

Pharmacological Targeting of Catalyzed Protein Folding: The Example of Peptide Bond *cis/trans* Isomerases

F. Edlich · G. Fischer (✉)

Max-Planck Research Unit for Enzymology of Protein Folding, Weinbergweg 22,
06120 Halle/Saale, Germany
fischer@enzyme-halle.mpg.de

1	Introduction	360
2	Cyclophilins	364
2.1	Immunosuppression	364
2.2	Viral Infections	366
2.3	Parasitic Infections	369
2.4	Malignancies	369
2.5	Ischemia/Reperfusion Injury	370
2.6	Amyotrophic Lateral Sclerosis	371
2.7	Inflammation	372
2.8	Allergy	373
3	FKBPs	373
3.1	Immune Response	374
3.2	Skin Disorders	376
3.3	Neuropathies	376
3.4	Inherited Disease	378
3.5	Pathogenic Microorganisms	378
3.6	Steroid Responsiveness	379
3.7	Malignancies	381
3.8	Arylhydrocarbon (Dioxin) Responsiveness	383
3.9	Cardiovascular Disorders	384
4	Parvulins	386
4.1	Malignancies	386
4.2	Neuropathies	388
	References	389

Abstract Peptide bond isomerases are involved in important physiological processes that can be targeted in order to treat neurodegenerative disease, cancer, diseases of the immune system, allergies, and many others. The folding helper enzyme class of Peptidyl-Prolyl-*cis/trans* Isomerases (PPIases) contains the three enzyme families of cyclophilins (Cyps), FK506 binding proteins (FKBPs), and parvulins (Pars). Although they are structurally unrelated, all PPIases catalyze the *cis/trans* isomerization of the peptide bond preceding the proline in a polypeptide chain. This process not only plays an important role in de novo protein folding, but also in isomerization of native proteins. The native state isomerization plays a role in physiological processes by influencing receptor ligand recognition or isomer-

specific enzyme reaction or by regulating protein function by catalyzing the switch between native isomers differing in their activity, e.g., ion channel regulation. Therefore elucidating PPIase involvement in physiological processes and development of specific inhibitors will be a suitable attempt to design therapies for fatal and deadly diseases.

Keywords Peptide bond isomerases · PPIases · FKBP · Cyclophilin · Parvulin

1

Introduction

In principle, the native conformation of a protein is determined by its amino acid sequence since many isolated proteins can be denatured and refolded in vitro in the absence of other cellular components. However, it is now clear that assembly and folding of polypeptides in vivo involves helper proteins that assist in protein folding. The spatiotemporal characteristics of the various conformational states or folding states of a protein dictate important events in cell life under normal and pathophysiological conditions. Physically, protein folding is a complex process whereby a polypeptide chain can adopt a huge number of conformations that may differ greatly from its native folding state, but represent, as well, a transiently stable form of protein. These different folding intermediates can be seen as a polypeptide chain on an energy landscape containing many energy minima. Obviously, the connection of different energy minima requires folding pathways in order to allow adoption of the native protein fold (Dobson et al. 1998). Notably, the same rules apply for de novo protein folding and conformational changes in native proteins. Therefore chain reshuffling by protein–protein and protein–ligand interactions, de novo protein folding, and refolding of denatured proteins are based on similar molecular principles (Tsai et al. 1999).

Protein-mediated folding assistance was found to exploit many biochemical mechanisms resulting in different modes of action. It includes folding in cavities, enzymatically catalyzed folding and coupled chain holding-chain folding cycles. The pathways of assisted folding play a key role in cell response to physiological signals, and effectors of the helper proteins involved represent a potential for therapeutic intervention for many human diseases. In the last two decades, different families of folding helper proteins have been characterized that proved to be highly conserved during evolution. Among them, the folding helper enzymes act catalytically to control the folding dynamics and the products of folding. Both protein disulfide isomerases and peptide bond *cis/trans* isomerases form the sole examples of folding helper enzymes known to date. Particular attention is given to peptide bond *cis/trans* isomerases because peptide bond *cis/trans* isomerization plays an obligatory role for protein folding, whereas disulfide bond formation does not.

Notably, most rotations about covalent bonds, events that dictate the progress of the folding reaction, proved intrinsically to be very fast and do not need fur-

ther rate acceleration by either external factors or intramolecular assistance. Potentially, external influences on rotational rates could be mediated by a wide variety of physical and chemical means, such as enzyme catalysis, catalysis by low-molecular-mass compounds, heat, mechanical forces, and supportive microenvironments. Acid-base catalysis, torsional strain or proximity, and field effects are major forces with the potential to act in intramolecular assistance or enzyme catalysis. Among the folding helper enzymes only peptide bond *cis/trans* isomerases are able to catalyze conformational interconversions in unfolded, partially folded, and native states of proteins (Fischer and Aumuller 2003). In all cases, enzymatic rate enhancement requires a particular substrate structure usually characterized by a combination of primary and secondary binding sites. In fact, on the basis of accelerated bond rotation, peptide bond *cis/trans* isomerases can be characterized as conformases. Currently known enzymes show specificity for the peptide bond preceding either a proline residue (prolyl peptide bond) or a variety of secondary amide peptide bonds. These enzymes termed peptidyl prolyl *cis/trans* isomerases (PPIases) and the secondary amide peptide bond *cis/trans* isomerases (APIases), can be divided into subfamilies, some of which are characterized by their ability to recruit secondary binding sites of the substrate polypeptide chain for catalysis. Current knowledge on PPIases goes back to first observations in 1984 (Fischer et al. 1984), whereas the discovery of APIases is a more recent event (Schiene-Fischer et al. 2002).

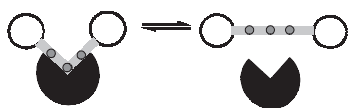
Extensive studies of PPIases have provided reasonable knowledge of the mechanisms determining the relationship between prolyl isomerization in proteins and cell signaling.

It was shown that PPIases not only increase the rate of the slow kinetic phases in the refolding of denatured proteins, but also accelerate efficiently the interconversion between native state isomers of proteins. Regardless of their sequential context, the *cis* and *trans* prolyl bond isomers in unstructured, partially structured, or even native proteins exhibit energetic differences, which tend to be small, thus leading to comparable levels of isomers in solution.

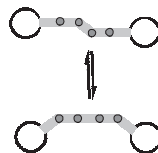
Given that one isomer is not reactive, coupling of this equilibrium to a fast subsequent reaction step must cause a transient discrimination of an isomer in the overall reaction (isomer-specificity, Fig. 1a). Isomer-specific reactivity differences have been demonstrated in many bioreactions, including enzyme catalyzed phosphate transfer, proteolysis, protein folding, and receptor/ligand recognition (Fischer and Aumuller 2003). In addition, peptide bond stiffness, which is reduced under PPIase catalysis, may be a reactivity determining molecular parameter in polypeptides (Fig. 1b). Furthermore, the switch-like character of peptide bond *cis/trans* isomerization allows for chemomechanical coupling in proteins (Fig. 1c) (Fischer 1994; Tchaicheeyan 2004).

In the years following the discovery of the PPIase in pig kidney, cyclophilins, FK506-binding proteins, and parvulins have been characterized, which now collectively form the enzyme class of PPIases (EC 5.2.1.8.). Prolyl bonds usually

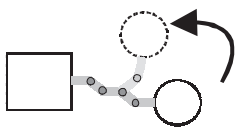
A) Isomer-specificity of polypeptide recognition



B) Segmental flexibilization in the context of the rigid backbone



C) Mechanical force generator for structural distortion



D) One-bond folding energy trap in native proteins

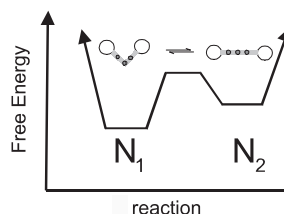


Fig. 1a–d Biochemical mechanisms underlying the physiological role of peptidyl prolyl bond *cis/trans* isomerizations of proteins. **a** Proteins (dark gray) capable of isomer-specific recognition of a polypeptide ligand (gray). An extreme manifestation of isomer specificity is seen if one prolyl isomer releases free binding energy (left), while the other isomer is not able to do so (right). Examples have been found in protein folding, enzyme catalysis, and receptor/ligand interactions. **b** Local control of chain flexibility in the context of the rigid polypeptide backbone. A decrease in energy barrier to rotation of a prolyl bond has the conformational consequence of promoting nearly unconstrained motion of adjacent covalent bonds similar to those present in alkanes. This process might play a role in protein folding. **c** Mechanical force generator for structural distortion. The *cis/trans* isomerization of a single peptidyl-prolyl bond may confer to global structural rearrangements changing the relative positions of a moving domain (circle) to a static domain (rectangle). This process is thought to play a role in allosteric regulation of proteins. In addition, peptidyl prolyl bond *cis/trans* isomerization addresses, on the submolecular level, a broad spectrum of actomyosin's functional characteristics (Tchaicheeyan 2004). **d** One-bond folding energy trap is important in conferring protein metastability. The native protein adopts *cis/trans* isomerism of prolyl bonds, which must lead to conformational polymorphism. Assuming that protein molecules populate the N_2 state, the higher energy implies kinetic instability. Conversion to the stable folding state may proceed very slowly for prolyl isomers

confer uncoupling of a bond rotation from the framework movement of the backbone and side chains by virtue of an exceptionally high torsion barrier. As a result, the time scale of the uncatalyzed interconversion between the prolyl *cis/trans* isomers ranges from several hundred milliseconds to a few hours under physiological conditions.

The singularity of prolyl bonds in peptide chains results from the N-alkylated amino acid proline forming an imidic peptide bond at its N-terminus. This property is exceptional since all other gene-coded amino acids form secondary amide peptide bonds. In both types of peptide bond, the nitrogen atom is able to delocalize its lone electron pair over the whole functional group. This results in a planar framework of electronic organization and the consequent partial double-bond character of the C-N bond, restricting the energy minima in peptide bond torsion. The free energy dependence of the peptide bond *cis/trans* isomerization in a chain fragment indicates just two minimum-energy states: the geometric isomers *cis* and *trans*. Both isomers are separated by the above-mentioned rotational barrier corresponding to the perpendicular high-energy intermediate state of rotation. While three-dimensional protein structures achieved by X-ray crystallography reveal prolyl bonds that adopt a conformational homogeneous state, either *cis* or *trans* conformation, solution NMR spectroscopy reveals a more complex picture. Many native proteins show limited conformational polymorphism, termed native state isomerization (Fig. 1d). These conformational states are mainly caused by the structural properties of a particular proline residue in the polypeptide chain. Protein conformational polymorphism often indicates the existence of peptide bond *cis/trans* isomers of different biological activity (Andreotti 2003).

The enzyme class of PPIases presently comprises three enzyme families that represent structurally and functionally distinct proteins. Enzyme families were named according to the affinity of prototypic enzymes for the immunosuppressive drugs cyclosporin A (CsA) and FK506, cyclophilins (Cyps), and FK506-binding proteins (FKBPs). "Immunophilin" is a term that collectively comprises members of both enzyme families. The parvulin family of PPIases has not been reported thus far to exhibit affinity for these immunosuppressive drugs. Heat shock proteins of the hsp70 family comprise the first examples of APIases (Schiene-Fischer et al. 2002).

Numerous biochemical investigations have led to the elucidation of four different modes of action, some of which were shown to represent auxiliary features of PPIases in cells (Fischer and Aumuller 2003). However, rate acceleration of prolyl isomerization plays a central role in their physiological function. Consequently, these enzymes may contribute to de novo protein folding as well as polypeptide chain rearrangements in native proteins. Logically, PPIases involved in de novo protein folding must show distinct characteristics as compared to enzymes involved in the interconversion between native states of proteins. To effectively perform this broad range of tasks, PPIase families, in particular FKBPs and cyclophilins, consist of many individual members of different molecular masses that encompass one or more PPIase domains complemented with other functional polypeptide segments (Galat 2004a, 2004b). In addition, PPIase activity might be controlled by accessory cellular factors, such as second messengers or interacting proteins. Such molecular characteristics are expected to contribute to the regulatory function of the PPIase,

a situation reminiscent of the regulation of protein kinases and protein phosphatases.

Among the plethora of human PPIases, several members seem to be specifically involved in the interconversion of native protein conformers, which differ in their biological activity. However, it is reasonable to assume functional redundancy among paralogous PPIases. It should also be noted that complete depletion of the catalytic activity in human cells, which may be possible for lower eukaryotes, proved to be difficult (Dolinski et al. 1997; Wang et al. 2001).

Functional analyses of PPIases in cells, performed with PPIase variants, attenuated in their catalytic activity, protein knock-down by genetic means, or small cell-permeable inhibitors, allowed unequivocal identification of many signaling pathways that are based on catalyzed prolyl isomerization. Consequently, specific inhibition of PPIases might cause pathway blockade, which can have beneficial effects in pathologically altered cells, tissues and organs, by interfering with catalyzed folding processes.

However, it remains a challenge to identify differential inhibitors for the individual members of the PPIase families.

2 Cyclophilins

Members of this PPIase family are ubiquitously expressed throughout the human organism (Fischer and Aumuller 2003; Galat 2004a). In their catalytic domain, cyclophilins share high sequence similarity to the prototypic cyclophilin 18 (Cyp18, CypA), which has a molecular mass of 18 kDa. The immunosuppressive drug cyclosporine A (CsA) represents a tight-binding inhibitor for the PPIase activity of most human cyclophilins. For many human cyclophilins, additional polypeptide segments complete the full-length proteins. They were found to be located N-terminally and C-terminally to the catalytic core domain. Functionally, the extra domains and segments are coupled with intracellular targeting, RNA recognition, tetratricopeptide repeat (TPR)-mediated protein-protein interactions and macro-complex assembly of Ran-binding proteins.

2.1 Immunosuppression

Low-molecular-mass inhibitors of cyclophilins are utilized in prevention of allograft rejection in transplantation medicine, for treatment of autoimmune diseases, and for prophylaxis of graft-versus-host diseases (Arai and Vogelsang 2000; Gremese and Ferraccioli 2004). Prior binding of CsA derivatives to Cyp18 is one requirement for inhibition of T cell activation after antigenic stimulation and immunosuppressive activity (Fischer et al. 1989; Takahashi et al. 1989). A causal relationship between inhibition of the Cyp18 PPIase activity and T

cell replication does not exist (Navia 1996; Schreier et al. 1993). Instead, the protein phosphatase calcineurin appears to be involved in Ca^{2+} -dependent signal transduction pathways in, among others, T cells and mast cells. Cyp18 and FKBP12 probably mediate the immunosuppressive actions of CsA and FK506, respectively, by a gain-of-function effect of the immunosuppressive drug/immunophilin complex with and inhibiting the protein phosphatase activity of calcineurin (Friedman and Weissman 1991; Liu et al. 1991). Mechanistically, Cyp18 forms a binding platform for CsA, with a portion of the drug molecule remaining presented to calcineurin by the resulting complex. Probably the most important reason for the occurrence of gain-of-function effects is alteration in macrocyclic conformation of CsA when bound to its presenter PPIase. Downregulation of the NF-AT, NF κ B, and the c-jun N-terminal kinase (JNK) signaling pathways that determine expression of IL-2 and other cytokines is thought to play a key role in conferring susceptibility to CsA and FK506 effects on the growth of T cells involved in cellular immune response (Vogel et al. 2001). It has been reported that continuous treatment of transplant patients with CsA induces numerous side effects such as hypertension, encephalopathy, malignancy, neurotoxicity, hyperglycemia and chronic nephropathy. The most prominent CsA side effects are nephrotoxicity and oxidative stress causing a block in muscle differentiation that is mediated by ROS generation (Hong et al. 2002). In addition, CsA application causes nephrotoxicity by initiation of vacuolization and fatty change in tubular epithelial and endothelial cells, inhibition of cell growth, detachment, and cell death in a time- and dose-dependent manner (Ryffel et al. 1988). Besides its ability to interfere with calcineurin-mediated signaling, cyclosporins inhibit PPIase activity of many cellular cyclophilins in drug-treated patients. A variety of physiological processes exist where cyclophilins were shown to be involved (Bennett et al. 1998; Waldmeier et al. 2002, 2003; Wei et al. 2004; Yurchenko et al. 2002). Thus, inhibition of the PPIase activity of cyclophilins may be associated with the occurrence of adverse effects in long-term treatment with cyclosporins. In fact, calcineurin-independent pathways affect peripheral T cell deletion induced by either superantigens or anti-TCR $\alpha\beta$ mAb, and allergy-induced by superantigens, and this may be relevant to the development of immune tolerance (Prudhomme et al. 1995).

To add to the puzzle, the estimation of calcineurin activity in CsA-treated renal transplant patients raised doubts about the direct relationship between calcineurin inhibition and immunosuppression. In circulating lymphocytes of the immunosuppressed patients, calcineurin activity is only partially reduced (50%–85% of the control) (Batiuk et al. 1995). The calcineurin fraction resistant to inhibition is even larger for FK506 administration in several tissues. The serum CsA level can approximately predict clinical parameters of immunosuppression in individuals but is poorly correlated with the calcineurin activity of lymphocytes. It was hypothesized that partial calcineurin inhibition might account for both the immunosuppression and the immunocompetence

of CsA-treated patients (Batiuk et al. 1997). Sanglifehrin macrolides, which are structurally distinct from cyclosporins, offer a new approach for preventing clonal T cell expansion via tight binding to cyclophilins. Sanglifehrin A blocks T cell proliferation in response to IL-2 by inhibiting the appearance of activity in the cell cycle kinase cyclinE-Cdk2 (Zhang et al. 2001). Interestingly, a Cyp18-sanglifehrin complex does not inhibit the protein phosphatase calcineurin but is completely inactive in PPIase assays (Zenke et al. 2001). Sanglifehrin A was shown to inhibit mitochondrial cyclophilin, and is thus an inactivating agent of the mitochondrial permeability transition pore, which is highly involved in the promotion of apoptosis and necrosis (Clarke et al. 2002). In addition, Sanglifehrin A interferes with the differentiation of dendritic cells and promotes endocytosis of antigens (Steinschulte et al. 2003; Woltman et al. 2004).

Cyp18 can influence T cell activation on its own utilizing a pathway that does not require the application of cyclosporins. Knock-down of Cyp18 in mice promotes development of allergic diseases with elevated IgE and tissue infiltration by mast cells and eosinophils, which is driven by CD4(+) T helper type II (Th2) cytokines (Colgan et al. 2004). Cyp18 inhibits the interleukin-2 tyrosine kinase (Itk) that is a nonreceptor kinase, promoting T cell activation (Brazin et al. 2002; Mallis et al. 2002). Mechanistically, the PPIase interacts with the Src homology 2 (SH2) domain of the kinase known to control the activity of the neighboring catalytic domain. This interaction mediates a native state prolyl isomerization, causing a conformational change in the structure of Itk. Consequently, Cyp18 catalytically accelerates switching between the active and the inactive isomer of Itk. This process prevents T cell activation by cellular factors acting downstream of the Itk. Application of CsA prevents the interaction of Cyp18 and Itk.

2.2

Viral Infections

Cyp18 plays an important functional role in virus replication among the host cell proteins of human immunodeficiency virus type-1 (HIV-1) virions because it enhances viral infectivity (Luban et al. 1993). Other folding helper enzymes are also found in complex with viral proteins (Brenner and Wainberg 1999). Virions released from infected cells show a ratio of ten copies of p24^{gag} to one copy of Cyp18, which is a unique feature among the virions of retroviruses (Thali et al. 1994). The results of some studies underscore the importance of the Cyp18 population of the target cell vs Cyp18 packaged in virions (Ikeda et al. 2004). During early phases of replication, Cyp18 was found to associate with proline-rich segments of both the retroviral capsid protein p24^{gag} and the viral protein R (Vpr) (Zander et al. 2003).

Consequently, blocking the host cell Cyp18–provirus interaction by cyclophilin inhibitors, such as immunosuppressive and nonimmunosuppressive CsA derivatives or reducing the cellular concentration of Cyp18 by antisense

U7 snRNAs and siRNAs targeting Cyp18 was beneficial for limiting the virus load (Bartz et al. 1995; Liu et al. 2004; Thali et al. 1994). Indeed, previous analyses already suggested the beneficial effect of CsA treatment on the progression of disease and outcome of AIDS-related mortality (Huss et al. 1995). In another study, CsA restored normal CD4(+) T cell levels, both in terms of percentage and absolute numbers in AIDS patients (Rizzardi et al. 2002).

Recent data show that three isoforms of Cyp18 assemble with HIV-1 particles (Misumi et al. 2002). One of the three isoforms was found outside of the viral membrane. It was suggested that this Cyp18 isoform might play a role in the attachment of the virions to the surface of the target cell. It has been discussed that Cyp18 mediates HIV-1 attachment to a cell by targeting heparans on the cellular surface (Saphire et al. 1999). This observation is supported by data from groups investigating the role of Cyp18 and Cyp23 in response to inflammation (Yurchenko et al. 2002).

The two remaining isoforms were isolated as components of the viral membrane. They most likely play a role in the regulation of the HIV-1 p24^{gag} conformation (Misumi et al. 2002). They are included in the viral membrane as a result of interactions with Gag polyprotein when the virion is assembled and released from the host cell (Franke et al. 1994). Cyp18 binds p24^{gag} at three distinct sites. One is located in the N-terminal part of the protein around Gly₈₉-Pro₉₀ (Gamble et al. 1996; Howard et al. 2003) (Fig. 2). The interaction at this site certainly influences virion packaging.

In the C-terminal part of p24^{gag}, the other two interaction sites are at Gly₁₅₆-Pro₁₅₇ and Gly₂₂₃-Pro₂₂₄ and may play a role in destabilizing the capsid cone (Endrich et al. 1999). Viruses assembled in cells with low Cyp18 concentrations are less infectious than particles assembled at high Cyp18 concentrations (Liu et al. 2004).

Another aspect of the Cyp18-p24^{gag} interaction is the Cyp18-mediated evasion of the antiviral action of a human restriction factor that targets p24^{gag} soon after virus entry into the cell. This situation is revealed in owl monkey cells where restriction is released by capsid mutants or CsA that disrupt capsid interaction with Cyp18. The structures of viral capsid proteins largely determines restriction (Perron et al. 2004). It was suggested that HIV-1 co-opted Cyp18 to counteract restriction factors and that this adaptation can confer sensitivity to restriction in unnatural hosts (Towers et al. 2003). A chimerical protein containing a restriction factor and Cyp18 expressed in owl monkey was shown to account for postentry restriction of HIV-1 and block HIV-1 infection when transferred to otherwise infectable human or rat cells (Sayah et al. 2004).

Cyp18-catalyzed prolyl isomerization has been detected in capsid-derived oligopeptides and the N-terminal fragment of HIV-1 capsid at the Gly₈₉-Pro₉₀ site (Bosco et al. 2002). On the other hand, a Vpr-Cyp18 fusion protein variant, which lacks PPIase activity, rescues HIV-1 replication in a *trans* complementation assay (Saphire et al. 2002).

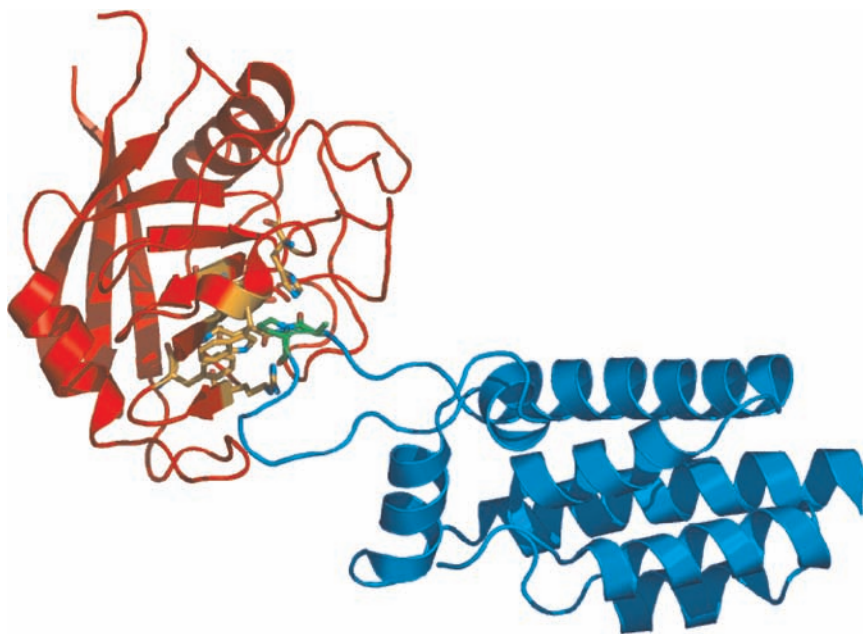


Fig. 2 Representation of the three-dimensional structure of the Cyp18/HIV-1 capsid complex (Howard et al. 2003). The prototypic cyclophilin folds a β -barrel structure containing eight antiparallel β -sheets and two amphipathic α -helices depicted in *red*. The PPIase-active site of Cyp18 is assembled by the amino acid side chains of R55, F60, Q63, F113, W121, and H126 (*orange*). The active site of Cyp18 binds to P90 and its preceding glycine residue (*green*) within the exposed loop of the capsid protein, displayed in *blue*

Interestingly, application of CsA and a nonimmunosuppressive CsA derivate to virus-infected quiescent cells preloaded with anti-CD25 immunotoxin diminishes virus production and thus suppress infectivity (Borvak et al. 1996).

In addition to data showing the importance of Cyp18 for HIV-1 replication, there is evidence that the cyclophilins are utilized by other viruses, as well. Because CsA dose-dependently inhibits herpes simplex virus production in resting monkey kidney cells, the involvement of cyclophilins in virus replication is obvious (Vahlne et al. 1992). A considerable variation in CsA sensitivity of replication has been obtained for serologically distinct types of vesicular stomatitis virus (VSV) in baby hamster kidney cells (Bose et al. 2003). Overexpression of a catalytically inactive Cyp18 variant parallels the CsA effects. It was shown that Cyp18 interacts with the nucleocapsid protein and is incorporated into VSV particles. These data imply that the processes that Cyp18 mediates in the VSV life cycle differ from its function in HIV-1. A calcineurin-independent effect of CsA has been reported for the replication of the hepatitis C virus and data from bone marrow transplantation patients and nontransplant patient populations confirm that CsA inhibits HCV replication (Pollard 2004; Watashi et al. 2003).

2.3

Parasitic Infections

CsA is an antiparasitic drug for many different parasitic infections whose mode of action might involve both parasite and host cell cyclophilins. Response to CsA treatment has already been observed for malaria, leishmaniasis, trypanosomiasis, schistosomiasis, and filariasis (Bell et al. 1996). In fact, *Bruugia malayi* is resistant to the antiparasitic activity of cyclosporin A (CsA), in accordance with the relatively low CsA affinity of parasitic cyclophilins (Ellis et al. 2000). On the other hand, the antihelmintic action of different cyclosporins against *Hymenolepis microstoma* does not correspond with the degree of cyclophilin binding and implies that a parasite surface component is the drug target (McLauchlan et al. 2000). Typically, survival to infection with *Trypanosoma cruzi* was 50% higher for CsA-treated mice than in non-treated animals (Calabrese et al. 2000). Immunosuppression and antimalarial activity of cyclosporin derivatives does not correlate in parasites. Therefore calcineurin is not involved in cyclosporin-sensitive pathogenesis (Bell et al. 1996). This study investigates whether complexing of CsA with parasite Cyp may account for its antihelmintic action.

2.4

Malignancies

Using differential display techniques, cDNA microarrays and proteome projects frequently revealed upregulation of Cyp18 in various types of cancer cells (Campa et al. 2003; Grzmil et al. 2004; Lim et al. 2002; Rey et al. 1999). The mitochondrial Cyp22 is specifically upregulated in human tumors of the breast, ovary, and uterus (Schubert and Grimm 2004). Among the cyclophilins, Cyp40 is unique for its ability to sequester heat shock proteins and its involvement in the control of mitogenic signaling mediated by glucocorticoids (Renoir et al. 1995; Ward et al. 1999). Consequently, deletion of the Cyp40 gene promotes breast tumor progression in late developmental phases (Ward et al. 2001). Treatment of breast cancer cells with estradiol increases steady state concentrations of Cyp40 mRNA through both transcriptional and post-transcriptional mechanisms (Kumar et al. 2001). The function of Cyp40 in the steroid hormone receptor complex seems to be related to the role of FKBP51 and FKBP52 in that the unactivated receptor complex is stabilized, and assistance is provided for subcellular translocation processes upon steroid binding. In addition, Cyp40 seems required to form the functional peroxisome proliferator-activated receptor α (PPAR α) as well. This complex might play a role in the development of liver cancer after chronic exposure to peroxisome proliferators, including certain industrial and pharmaceutical chemicals, and in mitogenic or apoptotic regulation of growing tumors (Miller et al. 2000).

Both Cyp40 and FKBP5 have been found in complex with Hsp90 and p53 via their tetratricopeptide repeat (TPR) domains and dynein with their PPIase domains. This heterocomplex forms a functional link between the PPIases and p53 transport into the nucleus in colon carcinoma cells (Galigniana et al. 2004).

A Cyp18 isoform sharing about 84% sequence identity with Cyp18 were identified to be encoded in the q21–23 region of chromosome 1, which has been recognized as being extensively transcribed during the development of metastases in liver, breast, and bladder cancer (Meza-Zepeda et al. 2002; Nilsson et al. 2004). The gene product was designated chromosome one amplified sequence 2 (COAS2), but has not yet been characterized enzymatically.

Immunosuppressive drugs could promote tumor progression due to calcineurin inhibition, but immune response-independent mechanisms have also been suggested (London et al. 1995; Van de Vrie et al. 1997). In contrast, rapamycin, another immunosuppressive agent acting through a calcineurin/cyclophilin-independent mechanism, exhibits potent anti-tumor activity. It was shown that CsA could induce cancer cell-typical phenotypic changes in normal cells. The host immune defense does not account for this effect. The CsA-mediated enhancement of tumor growth in immunodeficient SCID-beige mice can be prevented by simultaneous treatment with mAb directed against transforming growth factor- β (TGF- β) (Hojo et al. 1999).

A 166-kDa cyclophilin (NKTR), which harbors a N-terminal cyclophilin domain followed by a long C-terminal extension, is an important determinant of natural killer (NK) cells. A signaling pathway has been described that utilizes this cyclophilin in MHC unrestricted killing of tumor cells (Giardina et al. 1995). This protein belongs to the large subfamily of SR cyclophilins thought to be involved in cell cycle regulation (Dubourg et al. 2004).

Cyclosporin A and its nonimmunosuppressive derivative [3'-Keto-Bmt¹]-[Val²]-cyclosporin (PSC 833) has been shown to increase the sensitivity of multidrug-resistant (MDR) cells to chemotherapeutic agents used in anti-tumor treatment (Boesch et al. 1991). Labeling of the cyclosporin derivatives demonstrated the binding to and blocking of the human MDR1 P-glycoprotein, a transmembranous adenosine 5'-triphosphate binding cassette (ABC) transporter (Loor et al. 2002).

2.5

Ischemia/Reperfusion Injury

Mitochondria play a major role in ischemic cell death in peripheral organs and the central nervous system, because activation of the mitochondrial permeability transition pore (mtPTP) causes apoptosis and necrosis in injured tissue (Li et al. 2004). During pore opening, the mitochondrial inner membrane becomes freely permeable to solutes of less than 1.5 kDa. The mtPTP is a protein complex consisting of a cyclophilin (Cyp22, CypD), the voltage-dependent anion channel, members of the pro- and anti-apoptotic Bax/Bcl-2 protein family,

and the adenine nucleotide (ADP/ATP) translocator. Calcium-triggered functional switching of the mtPTP from a specific transporter to a nonspecific pore is facilitated by the binding of Cyp22 to the adenine nucleotide (ADP/ATP) translocator (probably on proline residue 61) (Halestrap and Brenner 2003). Overexpression of Cyp22 had opposite effects on apoptosis and necrosis, obtaining suppression of apoptosis at high Cyp22 levels (Li et al. 2004; Schubert and Grimm 2004).

On the other hand, permeability transition induced by calcium ions was powerfully inhibited by CsA and nonimmunosuppressive CsA derivatives at nanomolar concentrations, indicating a potential anti-apoptotic drug effect due to inhibition of Cyp22 enzyme activity (Griffiths and Halestrap 1995; Hansson et al. 2004). Consequently, studies suggest preservation of mitochondrial integrity in animal stroke models, in injured rat liver and heart as well as neuroprotective effects of CsA in animal models (LeDucq et al. 1998; Waldmeier et al. 2003; Yu et al. 2004). However, different cyclophilins are thought to be involved in the beneficial CsA effects (Li et al. 2004).

Using the calcineurin-inactive drug sangliferin new light was shed on Cyp22 function in the mtPTP complex (Clarke et al. 2002). Sangliferin inhibits mtPTP activation but not formation of an adenine nucleotide (ADP/ATP) translocator–Cyp22 complex. Therefore, proteins assembling the mtPTP probably undergo structural changes catalyzed by Cyp22 that do not correlate with complex formation but with the pore function. Despite similar K_i values for inhibition of the PPIase activity of Cyp22 *in vitro*, sangliferin binds more tightly to mitochondria than CsA. The sigmoidal dose-response curves obtained for the pore inhibition by sangliferin allowed assessment of pore complex composition and detection of cooperative interactions between the pore components during opening.

2.6

Amyotrophic Lateral Sclerosis

Hyperactive, mutant Cu/Zn superoxide dismutase-1 (SOD), which can be linked to familial amyotrophic lateral sclerosis (familial ALS), induces apoptosis of neuronal cells in culture because of increase in reactive oxygen species (Lee et al. 1999). Overexpressed wild type Cyp18, but not a PPIase-inactive Cyp18 variant, protected cells from death after hyperactive SOD variant expression. This result indicates involvement of misfolding reactions of unknown client proteins in the loss of motor neurons. Application of CsA enhanced neuronal cell death. Silencing of SOD hyperactivity by RNAi protects against CsA effects. Consequently, CsA was not shown to be beneficial in patients with allergic contact dermatitis, multiple sclerosis, or amyotrophic lateral sclerosis (Faulds et al. 1993). It is somewhat surprising that CsA treatment is also able to attenuate degeneration and cell death of injured neurons in mouse brain tissue (Karlsson et al. 2004). Results showing that CsA in doses of (10.0 mg kg⁻¹) can prevent ax-

otomized neonatal motor neuron death are counter to the expectations drawn from the SOD variant-based approach (Iwasaki et al. 2002). Interestingly, the downregulation of another PPIase, the multidomain enzyme FKBP52, was also found to be important for pathogenesis of ALS (Manabe et al. 2002).

2.7

Inflammation

Dependent on its concentration level, prototypic Cyp18 acts as proinflammatory molecule in many diseases. The protein mediates endothelial cell proliferation, migration, invasive capacity, and tubulogenesis at low concentrations. Opposite effects have been observed at high Cyp18 concentrations (Kim et al. 2004). Increased concentrations of Cyp18 are found in the blood stream of patients with inflammatory diseases. Cyp18 acts chemotactically to leukocytes that are found in increased levels, as well (Xu et al. 1992). Cyp18 and the highly homologous Cyp23 are also found in high concentrations in the serum of patients with rheumatic arthritis and sepsis (Billich et al. 1997; Tegeder et al. 1997). The chemotactic action of PPIases depends on the free accessibility of the active site of the cyclophilins, because application of PPIase inhibitors can completely suppress the effect. Most likely, the cyclophilins are exocytosed to the plasma during inflammation and act as a chemotaxis factor. Once in the blood stream, the cyclophilins might bind with high affinity to heparin sulfate proteoglycans and thus induce chemotaxis for neutrophils and T cells (De Ceuninck et al. 2003).

Upon stimulation of arterial endothelia cells by lipopolysaccharides, Cyp18 is expressed and secreted (Coppinger et al. 2004; Kim et al. 2004). Smooth muscle cells and macrophages show similar effects in response to stimulation by lipopolysaccharides and oxidative stress, suggesting a role in the pathogenesis of inflammatory diseases, such as arteriosclerosis (Jin et al. 2000, 2004).

Heat and chronic hypoxia mediate overexpression of Cyp18 up to three-fold in myogenic cells (Andreeva et al. 1997). Furthermore, it was found that hypoxic cardiac muscle cells overexpress the cell surface receptor CD147, considered to be the Cyp18 receptor (Seko et al. 2004). Cyp18 signaling via CD147 involves the Cyp18 active site that probably binds to the Pro₁₈₀-Gly₁₈₁ moiety of CD147 (Yurchenko et al. 2002). CD147 signaling culminates in ERK activation. PPIase-inactive Cyp18 mutants failed to initiate the signaling cascade. Cyp23 is able to promote CD147 signaling, as well (Yurchenko et al. 2001). However, the mechanism by which cyclophilins protect cells against oxidative stress remains to be discovered. After chemical modifications, the systemically active cyclosporin A can be converted to a drug that after topical application is beneficial in cutaneous inflammation (Rothbard et al. 2000).

Characteristic features of asthma are allergic bronchial inflammation and airway hyperresponsiveness, with increased numbers of eosinophils and activated T cells in the airways. The efficacy and the pharmacological profile

of cyclosporin A treatment in patients with chronic severe asthma argues for T cell involvement, but a direct effect on the pro-inflammatory function of cyclophilins can also be assumed (Kon and Kay 1999). For the allergen-induced late asthmatic reaction, inhibitory effects on eosinophil-associated cytokines and chemokines have been discussed. The beneficial effect of CsA may also be the result of a reduced accumulation of eosinophils (Khan et al. 2000). Blocking the chemotactic action of Cyp18 might contribute to the latter effect.

2.8

Allergy

FK506, CsA, and CsA derivatives attenuate IgE-mediated histamine release from human basophils according to their relative immunosuppressive potency (Sperr et al. 1997). Exposure of cyclophilins, which form a pan-allergen family, to sensitized individuals could lead to the release of anaphylactogenic mediators due to cross-linking of IgE bound to the high-affinity surface protein Fc(ϵ)RI (Fluckiger et al. 2002). FK506 effects on Fc(ϵ)RI-mediated exocytosis of preformed mediators directly correlates with the amount of calcineurin B subunits in the cytosol of mast cells and basophils (Hultsch et al. 1998a, 1998b). Prototypic cyclophilins of carrots, birch, *A. fumigatus*, *E. granulosus*, and *basidiospores* belong to the allergens that share a high degree of sequence identity with the corresponding human protein. In fact, serum of individuals sensitized against mold proteins shows autoreactivity against human Cyp18 (Appenzeller et al. 1999).

3

FKBPs

Members of the FKBP family have rather different molecular masses in humans. Overall, catalytic domains in FKBP are much less conserved than the catalytic domains within the cyclophilin family of PPIases. Both PPIase families exhibit significant differences in their catalytic mechanisms and substrate specificities (Fanghanel and Fischer 2004). The domain composition is also more diverse, ranging from a multiplicity of catalytic domains, EF hands, calmodulin binding sites to TPR motifs (Fischer and Aumuller 2003). Among the 16 different FKBP in humans, there are members that are crucial in apoptosis (Edlich et al. 2005), receptor signaling (Pratt et al. 1999), calcium homeostasis (Schiene-Fischer and Yu 2001), and spermatogenesis (Crackower et al. 2003). Most human FKBP, if not all, bind to and become inhibited by FK506, with K_i values ranging from high picomolar to high nanomolar levels. The three-dimensional structure of the inhibitory complex with FBBP12-FK506 complex might identify the catalytic site of FKBP12 at the FK506 position in the complex (Fig. 3). As in the case of cyclophilins, differ-

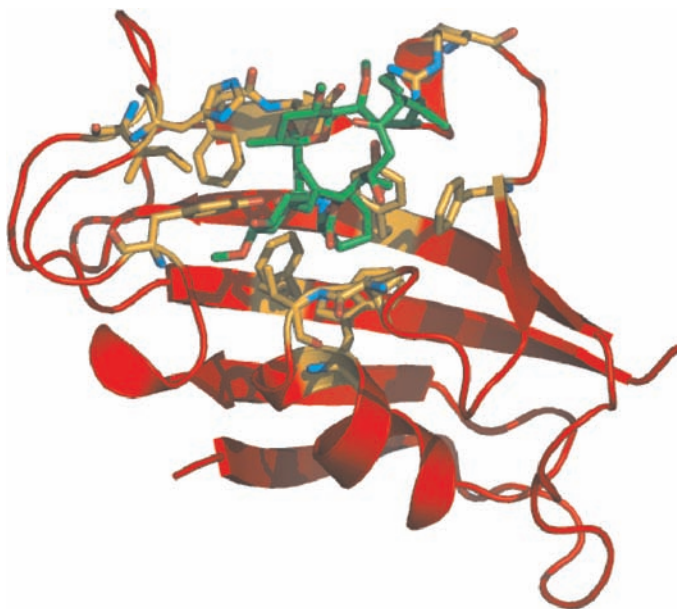


Fig. 3 Representation of the three-dimensional structure of the inhibitory FK506/FKBP12 complex (Van Duyne et al. 1991). The global fold of the prototypic FKBP12 (*red*) is assembled by five antiparallel β -sheets and an α -helix. The peptidomacrolide FK506 (*green*) binds to the putative active site of FKBP12. The interaction of FKBP12 to FK506 is mediated by the residues Y26, F36, D37, R42, F46, V55, J56, W59, Y82, H87, I91, and F99, depicted in *orange*, which are thought to assemble the active site of this PPIase. The pipecolinyl moiety of FK506 penetrates the prolyl binding pocket of FKBP12

ential low-molecular-mass inhibitors for the human members of the FKBP family are still lacking.

3.1 Immune Response

Unlike Cyp18, FKBP12 utilizes two different modes of action to contribute to drug-mediated immunosuppression. First, human FKBP, like cyclophilins, belong to the immunophilins because the endogenous FKBP12 of T cells binds the microbial drug FK506 (also known as tacrolimus), with subsequent block of antigen-stimulated T cell replication due to calcineurin targeting. Inhibition of the protein phosphatase activity of calcineurin by the FKBP12-FK506 complex is thought to form the biochemical basis of immunosuppression (Liu et al. 1991). Complex formation between calcineurin and FKBP12-FK506 does not directly interfere with the catalytic site of calcineurin (Griffith et al. 1995). Instead, complex formation hinders proteinaceous calcineurin substrates from entering the active site by applying steric constraints to binding of protein

substrates (Fig. 4). Notably, the active FK506 conformer competitively inhibits the PPIase activity of FKBP12 with a K_i value of 0.5 nM (Zarnt et al. 1995). The dissociation constants of the ternary CaN/FKBP/FK506 complex range from 88 nM to 27 μ M when different FKBP s are allowed to share the ternary complex (Edlich, unpublished observations).

Secondly, formation of a tight complex of the peptidomacrolide rapamycin (also known as sirolimus) with endogenous FKBP12 inhibits T cell proliferation by blocking protein synthesis and arresting the cell cycle in the G1 phase. Similar to the Cyp18-CsA combination, FKBP12-attached rapamycin experiences gain-of-function that leads to inhibition of mTOR kinase, a downstream effector of the phosphatidylinositol 3-kinase (PI3 K)/Akt (protein kinase B) signaling pathway. mTOR plays an important role in RNA stability and transcription, and controls the translation machinery, in response to amino acids and growth factors, via activation of p70 ribosomal S6 kinase, and inhibition of the eIF-4E binding protein (Fingar and Blenis 2004; Kahan 2004).

Given the common inhibition of many PPIases in the cell, CsA, FK506, and rapamycin may share similar side effects in patients (Hong et al. 2002; Mihatsch et al. 1998).

The multidomain enzyme FKBP52 was shown to interact with the peroxisomal phytanoyl-CoA α -hydroxylase, a protein that has homology to the LN1 sup-

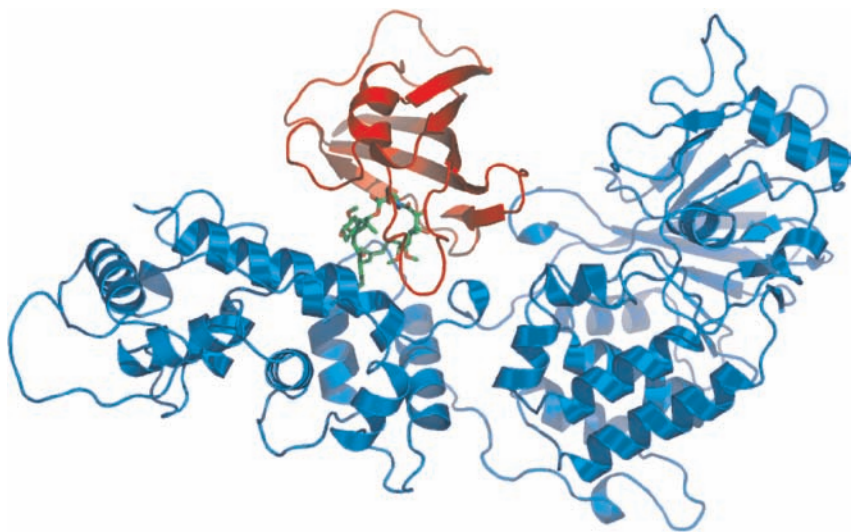


Fig. 4 Three-dimensional structure of the CaN/FK506/FKBP12 complex (Griffith et al. 1995). The protein phosphatase CaN is assembled from two subunits (*blue*), the catalytic subunit CaNA and the regulatory subunit CaNB. FKBP12 is displayed in *red*. After FKBP12 binding, FK506 (*green*) experiences gain of function enabling the resulting complex to form an inhibitory CaN complex. Both FKBP12 and FK506 participate in the interaction with calcineurin. Neither FK506 nor FKBP12 alone are able to inhibit CaN

pressor of progression of the human autoimmune disease lupus erythematosus (Chambrud et al. 1999). It was shown that the N-terminal PPIase-domain of FKBP52 mediates the interaction. A physiological role of this interaction has not yet been elucidated. FKBP12-directed autoantibodies, which prevent the formation of the inhibitory FKBP12–FK506–calcineurin complex, have been frequently found in serum of patients with autoimmune diseases (Shinkura et al. 1999).

3.2

Skin Disorders

The therapeutic effects of FKBP active site ligands to inflammatory skin disorders suggest a major role of FKBP in functional modulations of epidermal cells. Cyclosporin A, rapamycin and FK506 were found to exhibit differential effects on T cell and keratinocyte proliferation. Recent reports link between FK506 application in basal epidermal keratinocytes and therapeutic success in skin disease such as psoriasis (Al-Daraji et al. 2002; Panhans-Gross et al. 2001). Ongoing autoreactive Th-1 response of psoriatic epidermis has genetically determined immunogenic and inflammatory components. It is thought that the calcineurin inhibition in T cells may account in epidermal keratinocytes for the block of cell division causing psoriasis or atopic dermatitis (Hultsch et al. 1998b). A topically active FK506 derivative (pimecrolimus) with reduced system exposure and thus increased immunological safety has been launched for therapeutic application in atopic dermatitis, psoriasis, and allergic contact dermatitis (Marsland and Griffiths, 2004).

3.3

Neuropathies

Analyses of the neurotrophic and neuroprotective properties of FK506 and CsA show convincingly that inhibition of enzyme catalyzed prolyl isomerizations is a major factor in neuronal cell signaling under physiological conditions, but the nature of the PPIase substrates is still unknown (Brecht et al. 2003; Christner et al. 2001; Gold 1997; Hamilton et al. 1997; Steiner et al. 1997). Besides protection and regeneration following nerve fiber injury, FK506 and derivatives have further beneficial effects including alteration of neurotransmitter release (Steiner et al. 1996), protection against ischemic brain injury (Sharkey and Butcher 1994; Shichinohe et al. 2004), attenuation of glutamate neurotoxicity in vitro (Dawson et al. 1993), prevention of N-methyl-D-aspartate (NMDA)-receptor desensitization (Tong et al. 1995), modulation of long-term potentiation (LTP) (Terashima et al. 2000), and the blockage/prevention of long-term depression (LTD) in the rat hippocampus (Hodgkiss and Kelly 1995) as well as prevention of LTP and LTD in the visual cortex (Funauchi et al. 1994).

Multiple beneficial factors might contribute to the neuroprotective effects of PPIase-inhibitory FKBP ligands, including anti-apoptotic properties and activation of neurotrophic factors (Avramut et al. 2001; Lyons et al. 1994).

Selective enrichment in neurons of the peripheral and central nervous system is the hallmark of the neuronal FKBP, occasionally termed neuroimmunophilins (Steiner et al. 1992). The involvement of at least one FKBP in the regulation of neuronal cell death was identified by *in vivo* experiments studying the effect of FK506 application (Lyons et al. 1994). It has been observed, as well, that FK506 binding of neuronal cell protein and FKBP expression levels are significantly increased following the time course of neuronal-damaging processes, such as ischemia (Araki et al. 1998; Avramut and Achim 2003). Nonimmunosuppressive FKBP inhibitors, such as JNJ460, GPI1046, V10,367, GPI1048, and GPI1485, to name the most prominent candidates, have already been used in preclinical or clinical studies for treatment of Parkinson's disease (Birge et al. 2004; Gold et al. 2004; Steiner et al. 1997). In a mouse model, systemic administration of GPI1046 resulted in sparing of dopaminergic neurons and fibers. Schwann cells were found to be crucial components for the neurotrophic effects of the FKBP inhibitors (Birge et al. 2004). However, GPI1046 did not provide convincing evidence supporting neuroprotection in methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)-treated rhesus monkeys after oral administration probably resulting from pharmacokinetics and pharmacodynamics (Emborg et al. 2001). Treatment with FKBP inhibitors resulted in dramatic recovery of penile erectile function after unilateral and bilateral cavernous nerve injury in a rat model. Therefore, FKBP inhibitors offer a new potential for patients who suffer nerve damage as a result of or during prostate surgery (Burnett and Becker 2004; Sezen et al. 2001).

Despite its abundance in neuronal tissues, inhibition of FKBP12 is unlikely to be functionally significant (Costantini et al. 2001; Tanaka et al. 2002).

Identification of the drug-targeted neuroimmunophilin from the whole collection of human FKBP has advanced slowly. Unlike CsA and FK506, rapamycin was rarely shown to promote neuroregeneration and neuroprotection (Alemdar et al. 2004; Parker et al. 2000). In many instances, it proved to be inactive on its own but was shown to antagonize the neurotrophic effects of FK506 in some models (Costantini and Isacson 2000; Dawson et al. 1993; Sharkey and Butcher 1994). Thus, FKBP responsible for the interaction with neurotrophic FKBP ligands can be identified among the rapamycin-insensitive PPIases.

Inhibition constants of rapamycin for several FKBP have been collected (Christner et al. 2001). Because the K_i values for FKBP12, FKBP13, FKBP25, and FKBP52 were found to be in the low nanomolar range, the targeted FKBP must reside among the remaining members of the human FKBP. FKBP52, which has already been discussed as mediating neurotrophic actions of neuroimmunophilin ligands (Gold et al. 1999) must be excluded using the K_i criterion.

However, there is one promising candidate FKBP that might regulate neuronal cell death. The multidomain protein FKBP38 is predominantly expressed in brain tissue and shows a secondary messenger-regulated PPIase activity that can be efficiently inhibited by FK506 and its nonimmunosuppressive derivatives, but shows 1,000-fold lower affinity for rapamycin when compared to the rapamycin–FKBP12 complex (Edlich et al., 2005). FKBP38 has been reported to be involved in cell size control. In addition, it shows considerable antimetastatic activity in mice tumor models (Fong et al. 2003; Rosner et al. 1997). Furthermore, the protein interacts functionally with the anti-apoptotic Bcl-2 (Edlich et al., 2005). Interestingly FKBP38 is required in hedgehog signal transduction during central nervous system development by antagonizing the secreted sonic hedgehog morphogen in neural tissues (Bulgakov et al. 2004).

3.4

Inherited Disease

There are several FKBP genes reported to have a defective function in inherited diseases, such as the Williams Beuren syndrome and Lebers congenital amaurosis for FKBP36 (FKBP6) and FKBP44 (AIPL1) mutations, respectively (Meng et al. 1998; Ramamurthy et al. 2003; Sohocki et al. 2000). Lebers congenital amaurosis is diagnosed early in life with severely impaired vision or blindness, nystagmus, and an abnormal or flat electroretinogram. The onset of disease might be linked to the regulation of cell cycle progression during photoreceptor maturation via a FKBP44–NUB1 (NEDD8 ultimate buster 1) interaction (Akey et al. 2002).

The observed overlap of clinical phenotypes of autosomal dominant supralvalvar aortic stenosis (SVAS) and Williams syndrome do not correlate with a common loss of FKBP6 gene integrity (Morris et al. 2003). Similar to wheat FKBP73, FKBP36 deletion results in defect spermatogenesis, suppressed testis development, and male-sterile organisms (Crackower et al. 2003; Kurek et al. 2002). Fertility and meiosis are normal in mutant females devoid of FKBP36. These findings suggest a novel strategy for the development contraceptive drugs based on the inhibition of FKBP36.

3.5

Pathogenic Microorganisms

Multidomain FKBPs of the Mip-type have been identified as virulence factors of many human pathogens including *Legionella pneumophila*, *Neisseria meningitidis*, *Chlamydia trachomatis*, *Coxiella burnetii*, *Trypanosoma cruzi*, *Aeromonas hydrophila*, and *Salmonella typhimurium*. For example, *Legionella pneumophila*, the causative agent of Legionnaires disease, is able to parasitize human lung macrophages and to cause this severe form of pneumonia.

The three-dimensional structures of Mip proteins from *Legionella pneumophila* (LpFKBP25) and *Trypanosoma cruzi* (TcFKBP18.8) reveal that each monomer has a FKBP-like catalytic core domain attached to a very long α -helical rod N-terminal to the core domain (Pereira et al. 2002; Riboldi-Tunncliffe et al. 2001).

Monoclonal antibodies raised against (LpFKBP25) significantly inhibit the early establishment and initiation of an intracellular infection of the bacteria in *Acanthamoeba castellanii*, the natural host, and in the human U937 macrophages (Helbig et al. 2003). Utilizing its PPIase site, LpFKBP25 greatly enhances bacterial infectivity in animal models. Consequently, FK506 has been found to attenuate intracellular infections with *Chlamydia trachomatis* (Lundemose et al. 1993) and *Trypanosoma cruzi* (Moro et al. 1995). The mechanism by which Mip-catalyzed prolyl isomerization facilitates intracellular infections is still unknown. Typically, deletion of a single FKBP gene in *Flavobacterium johnsoniae* gave rise to phenotypic alterations, such as abolishing cell motility and inability to digest biopolymers that might have implications for understanding Mip-mediated bacterial virulence (McBride and Braun, 2004).

3.6

Steroid Responsiveness

Glucocorticoid treatment affects many diseases through a spectrum of anti-inflammatory and immunosuppressive effects, interferences in the growth factor-mediated pathways, as well as responses in the hematopoietic system and the calcium phosphate turnover. In addition to intrinsic functions such as steroid and DNA binding, proteins of the glucocorticoid receptor superfamily assemble nonreceptor proteins associated with the unactivated forms of the receptor, and among them, PPIases appear to play a crucial role in receptor function. Unactivated glucocorticoid receptors form hetero-oligomeric complexes of different composition that are located in the peripheral cytoplasm in order to detect their ligands. Upon ligand binding, the receptor molecule is translocated into the nucleus forming homodimers. The receptor homodimers induce transcription of several target genes (Fig. 5).

Two large human FKBP, FKBP51 (also designated FKBP5, FKBP54) and FKBP52 (also designated FKBP4, FKBP56, FKBP59, p59), are intimately linked to steroid hormone signaling.

Both proteins possess two FKBP-like domains, and two putative calmodulin-binding sites adjacent to three TPR (tetratricopeptide repeat) domains. One molecule of FKBP51 or FKBP52 is involved in the formation of unactivated receptor complex in the cytosol that is capable of steroid hormone recognition and promotion of downstream signaling. FKBP52 develops affinity to client proteins in the presence of Hsp90 that assembles via the TPR motifs of the FKBP. Besides the steroid receptors Hsp90 and FKBP52, further components serve to assemble the unactivated receptor complex such as FKBP51, p23, Hsp70,

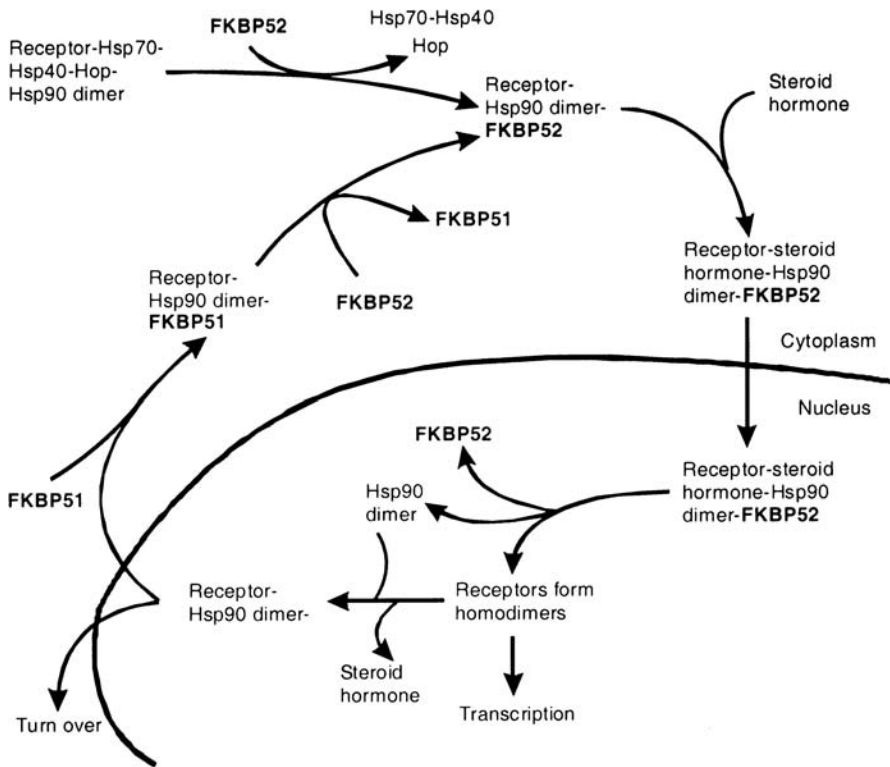


Fig. 5 Schematic representation of the involvement of multidomain FKBP in steroid hormone signaling. FKBP52 assembles with the receptor complex in order to stabilize the unactivated receptor complex in the periphery of the cell. It takes part in the receptor maturation altering proteins, assembling the hormone receptor complex. Upon steroid binding, the receptor is translocated into the nucleus, where it disassembles in order to allow dimerization of the ligand-bound receptor molecules that induce transcription. FKBP52 is believed to participate in receptor complex translocation. Once the receptor dimer disassembles, the receptor complex with Hsp90 translocates to the cytosol, where FKBP51 might regulate the transport of the receptor complex to the periphery of the cytosol

Hsp40, protein phosphatase 5, cyclophilin 40, Hip, and Hop. A conserved functional element of most protein components is their sequence similarity to already established peptide bond *cis/trans* isomerases (Fischer and Aumuller 2003; Lee et al. 2004; Schiene-Fischer et al. 2002; Silverstein et al. 1997). Among them, FKBP52 maintains the glucocorticoid receptor in a high-affinity state while FKBP51 decreases steroid affinity if it is present in the unactivated receptor complex (Denny et al. 2000). An increase in steroid affinity requires both the Hsp90-binding ability and the prolyl isomerase activity of FKBP52 (Riggs et al. 2003). An early step in receptor response to steroids might be associated with an exchange process between FKBP51 and FKBP52, which

subsequently releases affinity to dynein and receptor trafficking only in the FKBP52-bound state (Davies et al. 2002).

In addition, the expression of FKBP51 is specifically enhanced by glucocorticoids, progestins, and androgens. This effect might attenuate the cellular response to the steroid hormones (Hubler et al. 2003).

A role for FKBP52 in subcellular trafficking has also been considered, because dynein co-purifies and microtubules co-localizes with FKBP52 and anchors the receptor in the cytosol (Czar et al. 1994; Galigniana et al. 2004). The PPIase site of FKBP52 is responsible for interaction with dynein.

Recent research shows that both receptor-associated FKBP5s can play a role in other disorders based on steroid hormone signaling, such as the androgen insensitivity syndrome (AIS) or hypospadias. For example, polymorphisms in the FKBP5 gene, encoding FKBP51, were identified as causing more frequent depression periods, rapidly responding to antidepressant treatment (Binder et al. 2004).

Cross-talk between Hsp90 and FKBP52 has been observed for the interaction of FKBP52 with the single-stranded D-sequence binding protein of the adeno-associated virus type 2 that is important for the prevention of viral replication (Zhong et al. 2004). Adeno-associated virus 2 (AAV) vectors are currently in use in phase I/II clinical trials for gene therapy of cystic fibrosis and hemophilia B.

Differential gene expression following androgen-deprivation of an androgen-dependent prostate tumor xenograft revealed decreased expression of FKBP51. When the tumor was grown to an androgen-independent state, FKBP51 increased to its original level (Amler et al. 2000).

3.7

Malignancies

Biochemical investigations have led to the elucidation of two different molecular mechanisms by which FKBP might contribute to the control of malignant growth and proliferation.

First, several studies have suggested a regulatory role for FKBP5s in the cell cycle and tumor progression (Aghdasi et al. 2001; Fong et al. 2003). In yeast, the Hmo1 protein, which is likely to be the homolog of HMG1/2 in higher organisms, plays an important role in genome maintenance, because it binds single-stranded DNA and unwinds DNA in the presence of eukaryotic DNA topoisomerase I (Dolinski and Heitman 1999). FKBP52 has been shown to be a direct interaction partner of the gene product of the *c-Myc* protooncogene by Northern blotting (Coller et al. 2000). The overexpression of two PPIases, Cyp40 and FKBP52, coupled with relative differences in their expression pattern among individual tumors, may have important functional implications for steroid response in breast cancer (Ward et al. 1999). Similarly, the function of the aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1, FKBP44) might conceivably involve cell cycle control, because it participates in cell cycle

progression via the NUB1 (NEDD8 ultimate buster 1)/NEDD8 pathway (Akey et al. 2002).

Promotion of malignant astrocytoma-induced angiogenesis characterizes the differential expression patterns of angiogenesis related genes in malignant high-grade astrocytomas. In a microarray of 133 angiogenesis-related genes, FKBP12 is among the few significantly overexpressed genes, and 9 of 21 (43%) genes overexpressed by high-grade astrocytomas were genes associated with either FKBP or hypoxia-inducible transcription factor 2 α (Khatua et al. 2003). Among the proteins with anti-invasive and antimetastatic functions, FKBP38 and FKBP12 have been identified using systemic gene transfer in tumor-bearing mice. Syndecan and matrix metalloproteinase 9 were thought to mediate the antitumor activities of these enzymes (Fong et al. 2003).

Reduced cell growth and cyclin D1 level in fibroblasts from FKBP12-deficient (FKBP12^{-/-}) mice corresponds to cell cycle arrest in G₁ phase and to the fact that these cells can be rescued by FKBP12 transfection (Aghdasi et al. 2001). Generally, proteins associated with G1 regulation have been shown to play a key role in proliferation, differentiation, and oncogenic transformation as well as apoptosis, and represent promising targets for cancer treatment.

The mechanism suggested for the FKBP12-driven cell cycle is based on the regulation of TGF- β receptor signaling. The prototypic FKBP12 interacts with the transforming growth factor β (TGF β) (Wang et al. 1994), acting as a negative regulator of TGF β receptor endocytosis (Aghdasi et al. 2001; Yao et al. 2000). The immunophilin binds the unphosphorylated form of the TGF β receptor type I (Huse et al. 1999). Within the complex FKBP12 caps the phosphorylation site of the receptor and further stabilizes the inactive conformation of the receptor (Huse et al. 1999). Even though FKBP12 binds directly to the GS2 helix in the receptor molecule, the conformation of the GS2 region is also stable in the absence of FKBP12 and not induced by its binding (Huse et al. 1999). GS loop phosphorylation induces FKBP12 dissociation. Recent data points to a function of FKBP12 as buffer in TGF β signaling, stabilizing and prolonging the lifetime of the dephosphorylated receptor (Wang and Donahoe 2004). This function might become important in preventing ligand-independent activation of the receptor at very high concentrations of the receptor molecules.

Similar effects were observed with the epidermal growth factor (EGF) receptor that utilizes the PPIase activity of cytosolic FKBP12 to downregulate receptor tyrosine autophosphorylation (Lopez-Illasaca et al. 1998). Inhibition of FKBP12 through FK506 and rapamycin leads to stimulation of EGF receptor autophosphorylation.

Secondly, the peptidomacrolide rapamycin is a major player in antitumor activities mediated by FKBP. Both rapamycin and CCI-779, an ester analog of rapamycin with improved pharmaceutical properties and solubility in water, have demonstrated impressive activity against a broad range of human cancers growing in tissue culture and in human tumor xenograft models (Hidalgo and Rowinsky 2000). Problems might arise from genetic mutations or com-

pensatory changes in tumor cells that could enable cells to escape rapamycin action (Huang and Houghton 2001). Wyeth Research (USA) is developing CCI-779 as a anticancer drug. By November 2001, phase III trials had been initiated. In October 2001, filing was predicted for 2003, with a potential launch in 2005 (Elit 2002).

The FKBP12/rapamycin (CCI-779) complexes are probably the biologically relevant species because these drugs experience gain-of-function when bound to the FKBP. The drug-targeted protein is the serine/threonine protein kinase TOR (target of rapamycin) (Sabers et al. 1995).

The protein kinase TOR is an essential protein in eukaryotes that acts as a gatekeeper for the progression from G1 to S phase in cell cycle and is therefore a central regulator of cell growth and proliferation (Brunn et al. 1997). In fact, rapamycin-induced apoptosis in tumor cells is a consequence of continued G₁ progression during mTOR inhibition (Huang et al. 2001).

The protein controls cap-dependent translation initiation by inactivating eukaryotic initiation factor 4E binding proteins in response to mitogen, nutrient, and energy levels (Bjornsti and Houghton 2004; Chen and Fang 2002).

Under normal conditions, TOR is precisely regulated interacting with a protein, called raptor (regulatory associated protein of TOR). The raptor-TOR interaction changes TOR affinity to its substrates in dependence to amino acid and ATP levels (Oshiro et al. 2004). It is now controversially discussed whether the FKBP12-rapamycin complex changes the conformation of TOR, masks the active site for TOR substrates or mimics directly a substrate of the protein kinase activity, but there is growing evidence that the FKBP12-rapamycin complex inhibits the raptor-TOR interaction. The disruption of this interaction may uncouple TOR from its substrates rather than inhibiting the intrinsic kinase activity (Oshiro et al. 2004).

Importantly, application of rapamycin, a product of the bacteria *Streptomyces hygroscopicus*, as well as its synthetic analogous to early mouse embryos resulted in the same "flat top" phenotype that is developed by TOR mutant mice, causing death after less than 2 weeks (Bjornsti and Houghton 2004). These results imply that application of rapamycin leads to complete inhibition of TOR and therefore affects cell cycle progression. Other results suggest either that rapamycin reversibly acts in living cells or that the cellular effects of rapamycin are not mediated through global inhibition of mTOR kinase activity (Edinger et al. 2003).

3.8

Arylhydrocarbon (Dioxin) Responsiveness

The arylhydrocarbon (dioxin) receptor (AhR) is a ligand-inducible transcriptional activator that exhibits structural and functional similarities to steroid hormone receptors. It is a cytosolic receptor complex that can detect 2,3,7,8-tetrachlorodibenzo-p-dioxin in order to start transcription of xenobiotic re-

sponse genes encoding drug-metabolizing enzymes. Induced enzymes include those required for carcinogen activation. Furthermore, AhR ligands are involved in cardiovascular diseases through lipid peroxidation and endothelium dysfunction.

The cytosolic AhR assembles the ligand-binding subunit, a dimer of Hsp90 and FKBP37.7 (XAP2, AIP, ARA9) that dissociate subsequent to 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment (Carver and Bradfield 1997; Ma and Whitlock 1997). To molecularly characterize FKBP37.7, three TPR (tetratricopeptide repeat) motifs follow a single FKBB-like domain (Petrulis and Perdew 2002). In a dioxin-responsive element reporter gene assay the presence of FKBP37.7 in endogenous AhR complexes causes twofold higher signal for the luciferase activity signal (Meyer et al. 1998) and FKBP37.7 stabilizes the ternary receptor complex molecule. In addition, it diminishes receptor degradation by protection against ubiquitination (Kazlauskas et al. 2000). Furthermore, interactions between FKBP37.7 and Hsp90 with the AhR subunit proved to be similar to those observed within the glucocorticoid receptor complexes, pointing to a common mode of action. However, the number of peptide bond *cis/trans* isomerases involved in receptor signaling is quite different in both cases. It raises the question whether there is a yet unidentified folding helper enzyme that adds functionally and physically to FKBP37.7 in the cytosolic AhR. Recently, an anchoring function of FKBP37.7 for the ligand-free receptor complex to cytoskeletal structures of the actin network has been demonstrated (Berg and Pongratz 2002). In addition, FKBP37.7 inhibits the ligand-independent shuttling of the receptor into the nucleus by the a conformational alteration of the nuclear localization signal of the AhR that prevents binding of importin- β (Petrulis et al. 2003). For a nuclear receptor, termed peroxisome proliferator-activated receptor alpha (PPAR α), the presence of FKBP37.7 in a ternary complex with Hsp90 and the receptor protein was also shown. In humans, this member of the nuclear receptor superfamily regulates energy homeostasis by controlling the lipid metabolism (Sumanasekera et al. 2003).

3.9

Cardiovascular Disorders

Prototypic FKBP, such as FKBP12 and FKBP12.6, belong to the receptor-associated folding helper enzymes (Schiene-Fischer and Yu 2001). For example, they co-purify and physically interact with intracellular Ca²⁺ release channels, the ryanodine receptors (RyR), and contribute to the regulation of the release of intracellular Ca²⁺ stores (Jayaraman et al. 1992). FKBP12 and RyR1 assemble in the skeletal receptor in a 4:1 molar ratio of FKBP12-tetrameric receptor protein; the cardiac RyR2 contains FKBP12.6 (Marks et al. 2002). Receptor-binding affinities of FKBP have been found in the high nanomolar range (Jeyakumar et al. 2001). The heterocomplex dissociation by FK506

titration reveals that the active site of FKBP12 is involved in the interaction. Binding of FKBP12 to RyRs are thought to stabilize the close state of the Ca^{2+} channel, removal of FKBP makes the channel leaky. However, there are conflicting reports about the involvement of a catalyzed prolyl isomerization in the FKBP-mediated regulation of the open probability and mean open time of terminal cisternae (Marks 1996; Timerman et al. 1995). Additional control of RyR1 channel function is provided by protein kinase A-mediated phosphorylation, preventing FKBP12 binding to the hyperphosphorylated receptor. Under these conditions, impaired sarcoplasmic Ca^{2+} release and early fatigue in the skeletal muscle results in heart failure among test animals (Reiken et al. 2003a). Generally, prototypic FKBP deficiency and failure of RyR mutants to bind prototypic FKBP have similar phenotypic responses, such as arrhythmogenic right ventricular dysplasia/cardiomyopathy type 2 and stress-induced polymorphic ventricular tachycardia (Tiso et al. 2002). The phenotype of FKBP12.6-deficient mice is consistent with this model (Wehrens et al. 2003).

In patients with heart failure, improved cardiac muscle function under treatment with blockers of β -adrenergic receptor was associated with restoration of normal FKBP12.6 levels in the RyR2 heterocomplex and RyR2 channel function (Reiken et al. 2003b).

FKBP12.6 stabilizes RyR2, preventing aberrant activation of the channel during the resting phase of the cardiac cycle. FKBP12.6^{-/-} mice consistently exhibited exercise-induced cardiac ventricular arrhythmias that caused sudden cardiac death. Cyclic ADP ribose (cADPR) was reported to be a ligand for FKBP12.6 in islet RyR, and the binding of cADPR to FKBP12.6 dissociates it from the channel, causing Ca^{2+} release (Noguchi et al. 1997). However, cADPR neither binds to nor inhibits recombinant FKBP12.6 (Edlich and Fanghänel, unpublished results).

In conclusion, FKBP12.6 plays a role in the regulation of calcium homeostasis, which is of importance for heart muscle contraction. When RyR2 is hyperphosphorylated, FKBP12.6 is depleted from the receptor. Similarly, mutations in RyR2 that lead to FKBP12.6-deficient receptors cause intracellular calcium leakage triggering fatal cardiac arrhythmia failure and exercise-induced cardiac death (Lehnart et al. 2003, 2004; Wehrens et al. 2004). Several attempts show that the application of numerous drugs can improve FKBP-mediated regulation of ryanodine receptors (Doi et al. 2002; Kohno et al. 2003).

It appears that a different function of FKBP12 plays a role in the cardiac growth and chamber maturation. Transcripts of the cardiac restricted cytokine bone morphogenic protein 10 (BMP10) are upregulated in hypertrabeculated hearts of FKBP12^{-/-} mouse embryos. BMP10 upregulation may be causative of congenital heart disease, such as ventricular septal defect (VSD), myocardium noncompaction, and ventricular hypertrabeculation (Chen et al. 2004).

4

Parvulins

Parvulins constitute the third PPIase family that, unlike enzymes of the other two families, cyclophilins and FKBP, do not express affinity for immunosuppressive drugs (Rahfeld et al. 1994). In humans, Par14 and Par18 (also known as Pin1) represent the only members of the parvulin family. Among PPIases Pin1 provides a rare example of relatively high substrate specificity, because polypeptide chains require a pSer(pThr)-Pro-moiety (where p denotes phosphoesterification) if orderly Pin1 catalysis is to occur (Ranganathan et al. 1997; Zhou et al. 2000). Pin1 contains an N-terminal group IV WW domain and a C-terminal parvulin-like catalytic domain connected by a flexible linker (Ranganathan et al. 1997). The mechanism by which Pin1 exerted its critical role in the cell cycle became obvious by combining in vitro studies on isomer-specific dephosphorylation by protein phosphatase 2a (PP2a) with dephosphorylation studies in *Xenopus* mitotic extracts, and rescue experiments in yeast (Lu et al. 2002; Zhou et al. 2000). For example, the cell cycle regulatory protein phosphatase Cdc25C contains the pThr48-Pro and pThr67-Pro moieties that represent critical regulatory phosphorylation sites. The prolyl bond conformation can be either *cis* or *trans* or a mixture of both. For PP2a the inability to dephosphorylate *cis* pThr(Ser)-Pro moieties can be detected in vivo by a reciprocal genetic interactions in temperature-sensitive PP2a-deficient *PPH* and the Pin1-homolog-deficient *ESS1/PTF1* mutant strains of budding yeast and in vitro using oligopeptide dephosphorylation experiments. Despite the presence of sufficient activity of PP2a a fraction of Cdc25C containing *cis* pThr-Pro is left over until it slowly isomerizes to the *trans* isomer. This isomerization is accelerated markedly in the presence of Pin1. Transgenic expression of point-mutated variants of Pin1 and isolated Pin1 domains in yeast indicates that the enzymatically active PPIase domain is necessary and sufficient to carry out conformational tuning of a PP2a substrate to become dephosphorylated in time. The group IV WW domain alone, although exhibiting affinity to the pThr(Ser)-Pro containing polypeptide chains, cannot assist in this function (Fig. 6).

In contrast, Par14 has some specificity for arginine preceding the proline residue (Uchida et al. 1999). This enzyme seems to localize to the nucleus and the N-terminal extension of the catalytic domain might mediate interactions with the preribosomal nucleoprotein complex (Fujiyama et al. 2002).

4.1

Malignancies

Pin1 was identified in its involvement in the *Aspergillus* mitotic kinase NIMA pathway and thus prevention of NIMA-induced mitotic cell death (Lu et al. 1996). Depletion of Pin1 induces mitotic arrest, whereas HeLa cells overexpressing Pin1 arrest in the G2 phase of the cell cycle. While expression is at

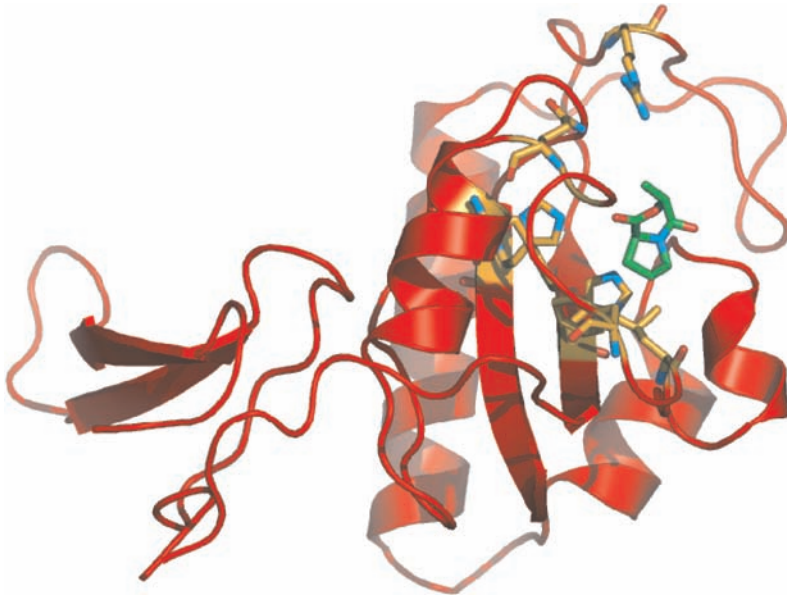


Fig. 6 Representation of the global fold of Pin1 (*red*) harboring a parvulin-like PPIase domain (*right*), which consists of two antiparallel β -sheets and four α -helices, and a WW domain (*left*); both domains are connected by a flexible hinge region (Ranganathan et al. 1997). The structure includes the dipeptide Ala-Pro bound to the active site of Pin1. The parvulin-type PPIase site is formed by the amino acid residue side chains of H59, L122, F125, M130, Q131, F134, and H157 (*orange*). The R68 residue (*orange*) completes the substrate binding pocket of Pin1. The WW domain is described to mediate phosphorylation-dependent protein–protein interactions

low level in most normal tissues, Pin1 is highly expressed in many different human cancers including prostate, lung, ovary, cervical, and brain tumors and melanoma (Bao et al. 2004; Miyashita et al. 2003). In breast cancer cells, early transformed properties correlate with the Pin1 content of the cells, because they are effectively suppressed by Pin1 deletion (Wulf et al. 2004). In conclusion, depletion of pThr(pSer)-Pro-specific PPIase activity point mutations, gene deletion, or expression of antisense RNA induce mitotic arrest and apoptosis in budding yeast and human tumor cell lines (Hani et al. 1999; Rippmann et al. 2000; Wu et al. 2000).

The probably best studied Pin1 substrate in the regulation of mitosis is Cdc25C (Crenshaw et al. 1998; Patra et al. 1999; Yaffe and Cantley 1999). Pin1 induces a structural change in Cdc25C and directly affects the phosphatase activity of Cdc25C by triggering the isomer-specific Cdc25C dephosphorylation by PP2A. This protein phosphatase dephosphorylates Cdc25C, which probably exhibits native state conformational polymorphism with regard to pSer(pThr)-Pro moieties, only if the protein conformer has *trans* pSer(pThr)-Pro moieties.

Pin1 facilitates dephosphorylation through catalyzed *cis/trans* isomerizations. Furthermore, Pin1 can antagonize the Cdc2-associated proteins Suc1/Cks1 by binding the same site in Cdc25C (Landrieu et al. 2001). Pin1 has also been reported to bind to Cdc25C motifs that play a crucial role in Cdc2 activation and subsequently for G2-M transition in the cell cycle.

Among the many tumor-growth-related substrates of Pin1 (Lu, 2004), p53 interacts with Pin1 in response to p53 phosphorylation on a number of Ser-Pro and Thr-Pro moieties. On p53 targeting, Pin1 generates conformational changes in p53, enhancing its transactivation activity. Genotoxicity-induced phosphorylation of p53 causes Ser(Thr) phosphoester sites, the prolyl bond *cis/trans* interconversion of which requires Pin1 catalyzed conformational interconversions in order for the p53-mediated tumor suppression to occur (Wulf et al. 2002; Zacchi et al. 2002). Consequently, Pin1 depletion reduces the stress response of p53 function. Interestingly, another p53 family member, the cAbl-linked p73, responds to Pin1 depletion by protein destabilization in the presence and the absence of DNA (Mantovani et al. 2004). Anticancer drugs, such as cisplatin and adriamycin, exert their cytotoxicity in p73-dependent manner characterized by activating the mismatch-repair-dependent apoptosis pathway. These data converge on prolyl bond isomerization since cAbl activates proline-directed p38 MAP kinase to phosphorylate Thr-Pro sites within p73. The p38-mediated protein phosphorylation depends on the isomeric state of the Thr-Pro, and could be catalyzed by PPIases (Weiwad et al. 2004). The mammalian germ cell development and spermatogenesis is discussed in the context of the interplay between Pin1, and the Ras/MEK/MAPK pathway may also explain the effects of Pin1 deletion observed in primordial germ cell proliferation during embryonic development, along with degenerative or proliferative defects in the adult testis, retina, mammary gland, and brain in mice (Atchison and Means 2004).

Another cancer-relevant finding shows that the dephosphorylation of the proto-oncogenic transcription factor c-Myc by protein phosphatase 2a is assisted by Pin1, and Pin1 facilitates c-My protein degradation (Yeh et al. 2004).

Summarizing these studies demonstrates that Pin1-specific PPIase inhibitors may represent potential lead structures for therapeutic intervention in malignancies.

4.2

Neuropathies

Among the many Pin1-targeted phosphoproteins potentially present in neurons, microtubule-associated tau-protein is a prominent member, whose dephosphorylation restores its ability to stabilize microtubules. Mice with Pin1 knock-out suffer from many age-dependent neurological deficits, such as motor and behavioral problems, tau hyperphosphorylation, tau filament formation and neuronal degeneration (Liou et al. 2002). Neuronal apoptosis fol-

lowing nuclear depletion of Pin1 was reported to be a contributing factor to frontotemporal dementias (Thorpe et al. 2004). The involvement of Pin1 in the G0/G1 transition in neurons makes its presence crucial to neuronal apoptosis (Hamdane et al. 2002). The fraction of soluble Pin1 is reduced in the brains of Alzheimer's disease patients (Lu et al. 1999). Rather, it consistently associates with the pThr231 residues of the various hyperphosphorylated tau proteins that characterize degenerating neurons. Surprisingly, Pin1 may restore the tubulin polymerization function of these hyperphosphorylated tau species (Lu et al. 2002). According to its biochemical role, Pin1 can restore tau function by facilitating its enzymatic dephosphorylation and the subsequent recovery of microtubule assembly (Lu et al. 1999; Zhou et al. 2000). In fact, neurons containing Pin1 granules were devoid of neurofibrillary tangles in Alzheimer patients' brains (Holzer et al. 2002).

References

- Aghdasi B, Ye KQ, Resnick A, Huang A, Ha HC, Guo X, Dawson TM, Dawson VL, Snyder SH (2001) FKBP12, the 12-kDa FK506-binding protein, is a physiologic regulator of the cell cycle. *Proc Natl Acad Sci U S A* 98:2425–2430
- Akey DT, Zhu X, Dyer M, Li A, Sorensen A, Blackshaw S, Fukuda-Kamitani T, Daiger SP, Craft CM, Kamitani T, Sohocki MM (2002) The inherited blindness associated protein AIPL1 interacts with the cell cycle regulator protein NUB1. *Hum Mol Genet* 11:2723–2733
- Al-Daraji WI, Grant KR, Ryan K, Saxton A, Reynolds NJ (2002) Localization of calcineurin/NFAT in human skin and psoriasis and inhibition of calcineurin/NFAT activation in human keratinocytes by cyclosporin A. *J Invest Dermatol* 118:779–788
- Alemdar AY, Sadi D, McAlister VC, Mendez I (2004) Liposomal formulations of tacrolimus and rapamycin increase graft survival and fiber outgrowth of dopaminergic grafts. *Cell Transplant* 13:263–271
- Amler LC, Agus DB, LeDuc C, Sapinoso ML, Fox WD, Kern S, Lee D, Wang V, Leysens M, Higgins B, Martin J, Gerald W, Dracopoli N, Cordon-Cardo C, Scher HI, Hampton GM (2000) Dysregulated expression of androgen-responsive and nonresponsive genes in the androgen-independent prostate cancer xenograft model CWR22-R1. *Cancer Res* 60:6134–6141
- Andreeva L, Motterlini R, Green CJ (1997) Cyclophilins are induced by hypoxia and heat stress in myogenic cells. *Biochem Biophys Res Commun* 237:6–9
- Andreotti AH (2003) Native state proline isomerization: an intrinsic molecular switch. *Biochemistry* 42:9515–9524
- Appenzeller U, Meyer C, Menz G, Blaser K, Cramer R (1999) IgE-mediated reactions to autoantigens in allergic diseases. *Int Arch Allergy Immunol* 118:193–196
- Arai S, Vogelsang GB (2000) Management of graft-versus-host disease. *Blood Rev* 14:190–204
- Araki T, Kato H, Shuto K, Itoyama Y (1998) Post-ischemic alterations in [3H]FK506 binding in the gerbil and rat brains. *Metab Brain Dis* 13:9–19
- Atchison FW, Means AR (2004) A role for Pin1 in mammalian germ cell development and spermatogenesis. *Front Biosci* 9:3248–3256
- Avramut M, Achim CL (2003) Immunophilins in nervous system degeneration and regeneration. *Current Topics Med Chem* 3:1376–1382

- Avramut M, Zeevi A, Achim CL (2001) The immunosuppressant drug FK506 is a potent trophic agent for human fetal neurons. *Brain Res Develop Brain Res* 132:151–157
- Bao L, Kimzey A, Sauter G, Sowadski JM, Lu KP, Wang DG (2004) Prevalent overexpression of prolyl isomerase Pin1 in human cancers. *Am J Pathol* 164:1727–1737
- Bartz SR, Hohenwarter E, Hu MK, Rich DH, Malkovsky M (1995) Inhibition of human immunodeficiency virus replication by nonimmunosuppressive analogs of cyclosporin A. *Proc Natl Acad Sci U S A* 92:5381–5385
- Batiuk TD, Kung LN, Halloran PF (1997) Evidence that calcineurin is rate-limiting for primary human lymphocyte activation. *J Clin Invest* 100:1894–1901
- Batiuk TD, Pazderka F, Halloran PF (1995) Cyclosporine-treated renal transplant patients have only partial inhibition of calcineurin phosphatase activity. *Transplant Proc* 27:840–841
- Bell A, Roberts HC, Chappell LH (1996) The antiparasite effects of cyclosporin A—possible drug targets and clinical applications. *Gen Pharmacol* 27:963–971
- Bennett PC, Singaretnam LG, Zhao WQ, Lawen A, Ng KT (1998) Peptidyl-prolyl-cis/trans-isomerase activity may be necessary for memory formation. *FEBS Lett* 431:386–390
- Berg P, Pongratz I (2002) Two parallel pathways mediate cytoplasmic localization of the dioxin (Aryl hydrocarbon) receptor. *J Biol Chem* 277:32310–32319
- Billich A, Winkler G, Aschauer H, Rot A, Peichl P (1997) Presence of cyclophilin a in synovial fluids of patients with rheumatoid arthritis. *J Exp Med* 185:975–980
- Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Kunzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Kohnlein O, Dabitz H, Bruckl T, Muller N, Pfister H, Lieb R, Mueller JC, Lohmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B (2004) Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet* 36:1319–1325
- Birge RB, Wadsworth S, Akakura R, Abeysinghe H, Kanojia R, MacIelag M, Desbarats J, Escalante M, Singh K, Sundarababu S, Parris K, Childs G, August A, Siekierka J, Weinstein DE (2004) A role for Schwann cells in the neuroregenerative effects of a non-immunosuppressive FK506 derivative, JNJ460. *Neuroscience* 124:351–366
- Bjornsti MA, Houghton PJ (2004) The TOR pathway: a target for cancer therapy. *Nat Rev Cancer* 4:335–348
- Boesch D, Muller K, Pourtier-Manzanedo A, Loor F (1991) Restoration of daunomycin retention in multidrug-resistant P388 cells by submicromolar concentrations of SDZ PSC 833, a nonimmunosuppressive cyclosporin derivative. *Exp Cell Res* 196:26–32
- Borvak J, Chou CS, Van Dyke G, Rosenwirth B, Vitetta ES, Ramilo O (1996) The use of cyclosporine, FK506, and SDZ NIM811 to prevent CD25-quiescent peripheral blood mononuclear cells from producing human immunodeficiency virus. *J Infect Dis* 174:850–853
- Bosco DA, Eisenmesser EZ, Pochapsky S, Sundquist WI, Kern D (2002) Catalysis of cis/trans isomerization in native HIV-1 capsid by human cyclophilin A. *Proc Natl Acad Sci U S A* 99:5247–5252
- Bose S, Mathur M, Bates P, Joshi N, Banerjee AK (2003) Requirement for cyclophilin A for the replication of vesicular stomatitis virus New Jersey serotype. *J Gen Virol* 84:1687–1699
- Brazin KN, Mallis RJ, Fulton DB, Andreotti AH (2002) Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase cyclophilin A. *Proc Natl Acad Sci U S A* 99:1899–1904

- Brecht S, Schwarze K, Waetzig V, Christner C, Heiland S, Fischer G, Sartor K, Herdegen T (2003) Changes in peptidyl-prolyl cis/trans isomerase activity and FK506 binding protein expression following neuroprotection by FK506 in the ischemic rat brain. *Neuroscience* 120:1037–1048
- Brenner BG, Wainberg MA (1999) Heat shock protein-based therapeutic strategies against human immunodeficiency virus type 1 infection. *Infect Dis Obstet Gynecol* 7:80–90
- Brunn GJ, Fadden P, Haystead TAJ, Lawrence JC (1997) The mammalian target of rapamycin phosphorylates sites having a (Ser/Thr)-pro motif and is activated by antibodies to a region near its cooh terminus. *J Biol Chem* 272:32547–32550
- Bulgakov OV, Eggenschwiler JT, Hong DH, Anderson KV, Li TS (2004) FKBP8 is a negative regulator of mouse sonic hedgehog signaling in neural tissues. *Development* 131:2149–2159
- Burnett AL, Becker RE (2004) Immunophilin ligands promote penile neurogenesis and erection recovery after cavernous nerve injury. *J Urol* 171:495–500
- Calabrese KS, Paradela A, do Valle TZ, Tedesco RC, Silva S, Mortara RA, da Costa SCG (2000) Study of acute chagasic mice under immunosuppressive therapy by cyclosporin A: modulation and confocal analysis of inflammatory reaction. *Immunopharmacology* 47:1–11
- Campa MJ, Wang MZ, Howard B, Fitzgerald MC, Patz EF (2003) Protein expression profiling identifies macrophage migration inhibitory factor and cyclophilin A as potential molecular targets in non-small cell lung cancer. *Cancer Res* 63:1652–1656
- Carver LA, Bradfield CA (1997) Ligand-dependent interaction of the aryl hydrocarbon receptor with a novel immunophilin homolog in vivo. *J Biol Chem* 272:11452–11456
- Chambraud B, Radanyi C, Camonis JH, Rajkowski K, Schumacher M, Baulieu EE (1999) Immunophilins, refsum disease, and lupus nephritis: The peroxisomal enzyme phytanoyl-CoA alpha-hydroxylase is a new FKBP-associated protein. *Proc Natl Acad Sci U S A* 96:2104–2109
- Chen HY, Shi S, Acosta L, Li WM, Lu J, Bao SD, Chen ZA, Yang ZC, Schneider MD, Chien KR, Conway SJ, Yoder MC, Haneline LS, Franco D, Shou WN (2004) BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. *Development* 131:2219–2231
- Chen J, Fang YM (2002) A novel pathway regulating the mammalian target of rapamycin (mTOR) signaling. *Biochem Pharmacol* 64:1071–1077
- Christner C, Herdegen T, Fischer G (2001) FKBP ligands as novel therapeutics for neurological disorders. *Mini-Rev Med Chem* 1:377–397
- Clarke SJ, McStay GP, Halestrap AP (2002) Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A. *J Biol Chem* 277:34793–34799
- Colgan J, Asmal M, Neagu M, Yu B, Schneidkraut J, Lee Y, Sokolskaja E, Andreotti A, Luban J (2004) Cyclophilin A regulates TCR signal strength in CD4(+) T cells via a proline-directed conformational switch in Itk. *Immunity* 21:189–201
- Coller HA, Grandori C, Tamayo P, Colbert T, Lander ES, Eisenman RN, Golub TR (2000) Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. *Proc Natl Acad Sci U S A* 97:3260–3265
- Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, McRedmond JP, Cahill DJ, Emili A, Fitzgerald DJ, Maguire PB (2004) Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood* 103:2096–2104

- Costantini LC, Cole D, Chaturvedi P, Isacson O (2001) Immunophilin ligands can prevent progressive dopaminergic degeneration in animal models of Parkinson's disease. *Eur J Neurosci* 13:1085–1092
- Costantini LC, Isacson O (2000) Immunophilin ligands and GDNF enhance neurite branching or elongation from developing dopamine neurons in culture. *Exp Neurol* 164:60–70
- Crackower MA, Kolas NK, Noguchi J, Sarao R, Kikuchi K, Kaneko H, Kobayashi E, Kawai Y, Kozieradzki I, Landers R, Mo R, Hui CC, Nieves E, Cohen PE, Osborne LR, Wada T, Kunieda T, Moens PB, Penninger JM (2003) Essential role of Fkbp6 in male fertility and homologous chromosome pairing in meiosis. *Science* 300:1291–1295
- Crenshaw DG, Yang J, Means AR, Kornbluth S (1998) The mitotic peptidyl-prolyl isomerase, Pin1, interacts with Cdc25 and Plx1. *EMBO J* 17:1315–1327
- Czar MJ, Owensgrillo JK, Yem AW, Leach KL, Deibel MR, Welsh MJ, Pratt WB (1994) The Hsp56 immunophilin component of untransformed steroid receptor complexes is localized both to microtubules in the cytoplasm and to the same nonrandom regions within the nucleus as the steroid receptor. *Mol Endocrinol* 8:1731–1741
- Davies TH, Ning YM, Sanchez ER (2002) A new first step in activation of steroid receptors—hormone-induced switching of FKBP51 and FKBP52 immunophilins. *J Biol Chem* 277:4597–4600
- Dawson TM, Steiner JP, Dawson VL, Dinerman JL, Uhl GR, Snyder SH (1993) Immunosuppressant Fk506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. *Proc Natl Acad Sci U S A* 90:9808–9812
- De Ceuninck F, Allain F, Caliez A, Spik G, Vanhoutte PM (2003) High binding capacity of cyclophilin B to chondrocyte heparan sulfate proteoglycans and its release from the cell surface by matrix metalloproteinases: possible role as a proinflammatory mediator in arthritis. *Arthritis Rheumatism* 48:2197–2206
- Denny WB, Valentine DL, Reynolds PD, Smith DF, Scammell JG (2000) Squirrel monkey immunophilin FKBP51 is a potent inhibitor of glucocorticoid receptor binding. *Endocrinology* 141:4107–4113
- Dobson CM, Sali A, Karplus M (1998) Protein folding—a perspective from theory and experiment. *Angewandte Chemie International Edition in English* 37:868–893
- Doi M, Yano M, Kobayashi S, Kohno M, Tokuhisa T, Okuda S, Suetsugu M, Hisamatsu Y, Ohkusa T, Matsuzaki M (2002) Propranolol prevents the development of heart failure by restoring FKBP12.6-mediated stabilization of ryanodine receptor. *Circulation* 105:1374–1379
- Dolinski K, Muir S, Cardenas M, Heitman J (1997) All cyclophilins and Fk506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 94:13093–13098
- Dolinski KJ, Heitman J (1999) Hmo1p, a high mobility group 1/2 homolog, genetically and physically interacts with the yeast FKBP12 prolyl isomerase. *Genetics* 151:935–944
- Dubourg B, Kamphausen T, Weiwad M, Jahreis G, Feunteun J, Fischer G, Modjtahedi N (2004) The human nuclear SRcyp is a cell cycle-regulated cyclophilin. *J Biol Chem* 279:22322–22330
- Edinger AL, Linardic CM, Chiang GG, Thompson CB, Abraham RT (2003) Differential effects of rapamycin on mammalian target of rapamycin signaling functions in mammalian cells. *Cancer Res* 63:8451–8460
- Edlich F, Weiwad M, Erdmann F, Fanghanel J, Jarczowski F, Rahfeld JU, Fischer G (2005) Bcl-2 regulator FKBP38 is activated by Ca^{2+} /calmodulin. *EMBO J* (in press)
- Elit L (2002) CCI-779 Wyeth. *Curr Opin Invest Drugs* 3:1249–1253
- Ellis PJ, Carlow CK, Ma D, Kuhn P (2000) Crystal structure of the complex of brugia malayi cyclophilin and cyclosporin A. *Biochemistry* 39:592–598

- Emborg ME, Shin P, Roitberg B, Sramek JG, Chu Y, Stebbins GT, Hamilton JS, Suzdak PD, Steiner JP, Kordower JH (2001) Systemic administration of the immunophilin ligand GPI 1046 in MPTP-treated monkeys. *Exp Neurol* 168:171–182
- Endrich MM, Gehrig P, Gehring H (1999) Maturation-induced conformational changes of HIV-1 capsid protein and identification of two high affinity sites for cyclophilins in the C-terminal domain. *J Biol Chem* 274:5326–5332
- Fanghanel J, Fischer G (2004) Insights into the catalytic mechanism of peptidyl prolyl cis/trans isomerases. *Front Biosci* 9:3453–3478
- Faulds D, Goa KL, Benfield P (1993) Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs* 45:953–1040
- Fingar DC, Blenis J (2004) Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 23:3151–3171
- Fischer G (1994) Peptidyl-prolyl cis/trans isomerases and their effectors. *Angew Chem International Edition in English*. 33:1415–1436
- Fischer G, Aumuller T (2003) Regulation of peptide bond cis/trans isomerization by enzyme catalysis and its implication in physiological processes. *Rev Physiol Biochem Pharmacol* 148:105–150
- Fischer G, Bang H, Mech C (1984) Nachweis einer Enzymkatalyse für die cis-trans-Isomerisierung der Peptidbindung in prolinhaltigen Peptiden. *Biomed Biochim Acta* 43:1101–1111
- Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX (1989) Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 337:476–478
- Fluckiger S, Fijten H, Whitley P, Blaser K, Crameri R (2002) Cyclophilins, a new family of cross-reactive allergens. *Eur J Immunol* 32:10–17
- Fong S, Mounkes L, Liu Y, Maibaum M, Alonzo E, Desprez PY, Thor AD, Kashani-Sabet M, Debs RJ (2003) Functional identification of distinct sets of antitumor activities mediated by the FKBP gene family. *Proc Natl Acad Sci U S A* 100:14253–14258
- Franke EK, Yuan HEH, Luban J (1994) Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* 372:359–362
- Friedman J, Weissman I (1991) Two cytoplasmic candidates for immunophilin action are revealed by affinity for a new cyclophilin: one in the presence and one in the absence of CsA. *Cell* 66:799–806
- Fujiyama S, Yanagida M, Hayano T, Miura Y, Isobe T, Fujimori F, Uchida T, Takahashi N (2002) Isolation and proteomic characterization of human Parvulin-associating preribosomal ribonucleoprotein complexes. *J Biol Chem* 277:23773–23780
- Funauchi M, Haruta H, Tsumoto T (1994) Effects of an inhibitor for calcium/calmodulin-dependent protein phosphatase, calcineurin, on induction of long-term potentiation in rat visual cortex. *Neurosci Res* 19:269–278
- Galat A (2004a) Function-dependent clustering of orthologues and paralogues of cyclophilins. *Proteins* 56:808–820
- Galat A (2004b) A note on clustering the functionally-related paralogues and orthologues of proteins: a case of the FK506-binding proteins (FKBPs). *Comput Biol Chem* 28:129–140
- Galigiana MD, Harrell JM, O'Hagen HM, Ljungman M, Pratt WB (2004) Hsp90-binding immunophilins link p53 to dynein during p53 transport to the nucleus. *J Biol Chem* 279:22483–22489
- Gamble TR, Vajdos FF, Yoo SH, Worthylake DK, Houseweart M, Sundquist WI, Hill CP (1996) Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* 87:1285–1294

- Giardina SL, Anderson SK, Sayers TJ, Chambers WH, Palumbo GA, Young HA, Ortaldo JR (1995) Selective loss of NK cytotoxicity in antisense Nk-*Tr1* rat Lgl cell lines—abrogation of antibody-independent tumor and virus-infected target cell killing. *J Immunol* 154:80–87
- Gold BG (1997) FK506 and the role of immunophilins in nerve regeneration. *Mol Neurobiol* 15:285–306
- Gold BG, Densmore V, Shou W, Matzuk MM, Gordon HS (1999) Immunophilin FK506-binding protein 52 (not FK506-binding protein 12) mediates the neurotrophic action of FK506. *J Pharmacol Exp Therap* 289:1202–1210
- Gold BG, Voda J, Yu XL, McKeon G, Bourdette DN (2004) FK506 and a nonimmunosuppressant derivative reduce axonal and myelin damage in experimental autoimmune encephalomyelitis: neuroimmunophilin ligand-mediated neuroprotection in a model of multiple sclerosis. *J Neurosci Res* 77:367–377
- Gremese E, Ferraccioli GF (2004) Benefit/risk of cyclosporine in rheumatoid arthritis. *Clin Exp Rheumatol* 22:S101–S107
- Griffith JP, Kim JL, Kim EE, Sintchak MD, Thomson JA, Fitzgibbon MJ, Fleming MA, Caron PR, Hsiao K, Navia MA (1995) X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12–FK506 complex. *Cell* 82:507–522
- Griffiths EJ, Halestrap AP (1995) Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J* 307:93–98
- Grzmil M, Voigt S, Thelen P, Hemmerlein B, Helmke K, Burfeind P (2004) Up-regulated expression of the MAT-8 gene in prostate cancer and its siRNA-mediated inhibition of expression induces a decrease in proliferation of human prostate carcinoma cells. *Int J Oncol* 24:97–105
- Halestrap AP, Brenner C (2003) The adenine nucleotide translocase: a central component of the mitochondrial permeability transition pore and key player in cell death. *Curr Med Chem* 10:1507–1525
- Hamdane M, Smet C, Sambo AV, Leroy A, Wieruszeski JM, Delobel P, Maurage CA, Ghestem A, Wintjens R, Begard S, Sergeant N, Delacourte A, Horvath D, Landrieu I, Lip-pens G, Buee L (2002) Pin1: a therapeutic target in Alzheimer neurodegeneration. *J Mol Neurosci* 19:275–287
- Hamilton GS, Huang W, Connolly MA, Ross DT, Guo H, Valentine HL, Suzdak PD, Steiner JP (1997) Fkbp12-binding domain analogues of Fk506 are potent, nonimmunosuppressive neurotrophic agents in vitro and promote recovery in a mouse model of Parkinson's disease. *Bioorg Med Chem Lett* 7:1785–1790
- Hani J, Schelbert B, Bernhardt A, Domdey H, Fischer G, Wiebauer K, Rahfeld JU (1999) Mutations in a peptidylprolyl-*cis/trans*-isomerase gene lead to a defect in 3'-end formation of a pre-mRNA in *Saccharomyces cerevisiae*. *J Biol Chem* 274:108–116
- Hansson MJ, Mattiasson G, Mansson R, Karlsson J, Keep MF, Waldmeier P, Ruegg UT, Dumont JM, Besseghir K, Elmer E (2004) The nonimmunosuppressive cyclosporin analogs NIM811 and UNIL025 display nanomolar potencies on permeability transition in brain-derived mitochondria. *J Bioenergetics Biomembranes* 36:407–413
- Helbig JH, König B, Knospe H, Bubert B, Yu C, Luck CP, Riboldi-Tunncliffe A, Hilgenfeld R, Jacobs E, Hacker J, Fischer G (2003) The PPIase active site of *Legionella pneumophila* Mip protein is involved in the infection of eukaryotic host cells. *Biol Chem* 384:125–137
- Hidalgo M, Rowinsky EK (2000) The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene* 19:6680–6686
- Hodgkiss JP, Kelly JS (1995) Only 'de novo' long-term depression (LTD) in the rat hippocampus in vitro is blocked by the same low concentration of FK506 that blocks LTD in the visual cortex. *Brain Res* 705:241–246

- Hoyo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, Shimbo T, Suthanthiran M (1999) Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 397:530–534
- Holzer M, Gartner U, Stobe A, Hartig W, Gruschka H, Bruckner MK, Arendt T (2002) Inverse association of Pin1 and tau accumulation in Alzheimer's disease hippocampus. *Acta Neuropathol (Berl)* 104:471–481
- Hong F, Lee J, Song JW, Lee SJ, Ahn H, Cho JJ, Ha J, Kim SS (2002) Cyclosporin A blocks muscle differentiation by inducing oxidative stress and inhibiting the peptidyl-prolyl-cis-trans isomerase activity of cyclophilin A: cyclophilin A protects myoblasts from cyclosporin A-induced cytotoxicity. *FASEB J* 16:1633–1635
- Howard BR, Vajdos FF, Li S, Sundquist WI, Hill CP (2003) Structural insights into the catalytic mechanism of cyclophilin A. *Nat Struct Biol* 10:475–481
- Huang S, Houghton PJ (2001) Mechanisms of resistance to rapamycins. *Drug Resistance Updates* 4:378–391
- Huang S, Liu LN, Hosoi H, Dilling MB, Shikata T, Houghton PJ (2001) p53/p21(CIP1) cooperate in enforcing rapamycin-induced G(1) arrest and determine the cellular response to rapamycin. *Cancer Res* 61:3373–3381
- Hubler TR, Denny WB, Valentine DL, Cheung-Flynn J, Smith DE, Scammell JG (2003) The FK506-binding immunophilin FKBP51 is transcriptionally regulated by progesterone and attenuates progesterone responsiveness. *Endocrinology* 144:2380–2387
- Hultsch T, Brand P, Lohmann S, Saloga J, Kincaid RL, Knop J (1998a) Direct evidence that FK506 inhibition of FcepsilonRI-mediated exocytosis from RBL mast cells involves calcineurin. *Arch Dermatol Res* 290:258–263
- Hultsch T, Muller KD, Meingassner JG, Grassberger M, Schopf RE, Knop J (1998b) Ascomycin macrolactam derivative SDZ ASM 981 inhibits the release of granule-associated mediators and of newly synthesized cytokines in RBL 2H3 mast cells in an immunophilin-dependent manner. *Arch Dermatol Res* 290:501–507
- Huse M, Chen YG, Massague J, Kuriyan J (1999) Crystal structure of the cytoplasmic domain of the type I TGF beta receptor in complex with FKBP12. *Cell* 96:425–436
- Huss R, Hoy CA, Ottinger H, Grosse-Wilde H, Deeg HJ (1995) Cyclosporine-induced apoptosis in CD4+ T lymphocytes and computer-simulated analysis: modeling a treatment scenario for HIV infection. *Res Immunol* 146:101–108
- Ikeda Y, Ylinen LM, Kahar-Bador M, Towers GJ (2004) Influence of gag on human immunodeficiency virus type 1 species-specific tropism. *J Virol* 78:11816–11822
- Iwasaki Y, Ichikawa Y, Igarashi O, Iwamoto K, Kinoshita M, Ikeda K (2002) Neuroprotective actions of FK506 and cyclosporin A on motor neuron survival following neonatal axotomy. *Neurol Res* 24:573–576
- Jayaraman T, Brillantes AM, Timerman AP, Fleischer S, Erdjument-Bromage H, Tempst P, Marks AR (1992) FK506 binding protein associated with the calcium release channel (ryanodine receptor). *J Biol Chem* 267:9474–9477
- Jeyakumar LH, Ballester L, Cheng DS, McIntyre JO, Chang P, Olivey HE, Rollins-Smith L, Barnett JV, Murray K, Xin HB, Fleischer S (2001) FKBP binding characteristics of cardiac microsomes from diverse vertebrates. *Biochem Biophys Res Commun* 281:979–986
- Jin ZG, Lungu AO, Xie L, Wang M, Wong C, Berk BC (2004) Cyclophilin A is a proinflammatory cytokine that activates endothelial cells. *Arterioscler, Thromb Vasc Biol* 24:1186–1191
- Jin ZG, Melaragno MG, Liao DF, Yan C, Haendeler J, Suh YA, Lambeth JD, Berk BC (2000) Cyclophilin A is a secreted growth factor induced by oxidative stress. *Circ Res* 87:789–796
- Kahan BD (2004) Sirolimus: a ten-year perspective. *Transplant Proc* 36:71–75

- Karlsson J, Fong KS, Hansson MJ, Elmer E, Csiszar K, Keep MF (2004) Life span extension and reduced neuronal death after weekly intraventricular cyclosporin injections in the G93A transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosurg* 101:128–137
- Kazlauskas A, Poellinger L, Pongratz I (2000) The immunophilin-like protein XAP2 regulates ubiquitination and subcellular localization of the dioxin receptor. *J Biol Chem* 275:41317–41324
- Khan LN, Kon OM, Macfarlane AJ, Meng Q, Ying S, Barnes NC, Kay AB (2000) Attenuation of the allergen-induced late asthmatic reaction by cyclosporin A is associated with inhibition of bronchial eosinophils, interleukin-5, granulocyte macrophage colony-stimulating factor, and eotaxin. *Am J Resp Crit Care Med* 162:1377–1382
- Khatua S, Peterson KM, Brown KM, Lawlor C, Santi MR, LaFleur B, Dressman D, Stephan DA, MacDonald TJ (2003) Overexpression of the EGFR/FKBP12/HIF-2 α pathway identified in childhood astrocytomas by angiogenesis gene profiling. *Cancer Res* 63:1865–1870
- Kim SH, Lessner SM, Sakurai Y, Galis ZS (2004) Cyclophilin A as a novel biphasic mediator of endothelial activation and dysfunction. *Am J Pathol* 164:1567–1574
- Kohno M, Yano M, Kobayashi S, Doi M, Oda T, Tokuhisa T, Okuda S, Ohkusa T, Matsuzaki M (2003) A new cardioprotective agent, JTV519, improves defective channel gating of ryanodine receptor in heart failure. *Am J Physiol Heart Circ Physiol* 284:H1035–H1042
- Kon OM, Kay AB (1999) Anti-T cell strategies in asthma. *Inflamm Res* 48:516–523
- Kumar P, Mark PJ, Ward BK, Minchin RF, Ratajczak T (2001) Estradiol-regulated expression of the immunophilins cyclophilin 40 and FKBP52 in MCF-7 breast cancer cells. *Biochem Biophys Res Commun* 284:219–225
- Kurek I, Dulberger R, Azem A, Tzvi BB, Sudhakar D, Christou P, Breiman A (2002) Deletion of the C-terminal 138 amino acids of the wheat FKBP73 abrogates calmodulin binding, dimerization and male fertility in transgenic rice. *Plant Mol Biol* 48:369–381
- Landrieu I, Odaert B, Wieruszeski JM, Drobecq H, Rousselot-Pailley P, Inze D, Lippens G (2001) p13(SUC1) and the WW domain of PIN1 bind to the same phosphothreonine-proline epitope. *J Biol Chem* 276:1434–1438
- LeDucq N, Delmas-Beauvieux MC, Bourdel-Marchasson I, Dufour S, Gallis JL, Canioni P, Diolez P (1998) Mitochondrial permeability transition during hypothermic to normothermic reperfusion in rat liver demonstrated by the protective effect of cyclosporin A. *Biochem J* 336:501–506
- Lee JP, Palfrey HC, Bindokas VP, Ghadge GD, Ma L, Miller RJ, Roos RP (1999) The role of immunophilins in mutant superoxide dismutase-1-linked familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 96:3251–3256
- Lee YS, Marcu MG, Neckers L (2004) Quantum chemical calculations and mutational analysis suggest heat shock protein 90 catalyzes trans-cis isomerization of geldanamycin. *Chem Biol* 11:991–998
- Lehnart SE, Huang F, Marx SO, Marks AR (2003) Immunophilins and coupled gating of ryanodine receptors. *Curr Topics Med Chem* 3:1383–1391
- Lehnart SE, Wehrens XH, Kushnir A, Marks AR (2004) Cardiac ryanodine receptor function and regulation in heart disease. *Ann N Y Acad Sci* 1015:144–159
- Li YM, Johnson N, Capano M, Edwards M, Crompton M (2004) Cyclophilin-D promotes the mitochondrial permeability transition but has opposite effects on apoptosis and necrosis. *Biochem J* 383:101–109
- Lim SO, Park SJ, Kim W, Park SG, Kim HJ, Kim YI, Sohn TS, Noh JH, Jung G (2002) Proteome analysis of hepatocellular carcinoma. *Biochem Biophys Res Commun* 291:1031–1037
- Liou YC, Ryo A, Huang HK, Lu PJ, Bronson R, Fujimori F, Uchida T, Hunter T, Lu KP (2002) Loss of Pin1 function in the mouse causes phenotypes resembling cyclin D1-null phenotypes. *Proc Natl Acad Sci U S A* 99:1335–1340

- Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL (1991) Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 66:807–815
- Liu SK, Asparuhova M, Brondani V, Ziekau I, Klimkait T, Schumperli D (2004) Inhibition of HIV-1 multiplication by antisense U7 snRNAs and siRNAs targeting cyclophilin A. *Nucleic Acids Res* 32:3752–3759
- London NJ, Farmery SM, Will EJ, Davison AM, Lodge JPA (1995) Risk of neoplasia in renal transplant patients. *Lancet* 346:403–406
- Loor F, Tiberghien F, Wenandy T, Didier A, Traber R (2002) Cyclosporins: structure-activity relationships for the inhibition of the human MDR1 P-glycoprotein ABC transporter. *J Med Chem* 45:4598–4612
- Lopez-Illasaca M, Schiene C, Kullertz G, Tradler T, Fischer G, Wetzker R (1998) Effects of Fk506-binding protein 12 and Fk506 on autophosphorylation of epidermal growth factor receptor. *J Biol Chem* 273:9430–9434
- Lu KP (2004) Pinning down cell signaling, cancer and Alzheimer's disease. *Trends Biochem Sci* 29:200–209
- Lu KP, Hanes SD, Hunter T (1996) A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature* 380:544–547
- Lu KP, Liou YC, Zhou XZ (2002) Pinning down proline-directed phosphorylation signaling. *Trends Cell Biol* 12:164–172
- Lu PJ, Zhou XZ, Shen M, Lu KP (1999) Function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science* 283:1325–1328
- Luban J, Bossolt KL, Franke EK, Kalpana GV, Goff SP (1993) Human immunodeficiency virus type-1 gag protein binds to cyclophilin-a and cyclophilin-B. *Cell* 73:1067–1078
- Lundemose AG, Rouch DA, Penn CW, Pearce JH (1993) The chlamydia-trachomatis Mip-like protein is a lipoprotein. *J Bacteriol* 175:3669–3671
- Lyons WE, George EB, Dawson TM, Steiner JP, Snyder SH (1994) Immunosuppressant FK506 promotes neurite outgrowth in cultures of PC12 cells and sensory ganglia. *Proc Natl Acad Sci U S A* 91:3191–3195
- Ma Q, Whitlock JP (1997) A novel cytoplasmic protein that interacts with the Ah Receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-P-dioxin. *J Biol Chem* 272:8878–8884
- Mallis RJ, Brazin KN, Fulton DB, Andreotti AH (2002) Structural characterization of a proline-driven conformational switch within the Itk SH2 domain. *Nat Struct Biol* 9:900–905
- Manabe Y, Warita H, Murakami T, Shiote M, Hayashi T, Omori N, Nagano I, Shoji M, Abe K (2002) Early decrease of the immunophilin FKBP52 in the spinal cord of a transgenic model for amyotrophic lateral sclerosis. *Brain Res* 935:124–128
- Mantovani F, Piazza S, Gostissa M, Strano S, Zacchi P, Mantovani R, Blandino G, Del Sal G (2004) Pin1 links the activities of c-Abl and p300 in regulating p73 function. *Mol Cell* 14:625–636
- Marks AR (1996) Cellular function of immunophilins. *Physiol Rev* 76:631–649
- Marks AR, Marx SO, Reiken S (2002) Regulation of ryanodine receptors via macromolecular complexes: a novel role for leucine/isoleucine zippers. *Trends Cardiovasc Med* 12:166–170
- Marsland AM, Griffiths CEM (2004) Therapeutic potential of macrocyclic immunosuppressants in dermatology. *Expert Opin Invest Drugs* 13:125–137
- McBride MJ, Braun TF (2004) GldI is a lipoprotein that is required for *Flavobacterium johnsoniae* gliding motility and chitin utilization. *J Bacteriol* 186:2295–2302

- McLauchlan PE, Roberts HC, Chappell LH (2000) Mode of action of cyclosporin A against *Hymenolepis microstoma* (Cestoda): relationship between cyclophilin binding and drug-induced damage. *Parasitology* 121:661–670
- Meng X, Lu X, Morris CA, Keating MT (1998) A novel human gene FKBP6 is deleted in Williams syndrome. *Genomics* 52:130–137
- Meyer BK, Praygrant MG, Vandenheuve JP, Perdew GH (1998) Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity. *Mol Cell Biol* 18:978–988
- Meza-Zepeda LA, Forus A, Lygren B, Dahlberg AB, Godager LH, South AP, Marenholz I, Lioumi M, Florenes VA, Maelandsmo GM, Serra M, Mischke D, Nizetic D, Ragoussis J, Tarkkanen M, Nesland JM, Knuutila S, Myklebost O (2002) Positional cloning identifies a novel cyclophilin as a candidate amplified oncogene in 1q21. *Oncogene* 21:2261–2269
- Mihatsch MJ, Kyo M, Morozumi K, Yamaguchi Y, Nickeleit V, Ryffel B (1998) The side-effects of cyclosporine-A and tacrolimus. *Clin Nephrol* 49:356–363
- Miller RT, Anderson SP, Corton JC, Cattley RC (2000) Apoptosis, mitosis and cyclophilin-40 expression in regressing peroxisome proliferator-induced adenomas. *Carcinogenesis* 21:647–652
- Misumi S, Fuchigami T, Takamune N, Takahashi I, Takama M, Shoji S (2002) Three isoforms of cyclophilin A associated with human immunodeficiency virus type 1 were found by proteomics by using two-dimensional gel electrophoresis and matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Virol* 76:10000–10008
- Miyashita H, Mori S, Motegi K, Fukumoto M, Uchida T (2003) Pin1 is overexpressed in oral squamous cell carcinoma and its levels correlate with cyclin D1 overexpression. *Oncol Rep* 10:455–461
- Moro A, Ruizcabello F, Fernandezcano A, Stock RP, Gonzalez A (1995) Secretion by *Trypanosoma cruzi* of a peptidyl-prolyl cis-trans isomerase involved in cell infection. *EMBO J* 14:2483–2490
- Morris CA, Mervis CB, Hobart HH, Gregg RG, Bertrand J, Ensing GJ, Sommer A, Moore CA, Hopkin RJ, Spallone PA, Keating MT, Osborne L, Kimberley KW, Stock AD (2003) GTF21 hemizygoty implicated in mental retardation in Williams syndrome: genotype-phenotype analysis of five families with deletions in the Williams syndrome region. *Am J Med Genet* 123A:45–59
- Navia MA (1996) Protein-drug complexes important for immunoregulation and organ transplantation. *Curr Opin Struct Biol* 6:838–847
- Nilsson M, Meza-Zepeda LA, Mertens F, Forus A, Myklebost O, Mandahl N (2004) Amplification of chromosome 1 sequences in lipomatous tumors and other sarcomas. *Int J Cancer* 109:363–369
- Noguchi N, Takasawa S, Nata K, Tohgo A, Kato I, Ikehata F, Yonekura H, Okamoto H (1997) Cyclic Adp-ribose binds to Fk506-binding protein 12.6 to release Ca^{2+} from islet microsomes. *J Biol Chem* 272:3133–3136
- Oshiro N, Yoshino K, Hidayat S, Tokunaga C, Hara K, Eguchi S, Avruch J, Yonezawa K (2004) Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes Cells* 9:359–366
- Panhans-Gross A, Novak N, Kraft S, Bieber T (2001) Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (FK506). *J Allergy Clin Immunol* 107:345–352
- Parker EM, Monopoli A, Ongini E, Lozza G, Babij CM (2000) Rapamycin, but not FK506 and GPI-1046, increases neurite outgrowth in PC12 cells by inhibiting cell cycle progression. *Neuropharmacology* 39:1913–1919

- Patra D, Wang SX, Kumagai A, Dunphy WG (1999) The xenopus Suc1/Cks protein promotes the phosphorylation of G(2)/M regulators. *J Biol Chem* 274:36839–36842
- Pereira PJB, Vega MC, Gonzalez-Rey E, Fernandez-Carazo R, Macedo-Ribeiro S, Gomis-Ruth FX, Gonzalez A, Coll M (2002) Trypanosoma cruzi macrophage infectivity potentiator has a rotamase core and a highly exposed alpha-helix. *EMBO Rep* 3:88–94
- Perron MJ, Stremmler M, Song B, Ulm W, Mulligan RC, Sodroski J (2004) TRIM5 alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc Natl Acad Sci U S A* 101:11827–11832
- Petrulis JR, Kusnadi A, Ramadoss P, Hollingshead B, Perdew GH (2003) The hsp90 co-chaperone XAP2 alters importin beta recognition of the bipartite nuclear localization signal of the Ah receptor and represses transcriptional activity. *J Biol Chem* 278:2677–2685
- Petrulis JR, Perdew GH (2002) The role of chaperone proteins in the aryl hydrocarbon receptor core complex. *Chem Biol Interact* 141:25–40
- Pollard S (2004) Calcineurin inhibition and disease recurrence in the hepatitis C virus-positive liver transplant recipient. *Liver Int* 24:402–406
- Pratt WB, Silverstein AM, Galigniana MD (1999) A model for the cytoplasmic trafficking of signalling proteins involving the hsp90-binding immunophilins and p50(cdc37). *Cell Signal* 11:839–851
- Prudhomme GJ, Vanier LE, Bocarro DC, Stecroy H (1995) Effects of cyclosporin A, rapamycin, and Fk520 on peripheral T-cell deletion and anergy. *Cell Immunol* 164:47–56
- Rahfeld JU, Rucknagel KP, Schelbert B, Ludwig B, Hacker J, Mann K, Fischer G (1994) Confirmation of the existence of a third family among peptidyl-prolyl cis/trans isomerases. Amino acid sequence and recombinant production of parvulin. *FEBS Lett* 352:180–184
- Ramamurthy V, Roberts M, van den Akker F, Niemi G, Reh TA, Hurley JB (2003) AIPL1, a protein implicated in Leber's congenital amaurosis, interacts with and aids in processing of farnesylated proteins. *Proc Natl Acad Sci U S A* 100:12630–12635
- Ranganathan R, Lu KP, Hunter T, Noel JP (1997) Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell* 89:875–886
- Reiken S, Lacampagne A, Zhou H, Kherani A, Lehnart SE, Ward C, Huang F, Gaburjakova M, Gaburjakova J, Rosemblyt N, Warren MS, He KL, Yi GH, Wang J, Burkhoff D, Vassort G, Marks AR (2003a) PKA phosphorylation activates the calcium release channel (ryanodine receptor) in skeletal muscle: defective regulation in heart failure. *J Cell Biol* 160:919–928
- Reiken S, Wehrens XHT, Vest JA, Barbone A, Klotz S, Mancini D, Burkhoff D, Marks AR (2003b) beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation* 107:2459–2466
- Renoir JM, Mercierbodard C, Hoffmann K, Leblanc S, Ning YM, Sanchez ER, Handschumacher RE, Baulieu EE (1995) Cyclosporin A potentiates the dexamethasone-induced mouse mammary tumor virus-chloramphenicol acetyltransferase activity in Lmcat cells—a possible role for different heat shock protein-binding immunophilins in glucocorticosteroid receptor-mediated gene expression. *Proc Natl Acad Sci U S A* 92:4977–4981
- Rey O, Baluda MA, Park NH (1999) Differential gene expression in neoplastic and human papillomavirus-immortalized oral keratinocytes. *Oncogene* 18:827–831
- Riboldi-Tunnicliffe A, Konig B, Jessen S, Weiss MS, Rahfeld J, Hacker J, Fischer G, Hilgenfeld R (2001) Crystal structure of Mip, a prolylisomerase from Legionella pneumophila. *Nat Struct Biol* 8:779–783

- Riggs DL, Roberts PJ, Chirillo SC, Cheung-Flynn J, Prapapanich V, Ratajczak T, Gaber R, Picard D, Smith DF (2003) The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling in vivo. *EMBO J* 22:1158–1167
- Rippmann JF, Hobbie S, Daiber C, Guilliard B, Bauer M, Birk J, Nar H, Garin-Chesa P, Rettig WJ, Schnapp A (2000) Phosphorylation-dependent proline isomerization catalyzed by Pin1 is essential for tumor cell survival and entry into mitosis. *Cell Growth Differ* 11:409–416
- Rizzardi GP, Harari A, Capiluppi B, Tambussi G, Ellefsen K, Ciuffreda D, Champagne P, Bart PA, Chave JP, Lazzarin A, Pantaleo G (2002) Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy. *J Clin Invest* 109:681–688
- Rosner M, Solberg Y, Turetz J, Belkin M (1997) Neuroprotective therapy for Argon-LASER induced retinal injury. *Exp Eye Res* 65:485–495
- Rothbard JB, Garlington S, Lin Q, Kirschberg T, Kreider E, McGrane PL, Wender PA, Khavari PA (2000) Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. *Nat Med* 6:1253–1257
- Ryffel B, Foxwell BM, Gee A, Greiner B, Woerly G, Mihatsch MJ (1988) Cyclosporine—relationship of side effects to mode of action. *Transplantation* 46:90S–96S
- Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, Abraham RT (1995) Isolation of a protein target of the Fkbp12-rapamycin complex in mammalian cells. *J Biol Chem* 270:815–822
- Saphire ACS, Bobardt MD, Gallay PA (1999) Host cyclophilin A mediates HIV-1 attachment to target cells via heparans. *EMBO J* 18:6771–6785
- Saphire ACS, Bobardt MD, Gallay PA (2002) A trans-complementation rescue of cyclophilin A-deficient viruses reveals that the requirement for cyclophilin A in human immunodeficiency virus type 1 replication is independent of its isomerase activity. *J Virol* 76:2255–2262
- Sayah DM, Sokolskaja E, Berthoux L, Luban J (2004) Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* 430:569–573
- Schiene-Fischer C, Habazettl J, Schmid FX, Fischer G (2002) The hsp70 chaperone DnaK is a secondary amide peptide bond cis-trans isomerase. *Nat Struct Biol* 9:419–424
- Schiene-Fischer C, Yu C (2001) Receptor accessory folding helper enzymes: the functional role of peptidyl prolyl cis/trans isomerases. *FEBS Lett* 495:1–6
- Schreier MH, Baumann G, Zenke G (1993) Inhibition of T-cell signaling pathways by immunophilin drug complexes: are side effects inherent to immunosuppressive properties? *Transplant Proc* 25:502–507
- Schubert A, Grimm S (2004) Cyclophilin D, a component of the permeability transition-pore, is an apoptosis repressor. *Cancer Res* 64:85–93
- Seko Y, Fujimura T, Taka H, Mineki R, Murayama K, Nagai R (2004) Hypoxia followed by reoxygenation induces secretion of cyclophilin A from cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 317:162–168
- Sezen SF, Hoke A, Burnett AL, Snyder SH (2001) Immunophilin ligand FK506 is neuroprotective for penile innervation. *Nat Med* 7:1073–1074
- Sharkey J, Butcher SP (1994) Immunophilins mediate the neuroprotective effects of Fk506 in focal cerebral ischaemia. *Nature* 371:336–339
- Shichinohe H, Kuroda S, Abumiya T, Ikeda J, Kobayashi T, Yoshimoto T, Iwasaki Y (2004) FK506 reduces infarct volume due to permanent focal cerebral ischemia by maintaining BAD turnover and inhibiting cytochrome c release. *Brain Res* 1001:51–59

- Shinkura N, Ikai I, Yamauchi A, Hirose T, Kawai Y, Inamoto T, Ozaki S, Iwai M, Bona C, Yamaoka Y (1999) Autoantibodies to FK506 binding protein 12 (FKBP12) in autoimmune diseases. *Autoimmunity* 29:159–170
- Shirane M, Nakayama KI (2003) Inherent calcineurin inhibitor FKBP38 targets Bcl-2 to mitochondria and inhibits apoptosis. *Nat Cell Biol* 5:28–37
- Silverstein AM, Galigniana MD, Chen MS, Owens-Grillo JK, Chinkers M, Pratt WB (1997) Protein phosphatase 5 is a major component of glucocorticoid receptor.hsp90 complexes with properties of an FK506-binding immunophilin. *J Biol Chem* 272:16224–16230
- Sohocki MM, Bowne SJ, Sullivan LS, Blackshaw S, Cepko CL, Payne AM, Bhattacharya SS, Khaliq S, Qasim Mehdi S, Birch DG, Harrison WR, Elder FF, Heckenlively JR, Daiger SP (2000) Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis. *Nat Genet* 24:79–83
- Sperr WR, Agis H, Semper H, Valenta R, Susani M, Sperr M, Willheim M, Scheiner O, Liehl E, Lechner K, Valent P (1997) Inhibition of allergen-induced histamine release from human basophils by cyclosporine A and FK506. *Int Arch Allergy Immunol* 114:68–73
- Steiner JP, Connolly MA, Valentine HI, Hamilton GS, Dawson TM, Hester L, Snyder SH (1997) Neurotrophic Actions of nonimmunosuppressive analogues of immunosuppressive drugs FK506, rapamycin and cyclosporin A. *Nat Med* 3:421–428
- Steiner JP, Dawson TM, Fotuhi M, Glatt CE, Snowman AM, Cohen N, Snyder SH (1992) High brain densities of the immunophilin FKBP colocalized with calcineurin. *Nature* 358:584–587
- Steiner JP, Dawson TM, Fotuhi M, Snyder SH (1996) Immunophilin regulation of neurotransmitter release. *Mol Med* 2:325–333
- Steinschulte C, Taner T, Thomson AW, Bein G, Hackstein H (2003) Cutting edge: sanglifehrin A, a novel cyclophilin-binding immunosuppressant blocks bioactive IL-12 production by human dendritic cells. *J Immunol* 171:542–546
- Sumanasekera WK, Tien ES, Turpey R, Vanden Heuvel JP, Perdew GH (2003) Evidence that peroxisome proliferator-activated receptor alpha is complexed with the 90-kDa heat shock protein and the hepatitis virus B X-associated protein 2. *J Biol Chem* 278:4467–4473
- Takahashi N, Hayano T, Suzuki M (1989) Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337:473–475
- Tanaka K, Fujita N, Higashi Y, Ogawa N (2002) Neuroprotective and antioxidant properties of FKBP-binding immunophilin ligands are independent on the FKBP12 pathway in human cells. *Neurosci Lett* 330:147–150
- Tchaicheeyan O (2004) Is peptide bond cis/trans isomerization a key stage in the chemomechanical cycle of motor proteins? *FASEB J* 18:783–789
- Tegeder I, Schumacher A, John S, Geiger H, Geisslinger G, Bang H, Brune K (1997) Elevated serum cyclophilin levels in patients with severe sepsis. *J Clin Immunol* 17:380–386
- Terashima A, Taniguchi T, Nakai M, Yasuda M, Kawamata T, Tanaka C (2000) Rapamycin and FK506 induce long-term potentiation by pairing stimulation via an intracellular Ca^{2+} signaling mechanism in rat hippocampal CA1 neurons. *Neuropharmacology* 39:1920–1928
- Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh CT, Sodroski J, Gottlinger HG (1994) Functional association of cyclophilin a with HIV-1 virions. *Nature* 372:363–365
- Thorpe JR, Mosaheb S, Hashemzadeh-Bonehi L, Cairns NJ, Kay JE, Morley SJ, Rulten SL (2004) Shortfalls in the peptidyl-prolyl cis-trans isomerase protein Pin1 in neurons are associated with frontotemporal dementias. *Neurobiol Dis* 17:237–249

- Timerman AP, Wiederrecht G, Marcy A, Fleischer S (1995) Characterization of an exchange reaction between soluble FKBP-12 and the FKBP-ryanodine receptor complex. Modulation by FKBP mutants deficient in peptidyl-prolyl isomerase activity. *J Biol Chem* 270:2451–2459
- Tiso N, Salamon M, Bagattin A, Danieli GA, Argenton F, Bortolussi M (2002) The binding of the RyR2 calcium channel to its gating protein FKBP12.6 is oppositely affected by ARVD2 and VTSIP mutations. *Biochem Biophys Res Commun* 299:594–598
- Tong G, Shepherd D, Jahr CE (1995) Synaptic desensitization of NMDA receptors by calcineurin. *Science* 267:1510–1512
- Towers GJ, Hatzioannou T, Cowan S, Goff SP, Luban J, Bieniasz PD (2003) Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat Med* 9:1138–1143
- Tsai CJ, Ma BY, Nussinov R (1999) Folding and binding cascades: shifts in energy landscapes. *Proc Natl Acad Sci U S A* 96:9970–9972
- Uchida T, Fujimori F, Tradler T, Fischer G, Rahfeld JU (1999) Identification and characterization of a 14 kDa human protein as a novel parvulin-like peptidyl prolyl cis/trans isomerase. *FEBS Lett* 446:278–282
- Vahlne A, Larsson PA, Horal P, Ahlmen J, Svennerholm B, Gronowitz JS, Olofsson S (1992) Inhibition of herpes simplex virus production in vitro by cyclosporin A. *Arch Virol* 122:61–75
- Van de Vrie W, Marquet RL, Eggermont AM (1997) Cyclosporin A enhances locoregional metastasis of the CC531 rat colon tumour. *J Cancer Res Clin Oncol* 123:21–24
- Van Duyne GD, Standaert RF, Karplus PA, Schreiber SL, Clardy J (1991) Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. *Science* 252:839–842
- Vogel KW, Briesewitz R, Wandless TJ, Crabtree GR (2001) Calcineurin inhibitors and the generalization of the presenting protein strategy. *Adv Protein Chem* 56:253–291
- Waldmeier PC, Feldtrauer JJ, Qian T, Lemasters JJ (2002) Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. *Mol Pharmacol* 62:22–29
- Waldmeier PC, Zimmermann K, Qian T, Tintelnot-Blomley M, Lemasters JJ (2003) Cyclophilin D as a drug target. *Curr Med Chem* 10:1485–1506
- Wang P, Cardenas ME, Cox GM, Perfect JR, Heitman J (2001) Two cyclophilin A homologs with shared and distinct functions important for growth and virulence of *Cryptococcus neoformans*. *EMBO Rep* 2:511–518
- Wang TW, Donahoe PK (2004) The immunophilin FKBP12: a molecular guardian of the TGF-beta family type I receptors. *Front Biosci* 9:619–631
- Wang TW, Donahoe PK, Zervos AS (1994) Specific interaction of type I receptors of the Tgf-beta family with the immunophilin Fkbp-12. *Science* 265:674–676
- Ward BK, Kumar P, Turbett GR, Edmondston JE, Papadimitriou JM, Laing NG, Ingram DM, Minchin RF, Ratajczak T (2001) Allelic loss of cyclophilin 40, an estrogen receptor-associated immunophilin, in breast carcinomas. *J Cancer Res Clinical Oncol* 127:109–115
- Ward BK, Mark PJ, Ingram DM, Minchin RF, Ratajczak T (1999) Expression of the estrogen receptor-associated immunophilins, cyclophilin 40 and FKBP52, in breast cancer. *Breast Cancer Res Treat* 58:267–280
- Watashi K, Hijikata M, Hosaka M, Yamaji M, Shimotohno K (2003) Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. *Hepatology* 38:1282–1288
- Wehrens XH, Lehnart SE, Reiken SR, Deng SX, Vest JA, Cervantes D, Coromilas J, Landry DW, Marks AR (2004) Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. *Science* 304:292–296

- Wehrens XHT, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song LS, Rosemblyt N, D'Armiento JM, Napolitano C, Memmi M, Priori SG, Lederer WJ, Marks AR (2003) FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell* 113:829–840
- Wei L, Steiner JP, Hamilton GS, Wu YQ (2004) Synthesis and neurotrophic activity of nonimmunosuppressant cyclosporin A derivatives. *Bioorg Med Chem Lett* 14:4549–4551
- Weiwad M, Werner A, Rucknagel P, Schierhorn A, Kullertz G, Fischer G (2004) Catalysis of proline-directed protein phosphorylation by peptidyl-prolyl cis/trans isomerases. *J Mol Biol* 339:635–646
- Woltman AM, Schlagwein N, van der Kooij SW, van Kooten C (2004) The novel cyclophilin-binding drug sanglifehrin A specifically affects antigen uptake receptor expression and endocytic capacity of human dendritic cells. *J Immunol* 172:6482–6489
- Wu X, Wilcox CB, Devasahayam G, Hackett RL, Arevalo-Rodriguez M, Cardenas ME, Heitman J, Hanes SD (2000) The Ess1 prolyl isomerase is linked to chromatin remodeling complexes and the general transcription machinery. *EMBO J* 19:3727–3738
- Wulf G, Garg P, Liou YC, Iglehart D, Lu KP (2004) Modeling breast cancer in vivo and ex vivo reveals an essential role of Pin1 in tumorigenesis. *EMBO J* 23:3397–3407
- Wulf GM, Liou YC, Ryo A, Lee SW, Lu KP (2002) Role of Pin1 in the regulation of p53 stability and p21 transactivation, and cell cycle checkpoints in response to DNA damage. *J Biol Chem* 277:47976–47979
- Xu Q, Leiva MC, Fischkoff SA, Handschumacher RE, Lyttle CR (1992) Leukocyte chemotactic activity of cyclophilin. *J Biol Chem* 267:11968–11971
- Yaffe MB, Cantley LC (1999) Signal transduction. Grabbing phosphoproteins. *Nature* 402:30–31
- Yao DY, Dore JJE, Leof EB (2000) FKBP12 is a negative regulator of transforming growth factor-beta receptor internalization. *J Biol Chem* 275:13149–13154
- Yeh E, Cunningham M, Arnold H, Chasse D, Monteith T, Ivaldi G, Hahn WC, Stukenberg PT, Shenolikar S, Uchida T, Counter CM, Nevins JR, Means AR, Sears R (2004) A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nat Cell Biol* 6:308–318
- Yu G, Hess DC, Borlongan CV (2004) Combined cyclosporine-A and methylprednisolone treatment exerts partial and transient neuroprotection against ischemic stroke. *Brain Res* 1018:32–37
- Yurchenko V, O'Connor M, Dai WW, Guo HM, Toole B, Sherry B, Bukrinsky M (2001) CD147 is a signaling receptor for cyclophilin B. *Biochem Biophys Res Commun* 288:786–788
- Yurchenko V, Zybarth G, O'Connor M, Dai WW, Franchin G, Hao T, Guo HM, Hung HC, Toole B, Gallay P, Sherry B, Bukrinsky M (2002) Active site residues of cyclophilin A are crucial for its signaling activity via CD147. *J Biol Chem* 277:22959–22965
- Zacchi P, Gostissa M, Uchida T, Salvagno C, Avolio F, Volinia S, Ronai Z, Blandino G, Schneider C, Del Sal G (2002) The prolyl isomerase Pin1 reveals a mechanism to control p53 functions after genotoxic insults. *Nature* 419:853–857
- Zander K, Sherman MP, Tessmer U, Bruns K, Wray V, Prechtel AT, Schubert E, Henklein P, Luban J, Neidleman J, Greene WC, Schubert U (2003) Cyclophilin A interacts with HIV-1 Vpr and is required for its functional expression. *J Biol Chem* 278:43202–43213
- Zarnt T, Lang K, Bartscher H, Fischer G (1995) Time-dependent inhibition of peptidylprolyl cis-trans-isomerases by Fk506 is probably due to cis-trans isomerization of the inhibitors imide bond. *Biochem J* 305:159–164

- Zenke G, Strittmatter U, Fuchs S, Quesniaux VF, Brinkmann V, Schuler W, Zurini M, Enz A, Billich A, Sanglier JJ, Fehr T (2001) Sanglifehrin A, a novel cyclophilin-binding compound showing immunosuppressive activity with a new mechanism of action. *J Immunol* 166:7165–7171
- Zhang LH, Youn HD, Liu JO (2001) Inhibition of cell cycle progression by the novel cyclophilin ligand sanglifehrin A is mediated through the NF kappa B-dependent activation of p53. *J Biol Chem* 276:43534–43540
- Zhong L, Qing KY, Si Y, Chen LY, Tan MQ, Srivastava A (2004) Heat-shock treatment-mediated increase in transduction by recombinant adeno-associated virus 2 vectors is independent of the cellular heat-shock protein 90. *J Biol Chem* 279:12714–12723
- Zhou XZ, Kops O, Werner A, Lu PJ, Shen M, Stoller G, Kullertz G, Stark M, Fischer G, Lu KP (2000) Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. *Mol Cell* 6:873–883

Molecular Chaperones in Health and Disease

Gaestel, M. (Ed.)

2006, X, 442 p. 34 illus., 5 illus. in color., Hardcover

ISBN: 978-3-540-25875-9