FKBP51 and FKBP52

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Immunophilins are a family of proteins whose signature domain is the peptidylprolyl-isomerase domain. High molecular weight immunophilins are characterized by the additional presence of tetratricopeptide-repeats (TPR) through which they bind to the 90-kDa heat-shock protein (Hsp90), and via this chaperone, immunophilins contribute to the regulation of the biological functions of several client-proteins. Among these Hsp90-binding immunophilins, there are two highly homologous members named FKBP51 and FKBP52 (FK506-binding protein of 51-kDa and 52-kDa, respectively) that were first characterized as components of the Hsp90-based heterocomplex associated to steroid receptors. Afterwards, they emerged as likely contributors to a variety of other hormone-dependent diseases, stress-related pathologies, psychiatric disorders, cancer, and other syndromes characterized by misfolded proteins. The differential biological actions of these immunophilins have been assigned to the structurally similar, but functionally divergent enzymatic domain.

FKBP51 FKBP52 Hsp90 dynein telomerase NF-κB neurodifferentiation cell differentiation

1. Introduction

Immunophilins comprise a family of proteins that show two main features: a) they have a specific sequence that usually has peptidyl-prolyl-(*cis/trans*)-isomerase (PPIase) activity, i.e., the reversible *cis/trans* interconversion of Xaa-Pro bonds (see **Figure 1**a); b) they also have the capability to bind immunosuppressive drugs to the same PPIase domain. The classic binding ligands are FK506 (tacrolimus), rapamycin (sirolimus) or cyclosporine A, and all these drug-protein interactions abolish the PPIase enzymatic activity when the isomerase function is present in the protein. Regardless of these two conventional properties, the common feature of the family is the existence of a relatively conserved sequence in most of the members, the PPIase domain, which represents the signature domain of the entire family. Most researchers in the field often indistinctly use either term (immunophilin or PPIase protein) since they were simultaneously originated during the early times when these proteins were discovered and characterized by the binding capacity for immunosuppressive drugs and the enzymatic activity of protein *cis/trans* isomerase. For human multidomain FKBPs (FK506-binding proteins) such as FKBP25, FKBP51, FKBP52, and FKBP65, good catalysis of the *cis/trans* isomerization of the peptidyl prolyl bond using oligopeptide substrates has already been demonstrated [1]. However, despite possessing a PPIase domain not all members show significant isomerase enzymatic activity (e.g. FKBP38, FKBPL, etc.) or it is negligible or absent (e.g. FKBP37, PP5, etc.). For the case of the cyclophilin subfamily (CyP), no PPIase activity has been demonstrated to date for some members such as CyP35, CyP54, CyP57 and CyP60.

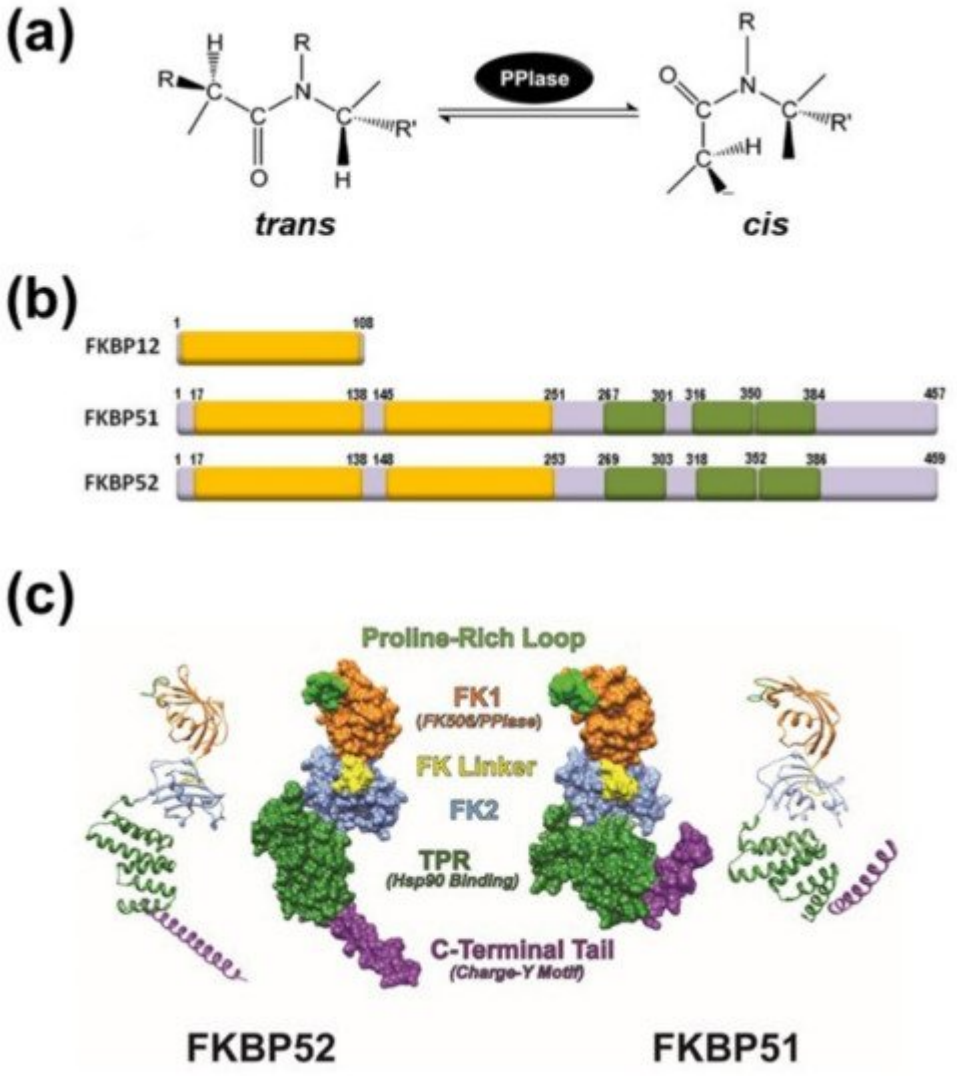

Figure 1. (a) Schematic representation of the peptidyl-prolyl isomerase (*cis/trans*) isomerization activity (PPIase). (b) Schematic structures of FK506-binding protein of 12-kDa (FKBP12) (acc.# AAA58476), FKBP51 (acc.# Q13451) and FKBP52 (acc.# NP_002005). The PPIase domain is depicted as yellow boxes. Only the FK1 domain (the extreme N-terminal of the protein) has PPIase activity. Tetratricopeptide repeats (TPRs) are shown in green color. (c) Ribbon and molecular surface depictions of hFKBP51 crystallo-graphic structure (right) and overlapping fragments encompassing the full length hFKBP52 crystallographic structure (left) are also shown. Note in both proteins that the FK1 domains (orange) containing the PPIase catalytic pocket and the Proline-Rich Loop (green) are connected to the FK2 domain (blue) by the FK Linker (yellow). Both proteins show TPR domains (purple) where Hsp90 binds. Structures were derived from the RCSB PDB (FKBP51: 1KT0; FKBP52: 1Q1C & 1P5Q) with Viewer-Lite 5.0 (Sharpened Productions Inc, Sioux City, IA, USA).

Based on the property to recognize different ligands, PPIases (EC 5.2.1.8) were classically grouped into two subfamilies [2]. They are named cyclophilins (or CyPs) when they bind cyclosporine A, or FKBPs when they bind FK506. Both drugs are not chemically-related. FK506 is a natural compound belonging to the family of macrolides that was first isolated from the bacterium *Streptomyces tsukubaensis* [3][4], whereas cyclosporine A is a cyclic

undecapeptide first isolated from the fungus *Trichoderma polysporum* [5] that contains a single D-amino acid rarely encountered in nature. Unlike most peptides, cyclosporine A is not synthesized by ribosomes [6].

2. General Aspects of FKPB51 and FKBP52

The immunosuppressive action of FK506 or cyclosporine A is responsibility of the drug binding to the smallest members of both classical immunophilin subfamilies, i.e. FKBP12, encoded by the *FKBP1A* gene, and cyclophilin A (CyPA), encoded by the *PP1A* gene. As consequence of these specific interactions, the liganded immunophilin (but not the drug or the protein alone) impairs the activity of calcineurin [7], a Ser/Thr-phosphatase also known as PP2B. Thus, the transcription factor NFAT (Nuclear Factor of Activated T-cells) remains phosphorylated in the cytoplasm of lymphocytes, and the production of interleukines and interferon-γ is consequently avoided (see [8] for a comprehensive review for this mechanism). On the other hand, members of the immunophilin family that possess higher molecular weight show more complex protein architecture because they have additional domains to the PPIase domain. One of the best characterized immunophilins is the 52-kDa FK506-binding protein, FKBP52 (gene name *FKBP4*)[9], which is the archetype of the Hsp90-binding subfamily (**Figure 1**b). In addition to the PPIase domain (also called FKBD1 or FK1 domain), FKBP52 shows three repetitions in tandem of a degenerative sequence of 34 amino acids named tetratricopeptide repeats (TPR), which has the capability to form associations with Hsp90 dimers [10].

The Hsp90-binding immunophilin FKBP52 shows a close-related partner, FKBP51 (gene name *FKBP5*), with whom it shares 75% similarity and 60% identity. Due to this high homology, but different conformational features (**Figure 1**c), both immunophilins usually compete one another for binding and functional properties of client-proteins, although this antagonistic effect shows exceptions in a few numbers of cases and their biological effects become redundant (see afterwards). Both immunophilins possess a short sequence of 7 to 9 amino acids that forms an FK-linker region. It connects the FK1 domain with the FK2 region. The linker sequence of FKBP52 is capped by a TEEED phosphorylation sequence that is substrate of casein kinase-2 (CK2). The resultant phosphorylation at Thr^{143} impairs the FKBP52•Hsp90 interaction [11] and also abrogates the normal regulation seen on steroid receptors by FKBP52. In the case of FKBP51, this loop is capped by FED, a conserved sequence in high molecular weight immunophilins, such that the phosphorylation of the site by CK2 does not occur. Nevertheless, these differences cannot account for the lack of receptor potentiation capability shown by FKBP51, which is most of the times an inhibitor of the biological actions of nuclear receptors.

As it was stated above, it is accepted that the FK1 domains of both immunophilins are the major structural elements responsible for the divergent properties of FKBP51 and FKBP52 on the steroid receptor action [12][13][14][15]. Nevertheless, while the PPIase domain is important in this regard, it appears that the enzymatic activity of prolyl-isomerase is not always essential [14][16]. The immunophilin is often part of a heterocomplex with Hsp90, such that the interaction with the chaperone also influences the FK1 domain of FKBPs [17]. The binding of Hsp90 to the immunophilin enables the FK1 domain to interact with the ligand binding domain of the glucocorticoid receptor (GR), thereby influencing the GR conformation and the steroid binding affinity [18]. Inasmuch as the conformations

of the FK1 domains of both FKBPs differ around the PPIase pocket, it is not surprising that this fact causes differential protein interactions with client-factors [19][20].

The structure of the FK2 region (second yellow box in **Figure 1**b) is similar to that of the FK1 region, but FK2 does not show enzymatic activity of isomerase and cannot recognize immunosuppressive ligands. When the FK2 region of FKBP51 was modified by point mutations, Hsp90 binding took place yet the mutant cannot integrate normally into the receptor heterocomplex [19]. Therefore, it might be possible that the mutation impairs key interactions not only with other members of the receptor heterocomplex, but also with the receptor itself. In contrast to the FK1 domain of FKBP52, the FK1 domain of FKBP51 does not show stimulating activity on steroid receptor activity, but random mutagenesis studies evidenced that two key point mutations in the FK1 domain of FKBP51 confer full receptor potentiation activity to this immunophilin equivalent to that observed for FKBP52 [21]. This suggestive finding is in line with the notion that both proteins may have diverged during evolution by only a very limited number of modified amino acid residues. Inasmuch as these two residues are in the proline-rich loop (see **Figure 1**c), it may be inferred that this region of FKBP52 is functionally relevant for the regulation of steroid receptor activity. Accordingly, it has recently been postulated that the loop serves as an interaction surface with the ligand binding domain of the receptor [22][23].

The TPR domain located at the C-terminal end of the immunophilin confers the capability to interact with the chaperone Hsp90 via the C-terminal sequence of the chaperone, the EEVD motif [10][24]. On the other hand, isothermal titration calorimetry studies performed with FKBP51 and FKBP52 demonstrated that the former immunophilin interacts with Hsp90 dimers with lower affinity (about one third) compared to FKBP52 [25]. Nonetheless, due to the influence of the relative abundance of each FKBPs in each cell type, solely the relative affinity of each immunophilin for Hsp90 cannot permit accurately predict the stoichiometry of the oligomeric complexes formed with the receptors.

3. Immunophilins Play a Key Role in Protein Trafficking

Steroid receptors bind their cognate ligands only if they are assembled with Hsp90 in oligomeric structures [26][27], this Hsp90-based complex being a biological on/off functional switch. This property agrees with the properties of Hsp90, a chaperone that at variance of others that prefer partially unfolded clients, favours substrates that possess a preserved tertiary structure. This is particularly notorious for some members of the steroid receptor family such as GR, mineralocorticoid receptor (MR), progesterone receptor (PR) and androgen receptor (AR) [28]. They are transcription factors with properties of phosphoproteins [29][30][31], and are activated by ligand binding. Steroid receptors are assembled with molecular chaperones and co-chaperones, including Hsp90, Hsp70, Hsp40, p23 and a TPR-domain protein, usually a high molecular weight immunophilin such as FKBP51, FKBP52, CyP40 or PP5. The interaction of TPR proteins with Hsp90 (and Hsp70) is conserved in nature and broadly distributed in both animal and plant kingdoms [32][33][34].

The discovery that the dynein/dynactin motor complex co-immunoprecipitates with GR [35] and MR [36] via its association to the PPIase domain of FKBP52 modified the classic view for the mechanism of steroid receptor

activation. In the absence of steroid, some receptors such as GR, MR or AR (which also interacts with dynein/dynactin, as it was further demonstrated [37]) are primarily cytoplasmic proteins. Upon steroid binding, they rapidly accumulate in the nucleus. Other receptors such as the estrogen receptor (ER) are constitutively nuclear even in the absence of steroid, but they are not statically confined to a cell compartment, but are continuously shuttling between cytoplasm and nucleus [38][39][40][41]. Classically, the driving force for soluble protein movement throughout the cytoplasm was always assumed to occur by simple diffusion, and steroid receptors were not the exception. The classic model for receptor activation posited in the '80s proposed that Hsp90 should dissociate from the receptor upon steroid binding to release the transcription factor from the cytoplasmic anchorage sites. When the key role for FKBP52 in the receptor retrograde movement was demonstrated, the classic dogma was replaced by a model where the entire receptor•Hsp90•FKBP52•dynein complex moves throughout the cytoplasm, translocates intact through the nuclear pore, and is finally dissociated in the nucleoplasm [36][42] (**Figure 2**). A direct corollary of this novel model is the prediction that receptor dimerization cannot be a cytoplasmic event because the association of the receptor with the Hsp90 chaperone complex (a key requirement for receptor retrotransport) blocks the dimerization domain; therefore, it may be predicted that the oligomeric chaperone complex should be released in the nucleoplasm after the translocation through the nuclear pore, which would allow receptor dimerization. This prediction was effectively demonstrated time after for the GR [43] and the MR [44] using different methodologies.

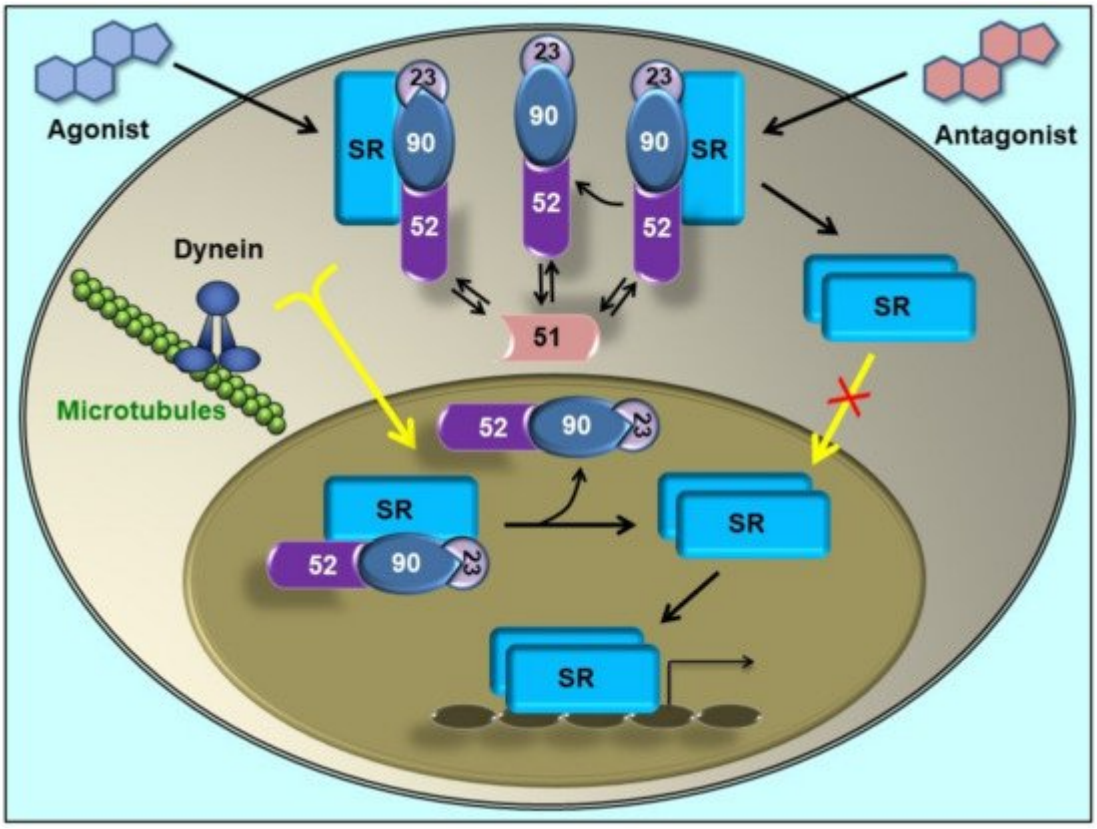

Figure 2. Steroid receptor (SR) retrotransport is affected by immunophilins. The mature form of SR forms complexes with a dimer of Hsp90, one molecule of Hsp70 (not depicted in this figure), the co-chaperone p23, and one TPR-domain immunophilin [45]. FKBP51 (unable to interact with dynein [12]) is associated to the empty SR.

When the steroid binds, FKBP51 is exchanged by FKBP52 due to a ligand-induced conformational change of the receptor [46]. In turn, FKBP52 recruits the dynein-dynactin motor complex to its PPIase domain [35]. The complex is rapidly ($T_{0.5}$= 5 min) transported to the nucleus on cytoskeleton tracks. SR '*transformation*' (i.e. the dissociation of the Hsp90-based complex) is a nuclear event [36]. Some antagonists can promote the release of Hsp90 in the cytoplasm, such that SR does not reach the nucleus [44]. Note that regardless of the SR primary localization, they are constantly cycling between the nuclear and cytoplasmic subcellular compartments [39][47], even when the final equilibrium may be displaced to a given cell compartment. When the steroid promotes the full nuclear accumulation of the SR, it still cycles. The disruption of the "transportosome" by any means (Hsp90-disrupting drugs, dynein inhibitors, overexpression of the PPIase or TPR domain, ATP depletion, low temperature, etc.) does not prevent SR movement, but it is one order of magnitude slower ($T_{0.5}$= 45–60 min) than the active mechanism. It is thought that this residual movement represents the diffusion of the complex through the crowded filamentous milieu [48] (Figure adapted from [49],with permission from the publisher).

On the other hand, the highly homologous partner FKBP51 does not bind dynein [12]. In line with this fact, it was demonstrated that FKBP51 favours its recruitment to unliganded receptor and is exchanged by FKBP52 when the steroid binds [46][50]. Such dynamic immunophilin exchange has biological relevance due to the differential action exerted by each co-chaperone on the final biological response of the receptor [51][52]. In agreement with the modern model, a very recent study by nuclear magnetic resonance (NMR) spectroscopy analysis [53] evidenced that the FK1 and FK2 domains populate respectively an ensemble of bound and unbound receptor conformation. Also, it was recently suggested that the $helix_{1-3}$ loop in the ligand binding domain of GR is responsible for the regulatory properties of both FKBPs [54]. In a different publication, the conformational transition of the FK1 domain of both immunophilins in their respective association with the GR was analysed [16], and the evidence showed that the interactions in the $\beta_{4–5}$ loop and the $\beta_{2–3a}$ strands tend to lock FKBP52 into a conformation that preferentially binds to a high affinity state of the steroid receptor, whereas FKBP51 shows preference for the empty receptor. These structural evidences agree with the biological behaviour of both immunophilins in intact cells. According to the novel model, the competitive balance between dynein-binding immunophilins versus non-dynein-binding TPR proteins associated to the receptor•Hsp90 complex may influence the basal subcellular redistribution of the receptor [36][48]. This could be achieved by regulation of the expression of a given immunophilin or by using drugs able to affect immunophilin function. Accordingly, the overexpression of the TPR domain of the immunophilin or whole FKBP51 protein retains higher amounts of steroid receptor in the cytoplasm [36], whereas drugs able to disrupt the interaction of FKBP51 with the receptor•Hsp90 complex like benztropine restores the expected subcellular localization of the receptor in ex vivo brain slices and primary neurons from mice [55].

Importantly, this Hsp90•FKBP-dependent trafficking machinery has also proved using other models [12][36][56][57][58][59], and has also been demonstrated for a great variety of factors such as the catalytic subunit of telomerase [60], the insect ecdysone receptor [61], or the diphtheria toxin [62], just to mention a few examples. Therefore, the discovery that immunophilins are involved in the "transportosome" molecular machinery has changed substantially the classic molecular model for the mechanism of action of steroid receptors posed heuristically in the literature, but never proved, and helped to explain the cytoplasmic transport of other soluble proteins that share the same chaperone machinery [61][62][63][64][65][66]. It is interesting to point out that the immunophilin-like protein

FKBPL/WISP39 shares the same properties as FKBP52 for the regulation of GR retrotransport, including the interaction with dynein motors [67]. It is possible that this type of functional redundancy may also occur with other members of the immunophilin family such as CyP40 and the immunophilin-like protein phosphatase PP5. These two proteins are known components of the Hsp90-based heterocomplex of steroid receptors and their PPIase domains also bind dynein [32].

Regarding the relative abundance of Hsp90-binding immunophilins able to interact with the motor protein in liganded steroid receptor•Hsp90 complexes, it has been shown that while empty GR, MR and PR preferentially associate with FKBP51 over FKBP52 and CyP40 [50][68][69], in the presence of steroid FKBP52 and PP5 are predominantly recruited (along with dynein). However it is CyP40 the dominant immunophilin for ERα•Hsp90 complexes [70], although FKBP52 is also recovered with this receptor. The AR also binds FKBP51 and FKBP52, also being retrotransported by dynein/dynactin motors [37]. Interestingly, the AR shows normal splicing variants that lack the hinge region [71], a short negatively charged segment that lies just C-terminal to the PPIase domain of FKBP52. Due to amino acid charge complementation, this region is the same that has been postulated as an FKBP52 interacting domain with steroid receptors [38][72]. As it may be predicted, the variants of these receptors are unable to interact with dynein, and cells expressing them are insensitive to taxane treatment [71]. Nevertheless, in the case of AR both immunophilins show a peculiar property when they are compared to other steroid receptors, i.e. they are functionally redundant from the transcriptional perspective.

Because the association of dynein with FKBPs has also been demonstrated in plant systems [32][33], the functional role of this complex seems to be preserved along the evolution. Importantly, the disruption of Hsp90 function is critical to abrogate receptor transport, which is not surprising if we consider that this chaperone is the main scaffold factor of the trafficking molecular machinery. The most relevant extrapolation of this property is the fact that those drugs able to interfere with the chaperone impair the biological action of the client-protein. This is the rational strategy for those ongoing clinical trials that are testing Hsp90 inhibitors for cancer treatment [73][74], particularly due to the broad governing functions shown by the chaperone to regulate most of the hallmark processes proper of malignancies [34] and proteinopathies [75]

Another important discovery in the immunophilin field related to the endocrine mechanism of action of steroid receptors was the hormone-dependent biological activity of the receptors, which is itself affected by the type of immunophilin present in the complex. New World primates have the characteristic to show plasma parameters of glucocorticoid resistance syndrome with high levels of plasma cortisol, but a normal GR. In 2001 it was demonstrated in squirrel monkeys that FKBP51 decreases both steroid binding capacity and transcriptional activity of the GR, properties that have been directly correlated with the very high levels of expression of endogenous FKBP51 in the cells of these primates [19][76][77][78]. On the other hand, the expression of FKBP52 is significantly lower. These observations indicated that a high level of FKBP51 expression contributes to glucocorticoid resistance. Later, the inhibitory action of FKBP51 on the GR-dependent response was also correlated with the expression of certain polymorphic isoforms of this immunophilin in neurons, this GR resistance being associated to the development of stress-related post-traumatic syndrome and other psychiatric disorders [79].

In contrast to FKBP51, it is regarded that FKBP52 enhances the biological response of the GR [80]. This was reported in a yeast reconstituted system where no endogenous immunophilins are expressed and after transfecting the GR, immunophilins and a gene reporter. In our own hands, we have also experienced greater transcriptional activity of GR in FKBP52-KO cells when the immunophilin was reintroduced by transfection, but not in wild type cells that seem to be unaffected by FKBP52 overexpression. This conundrum may be explained if it is reasoned that a basal expression of endogenous immunophilin is enough for the stimulatory effect, which could require sub-stoichiometric amounts of FKBP52. In line with this interpretation, it has also been reported that GR-regulated genes are not significantly affected by the loss of FKBP52 [49] in mice with targeted ablation of the FKBP52 gene, suggesting that the immunophilin may not be an essential global enhancer of GR transcription as it has always been considered. Nevertheless, the positive effect of FKBP52 on GR response in non-expressing cells is unquestionable and, under physiological conditions, it is clear that FKBP52 is required for an appropriate biological response of the receptor upon hormone stimulation.

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