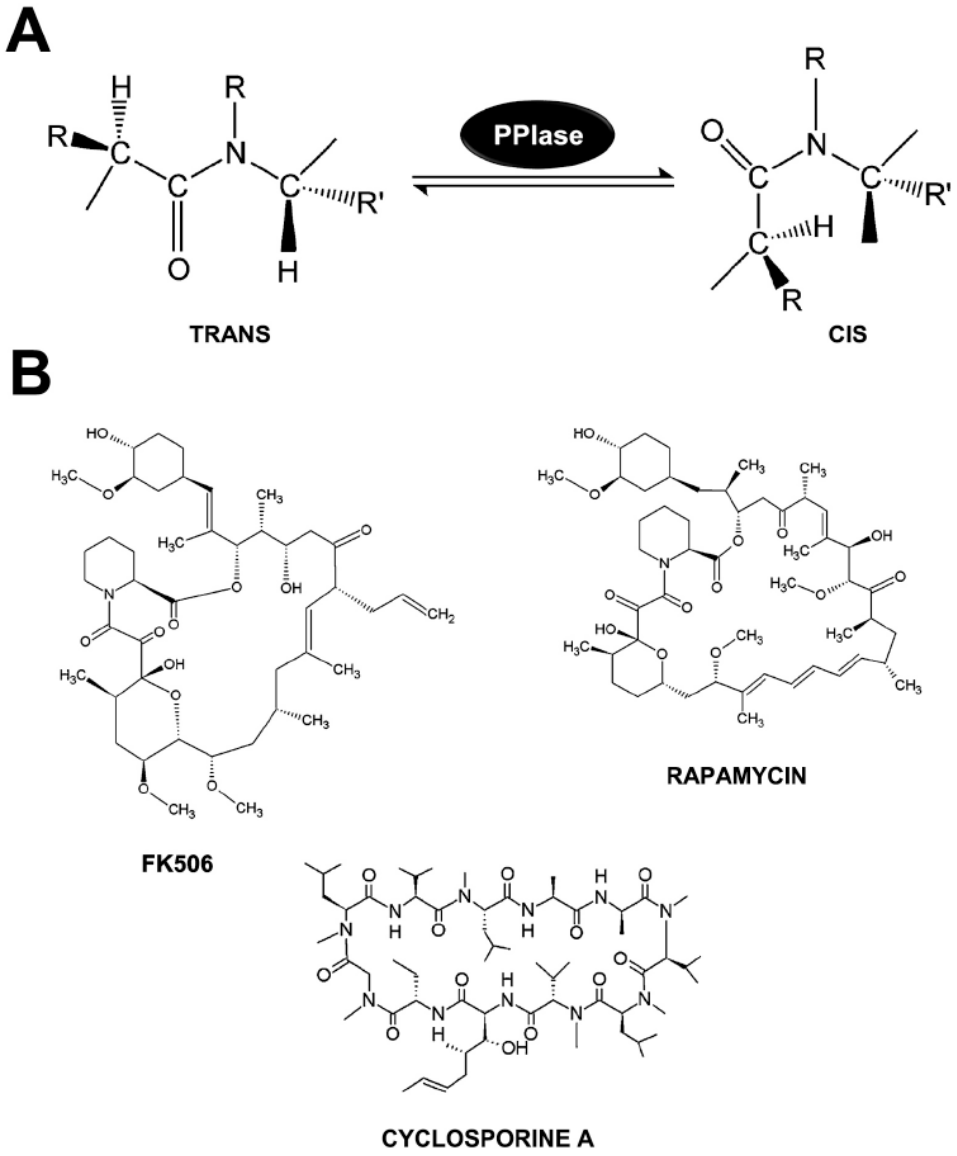

Fig. (1). Schematic representation of the *cis/trans* isomerization and structures of classical immunosuppressant drugs able to inhibit this activity. (A) The peptidyl-prolyl isomerase (PPIase)-catalyzed peptide bond *cis/trans* isomerization. **(B)** Chemical structures of the macrolide FK506 (tacrolimus), rapamycin (sirolimus) and cyclosporine A.

partner of 51-kDa, FKBP51 (gene name *fkbp5*), these FKBPs show other additional domains in addition to the active PPIase domain, also called FKBD1 or FK1 domain in FKBP proteins (see Fig. **2**). The best studied of these extra domains is the TPR (tetratricopeptide repeats) domain formed by sequences of 34 amino acids repeated in tandem through which they bind to 90-kDa heat-shock protein, Hsp90. The crystal structures of many TPR domain-containing proteins have been determined, showing TPR motifs as two anti-parallel α-helices packed in tandem arrays to form a structure with an amphipathic groove which can bind a target peptide (see [8] for a recent review). Most of the TPR-domain proteins were first characterized because of their ability to interact with steroid receptors via Hsp90, and since the crystallization of the TPR domain of the Ser/Thr phosphatase PP5 [9], the structures of several other steroid receptor-associated TPR-containing proteins have been solved. The four classical TPR domain IMMs related to steroid receptors are FKBP52, FKBP51, the cyclophilin of 40-kDa, CyP40 (gene name *ppID*), and the FKBP-like Ser/Thr phosphatase, PP5 (gene name *ppp5C*). All of them have their counterpart in plants [10], are highly ubiquitous, and are also able to form hetero-complexes (many of them still to be characterized) with several factors in addition to steroid receptors, although their biological functions and their molecular mechanism of action are still poorly understood.

Another important TPR-domain IMM is FKBP37 (gene name *AIP*), also known as XAP2/AIP. It was first discovered associated to AhR (aryl-hydrocarbon receptor, or "dioxin" receptor) associated to Hsp90. This IMM favors the biological actions of the receptor. FKBP37 is also able to interact and repress the biological activity of other members of the nuclear receptor superfamily, PPARα (peroxisome proliferator-activated receptor-α) [11], which modulates lipid metabolism, cell differentiation, and inflammation reactions.

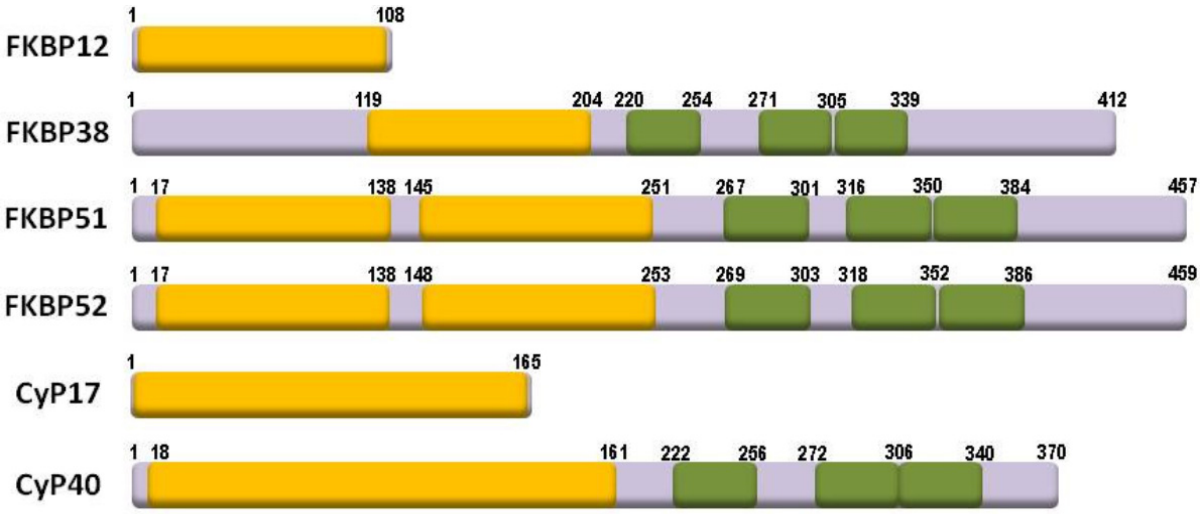

Fig. (2). Schematic structures of some IMMs. Numbers on the top represent the approximated limits of the N-terminal PPIase domain and the C-terminal TPR domains. Note that the two IMMs responsible for immunesuppression lack TPR domains and consequently, do not interact with Hsp90.

There are two more relevant TPR-domain IMMs whose biological roles have been elucidated more recently, and both are Hsp90-binding IMMs. One of them is FKBP38, which shows a mitochondrial localization signal and has been related to apoptosis (see [12] for a recent review). In spite of its almost identical three-dimensional structure of the PPIase domain with the immunosuppressive IMM FKBP12, FKBP38 lacks enzymatic activity and does not bind immunosuppressive drugs. The other relevant Hsp90-binding IMM is FKBP36, which is crucial to spermatogenesis since it is able to interact with components of the synaptonemal complex, and is also a natural inhibitor of GADPH activity [13]. GADPH is involved in the mechanism of vesicle transport from the endoplasmic reticulum to the Golgi and is also recruited by Rab2 to the vesicular-tubular clusters of the reticulum where it helps to form vesicles. Consequently, FKBP36 shows the additional potential to affect vesicle trafficking and the secretory pathways. To date, there are no small molecules able to recognize this IMM specifically.

IMMUNOPHILINS FOLD CLIENT PROTEINS

The *cis/trans* isomerization of Xaa-Pro bonds is one of the rate limiting steps of protein folding. Even though most nascent polypeptides emerge from the ribosome in the *trans*-conformation and retain that energetically favored state, about 5% to 7% of the proteins with structures of peptidyl-prolyl bonds (i.e., Xaa-Pro bonds) switch to the *cis*-conformation during folding, or during the transport and assembly [14]. The influence of CyPs and FKBPs on protein conformation, oligomeric states, and consequent biological activities cannot be explained on the sole basis of the PPIase activity alone. Actually, IMMs also act as molecular chaperones in an analogous manner to certain members of stress protein families. Thus, CyPs and FKBPs have been shown to influence the folding of a number of synthetic peptides and natural proteins such as collagen, chymotrypsin inhibitor, carbonic anhydrase, and ribonuclease T1, the latter considered the standard protein substrate of PPIases. The mechanistic steps of T1 enzymatic activity have been investigated structurally during refolding by NMR techniques. The results suggest that the presence of a non-native *trans*-prolyl bond at specific Pro[39] in a folding intermediate that may represent the targeted species for enzyme catalysis propagates the effect of prolyl isomerization not only to regions adjacent to the proline, but also to remote parts of the polypeptide chain.

Nonetheless, the exact catalytic mechanism of the PPIase activity still remains hazy. An interesting additional feature of IMMs is that, although both CyP and FKBP families can possess PPIase activity, the sequence and structure of the two families are dissimilar, though in both proteins the substrate and the inhibitory immunosuppressants compete for binding to the PPIase active site. Thus, the PPIase domain has become synonymous with drug-binding domain. The rest of the IMM sequence often plays a family-specific role, such as interacting with specific subsets of other proteins.

The mechanistic properties are not exclusive to PPIase domain proteins, but are somehow a reminiscence of the strategies adopted by other families of proteins such as Ser/Thr phosphatases. All members of this family catalyze the same basic reaction of dephosphorylating P-Ser and/or P-Thr residues. However, the phosphatases of the PPP class share a highly conserved sequence and structure in their catalytic core, whereas the metal-dependent PPM phosphatases have a very different sequence and novel protein folds. Finally, both high molecular weight IMMs and the IMM-like phosphatase contain protein-protein interaction domains outside the catalytic core, such as the TPR domains. Perhaps the best example is the IMM-like Ser/Thr phosphatase PP5, also a common Hsp90-binding factor associated with steroid receptor complexes.

IMMs are more abundant in the nervous system than in other tissues and have participation in several neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, prion protein diseases, polyglutamine repeat diseases (e.g., huntingtin in Huntington's disease) and amyotro-

phic lateral sclerosis (for a recent reviews see [15]). All of these diseases have been classified under the concept of "protein conformational disorders". Molecular chaperones that are able to constrain the structural freedom of misfolded proteins are essential for their processing and accumulation. In this regard, interaction of these factors with Hsp90-binding IMMs has recently revealed the importance of this protein family to regulate their overall stability. As it will be addressed later on, this property is particularly notorious for the case of tauopathies. *Tau* proteins pathologically aggregate in neurons, a process that is closely linked with cognitive deficits when they form higher-order aggregates. Recent studies by the Chad Dickey laboratory have demonstrated that TPR-domain IMMs were found to increase in forebrain neurons with age, and were linked with essential roles in *Tau* processing (see [16] for a recent review). Therefore, these IMMs are promising potential novel targets for developing the next generation of therapeutics.

FUNCTIONALLY DIVERGENT REGULATORS OF STEROID HORMONE RECEPTORS AND HORMONE-DEPENDENT PHYSIOLOGY

The large FK506-binding proteins FKBP51 and FKBP52 are IMMs that were first identified in complex with glucocorticoid receptor (GR) and progesterone receptor (PR)-heat shock protein 90 (Hsp90) complexes over twenty years ago [17, 18]. FKBP51 and FKBP52 share approximately 70% amino acid sequence similarity, yet they are functionally distinct. Biochemical and cellular studies firmly established FKBP52 as a specific, positive regulator of the androgen receptor (AR), PR, and GR signaling pathways [19]. The presence of FKBP52 results in an enhancement of receptor hormone binding affinity [20-22], and FKBP52 has been linked to the direct regulation of receptor nuclear-cytoplasmic shuttling [19, 23]. As a result, the expression of FKBP52 can enhance receptor-mediated expression of a reporter gene up to 60-fold in some cases. In contrast, FKBP51 has largely been characterized as a negative regulator of steroid hormone receptor activity [24], presumably through direct competition for binding to Hsp90 [25], and also due to transcriptional regulation effects (Erlejman AG and Galigniana MD, unpublished results).

The physiological consequences of FKBP regulation of steroid hormone receptor signaling pathways have been characterized in *fkbp51* (51KO) and *fkbp52*-deficient mice (52KO). As would be expected based on previous cellular studies, 52KO male mice are partially androgen-insensitive including ambiguous external genitalia and hypospadias, dysgenic prostate glands and seminal vesicles, and retention of nipples into adulthood [26, 27]. As a result 52KO male mice are infertile, although they do have normal testis that produce viable sperm. 52KO female mice are infertile due to uterine defects and implantation failure, a progesterone-dependent process [28]. Finally, phenotypes related to defects in GR signaling include increased susceptibility to high fat diet-induced hyperglycemia and hyperinsulinemia, hepatic steatosis, glucocorticoid resistance and behavioral alterations under basal and chronic stress conditions [29, 30]. Unlike the 52KO mice, 51KO mice display no overt morphological phenotypes but do display increased resilience to stress and depression [31-33]. Thus, although FKBP51 and FKBP52 have a high degree of similarity, they differentially affect hormone-dependent physiology.

FKBP51 AND FKBP52 STRUCTURE

Crystallographic structures have been solved for full-length FKBP51 and for overlapping fragments of FKBP52 (Fig. **3**) [34-36]. In addition to their high degree of similarity, both proteins adopt a similar fold. Both FKBP proteins contain a highly conserved TPR domain that consists of three tandem 34-amino acid degenerate direct repeats that adopt a helix-turn-helix conformation that stack to form a pocket for binding Hsp90. Additionally, FKBP51 and FKBP52 contain two N-terminal domains (FK1 and FK2) that have similarity to FKBP12. The FK1 domains of both proteins contain an active PPIase catalytic pocket in which the immunosuppressive drug FK506 binds. Although similar to FK1, FK2 lacks PPIase activity and drug binding.

As discussed above FKBP51 and FKBP52 are functionally distinct, despite their high degree of similarity at the amino acid sequence level, as well as at the structural level. One obvious structural difference that could contribute to functional differences is the orientation of the TPR domains. The FKBP51 TPR domain is in a more closed and kinked conformation facilitated by contacts with the FK2 domain, whereas the FKBP52 TPR domain is more open and extended. However, this structural difference must be viewed with caution in the absence of a full-length FKBP52 crystal structure. The following sections describe the various domains/regions/residues that have been found to be critical for FKBP52 regulation of steroid hormone receptor function, and compare and contrast these elements with those on FKBP51.

TETRATRICOPEPTIDE REPEAT DOMAIN (TPR)

All Hsp90-associated IMMs compete for binding the MEEVD sequence in the extreme C-terminus of Hsp90 by way of their TPR domains. In addition, recent studies identified a possible second TPR binding site in the N-terminal portion of Hsp90, suggesting that Hsp90 can accommodate multiple TPR protein interactions [37]. Indeed, recent evidence suggests that FKBP52 and the Hsp organizing protein (Hop) can be found in complex with Hsp90 together, although these studies were conducted in the absence of client protein [38]. Hsp90 is thought to continuously sample the pool of available TPR proteins and those interactions that ultimately affect Hsp90 client protein function are determined by which client protein is present in the complex [39].

FKBP52 specifically regulates AR, GR, and PR chaperone complexes and a single point mutation (K354A) within the TPR that significantly reduces FKBP52 binding to Hsp90 abrogates FKBP52 regulation of receptor function [40]. Thus, the FKBP52 TPR domain and its interaction with Hsp90 are necessary, but not sufficient for FKBP52 regulation of steroid hormone receptor function. An analogous mutation (K352A) in the FKBP51 TPR domain also abrogates FKBP51 binding to Hsp90 and, although FKBP51 does not regulate receptors in most systems tested, this mutant can serve as a tool to assess the need for Hsp90 binding in any FKBP51-dependent process [40]. The TPR domain is highly

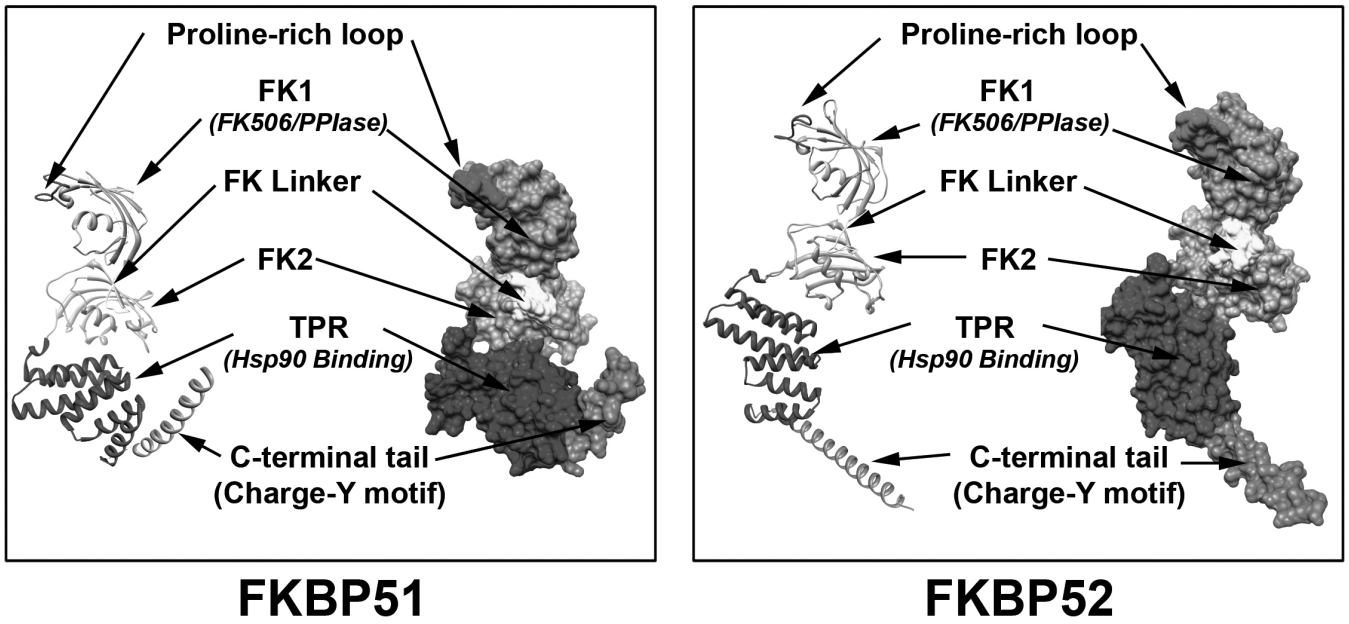

Fig. (3). FKBP51 and FKBP52 Structures and Functional Domains. Ribbon and molecular surface depictions of the human FKBP51 crystallographic structure (protein databank number 1KT0) and overlapping fragments encompassing the full length human FKBP52 crystallographic structure (protein databank numbers 1Q1C and 1P5Q) are shown. In both proteins the FK1 domain containing both the PPIase catalytic pocket and the Proline-Rich Loop is connected to the FK2 domain by way of the FK Linker domain. Both proteins also contain a tetratricopeptide repeat domain (TPR) that binds to Hsp90 and a C-terminal tail that also influences Hsp90 binding. UCSF Chimera version 1.5 was used to overlay the two partial FKBP52 structures and to generate the ribbon and molecular surface images.

conserved and studies using chimeric proteins demonstrated that the FKBP51 and FKBP52 TPR domains are functionally interchangeable [22]. Thus, the TPR domain does not contribute to FKBP51 and FKBP52 functional differences.

C-TERMINAL TAIL

Despite their high degree of similarity and the fact that the FKBP51 and FKBP52 TPR domains are functionally interchangeable, they display differences in Hsp90 binding affinities [41]. Sequences within the C-terminal 60 amino acids that are outside of the TPR domain likely contribute to these differences. A 20-amino acid consensus sequence motif that appears to be important for Hsp90 binding and is present in a large number of Hsp90 associated TPR-proteins has be defined [40]. This motif, termed the Charge-Y motif, is defined by the sequence -+-+XΦYXXMF, where – is glutamic acid or aspartic acid, + is lysine or arginine, Φ is a hydrophobic amino acid, and X is any amino acid. In addition to the Charge-Y motif, the extreme C-terminal 30 amino acids were shown to enhance the binding of FKBP51 to Hsp90, whereas these same residues in FKBP52 were shown to moderate binding to Hsp90 [40]. Thus, through differences in the C-terminal tail, FKBP51 and FKBP52 are thought to assume different conformations that affect binding to Hsp90.

FKBP12-LIKE DOMAIN 2 (FK2)

The FK2 domain has similarity to FK1, but lacks PPIase activity. Although FK2 is required, in that it contributes to the overall size and structure of the FKBPs, residues and/or regions within FK2 that are critical for FKBP52 regulation of steroid hormone receptor function have not yet been identified. However, FKBP51 and FKBP52 mutagenesis and chimera studies suggest that something exists within the FKBP52 FK2 domain that is critical for full receptor potentiating ability [21, 22]. In addition, deletion of three residues within the FKBP51 FK2 domain (D195, H196, and D197) results in a mutant that binds normally to Hsp90, but that does not integrate normally into PR•Hsp90 complexes [34]. Thus, specific residues and/or regions likely exist within FK2 that contribute directly to receptor regulation, possibly through influencing interactions with components of the receptor-chaperone complex or the receptor itself. Further studies are needed to define the obligate components within FK2 that are required for regulation of receptor activity.

FK LINKER

The FK2 domain is connected to the FK1 domain by way of a short (10 amino acids in FKBP52 and 8 amino acids in FKBP51), unstructured, solvent-accessible linker termed the FK Linker. The FKBP52 FK Linker contains a consensus casein kinase II (CKII) phosphorylation site (TEEED) that is absent in FKBP51 (the corresponding sequence in FKBP51 is FED), and CKII phosphorylation of T143 was demonstrated to abrogate FKBP52 binding to Hsp90 [42]. A second study did not observe a loss of Hsp90 binding, but demonstrated that T143 phosphorylation completely abrogated FKBP52 regulation of steroid hormone receptor function [43]. *In silico* modeling suggests that this loss of activity results from an allosteric reorientation of FK1 conformation upon T143 phosphorylation. As discussed below, the FK1

domain and the Proline-Rich Loop likely serve as a functionally important interaction surface. Thus, allosteric conformational changes in the FK1 domain induced by T143 phosphorylation could represent a physiologically relevant receptor regulatory mechanism by affecting FK1 interactions critical for receptor potentiation. An FKBP52 FK Linker mutant in which T143 was replaced with the F found in FKBP51 retained full receptor potentiating ability. Thus, although FKBP51 lacks a CKII phosphorylation site, this difference does not account for FKBP51 and FKBP52 functional differences.

FKBP12-LIKE DOMAIN 1 (FK1)

Unlike the FK2 domain, the FKBP52 FK1 domain contains a functional PPIase catalytic pocket and is absolutely critical for regulation of steroid hormone receptor function. In addition, receptor chimera studies demonstrated that FK1 regulation of receptor activity is localized to the hormone binding domain [22]. The logical hypothesis would be that the FK1 PPIase catalyzes a relevant proline substrate within the receptor hormone binding domain resulting in receptor conformational changes that favor hormone binding. Indeed, early studies suggested that PPIase was critical for function as a double point mutant, FKBP52-FD67DV, that abolishes PPIase activity also completely abrogates FKBP52 potentiation of receptor activity [22]. However, it was found that it is not the lack of PPIase activity in this mutant that causes a loss of receptor potentiation, but the fact that this mutant destabilizes the protein, which compromises FK1 conformation. A more detailed analysis of mutations within the PPIase catalytic pocket revealed that PPIase enzymatic activity is completely dispensable for FKBP52 regulation of receptor activity [21]. Thus, the FK1 domain as a whole, not the PPIase, is critical for function and likely serves as an interaction surface within the receptor-chaperone complex. This would also suggest that FKBP51 and FKBP52 functional differences result from sequence divergence within the FK1 domain. The FKBP51 FK1 domain is structurally similar to that of FKBP52 and also contains a functional PPIase catalytic pocket. However, analysis of random gain-of-function FKBP51 mutants identified critical residue differences in the Proline-Rich Loop that overhangs the PPIase catalytic pocket [21].

FK1 PROLINE-RICH LOOP

The Proline-Rich Loop is comprised of nine amino acids encompassing residues 116 to 124 in both FKBP51 and FKBP52. FKBP51 random mutagenesis studies demonstrated that two residue changes within this loop (A116V and L119P), together, confer full receptor potentiating ability to FKBP51 that is comparable to that observed with FKBP52 [21]. Additionally, analogous changes in the FKBP52 loop result in a loss of function. These findings suggest that the FK1 Proline-Rich Loop is not only critical for FKBP52 regulation of receptor activity, but also solely responsible for the divergent functions of FKBP51 and FKBP52. The prevailing hypothesis is that the FKBP52 FK1 domain, the Proline-Rich Loop in particular, serves as an interaction surface and receptor chimera studies suggest that the interaction partner is the receptor hormone binding domain. Precedence exists for Proline-Rich Loop interactions as a co-crystallographic structure of a related family member, FKBP12, bound to transforming growth factor β demonstrates that residues within the loop, including the residue analogous to P119 in FKBP52, directly participate in the interaction [44]. In addition, recent studies defined a putative FKBP52 interaction and/or regulatory surface on the androgen receptor hormone binding domain that overlaps with the recently characterized Binding Function 3 (BF3) surface [45, 46]. The BF3 surface is also the proposed binding site for the recently characterized FKBP52-specific AR inhibitor MJC13 [46]. Whether FKBP52 regulates this surface through direct interaction or indirectly through interactions with other components of the receptor-chaperone complex is currently unclear.

IMMUNOPHILINS AND CYTOSKELETON STABILITY

Confocal microscopy studies and biochemical fractionation demonstrated that FKBP52 is associated with microtubules [47, 48]. This IMM was present in the subcellular fractions containing either soluble proteins released from cells exposed to NP40 detergent, or proteins released from the cytoskeleton exposed to calcium ions (i.e., in microtubule-depolymerizing conditions). This association suggested a biological role for FKBP52 in the architecture of the cell, most likely as a chaperone of the filament network, but also a potential role in the physiology of the cell, for example, in transport mechanisms. Exposure of cells to FK506 and rapamycin did not modify significantly the co-localization with microtubules, indicating that the PPIase activity is not required for such interaction. During mitosis, FKBP52 segregates from the region of the chromosomes being mainly localized with the mitotic apparatus (centrosome, spindle and interzone separating the chromosomes) [47]. Then, it was shown that this IMM associates to dynein motors [49, 50], and more recently, it was postulated that FKBP52 can prevent tubulin polymerization [51].

Tau protein is a highly soluble microtubule-associated protein mostly found in neurons compared to non-neuronal human cells. It is accepted that *Tau* function is to modulate the stability of axonal microtubules, such that when *Tau* proteins are defective, they can result in neurological disorders such as Alzheimer's disease. A high percentage of prolines are common to most intrinsically disordered proteins, and *Tau* is no exception [52]. Nearly 10% of full-length *Tau* is composed of proline residues and more than 20% of the residues between I^{151} and Q^{244} are proline. Most of the known functions of *Tau* are mediated through microtubule binding domains distal to this proline-rich region. Recent studies have revealed that both IMMs, FKBP51 and FKBP52, interact directly with *Tau* protein [53, 54]. It has also been shown that *Tau* phosphorylation increases its binding affinity for FKBP52, whereas the overexpression of the IMM could prevent the accumulation of *Tau* protein induced by nerve growth factor. While FKBP52 appears to inhibit tubulin polymerization, which is triggered by Tau protein *in vitro* [53], FKBP51 stabilizes microtubules in a PPIase activity-dependent manner and also enhances the association of *Tau* with Hsp90, although the FKBP51/*Tau* interaction is not entirely dependent on the chaperone [54]. In this complex, phosphorylated *Tau* is in a *trans* configuration, and the PPIase activity of FKBP51 isomerizes *Tau* to a *cis* configura-

tion, which enhances dephosphorylation of *Tau* by the IMM-like phosphatase PP5. This is a critical event because dephosphorylated *Tau* is recycled to microtubules and stabilizes them. On the other hand, highly phosphorylated *Tau* exhibits reduced microtubule binding, leading to a loss of microtubule integrity.

Because both FKBP51 and FKBP52 possess PPIase activity and are capable of binding phosphorylated *Tau*, it is currently unclear why FKBP51 is selectively able to isomerize *Tau* and promote microtubule polymerization. One possible explanation could be the fact that both TPR-domain IMMs, as well as CyP40 and PP5, compete for a common Hsp90 binding-site in steroid receptor complexes [50, 55], so it would be possible that a similar competition may take place in the phospho-Tau•Hsp90 heterocomplex containing a TPR-domain co-chaperone. In turn, the qualitative composition of such heterocomplex may depend on the relative abundance of TPR proteins in a given cell, just like it has been postulated for steroid receptors [55-57]. Moreover, structural comparison of FKBP51 and FKBP52 revealed that the TPR domains have different spatial orientations [35].

IMMUNOPHILINS AND EARLY NEURONAL DIFFERENTIATION

FKBP51 and FKBP52 affect neurite length during neuronal differentiation. This may explain, at least in part, the observation that the IMM ligand FK506 exhibits potent neurotrophic effects [58, 59]. FK506 has been shown to promote neuroprotective and neuroregenerative effects in a number of injury models. The persistence of the capability to hasten nerve outgrowth by FK506-derivatives devoid of immunosuppressive effects indicated that both effects are independent [60], and experiments with mice where the *FKBP1A* gene was knocked-out clearly showed that such neurotrophic effect is not mediated by FK506•FKBP12 complexes [61]. Recently, it was demonstrated that the FKBP52•Hsp90•p23 complex forms a perinuclear structure in undifferentiated N2a neuroblastoma cells and hippocampal cells isolated from rat embryos [58]. This peculiar perinuclear structure undergoes a rapid subcellular redistribution along the cytoplasm, which is detectable within the first hour of stimulation with FK506. Importantly, FKBP52 is concentrated in terminal axons and arborization areas. In agreement with the potential relevance of this IMM, knock-down experiments showed that FKBP52 plays a key role in the architecture of these nuclear rings since these structures faded in most cells (if not all of them) where the expression of the IMM was abrogated. Importantly, both the rate of cell differentiation and neurite outgrowth were also inhibited. Therefore, there is a direct relationship between the disassembly of the chaperone complex and the progression of neuronal differentiation. The chaperones migrate to the cytoplasm and become associated with cytoskeletal structures, whereas the nuclear areas originally occupied by them in undifferentiated cells become transcriptionally active after chaperones redistribute.

In contrast to FKBP52, FKBP51 is not induced during differentiation, remains in the cell body and replaces FKBP52 in the annular structures of the nucleus complexed with Hsp70 [58]. While the overexpression of FKBP52 induces fast differentiation of N2a neuroblastoma cells and neurites are longer, the expected opposite action is observed after knocking-down FKBP52. On the other hand, FKBP51 overexpression decreases both the neurite length and the rate of cell differentiation, whereas its knock-down favors neurite outgrowth. In other words, it appears that both IMMs have antagonistic action during the very early steps of neuronal differentiation.

Surprisingly, microtubules of undifferentiated neurons show a diffuse cytoplasmic distribution, whereas they are arranged in the expected filamentary pattern after stimulation with FK506. Simultaneously, the Hsp90 co-chaperone p23 becomes associated to neurofilaments and seems to be dissociated from both Hsp90 and FKBP52. Importantly, after 5-6 days in culture with FK506 and no other trophic factor added to the medium, embryonic day 17 rat hippocampal neurons show a new redistribution of FKBP52 only, such that the IMM is concentrated back in nuclei but, in contrast to what was observed in the undifferentiated state, FKBP52 distributes in the nucleus in a diffuse manner and shows no particular arrangement. All these observations suggest an active role for the IMM•Hsp90•p23 complex in the arrangement of the neuronal cytoskeleton during the early steps of cell differentiation. The above-described observations by Quinta *et al.* [58, 59] apparently collide with those reported by Chambroud [51, 53] in that these studies reported that overexpression of FKBP52 reduced neurite outgrowth in response to 5 days in culture with nerve growth factor in PC12 pheochromocytoma cells always maintained in a medium with fetal serum. However, there are important experimental differences between both studies with respect to the cell type, length of stimulation in culture, and culture conditions that justify those differences.

S100A proteins constitute a large family of calcium binding proteins which are linked to many neurological disorders, inflammatory diseases, innate immunity, wound healing, and cancer processes [62]. Several S100A proteins can bind to the TPR domain of FKBP52 protein, which leads to the dissociation of the Hsp90•FKBP52 complex in the presence of calcium [63]. Apart from the possible crosstalk between S100A proteins and pro-angiogenic or anti-angiogenic factors, S100A4 and A13 have been reported to participate directly in the angiogenic process in other tissues. Because the common denominator able to affect the biological activity of S100A proteins in all these biological processes is calcium homeostasis, it could be implied that the function of FKBP52 can be regulated by calcium within the cell, just like Tau phosphorylation and pathology in Alzheimer disease are regulated. Moreover, FKBP52 contains a putative calmodulin binding domain at the C-terminus, the biological function of which is still enigmatic. Recently, FKBP52-dependent regulation of TRPC calcium channels was also reported in neurons [64].

FKBPS AND CANCER

There is strong evidence that, among the TPR-domain FK506-binding proteins, FKBP38, FKPB51, FKBP52, and FKBP65 are strongly related to cancer etiology and chemoresistance. It is known that FKBP38 inhibits mTOR [65], a signalling cascade that controls cell growth and proliferation, and is implicated in a wide range of human dis-

eases, including cancers [66]. Nevertheless, the functional consequences of this association remain as controversial as FKBP38 antiapoptotic action [67], which may also turn into proapoptotic effects [68].The reasons for these antagonistic properties of FKBP38 are unclear, although they may be related to the cellular context and type of stimuli. On the other hand, FKBP65 binds directly to c-Raf1 [69] and its low expression has been related to epithelial ovarian cancer cells [70]. Also, it could be involved in the initiation of colorectal carcinogenesis [71]. It is noteworthy that both IMMs (and c-Raf1) are Hsp90-interacting proteins, such that inhibition of this chaperone could potentially impair the biological actions of those associated proteins.

Regarding FKBP52, it has recently been reported that it is expressed at low levels in normal breast, bladder and testis, but it is overexpressed in malignant hepatoma [72], T cell leukemia [73], and hormone-dependent cancers [74]. Thus, it has been found highly expressed in breast cancer cell lines, in particular in ER-expressing cells [75, 76], as well as preinvasive and breast cancer tissues [77-79]. Interestingly, a recent study [79] identified Hsp90 client proteins sensitive to the specific ansamycin inhibitor geldanamycin in both tumour and healthy breast tissue, and FKBP52 was listed as one of the Hsp90 partner proteins associated to drug resistance. Importantly, several prostate cancer cell lines such as LNCaP, PC-3 and DU145 also show a general pattern of overexpression for FKBP52 compared to normal prostate epithelial cells [80] and prostate biopsy samples [81].

It is accepted that FKBP52 is an important positive regulator of AR in both cellular and animal models, and consequently represents an attractive target for the treatment of prostate cancer [24]. As it was discussed in a previous section, a recent study [46] showed that small molecules such as MJC13 that are predicted to bind the BF3 surface of the receptor (a region proposed to interact with FKBP52) impaired the dissociation of the AR•Hsp90•FKBP52 complex, resulting in an inhibition of AR nuclear translocation and AR-dependent gene expression.

FKBP51 was found to be expressed in most tissues, and it is also overexpressed in several cancer tissue samples and cancer cell lines (see [82] for a recent review). Perhaps due to its high homology with FKBP52, FKBP51 behaves as an antagonist of FKBP52 in most systems. In steroid receptor complexes, FKBP51 is usually an inhibitor of steroid binding and receptor-dependent transcriptional activity [19] with the only exception to date of AR-mediated response in prostate cells [80, 83]. FKBP51 has also been localized in mitochondria and associated with antiapoptotic effects [84], which may explain its relationship with aggressiveness and therapy resistance of some types of cancers. Therefore, it is a progressively emerging concept that FKBP51 is a critical protein for cell proliferation, antiapoptotic action, and carcinogenesis [74, 85-87]. More recently, the expression of certain FKBP51 isoforms have been related to depression and the development and resistance to treatment of post-traumatic stress disorders [88]. As a natural consequence, it is tempting to develop methods to detect both FKBP51 and FKBP52 as biomarkers of pathological processes, tumor invasiveness, and/or markers to predict resistance to treatments.

CONCLUDING REMARKS

Inasmuch as IMMs, FKBP51 and FKBP52, are implicated in a number of basic biological processes and signalling cascades, and are also directly involved in the development of health disorders ranging from endocrine and behavior alternations to cell cycle regulation and cancer, they are appealing pharmacological targets for therapeutic approaches. In this regard, ongoing efforts are aimed at the specificity of action of ligands able to discriminate between these two highly homologous proteins, which is, perhaps, the greatest challenge.

The technology roadmap for these FKBP-related signaling pathways is only beginning to be charted and includes from points of attack of FKBPs as cancer markers to neuroimmunophilins as novel targets for neuroprotective or neuroregenerative purposes. Nonetheless, despite some advances in the development of fluorescent probes to examine ligand specificity and a few drugs that are still in the experimental stage, concrete advances in the therapeutic role of IMM ligands is still a pending assignment.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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