

# FKBP Family Proteins: Immunophilins with Versatile Biological Functions

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## Key Words

Immunophilin • FK506-binding protein • Peptidylprolyl *cis/trans* isomerase • Immunophilin ligand • Neuroprotection • FK506 • Rapamycin

## Abstract

Immunophilins consist of a family of highly conserved proteins binding with immunosuppressive drugs such as FK506, rapamycin and cyclosporin A. FK506-binding protein (FKBP) is one of two major immunophilins and most of FKBP family members bind FK506 and show peptidylprolyl *cis/trans* isomerase (PPIase) activity. Small size FKBP family members contain only FK506-binding domain, while FKBP members with large molecular weights possess extra domains such as tetratricopeptide repeat domains, calmodulin binding and trans-membrane motifs. FKBP members are involved in several biochemical processes including protein folding, receptor signaling, protein trafficking and transcription. FKBP family proteins play important functional roles in the T-cell activation, when complexed with their ligands. The roles of immunophilins in protein transportation and apoptosis through their molecular interactions with receptors or proteins have emerged recently. Moreover, therapeutic implications of immunophilin ligands in treating neurodegenerative disorders have been accumulating. FK506 and its derivatives with no immunosuppressive activities bind to the conserved active sites of the canonical FKBP members such as FKBP12, which shows PPIase activity. These immunophilin ligands show variable efficacy in animal models for Parkinson's disease, dementia, and spinal cord injury, where the canonical immunophilins function as chaperones and are associated with the protein folding and modulation of oxidative stress. On the other hand, in the noncanonical FKBP members such as FKBP38, FK506-binding site is not conserved and shows neither PPIase activity nor affinity to FK506. Interestingly, the small molecule-mediated inhibition of the noncanonical member of FKBP family appears to cause neuronal protection and induce proliferation of neuronal stem cells in a rat focal cerebral ischemia model. Currently, the mechanisms of actions remain unclear. This review focuses on molecular characteristics of the canonical and noncanonical FKBP family members and the biological functions of their ligands in performing neuroprotective and neurotrophic activities.

## Introduction

FK506 (tacrolimus), rapamycin (sirolimus), and cyclosporine A (CsA) are well-known immunosuppressive drugs that bind to immunophilins, which exhibit peptidylprolyl *cis/trans* isomerase (PPIase) activity [1–3]. FK506-binding proteins (FKBPs) with various molecular weights are the principal intracellular targets for FK506 and rapamycin, while CsA binds to cyclophilins (CpN) [3]. The formation of FKBP/FK506 and CpN/CsA complexes inhibits not only the PPIase activity of FKBP and CpN, respectively, but also the phosphatase activity of the secondary target calcineurin (CaN), thereby preventing the dephosphorylation of NF-AT that is required for IL-2 gene expression and T-cell activation [4, 5]. On the other hand, rapamycin binds to FKBP12, but the FKBP/rapamycin complex interacts with mammalian target of rapamycin (mTOR) instead of CaN and exerts immunosuppressive activity [6] (fig. 1).

Despite the role of FKBPs and CpNs in modulating T cells, interestingly the immunophilins are more abundant in nervous tissues than in immune tissues, suggesting their biological significance in neurons [7]. Indeed these immunophilin ligands exert neuroprotective and neurotrophic effects. In this review, we provide an overview on the immunophilins in mediating diverse biological functions and examine their molecular characteristics by using examples of the canonical members such as FKBP12 and noncanonical members such as FKBP38, 51 and 52.

### FKBP as PPIase

In folded proteins there are two different conformations of peptide bonds, *cis* or *trans*. Most peptide bonds are found in *trans* conformation in folded proteins, whereas 6% of all Xaa-Pro peptide bonds show *cis* conformation [8]. In vitro studies have shown that energetically-hindered isomerization of Xaa-Pro bonds may limit refolding process of proteins [3]. The *cis/trans* peptidylprolyl isomerization is catalyzed by a super family of PPIase (EC 5.2.1.8) [9, 10]. PPIase of FKBPs are involved in the slow protein folding process and conserved in all organisms from *Archaea* bacteria to primate [3, 11]. *Cis/trans* interconversion of Xaa-Pro peptide bond occurs through binding of a peptide substrate in the hydrophobic binding pocket of the PPIase. The isomerization is facilitated by out-of-plane conformational change of amide bond, which is stabilized through a hydrogen bonding to the amino acid located in the hydrophobic binding cleft, resulting in stabilizing the transition state during the isomerization process [3]. PPIase interacts with a diverse range of intra- and extracellular targets, but the molecular interactions between PPIase and target proteins with Xaa-Pro sequences are weak. Thus, the amount of PPIase required for performing catalysis is usually high.

### FKBPs and Chaperone Activity

Chaperones are proteins that can recognize non-native proteins, prevent unwanted inter- and intramolecular interactions and influence the partitioning between the productive and unproductive folding steps. The chaperones are saved and excluded from the final structures of the folded proteins. There are many examples of FKBPs functioning as chaperones, such as

the mammalian FKBP52, the wheat FKBP73 and *Archaea* bacteria FKBP [3, 12]. Recently, human FKBP38 and *Plasmodium falciparum* FKBP35 (PfFKBP35) have been shown to exhibit chaperone activity [13, 14]. The chaperone activity of FKBP is not inhibited by FK506 or rapamycin, suggesting that this activity is independent of the PPIase activity.

### **FKBP12 and Its Function**

FKBP12, a prototype FKBP which is an extensively characterized member in FKBP family, contains only a single FK506-binding domain (FKBD) comprised of 108 amino acids. It is abundantly and ubiquitously expressed with PPIase activity. Human FKBP12 interacts with FK506 with a  $K_D$  of 0.4 nM or rapamycin with a  $K_D$  of 0.2 nM [15]. The complex formations of FKBP with the ligands enhance the stability of FKBP and the resulting complexes remain more resistant to proteolytic cleavage and create an appropriate binding surface for binding to CaN and mTOR, respectively [3, 16]. Interestingly, in the absence of FK506, FKBP12 binds to cellular targets such as modulate ryanodine receptors (RyRs), which is one of the major  $Ca^{2+}$ -releasing channels in the sarcoplasmic reticulum [12, 17, 18]. The molecular interaction between FKBP12 and RyRs stabilizes the RyR channel and modulates channel gating by increasing the number of RyRs at the full conductance level and thus mean open time [17, 19–21] and the removal of FKBP12 from RyR channels inhibits coupled gating [22], implying an important role of FKBP12 in modulating RyR complexes. FK506 or rapamycin causes a  $Ca^{2+}$  leakage in isolated endothelial cells and induces an intracellular  $Ca^{2+}$  leakage that may contribute to the pathogenesis of endothelial dysfunction and hypertension [23]. FKBP12 has also been demonstrated to tightly interact with the inositol 1,4,5-trisphosphate receptor (IP<sub>3</sub>R) which is activated through phosphorylation by protein A kinase and inactivated through dephosphorylation by CaN [12]. The binding of FKBP12 to IP<sub>3</sub>R enables it to interact with CaN and possibly modulate the receptor's phosphorylation status [24, 25].

FKBP12 also acts as a natural ligand for transforming growth factor- $\beta$  (TGF- $\beta$ ) that regulates a wide range of biological processes. FKBP12 binds to glycine- and serine-rich motif (GS motif) of TGF- $\beta$  receptor I (TGF- $\beta$ RI), capping its phosphorylation and further stabilizes the inactive conformation of TGF- $\beta$ RI [26]. The PPIase core domain of FKBP12 is important for the interaction and FK506 inhibits the interaction between the two proteins, suggesting that FK506 and TGF- $\beta$ RI share a common binding site on FKBP12. Activin, a member of TGF superfamily, induces the dissociation of FKBP12 from the Activin type I receptor (ALK4) and thus exerts its signal. FKBP12 interacts with another inhibitory molecule of Activin signal, Smad7, in an Activin-dependent manner [27] and associates again with ALK4 to suppress the Activin signal. FKBP12 has also an inhibitory effect on epidermal growth factor receptor autophosphorylation [28]. The PPIase activity and the hydrophobic drug-binding pocket of FKBP12 seem to be important for the interaction between FKBP12 and the receptors. Together, these data suggest that FKBP12 is involved in protein-protein interactions and regulates the activities of its cellular partners.

### **FKBPs with Multi-Domains**

Among FKBP family members, FKBP38, 51, and 52 all consist of a single FKBD followed by additional functional units [3]. FKBP38 is a multifunctional protein and contains FKBD,

tripartite TPR domain, putative calmodulin (CBD) and transmembrane (TM) motifs [29, 30] (fig. 1). FKBP38 helps anti-apoptotic proteins Bcl-2 and Bcl-X<sub>L</sub> to localize at the mitochondrial membrane and protects cells from apoptosis [14, 30]. How does FKBP38 interact with Bcl-2? Three-dimensional structural studies reveal that Bcl-2 contains a long flexible loop between Bcl-2 homology 3 and 4 (BH3 and BH4) regions [31]. FKBP38 binds to the flexible loop of Bcl-2 and protects Bcl-2 from degradation [32]. In HEK293 and MEF cells, however, FKBP38 can play an additional chaperone role for Bcl-2 to change its destination to ER membrane through its interaction with Presenilins 1 and 2 (PS1/2) and Bcl-2 [33]. FKBP38 interacts with HSP90 through TPR domain. The interaction between FKBP38 and HSP90 not only inhibits the PPIase activity of FKBP38 but also the interaction between FKBP38 and Bcl-2 [34]. When FKBP38 is knocked down by small interfering RNA, the level of Bcl2 protein is also significantly reduced, while no apparent change in the level of Bcl-2 mRNA is observed. This result suggests that the molecular interaction between FKBP38 and Bcl-2 is important for the function and stability of Bcl-2 and protects the anti-apoptotic protein from potential protein degradation pathways.

The phosphatase activity of CaN is inhibited by forming a ternary complex with FK506 and FKBP12 [35]. Interestingly, after the initial report showing FKBP38 as an endogenous inhibitor of CaN was made, contradictory observations about the inhibitory effect of human FKBP38 on CaN have been reported [30, 36]. A nuclear magnetic resonance spectroscopy study demonstrated that upon the addition of FK506, no apparent spectral changes were detected in 2D<sup>1</sup>H-<sup>15</sup>N heteronuclear single quantum correlation (HSQC) spectrum of FKBD of FKBP38, while chemical shift perturbations were observed in the HSQC spectrum of FKBP12 upon the addition of FK506 [14]. This study clearly suggests that FKBP38 has no affinity to FK506 and the mode of action differs from those of canonical FKBP family members. At least in vitro no intrinsic CaN inhibitory activity of FKBP38 was detected. However, since the observations were made in two different experimental conditions, whether FKBP38 functions as an endogenous inhibitor of CaN in the absence of FK506 remains to be further investigated.

Recently, Rheb, a Ras-like small guanosine triphosphatase (GTPase), in response to growth factor stimulation and nutrient availability, interacts directly with FKBP38 in the absence of rapamycin and prevents its association with mTOR in a guanosine 5-triphosphate (GTP)-dependent manner, suggesting that FKBP38 is an endogenous inhibitor of mTOR [37]. A previous study also suggests FKBP38 as an intrinsic inhibitor of CaN [30]. Together these data indicate that the molecular mechanism of the noncanonical FKBP family member FKBP38 is unique and pleiotropic.

Unlike FKBP38, larger-sized FKBP family members FKBP51 and 52 possess PPIase activity and bind FK506 [38–41]. These proteins contain a tandem FKBD separated by a short linker sequence. The N-terminal FKBDs of the larger FKBP family members are responsible for the PPIase- and ligand-binding activities. The C-terminal FKBDs are inactive in those activities. The latter domains contain an ATP/GTP-binding sequence [40, 42]. Thr143 residue located between the two FKBDs is phosphorylated by casein kinase-II [43]. Similarly, TPR domains are also important for their interactions with heat shock protein 90 (HSP90) [40]. The resulting FKBP51/ HSP90 or FKBP52/HSP90 complexes are associated with progesterone

receptor (PR) or glucocorticoid receptor (GR) and the ternary complexes migrate from the cytoplasm to the nucleus [44].

### **Structural Characteristics of FKBP**s

The prototype FKBP family member FKBP12 only contains a single FKBD (fig. 2), which is responsible for both PPIase- and FK506-binding activities. The structure of FKBP12 is characterized by an amphipathic five-stranded  $\beta$ -sheet. The  $\beta$ -sheet has a right-handed twist and wraps around the helix, and forms a hydrophobic pocket in which FK506 or rapamycin binds between the  $\alpha$ -helix and  $\beta$ -sheet (fig. 3a, c).

FKBP38, which is a noncanonical FKBP family member, unlike most canonical FKBP, lacks the conserved amino acid residues required for binding FK506 and for PPIase activity, suggesting its FK506-independent function [14, 29, 30]. FKBP38 also lacks the well-conserved Trp59 in FKBP12 which is important for the interaction with FK506 and has instead Leu residue at the corresponding position [14, 29]. NMR solution structures reveal that the FKBD of FKBP38 shows the overall structural similarity to that of FKBP12 [45, 46]. However, the hydrophobic packing and interactions could be unfavorably influenced by the multiple substitutions of the aromatic residues in FKBP12 to Leu in FKBP38, since the Leu provides a smaller van der Waal surface than that generated by aromatic residues (fig. 3b, d). This could be a molecular basis by which FKBP38 has no affinity to FK506.

It is interesting to note that FKBP38 appears to contain the secondary structure elements of an 'inactive' FKBP-type isomerase. In other words, it lacks significant loop insertion between  $\beta$ 3 and  $\beta$ 4. The larger molecular weight FKBP family proteins FKBP51 and 52 show unique structures featuring in tandem FKBDs and multiple TPR domains [41, 44]. Their N-terminal FKBD domains (referred to as FKBD1 in figure 2) possess PPIase activity and can bind FK506 and rapamycin. On the other hand, the C-terminal FKBP domains (referred to as FKBD2 in figure 2) neither possess measurable rotamase activity nor binds FK506 and rapamycin. The FKBD2 is mainly responsible for the molecular interactions between the immunophilins and proteins such as HSP90 and steroid receptors [40, 41, 47, 48]. Structures of FKBD1 and FKBD2 of FKBP51 [41] and FKBP52 [44] reveal that the amino acid substitutions at the active site and loop insertions flanking the binding pocket directly cause the loss of activity in the FKBD2. The structure of FKBP38 more closely resembles the C-terminal rather than the N-terminal FKBD domains of these two family members. However, the binding surface of FK506 is partially preserved in FKBP38 and not optimal for the full rotamase activity compared to canonical FKBP family members. FKBP38 retains a noncanonical rotamase activity, which is activated in response to  $\text{Ca}^{2+}$ /calmodulin mobilization, in an unknown mechanism [49].

### **Role of FKBP**s in Neuronal Cells

FKBP are abundantly present in neuronal tissues and their expressions are elevated after nerve injury [7, 50, 51], leading to examine the roles of FKBP ligands in neuronal cells. As alternative therapeutics to protein neurotrophins for the treatment of neurodegenerative diseases, immunosuppressive drugs have been studied and shown to have variable effects in

reversing neurodegenerative process and preventing apoptotic cell death in neuronal cells [52–54]. However, in contrast to neurotrophins, most immunophilin ligands are highly stable and can readily cross the blood-brain barrier, prompted the development of nonimmunosuppressive immunophilin ligands with potent and selective therapeutic activities. GPI-1046, one of nonimmunosuppressive immunophilin ligands, binds to FKBP12, and like FK506, it showed neuroprotective and neuroregenerative effects on primary cultures of midbrain dopaminergic neurons against both 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) toxicities [55], while exerting similar effects in rodent models [56, 57]. GPI-1485, the second-generation compound of GPI-1046, demonstrated substantial efficacy in preclinical and initial phases of clinical evaluation for Parkinson's disease (PD) model [58]. Another V-13,661, which does not bind FKBP12, can prevent a progressive dopaminergic axonal degeneration and neuronal death in a mouse model [59]. A recent study also demonstrates that the inhibition of the PPIase activity of FKBP38 by a small molecule inhibitor N-(N',N'-dimethylcarboxamidomethyl)cycloheximide shows neuroprotection as well as neural stem proliferation in a rat model for an acute focal cerebral ischemia [60], adding an additional diversity of the immunophilin-related neuroprotective mechanism.

How do these immunophilin ligands exert neurotrophic and neuroprotective functions? The canonical FKBP family members bind FK506 and the resulting FKBP/FK506 complexes inhibit CaN activity, leading to immunosuppression. However, the ligand binding does not appear to be required for the effects of the immunophilins on neurons, since both immunosuppressive and nonimmunosuppressive immunophilin ligands and also compounds that have no affinity to FKBP12 [59] or even FKBP12 knock-out mice still show efficacies in neurons [55]. These data suggest that neurotrophic and neuroprotective functions of immunophilin ligands can occur without forming complexes between immunophilin ligands and immunophilins. FKBP12 also binds to other chaperone proteins and receptors such as HSP90 [41], steroid receptor [17], and regulatory molecules [26, 30], suggesting important biological function of FKBP12 in protein-protein interaction in various signaling pathways. FKBP12 possess PPIase activity, function as chaperone proteins, and may aid protein folding of target molecules [52]. Or the neuroprotective properties of FKBP12 may be associated with modulation of oxidative stress in neural tissues [61]. Thus, the immunophilin ligands-mediated neuroprotective mechanism appears to be complex and might depend on combination of various external stimuli and cellular signaling pathways.

## **Conclusion**

FKBP12 in mammals and other organisms play important roles in various biochemical processes including protein folding, protein trafficking, and protein assembly. Accumulating evidence no doubt suggests that natural immunosuppressive drugs FK506, CsA, rapamycin and their analogs can promote neurite outgrowth and neuronal survival in in vitro and in vivo animal models and provide new therapeutic windows for neurodegenerative disorders such as PD. Several studies demonstrated that the complex formation between immunophilin ligands and immunophilin may not be necessary for neurotrophic and neuroprotective functions of immunosuppressive drugs [60], suggesting the presence of potential and novel mechanisms of actions of these drugs. Recent studies have shown that FKBP12, 38 and 52 are abundantly

expressed in the brain and participate in multiple cellular processes [60, 62–64]. With three-dimensional structural data available for FKBP family members, recent findings on FKBP family would certainly lead to the structure-based rational design of selective ligands that target specific FKBP family members in response to different external stimuli. The promising leads in this therapeutic approach would minimize the potential side effects associated with the lead ligands.

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## References

1. Harding MW, Galat A, Uehling DE, Schreiber SL: A receptor for the immunosuppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase. *Nature* 1989; 341: 758–760.
2. Siekierka JJ, Hung SH, Poe M, Lin CS, Sigal NH: A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* 1989; 341: 755–757.
3. Galat A: Peptidylprolyl *cis/trans* isomerases (immunophilins): biological diversity – targets – functions. *Curr Top Med Chem* 2003; 3: 1315–1347.
4. Huai Q, Kim HY, Liu Y, Zhao Y, Mondragon A, Liu JO, Ke H: Crystal structure of calcineurin-cyclophilin-cyclosporin shows common but distinct recognition of immunophilin-drug complexes. *Proc Natl Acad Sci USA* 2002; 99: 12037–12042.
5. Ke H, Huai Q: Structures of calcineurin and its complexes with immunophilins-immunosuppressants. *Biochem Biophys Res Commun* 2003; 311: 1095–1102.
6. Sharma VK, Li B, Khanna A, Sehajpal PK, Suthanthiran M: Which way for drug-mediated immunosuppression? *Curr Opin Immunol* 1994; 6: 784–790.
7. Dawson TM, Steiner JP, Lyons WE, Fotuhi M, Blue M, Snyder SH: The immunophilins, FK506 binding protein and cyclophilin, are discretely localized in the brain: relationship to calcineurin. *Neuroscience* 1994; 62: 569–580.
8. Fischer S, Michnick S, Karplus M: A mechanism for rotamase catalysis by the FK506-binding protein (FKBP). *Biochemistry* 1993; 32: 13830–13837.
9. Galat A: Sequence diversification of the FK506-binding proteins in several different genomes. *Eur J Biochem* 2000; 267: 4945–4959.
10. Maruyama T, Suzuki R, Furutani M: Archaeal peptidyl prolyl *cis-trans* isomerases (PPIases) update 2004. *Front Biosci* 2004; 9: 1680–1720.
11. Suzuki R, Nagata K, Yumoto F, Kawakami M, Nemoto N, Furutani M, Adachi K, Maruyama T, Tanokura M: Three-dimensional solution structure of an archaeal FKBP with a dual function of peptidyl prolyl *cis-trans* isomerase and chaperone-like activities. *J Mol Biol* 2003; 328: 1149–1160.
12. Breiman A, Camus I: The involvement of mammalian and plant FK506-binding proteins (FKBPs) in development. *Transgenic Res* 2002; 11: 321–335.
13. Monaghan P, Bell A: A *Plasmodium falciparum* FK506-binding protein (fkbp) with peptidyl-prolyl *cis-trans* isomerase and chaperone activities. *Mol Biochem Parasitol* 2005; 139: 185–195.
14. Kang CB, Feng L, Chia J, Yoon HS: Molecular characterization of FK-506 binding protein 38 and its potential regulatory role on the anti-apoptotic protein Bcl-2. *Biochem Biophys Res Commun* 2005; 337: 30–38.
15. Gothel SF, Marahiel MA: Peptidyl-prolyl *cis-trans* isomerases, a superfamily of ubiquitous folding catalysts. *Cell Mol Life Sci* 1999; 55: 423–436.
16. Harrar Y, Bellini C, Faure JD: FKBP: at the crossroads of folding and transduction. *Trends Plant Sci* 2001; 6: 426–431.
17. Brillantes AB, Ondrias K, Scott A, Kobrinisky E, Ondriasova E, Moschella MC, Jayaraman T, Landers M, Ehrlich BE, Marks AR: Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 1994; 77: 513–523.
18. Wang T, Donahoe PK: The immunophilin FKBP12: a molecular guardian of the TGF-13 family type I receptors. *Front Biosci* 2004; 9: 619–631.

19. Jayaraman T, Brillantes AM, Timerman AP, Fleischer S, Erdjument-Bromage H, Tempst P, Marks AR: FK506-binding protein associated with the calcium release channel (ryanodine receptor). *J Biol Chem* 1992; 267: 9474–9477.
20. Timerman AP, Ogunbumni E, Freund E, Wiederrecht G, Marks AR, Fleischer S: The calcium release channel of sarcoplasmic reticulum is modulated by FK-506-binding protein. Dissociation and reconstitution of FKBP-12 to the calcium release channel of skeletal muscle sarcoplasmic reticulum. *J Biol Chem* 1993; 268: 22992–22999.
21. Gaburjakova M, Gaburjakova J, Reiken S, Huang F, Marx SO, Rosemblyt N, Marks AR: FKBP12 binding modulates ryanodine receptor channel gating. *J Biol Chem* 2001; 276: 16931–16935.
22. Marx SO, Ondrias K, Marks AR: Coupled gating between individual skeletal muscle Ca<sup>2+</sup> release channels (ryanodine receptors). *Science* 1998; 281: 818–821.
23. Long C, Cook LG, Wu GY, Mitchell BM: Removal of FKBP12/12.6 from endothelial ryanodine receptors leads to an intracellular calcium leak and endothelial dysfunction. *Arterioscler Thromb Vasc Biol* 2007; 27: 1580–1586.
24. Cameron AM, Nucifora FC Jr, Fung ET, Livingston DJ, Aldape RA, Ross CA, Snyder SH: FKBP12 binds the inositol 1,4,5-trisphosphate receptor at leucine-proline (1400–1401) and anchors calcineurin to this FK506-like domain. *J Biol Chem* 1997; 272: 27582–27588.
25. Cameron AM, Steiner JP, Roskams AJ, Ali SM, Ronnett GV, Snyder SH: Calcineurin associated with the inositol 1,4,5-trisphosphate receptor-FKBP12 complex modulates Ca<sup>2+</sup> flux. *Cell* 1995; 83: 463–472.
26. Wang T, Donahoe PK, Zervos AS: Specific interaction of type I receptors of the TGF- $\beta$  family with the immunophilin FKBP-12. *Science* 1994; 265: 674–676.
27. Yamaguchi T, Kurisaki A, Yamakawa N, Minakuchi K, Sugino H: FKBP12 functions as an adaptor of the Smad7-Smurfl complex on activin type I receptor. *J Mol Endocrinol* 2006; 36: 569–579.
28. Lopez-Illasaca M, Schiene C, Kullertz G, Tradler T, Fischer G, Wetzker R: Effects of FK506-binding protein 12 and FK506 on autophosphorylation of epidermal growth factor receptor. *J Biol Chem* 1998; 273: 9430–9434.
29. Lam E, Martin M, Wiederrecht G: Isolation of a cDNA encoding a novel human FK506-binding protein homolog containing leucine zipper and tetratricopeptide repeat motifs. *Gene* 1995; 160: 297–302.
30. Shirane M, Nakayama KI: Inherent calcineurin inhibitor FKBP38 targets Bcl-2 to mitochondria and inhibits apoptosis. *Nat Cell Biol* 2003; 5: 28–37.
31. Petros AM, Medek A, Nettesheim DG, Kim DH, Yoon HS, Swift K, Matayoshi ED, Oltersdorf T, Fesik SW: Solution structure of the antiapoptotic protein Bcl-2. *Proc Natl Acad Sci USA* 2001; 98: 3012–3017.
32. Kang CB, Tai J, Chia J, Yoon HS: The flexible loop of bcl-2 is required for molecular interaction with immunosuppressant FK-506-binding protein 38 (FKBP38). *FEBS Lett* 2005; 579: 1469–1476.
33. Wang HQ, Nakaya Y, Du Z, Yamane T, Shirane M, Kudo T, Takeda M, Takebayashi K, Noda Y, Nakayama KI, Nishimura M: Interaction of presenilins with FKBP38 promotes apoptosis by reducing mitochondrial Bcl-2. *Hum Mol Genet* 2005; 14: 1889–1902.

34. Edlich F, Erdmann F, Jarczowski F, Moutty MC, Weiwad M, Fischer G: The Bcl-2 regulator FKBP38-calmodulin-Ca<sup>2+</sup> is inhibited by HSP90. *J Biol Chem* 2007; 282: 15341–15348.
35. Griffith JP, Kim JL, Kim EE, Sintchak MD, Thomson JA, Fitzgibbon MJ, Fleming MA, Caron PR, Hsiao K, Navia MA: X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12- FK506 complex. *Cell* 1995; 82: 507–522.
36. Weiwad M, Kullertz G, Schutkowski M, Fischer G: Evidence that the substrate backbone conformation is critical to phosphorylation by p42 MAP kinase. *FEBS Lett* 2000; 478: 39–42.
37. Bai X, Ma D, Liu A, Shen X, Wang QJ, Liu Y, Jiang Y: Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. *Science* 2007; 318: 977–980.
38. Lebeau MC, Myagkikh I, Rouviere-Fourmy N, Baulieu EE, Klee CB: Rabbit FKBP-59/HBI does not inhibit calcineurin activity in vitro. *Biochem Biophys Res Commun* 1994; 203: 750–755.
39. Wiederrecht G, Hung S, Chan HK, Marcy A, Martin M, Calaycay J, Boulton D, Sigal N, Kincaid RL, Siekierka JJ: Characterization of high molecular weight FK-506 binding activities reveals a novel FK-506-binding protein as well as a protein complex. *J Biol Chem* 1992; 267: 21753–21760.
40. Davies TH, Sanchez ER: FKBP52. *Int J Biochem Cell Biol* 2005; 37: 42–47.
41. Sinars CR, Cheung-Flynn J, Rimerman RA, Scammell JG, Smith DF, Clardy J: Structure of the large FK506-binding protein FKBP51, an HSP90-binding protein and a component of steroid receptor complexes. *Proc Natl Acad Sci USA* 2003; 100: 868–873.
42. Callebaut I, Renoir JM, Lebeau MC, Massol N, Burny A, Baulieu EE, Mornon JP: An immunophilin that binds M<sub>r</sub> 90,000 heat shock protein: main structural features of a mammalian p59 protein. *Proc Natl Acad Sci USA* 1992; 89: 6270–6274.
43. Miyata Y, Chambraud B, Radanyi C, Leclerc J, Lebeau MC, Renoir JM, Shirai R, Catelli MG, Yahara I, Baulieu EE: Phosphorylation of the immunosuppressant FK506-binding protein FKBP52 by casein kinase II: regulation of HSP90-binding activity of FKBP52. *Proc Natl Acad Sci USA* 1997; 94: 14500–14505.
44. Wu B, Li P, Liu Y, Lou Z, Ding Y, Shu C, Ye S, Bartlam M, Shen B, Rao Z: 3D structure of human FK506-binding protein 52: implications for the assembly of the glucocorticoid receptor/HSP90/immunophilin heterocomplex. *Proc Natl Acad Sci USA* 2004; 101: 8348–8353.
45. Kang CB, Ye H, Vivekanandan S, Simon B, Sattler M, Yoon HS: Backbone <sup>1</sup>H, <sup>13</sup>C, and <sup>15</sup>N resonance assignments of the N-terminal domain of FKBP38 (FKBP38NTD). *J Biomol NMR* 2006; 36(suppl 1): 37.
46. Maestre-Martinez M, Edlich F, Jarczowski F, Weiwad M, Fischer G, Lucke C: Solution structure of the FK506-binding domain of human FKBP38. *J Biomol NMR* 2006; 34: 197–202.
47. Chambraud B, Rouviere-Fourmy N, Radanyi C, Hsiao K, Peattie DA, Livingston DJ, Baulieu EE: Overexpression of p59-HBI (FKBP59), full length and domains, and characterization of PPIase activity. *Biochem Biophys Res Commun* 1993; 196: 160–166.
48. Denny WB, Prapapanich V, Smith DF, Scammell JG: Structure-function analysis of squirrel monkey FK506-binding protein 51, a potent inhibitor of glucocorticoid receptor activity. *Endocrinology* 2005; 146: 3194–3201.

49. Edlich F, Weiwad M, Erdmann F, Fanghanel J, Jarczowski F, Rahfeld JU, Fischer G: Bcl-2 regulator FKBP38 is activated by Ca<sup>2+</sup>/calmodulin. *EMBO J* 2005; 24: 2688–2699.
50. Lyons WE, George EB, Dawson TM, Steiner JP, Snyder SH: Immunosuppressant FK506 promotes neurite outgrowth in cultures of PC12 cells and sensory ganglia. *Proc Natl Acad Sci USA* 1994; 91: 3191–3195.
51. Lyons WE, Steiner JP, Snyder SH, Dawson TM: Neuronal regeneration enhances the expression of the immunophilin FKBP-12. *J Neurosci* 1995; 15: 2985–2994.
52. Poulter MO, Payne KB, Steiner JP: Neuroimmunophilins: a novel drug therapy for the reversal of neurodegenerative disease? *Neuroscience* 2004; 128: 1–6.
53. Zhang C, Steiner JP, Hamilton GS, Hicks TP, Poulter MO: Regeneration of dopaminergic function in 6-hydroxydopamine-lesioned rats by neuroimmunophilin ligand treatment. *J Neurosci* 2001; 21:RC156.
54. Pong K, Zaleska MM: Therapeutic implications for immunophilin ligands in the treatment of neurodegenerative diseases. *Curr Drug Targets CNS Neurol Disord* 2003; 2: 349–356.
55. Guo X, Dawson VL, Dawson TM: Neuroimmunophilin ligands exert neuroregeneration and neuroprotection in midbrain dopaminergic neurons. *Eur J Neurosci* 2001; 13: 1683–1693.
56. Ross DT, Guo H, Howorth P, Chen Y, Hamilton GS, Steiner JP: The small molecule FKBP ligand GPI 1046 induces partial striatal re-innervation after intranigral 6-hydroxydopamine lesion in rats. *Neurosci Lett* 2001; 297: 113–116.
57. Steiner JP, Hamilton GS, Ross DT, Valentine HL, Guo H, Connolly MA, Liang S, Ramsey C, Li JH, Huang W, Howorth P, Soni R, Fuller M, Sauer H, Nowotnik AC, Suzdak PD: Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. *Proc Natl Acad Sci USA* 1997; 94: 2019–2024.
58. Wu YQ, Wilkinson DE, Limburg D, Li JH, Sauer H, Ross D, Liang S, Spicer D, Valentine H, Fuller M, Guo H, Howorth P, Soni R, Chen Y, Steiner JP, Hamilton GS: Synthesis of ketone analogues of prolyl and pipercolyl ester FKBP12 ligands. *J Med Chem* 2002; 45: 3558–3568.
59. Costantini LC, Cole D, Chaturvedi P, Isacson O: Immunophilin ligands can prevent progressive dopaminergic degeneration in animal models of Parkinson's disease. *Eur J Neurosci* 2001; 13: 1085–1092.
60. Edlich F, Weiwad M, Wildemann D, Jarczowski F, Kilka S, Moutty MC, Jahreis G, Lucke C, Schmidt W, Striggow F, Fischer G: The specific FKBP38 inhibitor N-(N',N'-dimethylcarboxamidomethyl)-cycloheximide has potent neuroprotective and neurotrophic properties in brain ischemia. *J Biol Chem* 2006; 281: 14961–14970.
61. Tanaka K, Fujita N, Higashi Y, Ogawa N: Neuroprotective and antioxidant properties of FKBP-binding immunophilin ligands are independent on the FKBP12 pathway in human cells. *Neurosci Lett* 2002; 330: 147–150.
62. Christner C, Herdegen T, Fischer G: FKBP ligands as novel therapeutics for neurological disorders. *Mini Rev Med Chem* 2001; 1: 377–397.
63. Avramut M, Achim CL: Immunophilins and their ligands: Insights into survival and growth of human neurons. *Physiol Behav* 2002; 77: 463–468.

64. Steiner JP, Dawson TM, Fotuhi M, Glatt CE, Snowman AM, Cohen N, Snyder SH: High brain densities of the immunophilin FKBP colocalized with calcineurin. *Nature* 1992; 358: 584–587.

## List of Figures

- Figure 1 Immunophilin/immunosuppressant-mediated signal transduction pathways via the T-cell receptor/CD3 complex induce activation of protein tyrosine kinases (PTKs) and phospholipases, leading to the generation of inositol trisphosphate (IP<sub>3</sub>). Subsequently the receptor activation results in an increase in intracellular free calcium concentration, followed by the activation of calmodulin (CaM) and CaM-dependent enzymes. CaM-dependent phosphatase calcineurin (CaN) is a direct target of CsA/CpN and FK506/FKBP complexes. CaN is involved in T-cell activation through the dephosphorylation of critical phosphoprotein substrates such as NF-ATc. Simplified mechanism of action of rapamycin is also shown. Binding of growth factors, such as IL-2, to their receptors, induces PTK activity and activates MAP kinases. The FKBP12/rapamycin (Rapa) complex binds directly to the FKBP12-rapamycin-binding (FRB) domain of mTOR. This interaction results in the inhibition of activities of several downstream signaling processes as indicated.
- Figure 2 Shown are the schematic diagrams of function domains of FKBP12, 52 and 38. FKBD, TPR, and CBD domains are indicated. Prolyl hydroxylase 2 (PHD2)-interacting region and transmembrane (TM) motif of FKBP38 are shown as labeled. Other immunophilin-interacting proteins to FKBD and TPR domains are also shown.
- Figure 3 **a** Structure of FKBP12 bound to tacrolimus (FK506) (PDB ID: 1FKJ). FKBP12 is shown by ribbon representation. Residues conserved across human FKBP domains and relevant to substrate binding and catalysis are shown in stick representation and labeled. **b** Structure of FKBP38-FKBD (PDB ID: 2AWG) is shown. Most of the aromatic residues conserved for the ligand-binding site are replaced with Leu residue in FKBP38. Note the  $\beta$  'bulge' which is inserted between  $\beta 3$  and  $\beta 4$  in the FKBP12 structure is not present in the FKBP38 structure. **c, d** The ligand-binding surface of FKBP12 (**c**) and FKBP38 (**d**) are shown in surface representation; key residues surrounding the ligand-binding site are labeled. FK506 is shown in stick representation. The ligand surface in FKBP38 is partially occluded.

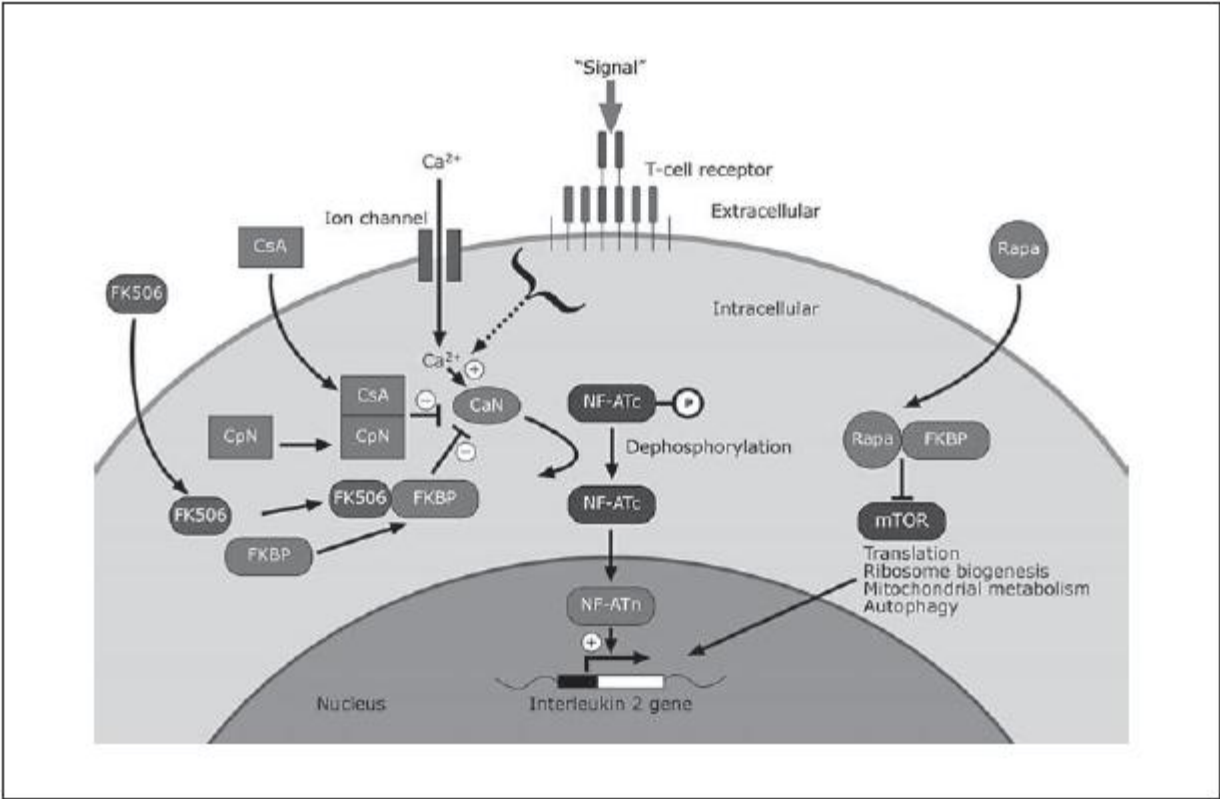


Figure 1

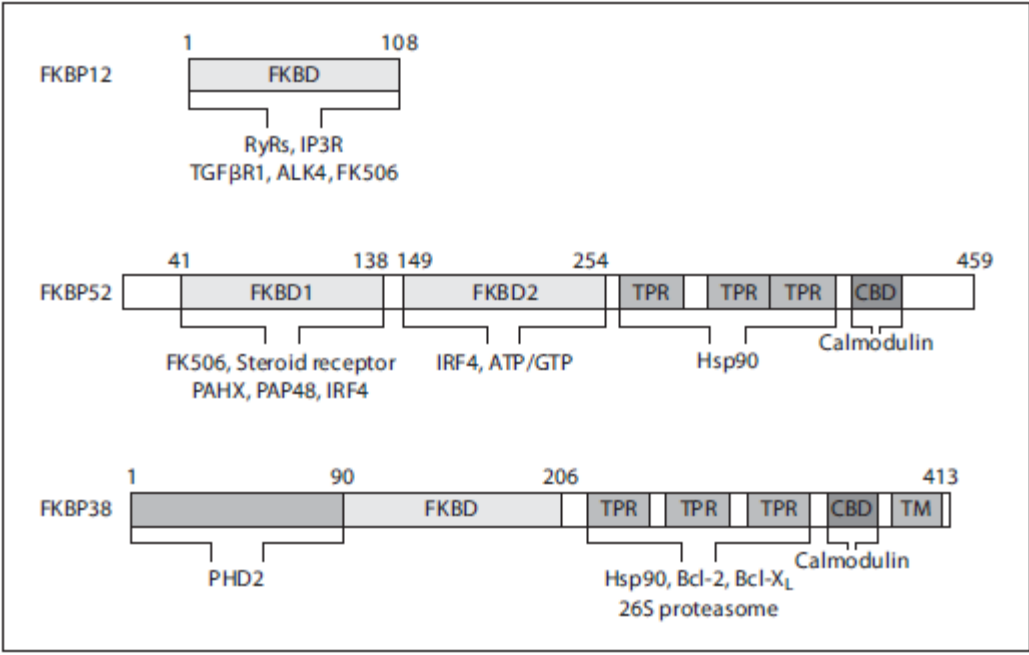


Figure 2

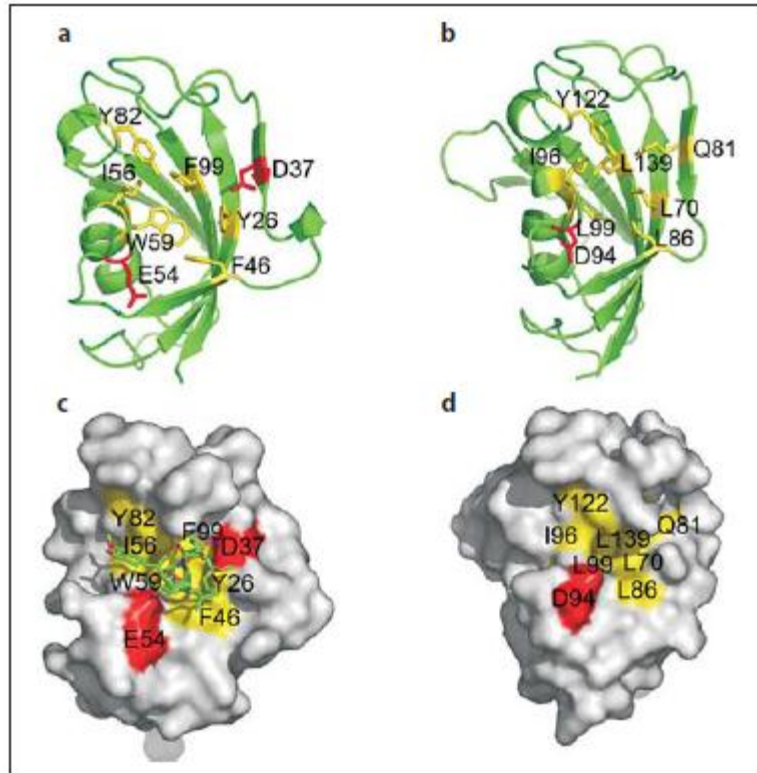


Figure 3