[Frontiers in Bioscience 9, 3453-3478, September 1, 2004]

INSIGHTS INTO THE CATALYTIC MECHANISM OF PEPTIDYL PROLYL *CIS/TRANS* ISOMERASES

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1. ABSTRACT

A large body of physiological, cell biological, kinetic and structural data about peptidyl prolyl *cis/trans* isomerases (PPIases) has been accumulated during the past 20 years, but despite the simplicity of the catalyzed reaction the question of how the enzyme action is performed is still not fully answered. In this review the center of attention is the molecular background of the catalytic mechanism of PPIases and the spontaneously occurring peptidyl prolyl *cis/trans* isomerization. We summarize and compare the available kinetic, structural and amino acid sequence data of all three PPIase families, the cyclophilins, FKBP and parvulins. Different catalytic mechanisms that have been suggested in the literature are discussed. A comprehensive comparison of enzyme active site structures reveals a hitherto unnoticed similarity between the three PPIase families and might suggest that PPIases utilize mechanisms that are more similar than previously suspected.

2. INTRODUCTION

The pioneering work of L. Pauling over half a century ago prepared the ground for understanding the atomic structure of proteins and the molecular basis of enzyme catalysis which should be especially valuable in studying catalysis of chemically simple reactions (1). Twenty years later, J. F. Brandts proposed that the slow phases observed in the unfolding kinetics of proteins might be due to the *cis*/*trans* isomerization of peptidyl prolyl bonds (the terms prolyl bond and prolyl isomerization are used throughout this review for the peptide bond preceding a proline residue and the peptidyl prolyl *cis/trans* isomerization, respectively) (2). An enzyme class discovered 9 years later with its first member isolated from pig kidney represented a powerful catalyst of prolyl isomerization operating on a level of one of the simplest chemical reactions: the rotation about a single bond (3). According to their substrate specificity these *cis/trans* isomerases were named peptidyl prolyl *cis*/*trans* isomerases (PPIases). These enzymes have opened up a broad new area into the investigations of assisted protein folding. Recently the secondary amid peptide bond *cis/trans* isomerases (APIases) have been found which are actively engaged in lowering the rotational barrier of a secondary amide peptide bond C(O)-NH- in oligopeptide- and protein substrates(4). Another 20 years have passed since the first PPIase was characterized and what have we learned about these enzymes in that time? A great deal of data has been published about the physiological significance and the enzyme mechanism of PPIases. The best characterized enzymes among the PPIases are human cyclophilin 18 (human Cyp18) and human FKBP12 (human FKBP12). Both are prototypic members of their corresponding PPIase families known as cyclophilins and FK506 binding proteins (FKBP). The family names derive from the ability of their respective members to bind to cyclosporine A or FK506, both highly active immunosuppressive compounds. A third PPIase family, the parvulins, was discovered in 1994 (5). The most prominent and best investigated member of this family is human Pin1 (human Pin1). The finding that cyclophilins and FKBP participate in immunosuppressive processes initiated a rush of scientific interest. Since then PPIases have been found to take part in a great number of physiological processes such as *in vivo* protein folding, heat shock response, transcription and translation, channel gating, virus assembly, signal transduction, tumor metastasis, pathogen virulence, cell cycle control and others (6-10). These topics have been reviewed exhaustively and shall not concern us here. In this review we want to focus on publications related to the catalytic mechanism and try to combine all available data to give a complete overview of this subject.

Despite the amount of data, the molecular basis of the PPIase mechanism is still only poorly understood.

For biochemical investigation, the simplicity of the reaction is a blessing and a curse at the same time. Since the reaction is so undemanding, evolution did not "bother" to equip prototypic PPIases with cofactors; which can often be exploited to unveil the enzyme mechanisms. Furthermore the difference between the substrate and the product state of a polypeptide chain is very subtle and therefore sometimes difficult to investigate. On the other hand the acceleration of the peptide bond rotation is already achieved by globular low-molecular-mass proteins, making these enzymes a perfect subject of structural investigations. Since the reaction is, at least for oligopeptide substrates, completely reversible, PPIases can be studied under equilibrium conditions, an advantage if one wants to employ NMR spectroscopic tools which often require long measuring times under biological conditions.

There exists no sequence homology between the three PPIase families, but they all catalyze the same chemical reaction. A fundamental question arises from this fact:

Do PPIases facilitate their enzymatic action by utilizing a common catalytic pathway?

3. MECHANISM OF CATALYZED PROLYL *CIS/TRANS* ISOMERIZATION

3.1. Non enzymatic acceleration of prolyl *cis/trans* isomerization

To fully understand the enzyme mechanism of PPIases, a profound knowledge of the chemical properties of prolyl bond isomers is unavoidable. Therefore a short excursion into the nonenzymatic acceleration of prolyl bond isomerization is necessary here. Although a full description requires a more complex explanation, the classical concept of „resonance stabilization", where the lone electron pair of the peptide bond nitrogen is delocalized over the entire peptide bond, is now widely accepted. This model explains many of the peptide bond features such as the limited number of stable ground state conformations, the high barrier to rotation about the C-N bond, a shortened C-N bond length, the kinetic stability of peptide bonds towards a nucleophilic attack and the isomer-sensitive carbonyl stretching frequencies in the IR-spectrum, and it defines the chemical means to lower the rotational barrier catalytically (Figure 1). Reviews on this topic are available (11, 12). Here we will focus on the data directly linked to the acceleration of the prolyl bond isomerization, supplemented in some cases with results obtained from secondary amides.

3.1.1. Environmental effects

The amide group shows a greater charge separation when planar than in its proposed orthogonal transition state, therefore the isomerization rate should be faster in less polar solvents. Accordingly the isomerization rate of N,N-dimethylacetamide (DMA) increases by a factor of 60 as the solvent is changed from water to cyclohexane (13). A similar increase in isomerization rates were observed for peptides containing 2-C substituted proline-like oxazolidine and thiazolidine derivatives, when the solvent was shifted from DMSO to $CDCl_3$ (14). Contrarily, it was found that the isomerization rate of Ac-GP-OMe did not correlate with the solvent dielectric constant or the solvent polarity but depended on the ability of the solvent to donate hydrogen bonds. Further analysis showed that the barrier to rotation is proportional to the strength of hydrogen bonds formed to the peptide bond oxygen. From the experimental results it was calculated that desolvation of the prolyl bond can reduce the free energy of activation by 1.3 kcal/mol, which is only little when compared with the decrease achieved by PPIases (15). A pH shift from 7.0 to 1.8 increases the rate of isomerization of DMA 130-fold (16). Although the preferred protonation site of a peptide bond is the carbonyl oxygen, the rotational barrier of amides can be lowered by a small but kinetically significant amount of an amide nitrogen protonated species. The direct participation of hydroxide ions to the acceleration of the cis/trans isomerization of DMA at pH 11.8 has been observed. The rate enhancement was attributed to the formation of a tetrahedral hemiorthoamide, in which the amide resonance is disrupted (16). Such extreme pH conditions are usually not found in biological systems under native conditions but reactive hydroxide species are frequently observed in enzymes utilizing Zn^{2+} ions like carbonic anhydrase (17). The findings that the isomerization rate of short proline containing peptides in buffered solutions is independent of the pH within the range of 5 to 9 apparently indicates the lack of general intermolecular acid/base catalysis (18). In this case the observed deuterium kinetic solvent isotope effect (KSIE) is nearly in unity and therefore it was postulated that no proton movement during transition state formation occurs (19).

Not mentioned in either of the categories above are effects on the isomerization rate of prolyl bonds due to modifications of the prolyl bond itself or of the two neighboring amino acid residues. In general, one finds that electron donating substituents near the carbonyl moiety accelerate the isomerization rate whereas a deceleration is observed when the electron density increases at the peptide nitrogen. Only a few modifications are of physiological relevance, such as hydroxylation of proline in position 4 or O-glycosylation and phosphorylation of serine or threonine side chains preceding proline. Proline homologues with differing ring size and heteroatom substitution in the proline ring influence the imidic rotational barrier of oligopeptides containing these analogues. For Aze, Pip, 4-Oxa and 2-Thz (Figure 2) a substantial acceleration of the *cis/trans* isomerization was detected (20). A similar effect was observed for fluoroproline derivatives (Ac-(4*R*)-FPro-OMe, Ac-(4*S*)-FPro-OMe and Ac-4,4-F_2Pro-OMe), where the inductive effect of the fluorine substitutions leads to a weakening of the Ac-Pro amide bond (21). Higher isomerization rates of the imide bond have also been reported for short peptides containing different 2-C substituted proline-like oxazolidine and thiazolidine derivatives (Figure 2) (14). On the other hand, the presence of 3-C alkylated proline derivatives in the chain leads to a marked decreasein the imidic isomerization rates in water. These effects were mainly attributed to sterical restrictions arising from these bulky side chains (22). O-glycosylation of serine residues preceding proline and hydroxyl substitution of proline were shown to have no effect on the isomerization rate, whereas serine or threonine

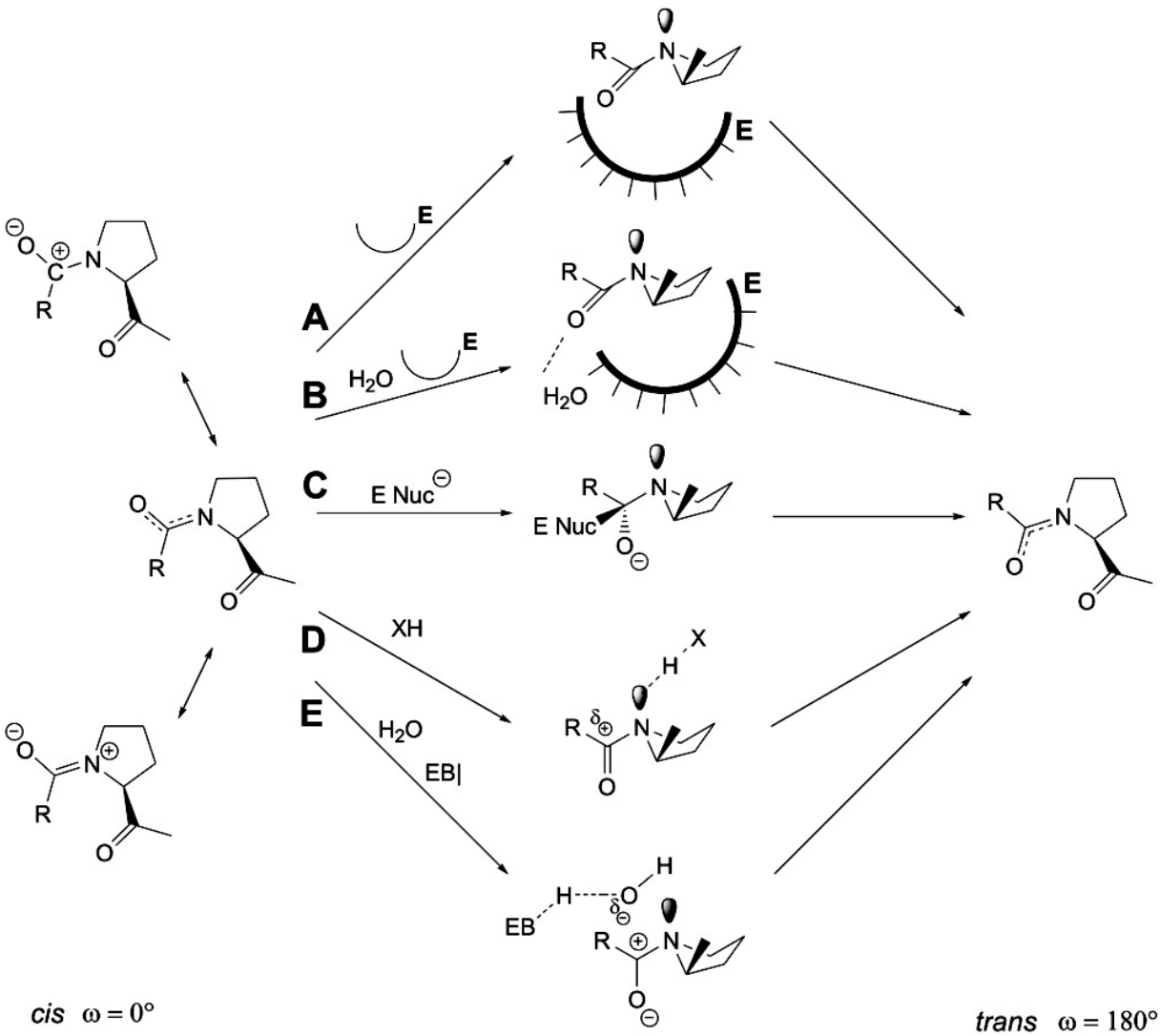

Figure 1. Canonical structures of the prolyl bond and possible catalytic mechanism for the *cis/trans* isomerization. Catalysis by the hydrophobic enzyme environment which stabilizes a more apolar twisted prolyl bond (A). Catalysis by a solvent assisted mechanism in which a enzyme bond water stabilizes the intermediate by a hydrogen bond to the carbonyl oxygen (B). Nucleophilic catalysis (C). Catalysis by an intra- or intermolecular protonation of the imide nitrogen (D). An active site structural water molecule assists bond rotation by electrostatic transition state stabilization (E).

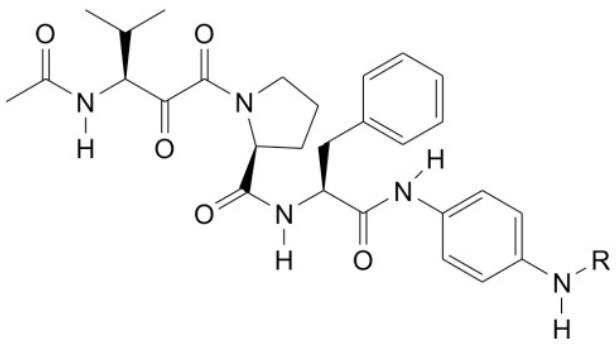

Figure 2. Haptens (R = $CO(CH_2)_3CO_2H$ or R = CHO) used to raise catalytic antibodies.

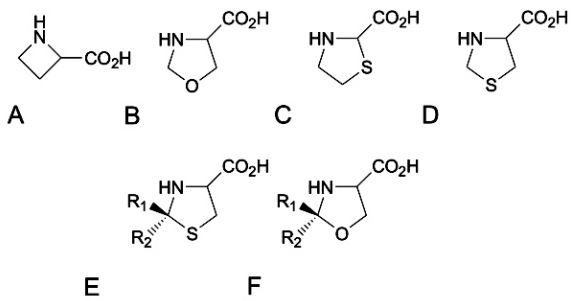

Figure 3. Proline analogues used to investigate the uncatalyzed prolyl *cis*/*trans* isomerization. A) (*S*)-azetidine-2-carboxylic acid, Aze; B) (*S*)-oxazolidine-4-carboxylic acid, 4-Oxa; C) (*R*)-thiazolidine-2-carboxylic acid, 2-Thz; D) *R*)-thiazolidine-4-carboxylic acid, 4-Thz; E) 2-C substituted thiazolidine derivatives, F) 2-C substituted oxazolidine derivatives.

phosphorylation decreased the isomerization rate up to 7 fold (22-25). The substitution of the prolyl bond carbonyl oxygen by sulfur in short peptides reduces the isomerization rate by a factor of about 100. This is in accordance with the resonance theory since the thioxo group increases the electron density along the C-N bond (26).

3.1.2. Intramolecular catalysis

Intramolecular catalysis of prolyl bond isomerization was first observed during the refolding reaction of denatured dihydrofolate reductase (27). Proline 66 undergoes a *trans* to *cis* isomerization catalyzed by the guanidinium group of arginine 44, which interacts with the imide nitrogen of the Gln65-Pro66 peptide bond. Analyzing protein structures available from the RCSB data bank revealed that in almost 6% of all presently available structures at least one arginine guanidinium group is within 4 Å of a proline imide nitrogen (Wille, G. *et al.*, in preparation) suggesting a general concept for accelerating proline-limited protein folding. It was also found that a hydrogen bond between the imide nitrogen and the adjacent amidic NH within a five-membered ring, a so called 5-NH- -N_a hydrogen bond, can accelerate *cis/trans* isomerization up to 260-fold, depending on the used solvent (28). A tenfold increase of the isomerization rate was reported for Cys-Pro bonds in disulfide bonded cyclic peptides (Ac-CPPC-NH_2 and Ac-TCPPCR-NH_2) as compared to the corresponding acyclic compounds. With the help of Monte Carlo molecular mechanics simulations it was concluded that the NH proton of the residue following Pro can establish a hydrogen bond to the sp^3 lone electron pair of the imide nitrogen in the transition state (29). An about tenfold increased rate of prolyl isomerization was found in peptides containing a His-Pro moiety after a pH jump from basic to acidic conditions. It was proposed that the protonation of the imidazol ring promotes the isomerization by either providing an intramolecular hydrogen bond or by localizing a positive charge close to the prolyl bond. Since the KSIE under acidic conditions was determined to be 2.0, it was concluded that a direct proton transfer from the protonated histidine moiety to the imide nitrogen during transition state formation occurs (30). The sequence specific catalytic effect to the neighbouring prolyl bond rotation suggests that His-Pro may play a general role in preventing proline-limited slow folding phases in proteins..

3.1.3. Intermolecular catalysis

Monoclonal antibodies raised against α-keto dicarbonyl containing haptens (Figure 3) were described to accelerate the *cis/trans* isomerization of short proline containing peptides and the refolding rate of RNase T1, which is limited by prolyl bond *cis/trans* isomerization. The authors suggested that the used α-keto dicarbonyl function might mimic a twisted amide bond of a PPIase bound substrate. It was further suggested that this hapten might result in antibodies capable of forming a tetrahedral adduct to the electrophilic carbonyl group of the α-keto functionality. However, such a specific catalytic mechanism was ruled out because no pH dependence or KSIE was observed and the small rate enhancement was comparable to that achieved by hydrophobic solvents alone (31, 32). Similar acceleration factors were observed for vesicle- and micelle-entrapped oligopeptides. Thermodynamic results as well as the effects of side chain variations of the investigated peptide substrates will be discussed in the FKBP section of this review (33).

3.2. PPIase catalyzed prolyl *cis/trans* isomerization

Among PPIases are single and multidomain enzymes, a review summarizing the domain structure of human PPIase is available (34), here we focus on the enzymatic activity and therefore only the function of PPIase domains will be discussed. Figure 1 shows five possible catalytic mechanisms to accelerate the *cis/trans* isomerization of prolyl bonds. Many of them have been proposed to be the driving force behind the catalytic power of PPIases but only few have been shown to be at least involved in the catalytic cycle of these enzymes.

3.2.1. Cyclophilins

After the development of an *in vitro* PPIase assay based on the isomer-specific proteolytic cleavage of peptide bonds it became feasible to investigate the catalytic mechanism of PPIases (3). Since then a number of different activity assays have been developed, including a protease free UV/Vis assay, dynamic NMR based methods, fluorescence based assays and protein folding/unfolding assays. A complete summary and descriptions of all available PPIase assays has been given in a recent review published by Fischer and Aumuller (34). In the last two decades a large number of cyclophilins from different organisms have been purified and kinetically characterized. Almost all cyclophilins investigated so far show a second order rate constant k_{cat}/K_M between 10^5 and 10^7 $M^{-1}s^{-1}$ when measured using the oligopeptide Suc-AAPF-pNA as substrate. Under optimal conditions the prototypic human Cyp18 is a perfectly evolved catalyst that approach the limit of diffusion control for enzyme catalyzed reactions; for the most suitable oligopeptide substrates it has a high turnover number ($k_{cat} > 600$ s^{-1}) combined with a low ground state affinity ($K_M > 80$ μM). Table 1 gives an overview of the catalytic constants (k_{cat}/K_M) for the *cis* to *trans* isomerization of cyclophilins, mostly estimated with the oligopeptide substrate Suc-AAPF-pNA.

To date the three dimensional structures of 11 cyclophilins from 9 different organisms have been determined (Table 2), most of these in complex with a tight-binding inhibitor such as CsA. All these cyclophilin domains consist of an eight-stranded antiparallel β-barrel capped at either end by two α-helices (Figure 4A). Despite the large quantity of structural data only three cyclophilins from different organisms, namely human Cyp18, *E.coli*

Table 1. Cyclophilins described as active using Suc-AAPF-pNA as substrate in the standard *in vitro* PPIase assays

Organism	Name as in publication or data bank/generic name	Swissprot	(k_{cat}/K_M) $M^{-1}s^{-1}$	References
A. nidulans	CypB / Cyp23	O94190	active	95
A. niger	CypA / Cyp19	O94184	active	96
A. niger	CypB / Cyp23	Q8X166	active	97
A. thaliana	AtCyp22	P34790	$5.7\ 10^6$	98
A. thaliana	AtCyp28	P34791	active	99
B. malayi	Cyp-1 / Cyp98	Q27450	$7.5\ 10^6$	100
B. malayi	Cyp-2 / Cyp19	Q17246	$1.23\ 10^7$	101
B. subtilis	PPiB / Cyp15	P35137	$1.1\ 10^6$	102
B. taurus	CypA / Cyp18	P04374	$1.3\ 10^7$	50
B. taurus	CypB / Cyp23	P80311	$3.0\ 10^6$	103
C. albicans	Cyp1 / Cyp18	P22011	active	104
C. elegans	cyp-1 / Cyp21	P52009	$7.0\ 10^4$	105
C. elegans	cyp-2 / Cyp18.5	P52010	$6.1\ 10^5$	105
C. elegans	cyp-3 / Cyp18.6	P52011	$3.6\ 10^5$	105
C. elegans	cyp-4 / Cyp59	P52012	$1.8\ 10^4$	105
C. elegans	cyp-5 / Cyp22	P52013	$7.4\ 10^4$	105
C. elegans	cyp-6 / Cyp21.9	P52014	$8.4\ 10^6$	105
C. elegans	cyp-8 / Cyp54	P52016	$1.95\ 10^4$	105
C. elegans	cyp-9 / Cyp36	Q09637	$1.5\ 10^4$	105
C. elegans	Cyp-10 / Cyp18	P52017	$1.9\ 10^4$	105
C. elegans	Cyp-11 / Cyp20	P52018	$1.5\ 10^4$	105
C. elegans	CeCyp-16 / Cyp25	Q9XXI7	$2\ 10^3$ a)	106
D. discoideum	CypE / Cyp17	Q9NI62	Active	107
D. melanogaster	Moca-CypA / Cyp113	Q8ISE5	$5.6\ 10^4$	108
D. immitis	Dicyp-3 / Cyp60	O61300	$3.95\ 10^5$	109
E. coli	CypA / Cyp20	P20752	$5.71\ 10^7$	110
E. coli	CypB / Cyp18	P23869	$6.74\ 10^7$	110
E. histolytica	EhCyp / Cyp18	O15729	active	111
H. cutirubrum	Cyp19	O50586	active	112
H. sapiens	NKCR_HUMAN / Cyp166	P30414	$7.5\ 10^5$	113
H. sapiens	Cyp 40	Q08752	$1.9\ 10^6$	114
H. sapiens	SnuCyp-20	O43447	active	115
H. sapiens	Cyp18	P05092	$1.3\ 10^7$ b)	116
H. sapiens	CypF / Cyp22	P30405	$2.3\ 10^7$	117
H. sapiens	CypB / Cyp23	P23284	$1.1\ 10^7$	117
L. esculentum	CypA / Cyp18	P21568	active	118
L. major	LmCyp19	O02614	$1.6\ 10^6$	119
L. pneumophila	lpCyp18	Q48822	$4.6\ 10^6$	120
N. crassa	CPH / Cyp24	P10255	$2.8\ 10^6$	121
N. crassa	NcCyP41	Q9P3X9	$6.5\ 10^5$	122
O. volvulus	OvCyp16	Q8IA80	$5.2\ 10^2$ c)	100
Orpinomyces sp.	CypB / Cyp22	Q01490	$9.3\ 10^6$	123
P. falciparum	PFCyP / Cyp25	Q8I6S4	active	124
P. falciparum	PFCyP / Cyp22	Q8IIK8	$2.3\ 10^6$	125
R. norvegicus	Matrin CYP / Cyp88	O55035	$1.0\ 10^6$	126
R. norvegicus	CypF / Cyp22	P29117	$0.9\ 10^6$	127
S. cerevisiae	Cpr1 / Cyp17	P14832	active	128
S. cerevisiae	Cpr3 / Cyp20	P25719	$5.8\ 10^6$	129
S. cerevisiae	Cpr6 / Cyp42	P53691	$5\ 10^5$	130
S. cerevisiae	Cpr7 / Cyp45	P47103	$7\ 10^4$	131
S. mansoni	SmCypB / Cyp23	Q26551	$8.2\ 10^5$	132
S. mansoni	SmCypA / Cyp31	Q26548	$3.65\ 10^5$	132
S. mansoni	Smp17.7	Q26565	active d)	133
S. pombe	SpCyp3 / Cyp19	O74729	$1.5\ 10^6$	134
S. chrysomallus	ScCYPA / Cyp18	Q06118	$3.73\ 10^6$	135
S. chrysomallus	ScCYPB / Cyp19	P77949	$7.5\ 10^6$	136
T. cruzi	TcCyP19	AI021872 e)	active	137
T. gondii	Cyp18.5	Q26994	active	138
T. gondii	Cyp20	Q26995	active	138
T.mentagrophytes	Cyp13	B019518	active	139
T. tridentatus	CypG / Cyp24	O44073	$1.8\ 10^5$ f)	140
T. inflatum	no name/ Cyp25	Q99009	active	141
V. faba	CypB / Cyp27	Q41651	active	142
X. laevis	XlCyp / Cyp17	AJ496795 g)	$1.1\ 10^7$	143
Z. mays	zmCyp18	P21569	$1.1\ 10^7$	144
Z. mays	CypB	Q10724 h)	$2.5\ 10^7$	144

The reported enzymatic constants were obtained as described in the respective publication. The generic name consists of the abbreviation of the respective PPIase family followed by the rounded molecular weight of the full length protein. a) Suc-AVPF-pNA was used as substrate, b) active site titration for this enzyme has been performed (145), c) Suc-ALPF-pNA was used as substrate, d) no data about the substrate used, e) no swissprot entry, SRS EMBL nucleotide accession number, f) Suc-AAPF-mca was used as substrate, g) no swissprot entry, SRS EMBL protein accession number, h) protein fragment, no sequence data available.

Table 2. Cyclophilin structures available in RCSB Data Bank

Organism	Name as used in publication	Number of structures	PDB identifier
H. sapiens	human Cyp18	43	1ak4, 1awq, 1awr, 1aws, 1awt, 1awu, 1bck, 1cwa, 1cwb, 1cwc, 1cwf, 1cwh, 1cwi, 1cwj, 1cwk, 1cwm, 1cwl, 1cwo, 1cya, 1cyb, 1fgl, 1m63, 1m9c, 1m9d, 1m9e, 1m9f, 1m9x, 1m9y, 1mf8, 1mik, 1nmk, 1oca, 1rmh, 1vbs, 1vbt, 2cpl, 2cyh, 2rma, 2rmb, 3cyh, 3cys, 4cyh, 5cyh
H. sapiens	hCypB	1	1cyn
H. sapiens	SnuCyp-20	2	1mzw, 1qoi
B. malayi	CYP-1	3	1a58, 1a33, 1c5f
M. musculus	CypC	1	2rmc
C. elegans	Cyp-3	2	1dyw 1e3b
C. elegans	Cyp-5	1	1hop
P. falciparum	Cyp18	2	1qng, 1qnh
B. taurus	Cyp40	2	1ihg, 1iip
E. coli	CypB	2	1clh, 1csa
E. coli	CypA	2	1lop, 2nul
S. cerevisiae	Cpr-1	1	1ist

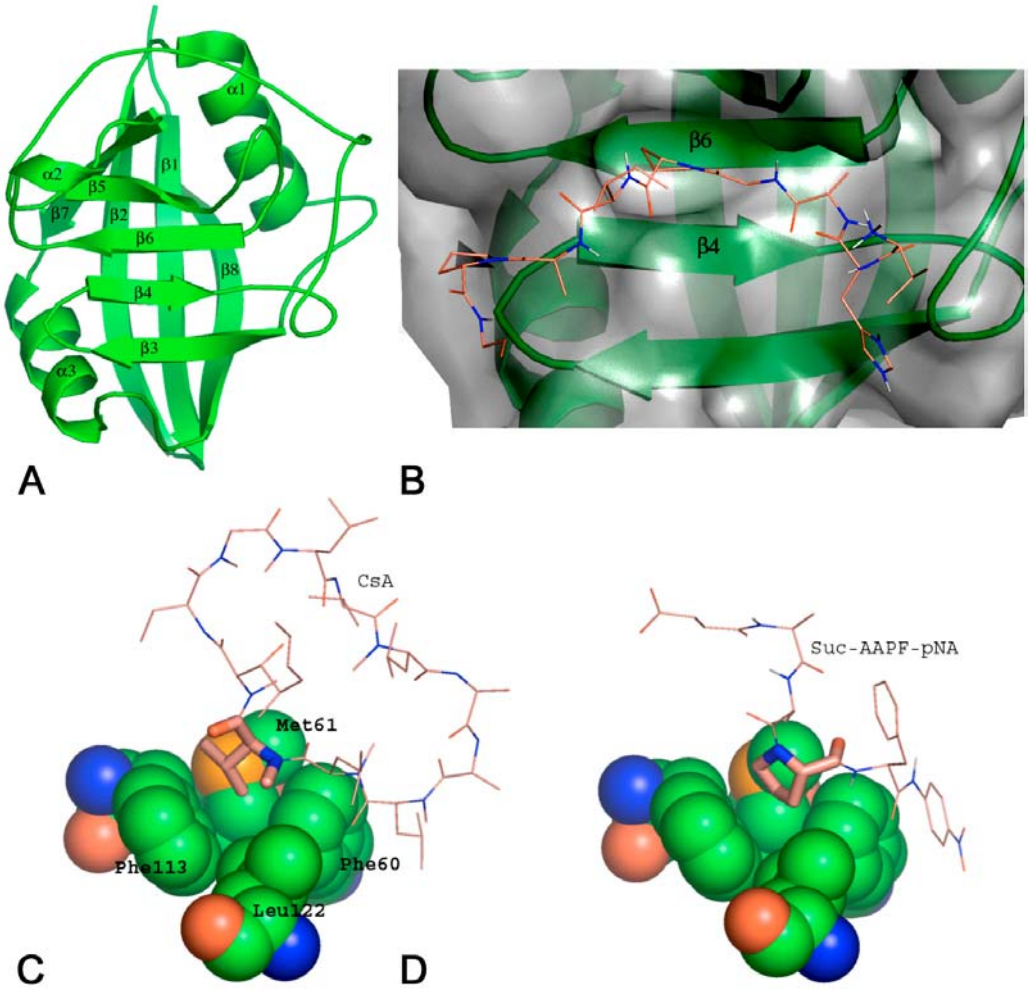

Figure 4. Ribbon representation of the global fold of cyclophilin domains, here shown for human Cyp18. The protein consists of a β-barrel formed by eight antiparallel β-sheets comprising residues 5 to 12 (β1), 15 to 24 (β2), 53 to 57 (β3), 61 to 64 (β4), 91 to 100 (β5), 112 to 115 (β6), 127 to 134 (β7) and 156 to 163 (β8) and two amphipathic α-helices (α1 residues 30 to 41 and α3 residues 136 to 145) and a short 3_1-helix (residues 120 to 122) (A). Two of the eight β-strands (β4, β6) form the cavity that accommodates the proline residue (B). This binding pocket is formed by four hydrophobic residues (Phe60, Met61, Phe113, and Leu122). The cavity accommodates the MeVal-11 moiety of the inhibitor CsA (C) as well as the prolyl moiety of a substrate (D).

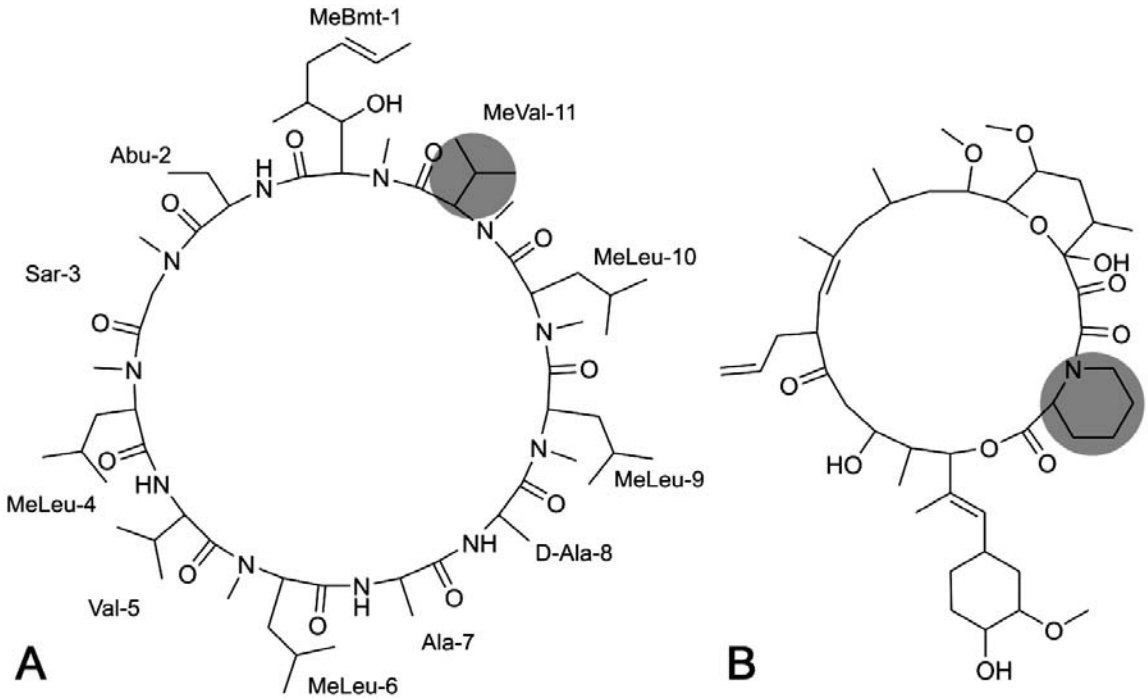

Figure 5. CsA (A) and FK506 (B); residues that penetrate the prolyl binding pocket (MeVal-11 of CsA and the pipecolinyl moiety of FK506) are indicated.

CypA and *C.elegance* Cyp3 have been co-crystallized with a substrate (35-42). The first view of the active site of human Cyp18 in complex with a substrate (Ac-AAPA-amc) was provided by Kallen *et al*. 1992. Amino acid residues of human Cyp18 in close contact (< 3.8 Å) with the substrate are R55, I57, F60, Q63, A101, N102, Q111, F113, L122, H126, and R148 (38). These residues form a channel on top of two antiparallel β-strands (Figure 4B). The binding pocket for the proline pyrrolidine ring is formed by residues F60, M61, F113 and L122 (Figure 4D). The same hydrophobic pocket is occupied by the MeVal-11 moiety of the tight binding inhibitor cyclosporin A (Figure 5A). The shape of the cavity is optimized to interact with a five-membered ring. The catalytic efficiency of human Cyp18 is greatly reduced if the prolyl moiety of peptides is replaced by four- or six-membered ring derivatives of proline, and completely abolished in cases of a secondary amide peptide bond (18, 43). This finding makes it clear that the correct spatial orientation of the proline residue in the transition state plays a crucial role in catalysis.

A pairwise amino acid sequence alignment of cyclophilin domains originating from cyclophilins that have been reported as active, shows that all residues comprising the binding pocket are conserved, with the exception of R148 (Table 3). The highest degree of variability is found at the three positions 57, 102 and 126. The exchange in position 57 of isoleucine with valine in six sequences is conservative, but nevertheless four out of these six cyclophilins posses a strongly reduced catalytic activity. The side chain of N102 is not involved in substrate interactions but the backbone atoms of this residue are in close contact with the substrate. The observed substitutions are tolerated accordingly; only OvCyp16, in which this residue is exchanged with a serine residue, possesses a low PPIase activity. The exchange of H126 with tyrosine found in four cyclophilin sequences appear not necessarily to impair the catalytic activity since both PPIases E.coli CypA and E.coli CypB are described as highly active. The same substitution seems to be responsible for the reduced activity of Cyp-10 from C. elegans. Mutational analysis of seven active site residues of human Cyp18 showed that only the variants R55A, F60A, and H126Q posses less than 1% residual activity (Table 4) (44). The phenylalanine residue in position 60 and arginine in position 55 are completely conserved among the cyclophilins. The side chain of F60 forms part of the prolyl binding pocket and is therefore essential for proper positioning of substrates. The importance of R55 for the catalytic power of human Cyp18 will be discussed later. On the basis of the first structure of a human Cyp18/substrate complex, it was suggested that H126 might attack the prolyl bond, but further refinement showed that H126 is not involved in any direct or indirect hydrogen bonds with the substrate (38, 45). It was pointed out that the hydrophobic interaction of this residue is essential for substrate binding (39). It was further concluded that the hydrophobic properties of H126 are more important than its ability to participate in hydrogen bonds, which was supported by the strongly reduced but still significant activity of the H126Q variant (Table 4).

The structural data in combination with kinetic experiments and site directed mutagenesis resulted in a variety of theories describing the molecular mechanism of cyclophilin catalysis. From the observed inactivation of

Table 3. Conserved active site residues of cyclophilins; activity has been measured using Suc-AAPF-pNA if not stated otherwise

Residue (human Cyp18 nomenclature)	Number of exchanges	Organism/Name as used in publication	Found exchange	(k_{cat}/K_M) $M^{-1}s^{-1}$	Swissprot entry
I57	6	*C.elegans* / Cyp-9	V	$1.5\ 10^4$	Q09637
		C.elegans / Cyp-16	V	$2\ 10^{3\ a}$	Q9XXI7
		R.norvegicus / Matrin CYP	V	$1.0\ 10^6$	O55035
		H.sapiens / NKCR_HUMAN	V	$7.5\ 10^5$	P30414
		S.cerevisiae / Cpr7	V	$7\ 10^4$	P47103
		D.melanogaster / Moca-CypA	V	$5.6\ 10^4$	Q8ISE5
N102	5	*E.coli* / CypA	T	$5.71\ 10^7$	P20752
		E.coli / CypB	T	$6.74\ 10^7$	P23869
		B.subtilis / PPiB	H	$1.1\ 10^6$	P35137
		L.pneumophila / Cyp18	R	$4.6\ 10^6$	Q48822
		O.volvulus / Cyp16	S	$5.2\ 10^{2\ a}$	Q8IA80
L122	1	*C.elegans* / Cyp-9	C	$1.5\ 10^4$	Q09637
H126	4	*E.coli* / CypA	Y	$5.71\ 10^7$	P20752
		E.coli / CypB	Y	$6.74\ 10^7$	P23869
		C.elegans / Cyp-10	Y	$1.9\ 10^4$	P52017
		P.falciparum / CyP	N	no number	Q8I6S4

a) Enzyme was inactive with Suc-AAPF-pNA, the value was obtained by using Suc-ALPF-pNA. Residues R55, F60, Q63, A101, Q111, and F113 were conserved throughout all sequences

Table 4. Relative activities of recombinantly expressed variants of human Cyp18

Variant	% rel. activity
WT	100
H54Q	15.0
R55A	0.1
F60A	0.32
Q111A	15.0
F113A	3.0
W121A	8.7
H126Q	0.53

human Cyp18 by sulfhydryl group modifying agents, an involvement of an active site thiol was proposed. The suggested mechanism assumed a nucleophilic attack of an activated sulfhydryl group to the prolyl bond carbonyl carbon resulting in hemithioorthoamides as a covalently bound tetrahedral intermediate (Figure 1, path C) (46). For this intermediate the resonance stabilization of the former peptide bond is destroyed, resulting in a greatly reduced rotational barrier about the C-N bond. This mechanism was supported by the observation that the kinetic secondary deuterium isotope effect of the cis to trans isomerization of Suc-AG(d_2)PF-pNA changed from k_H/k_D >1 to 0.91 ± 0.01 when cyclophilin was added to the reaction (47). A number of experiments disagree with this isotope effect derived mechanism. Site directed mutagenesis showed that all four cysteine residues (C52, C62, C115, and C161) of human Cyp18 are dispensable for activity (48). In accordance with these results is the observation that no cysteine is in the proximity of the substrate, the closest cysteine (C115) is, with 8 Å, too far away to build the proposed hemithioorthoamide intermediate (38).

Harrison and Stein suggested a mechanism which they called "catalysis by distortion", where the enzyme induces strain or distortion in the substrate by utilizing the binding energy provided by the enzyme/substrate complex formation (Figure 1, path A) (18, 19). Cyclophilin was proposed to tightly bind a transition state in which the prolyl bond is characterized by a partially rotated C-N imide bond. In agreement with this mechanism is the observed small enthalpy of activation (Δ H* =4.3 kcal/mol) and the relatively large negative entropy of activation (Δ S* = -47 eu). It should be pointed out that the Eyring plots used to calculate the activation parameters were nonlinear and that two interchangeable enzyme forms were proposed to account for it (49). This mechanism was also supported by a KSIE on k_{cat}/K_M and k_{cat} close to unity for the cyclophilin catalyzed isomerization of Suc-AAPF-pNA (19, 50) and a normal temperature independent secondary deuterium isotope effect of 1.12 ± 0.02 using Suc-AG(d_2)PF-pNA as substrate. The authors suggested that the transfer of the substrate into the hydrophobic active site results in a reduced hyperconjugation of the β-CH electrons generating the normal β-deuterium isotope effect

They also attribute a part of the catalytic power to the reduced stabilization of the polar resonance structure of the prolyl bond in the apolar active site. Structural analyses of human Cyp18 substrate complexes did not provide further proof for this theory. Neither in crystal structures nor in solution do short peptides such as Ala-Pro, Ac-AAPA-amc or Suc-AAPF-pNA adopt a twisted conformation (36, 37, 39, 40, 51). Further investigation using dynamic NMR spectroscopic techniques revealed that human Cyp18 binds to both ground state conformers with similar affinities (52). Studies using modified bicyclic lactam inhibitors and phosphinic Ala-Pro surrogates, which were thought to

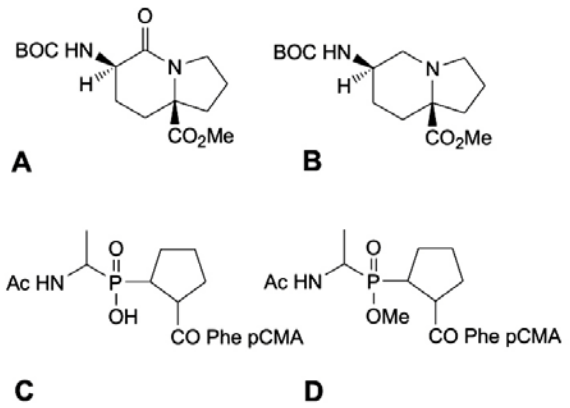

Figure 6. Compounds used as surrogates of the prolyl bond in their ground state (A) or a twisted transition state (B, C and D).

resemble the above mentioned twisted transition state, failed to display significant binding affinity towards the active site of human Cyp18 (Figure 6) (53, 54). Peptides harboring a thioxo prolyl bond or peptides with a reversed chirality at the Cα carbon of peptides in P1 and P1´ position (nomenclature according to Schechter and Berger (55)) can not activate the catalytic pathway of human Cyp18 (26, 56). This suggests that the pathway from ground state to transition state is sterically and electronically demanding, thereby ruling out the above mentioned enzyme mechanisms like ground state distortion and desolvation by binding to a hydrophobic cavity.

New grounds for speculation were given by a water molecule observed in a human Cyp18/Ala-Pro complex. This water molecule is hydrogen bonded to the Q63 active site residue of human Cyp18. Since the water is placed in the right orientation to stabilize a twisted transition state via a hydrogen bond to the carbonyl oxygen of the prolyl bond, the authors concluded that the human Cyp18 catalysis is "solvent-assisted" (Figure 1, path B). The necessary energy to distort the peptide bond should arise from thermodynamic fluctuations (39). Later it was found that the Ala-Pro dipeptide is not a substrate but rather a competitive inhibitor of human Cyp18 (37). The authors discussed that the additional charge of the free proline carboxyl group disturbs the active site of human Cyp18. It forces the guanidinium group of R55 away from the proline nitrogen and into an additional hydrogen bond with the unprotected C-terminus of the peptide. It could therefore be concluded that an additional amino acid residue C-terminally located to the proline might be a prerequisite for catalysis. The authors also suggested that the guanidinium group of R55 might be the hydrogen donor proposed earlier by Kofron et al., which lowers the rotational barrier of the prolyl bond by forming a hydrogen bond to the proline imide nitrogen (Figure 1, path D) (50). Consistent with this mechanism is the greatly reduced activity caused by the R55A mutation and the finding that intramolecular general acid catalysis by NH groups will lead to an increased isomerization rate of prolyl bonds (30, 44). The distance between the guanidinium NH2 group and the nitrogen of the prolyl bond ranges from 3.3 to 3.8 Å depending on the structure used and is therefore too far away to form a hydrogen bond. A shortening of this distance while the prolyl bond proceeds from ground to transition state was suggested (36, 41) and confirmed by a computational investigation (57, 58). Further support for this mechanism was provided by the observation that the R44 side chain of dihydrofolate reductase intramolecularly catalyzes the rate limiting step of folding by accelerating the *cis* to *trans* isomerization of the Q65-P66 peptide bond (Figure 7) (27). The spatial arrangement of the R44 guanidinium group and the imide nitrogen resembles the situation found in cyclophilin/substrate complexes. On the other hand, the observation that the peptide benzyl-Phe-Ala-Pro, which has the same free carboxy terminus as the dipeptide, acts as a substrate for human Cyp18 as shown by NMR spectroscopic line shape experiments casts doubt on this mechanism (59). Human Cyp18 activity (k_{cat}/K_M) has been shown to be pH dependent; a pKa value of 5.9 was calculated (26). The enzyme is almost two orders of magnitude more active at neutral or basic conditions than at pH 4.5. Since the observed decrease of activity originates mainly from a lowered k_{cat} value it is feasible that ionizable active site side chains are involved in catalysis. The guanidinium group of R55 is expected to be protonated within the pH range investigated thereby making this residue unlikely to be the source of the pH dependency.

All experiments mentioned so far utilized short peptides to gain information on the catalytic mechanism, whereas cyclophilins will act on proteins in biological systems. As it is obvious from the contradicting enzyme mechanisms derived from crystal structures harboring dipeptides compared to structures in complex with oligopeptides, it is necessary to ask whether peptides utilize the complete enzymatic machinery of cyclophilins. A new tool to help answer this question was provided by the interesting finding that human Cyp18 is incorporated into the human immunodeficiency type 1 virus (HIV) by interacting with the HIV Gag protein (60). Several structures showed that human Cyp18 binds specifically to a G89-P90 motive, which is situated within a solvent exposed loop of the capsid protein (35, 41, 61). The G89-P90 bond in this complex was shown to be in the *trans* conformation, unlike all other human Cyp18 peptide substrate complexes, where the prolyl bond was found exclusively in the *cis* conformation. An exchange of G89 to alanine resulted in a reduced binding affinity (62). The binding affinity depended not only on the amino acid moiety preceding the proline residue (P1 position) but also on the chain length of the substrate used, suggesting an extended substrate binding site of cyclophilin (63). NMR exchange spectroscopy experiments of the native N-terminal domain of the capsid protein in the presence of catalytic amounts of human Cyp18 showed clearly that the isomerization of the G89-P90 bond is catalyzed by human Cyp18 in the native state of the capsid protein (64). Structures of variants of the HIV capsid protein co-crystallized with human Cyp18 resulted in complexes in which both *cis* and *trans* conformations of the X-P90 bond occurred in crystallographically equivalent positions (41). The authors proposed a mechanism where the transition

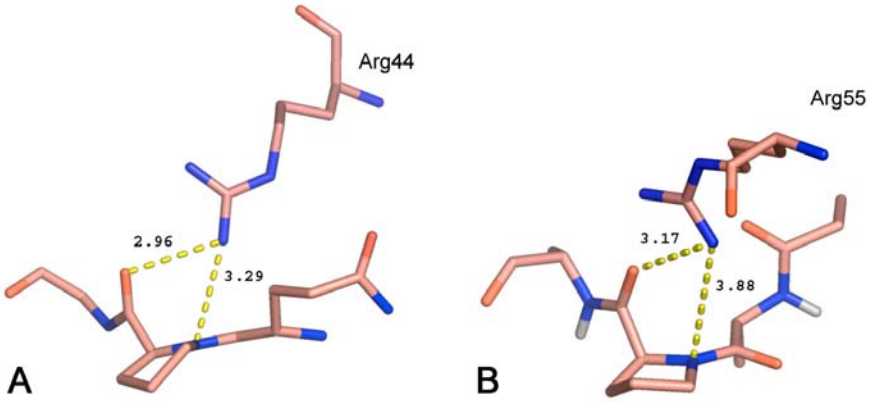

Figure 7. Distances from the NH_2 nitrogen of arginine to proline in DHFR and human Cyp18/substrate complexes. Panel A depicts the interactions in DHFR where R44 is involved in intramolecular catalysis of the Gln65-Pro66 bond cis/trans isomerization. Panel B shows the active site R55 residue of human Cyp18 which interacts with a ground state bound peptide substrate.

state deviates only minimally from the *cis* and *trans* ground states. During reaction progress the C-terminal part of the substrates including the proline remains fixed relative to the enzyme, while the carbonyl oxygen of the amino acid preceding proline performs a flip-over. This model is consistent with a computational analysis (57). However, it is inconsistent with NMR relaxation rate changes and an NMR line shape analysis of human Cyp18 main chain amides during catalysis, where a substantial movement of substrate and enzyme residues, especially in the C-terminal region of the tetrapeptide-4-nitroanilide substrate, was observed (52, 65).

3.3.2. FKBP

Even though FKBP are a conserved family of PPIases, their PPIase domains show a much higher degree of sequence variability than the cyclophilins. Some FKBP, like the *E.coli* trigger factor and *E.coli* SlyD can not be inhibited by FK506 or rapamycin. As described for higher molecular mass cyclophilins, the catalytic domain of FKBP was found to be complemented with other domains in several cases. That these additional domains can influence the PPIase activity by binding to their designated interacting proteins has been shown for human FKBP38 (Edlich F *et.al*, submitted). Some of the multidomain FKBP harbor up to four nonidentical FKBP PPIase domains within the same polypeptide chain. This domain copying is a feature not found within the cyclophilin family of PPIases. The functional significance of these multiple FKBP PPIase domains is still unclear, especially since it has been reported that like in the case of human FKBP51, human FKBP52, and rabbit FKBP59 the PPIase activity is mainly due to one single N-terminally located FKBP PPIase domain when tested with oligopeptide substrates (66-68). It remains to be discussed whether the inactivity of the other FKBP PPIase domains is due to the experimental conditions used, due to autoinhibitory segments within these proteins or if the inactivity is indeed an intrinsic property of these domains.

The majority of FKBP accelerate the *cis* to *trans* isomerization of the prolyl bonds with a second order rate constant between 10^4 and 10^7 M^{-1} s^{-1} (Table 5). Several FKBP exhibit k_{cat}/K_m values in the range of enzyme variants point mutated in the active site of human FKBP12 (Table 6). A few exceptions are found where only little or no activity could be detected (69-72). FKBP achieve the observed rate enhancement by lowering the rotational barrier by app. 6.6 kcal/mol (50, 73). In contrast to the uncatalyzed reaction, where the Eyring activation parameter is characterized by a large enthalpic contribution of $\Delta H^* = 18.9$ kcal/mol and a small entropic part of $\Delta S^* = -1.16$ eu , the FKBP catalyzed reaction is characterized by a large entropic ($\Delta S^* = -43.95$ eu) and small enthalpic ($\Delta H^* = 5.85$ kcal/mol) contribution to ΔG^* (74). The marked entropic contribution to the free energy of activation led the authors to the conclusion that the rate limiting step is a physical rather than a chemical one. They proposed that the enzyme selectively stabilizes a twisted amide by using the binding energy to compensate for the loss of amide resonance. In contrast to cyclophilins, the catalytic efficiency of FKBP depends strongly on the side chain in position P1 of the substrate. Peptides containing large hydrophobic residues like leucine or phenylalanine in this position show an up to 100- to 1000-fold increased second order rate constant compared to substrates with charged or small side chains in this position (73, 74). The difference is not due to an impaired substrate binding since the K_M value is not affected within the experimental error. It is rather an effect on k_{cat} which decreases more than 1000-fold when the leucine residue in the P1 position of Suc-ALPF-pNA is changed to glycine (73). These data in combination with the observed pH independence of the FKBP from pH 5 to 10, the solvent isotope effect close to unity and the reported, mainly entropic contribution to the activation parameter led to an enzyme mechanism called “catalysis by distortion” which had also been proposed for the cyclophilin catalyzed reaction. The energy necessary to destabilize the substrate was thought to arise from the binding energy. Within the active site the substrate is then distorted by electrostatic and/or geometric means towards the transition state. In addition to the induced strain, the transition state is further stabilized by the hydrophobic

Table 5. FKBP examined for their *in vitro* PPIase activity

Organism	Name as in publication/generic name	Swissprot	(k_{cat}/K_M) $M^{-1}s^{-1}$	Active site residues as found in a pair wise alignment with human FKBP12, +) identical residue, -) residue not present													X[a]	FK506 binding	Ref.
				Y 26	**F 36**	**D 37**	**R 42**	**F 46**	**F 48**	**Q 53**	**E 54**	**I 56**	**W 59**	**Y 82**	**H 87**	**F 99**			
H. sapiens	FKBP12 [b]	P20071	$1.6\ 10^6$														L	K_i=0.5 nM	146
H. sapiens	FKBP12.6	Q16645	$6.2\ 10^5$	+	+	+	+	+	+	+	+	+	F	+	+	+	L	K_d=0.55 nM	147 148
H. sapiens	FKBP13	P26885	$3.1\ 10^6$	+	+	+	Q	+	+	G	Q	+	+	+	A	+	L	K_i=74 nM	149
H. sapiens	FKBP25	Q00688	active	+	+	+	K	L	+	G	K	+	+	+	Q	+	A	IC_{50}=400 nM	150
A. thaliana	AtFKBP42	Q9LDC0	inactive	+	+	E	E	I	L	E	K	L	L	+	N	Y	L	no binding	69
B. malayi	FKBP13	O96335	$6.3\ 10^4$	+	+	+	+	+	+	G	Q	+	+	+	S	+	L	IC_{50} = 1.7 μM	151
B. taurus	FKBP12	P18203	$6.6\ 10^5$	+	+	+	+	+	+	+	+	+	+	+	+	+	L	K_i = 0.25 nM	50
B. taurus	FKBP25	P26884	$8\ 10^5$	+	+	+	K	L	+	G	K	+	+	+	Q	+	A	K_i = 160 nM	152
C. burnetii	CbMip / FKBP26	P51752	active	+	+	+	+	A	+	K	S	+	+	+	A	+	F	no number	153
C. tracho-matis	chl-mip / FKBP27	P26623	active	+	+	+	N	I	L	-	-	+	F	+	Q	+	F	no number	154
E. coli	trigger factor/FKBP48	P22257	$6.5\ 10^5$	V	+	T	K	+	L	G	R	+	F	F	G	S	L	no inhibition	155
E. coli	FKBP22	P39311	$1.3\ 10^6$	+	+	+	+	A	+	-	-	+	+	+	A	+	L	K_i = 25 nM	156
E. coli	SlyD/FKBP21	P30856	$1.4\ 10^4$	G	+	+	A	Y	Q	+	R	P	V	A	+	+	L	no inhibition	157
H. cutiru-brum	HcFKBP33	Q9P9H4	$9.7\ 10^5$	+	L	+	E	R	I	E	H	F	V	+	+	+	A	no inhibition	158
M. genita-lium	trigger factor / FKBP51	P47480	$7.2\ 10^3$	F	L	A	Q	Y	L	N	S	+	F	+	L	+	A	no inhibition	159
M. janna-schii	MjFKBP18	Q57726	$9.2\ 10^5$	I	Y	Y	Y	I	+	G	+	+	F	+	I	+	L	IC_{50} = 170 nM	71
M. janna-schii	MjFKBP26	Q58235	$6.4\ 10^2$	L	I	N	F	V	+	E	+	V	I	T	L	I	L	70% at 20 μM	71
M. thermo-autotrophicum	FKBP28.3	O27197	$3.6\ 10^2$	E	E	E	A	I	V	G	H	+	L	F	+	M	L	IC_{50} >20 μM	72
M. thermo-litho-trophicus	MtFK / FKBP17	O52980	$3.5\ 10^5$	D	A	G	+	L	+	G	Q	+	F	+	I	+	L	IC_{50}= 250 nM	160
N. crassa	NcFKBP22	O60046	$6.9\ 10^5$	+	+	+	+	+	+	G	Q	+	+	+	-	+	F	K_i = 4.5 nM	161
N. crassa	NcFKBP / FKBP13	P20080	active	+	+	+	+	L	+	G	Q	+	+	+	V	+	A	no numbers	162
N. menin-gitidis	NmFKBP / FKBP12	P25138	active	+	+	+	+	L	I	G	Q	+	+	+	A	+	A	no numbers	163
P. hori-koshii	PhFKBP29	no entry	$1.6\ 10^3$	D	I	Y	I	V	I	G	H	+	L	E	Q	Y	L	75% at 20 μM	164
S. cere-visiae	yFKBP12	P20081	$8.2\ 10^7$	+	+	+	+	+	C	G	Q	+	+	+	F	+	L	K_d = 0.9 nM	165
S. cere-visiae	yFKBP13	P32472	$5.4\ 10^7$	+	+	+	+	I	+	G	R	+	+	+	V	+	L	K_d = 18 nM	165
S. cere-visiae	yFKBP70	P38911	active	+	+	+	-	+	+	G	+	+	+	+	L	+	L	no numbers	166
S. frugi-perda	FKBP46 /	Q26486	$7.8\ 10^6$	+	M	F	K	+	+	K	+	+	+	+	S	+	L	IC_{50} = 5 μM	167
Thermo-coccus sp. KS-1	TcFK / FKBP18	O93778	$3.5\ 10^2$	+	L	V	Y	M	V	G	+	+	L	+	K	+	L	IC_{50} = 7 μM	70
V. faba	FKBP15	Q41649	active	+	+	+	+	I	+	G	Q	+	+	+	S	+	L	K_i = 30 nM	168
H. sapiens	hFKBP51 domain I	Q13451	$1.24\ 10^6$	+	+	+	+	+	+	G	Q	+	+	+	S	+	L	no numbers	169
H. sapiens	FKBP51 domain II	Q13451		L	+	+	+	+	V	H	D	P	I	F	K	Y			
H. sapiens	FKBP52 domain I	Q02790	$3.8\ 10^5$	+	+	+	+	+	+	G	+	+	+	+	S	+	L	K_i = 10 nM	170
H. sapiens	FKBP52 domain II	Q02790	inactive	L	+	+	+	+	I	N	D	P	L	F	K	Y		no numbers	171
M. musculus	FKBP51 domain I	Q64378	$4.8\ 10^5$	+	+	+	+	+	+	G	Q	+	+	+	+	+	L	K_i= 10-15 nM	148
M. musculus	FKBP51 domain II	Q64378		L	+	+	+	+	V	H	D	P	I	F	K	Y			
O. cuni-culus	rFKBP59 domain I	P27124	$1.2\ 10^6$	+	+	+	+	+	+	G	+	+	+	+	S	+	L	no numbers	66
O. cuni-culus	rFKBP59 domain II	P27124	$0.02\ 10^6$	L	+	+	+	+	V	L	D	P	L	F	K	Y	L	no numbers	66
A. thaliana	FKBP72 domain I	Q9M326	$1.5\ 10^4$	C	V	E	+	I	D	S	K	+	L	+	A	+		no number	172
A. thaliana	FKBP72 domain II	Q9M326		I	I	F	-	Y	+	S	+	P	L	L	L	+			
A. thaliana	FKBP72 domain III	Q9M326		+	Y	+	N	L	+	G	L	P	F	+	R	W			
M. musculus	FKBP60 domain I	Q9Z247	active	+	+	+	+	+	V	G	Q	+	M	+	V	+	L	no numbers	173
M. musculus	FKBP60 domain II	Q9Z247		+	+	+	+	Y	T	G	W	+	M	+	D	+			
M. musculus	FKBP60 domain III	Q9Z247		+	+	+	+	+	T	G	Y	+	M	+	R	+			
M. musculus	FKBP60 domain IV	Q9Z247		+	L	+	L	Y	I	G	Q	V	M	+	V	+			
M. musculus	FKBP65 domain I	Q61576	$6.5\ 10^5$	+	+	+	+	V	I	G	R	+	M	+	V	+	A	IC_{50} = 45 nM	174
M. musculus	FKBP65 domain II	Q61576		+	+	+	+	Y	T	G	W	+	M	+	Y	+			
M. musculus	FKBP65 domain III	Q61576		+	+	+	+	Y	T	G	Y	+	M	+	T	+			
M. musculus	FKBP65 domain IV	Q61576		+	L	F	Y	Q	I	N	K	+	L	H	A	+			

Activity was measured with the substrate Suc-AXPF-pNA. The reported enzymatic constants were obtained as described in the respective publication. All FKBP domains of multi domain FKBP have been aligned individually; the activity shown was determined for the full length protein, if not stated otherwise. The alignment of MjFKBP18, MjFKBP26 and FKBP 28.3 showed only very weak homologies, the given result should therefore be interpreted with caution. The statement "active" means that in the publication PPIase activity assays were performed but no (k_{cat}/K_M) values were provided. The generic name consists of the abbreviation of the respective PPIase family followed by the rounded molecular weight of the full length protein, a) X Stands for the amino acid proceeding proline in the used substrate (Suc-AXPF-pNA), b) active site titration for this protein has been performed (175).

Table 6. Catalytic activity of recombinantly expressed human FKBP12 active site variants determined using Suc-ALPF-pNA as substrate

Variant	k_{cat}/K_M $M^{-1}s^{-1}$	K_M	k_{cat}	References
Wt	$1.2\ 10^{6}$ b)	$5\ 10^{-4}$ M b)	600 s^{-1} b)	176
	$3.5\ 10^{6}$ a)			176
	$1.2 \pm 0.4\ 10^{6}$ c)			155
	$2.2 \pm 0.2\ 10^{6}$			177
	$3.6\ 10^{6}$ c	$0.9\text{-}1.3\ 10^{-3}$ M c)	1000-1300 s^{-1} c)	79
Y26F	$\sim 1\ 10^{5}$ a)			176
F36L	$\sim 4\ 10^{6}$ a)			176
D37V	$3\ 10^{5}$ a)			176
D37L	$6.6 \pm 0.4\ 10^{4}$ c)			155
R42A	$1.1 \pm 0.2\ 10^{6}$			177
F46L	$\sim 5\ 10^{6}$ a)			176
F48L	$\sim 0.9\ 10^{6}$ a)			176
Q53A	$1.8 \pm 0.4\ 10^{6}$			177
W59A	$\sim 1\ 10^{5}$ a)			176
Y82L	$3.6\ 10^{5}$ c)	$0.7\text{-}1.0\ 10^{-3}$ M c)	20-24 s^{-1} c)	79
Y82F	$2.6\ 10^{5}$ b)	$2.9\ 10^{-4}$ M b)	75 s^{-1} b)	176
Y26F/Y82F	$6.4\ 10^{4}$ b)	$7.5\ 10^{-4}$ M b)	48 s^{-1} b)	176
H87A	$1.9 \pm 0.2\ 10^{6}$			177
F99Y	$6.4\ 10^{4}$ c)			155

a) numbers estimated from diagram, assay performed at 15 °C, b) assay performed at 5 °C, c) assay performed at 10 °C

Table 7. FKBP structures available in the RCSB Data Bank

Organism	Name as used in publication	Number of structures	PDB identifier
B. taurus	FKBP12	2	1fkk, 1fkl
C. elegans	FKB-6	1	1r9h
E. coli	FKPA	3	1q6h, 1q6i, 1q6u
H. sapiens	FKBP12	33	1a7x, 1aui, 1b6c, 1bkf, 1bl4, 1d6o, 1d7h, 1d7i, 1d7j, 1eym, 1f40, 1fab, 1fkb, 1fkd, 1fkf, 1fkg, 1fkh, 1fki, 1fkj, 1fkr, 1fks, 1fkt, 1j4h, 1j4i, 1j4r, 1nsg, 1qpf, 1qpl, 1tco, 1fap, 2fke, 3fap, 4fap
H. sapiens	FKBP12.6	1	1c9h
H. sapiens	FKBP25	1	1pbk
H. sapiens	FKBP52	1	1n1a
H. sapiens	FKBP51	1	1kto
L. pneumophila	LpMip	1	1fd9
M. genitalium	Trigger factor	1	1hxv
M. thermolithotrophicus	MtFK	1	1ix5
O. cuniculus	FKBP59	2	1rot, 1rou
S. boliviensis	FKBP51	1	1kt1
S. cerevisiae	FKBP12	1	1yat
T. cruzi	TcMip	1	1jvw

environment of the active site (18). The normal secondary kinetic deuterium isotope effect observed for the specificity constant of the substrate Suc-AG(d_2)PF-pNA with FKBP12 has been used to argue in favor of a transition state characterized by a partial rotation about the C-N bond. However, since this -G-P- substrate might not be able to activate the complete machinery of the biocatalytic pathway, the mechanistic significance of the isotope effect remains questionable (73). A so called "unassisted conformational twist mechanism" was proposed on the basis of this isotope effect in combination with a slight inverse solvent isotope effect ($(k_{cat}/K_{M(H2O)})/(k_{cat}/K_{M(D2O)}) = 0.92$) (73). This mechanism is a modified version of the "assisted conformational twist mechanism" also suggested for cyclophilins, but it assumes that catalysis by FKBP does not require favorable hydrogen bonding to the lone electron pair of nitrogen from an active site residue.

The central question that needed to be addressed was what kinds of enzyme/substrate interactions were used by FKBP to lower the rotational barrier in a FKBP-like manner. Some answers could be found by investigating the FKBP structures reported. As many as 51 structures of FKBP from 12 different organisms have been determined (Table 7), none of them with a standard peptide substrate bound. They all show a very similar general fold which consist of a four to six stranded β-sheet wrapped around a short α-helix (Figure 8A). Inhibitor/enzyme complexes have been used to localize the active site of FKBP. The high resolution structure of the FK506/human FKBP12

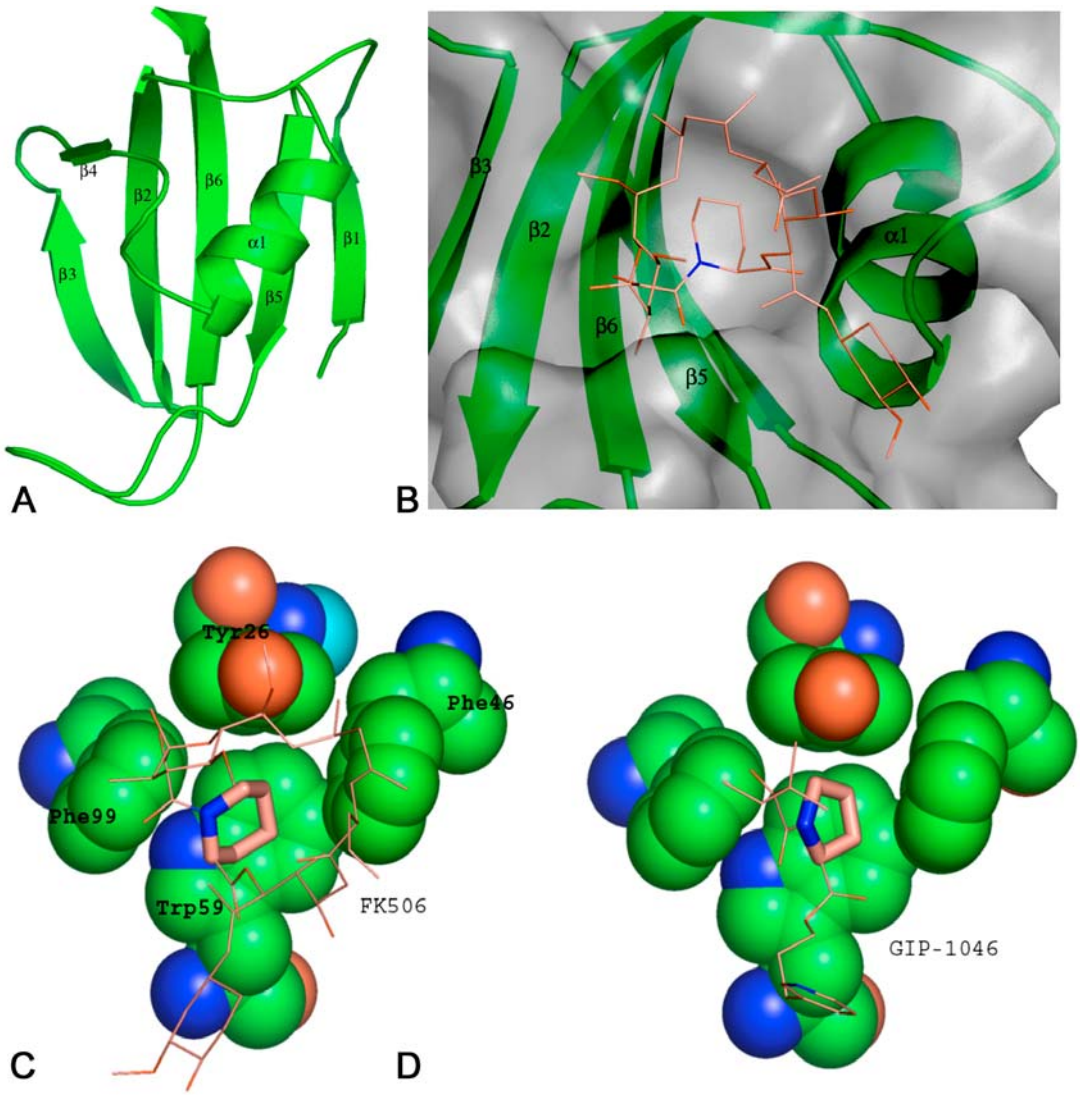

Figure 8. General fold of a FKBP domain, which is exemplarily shown for human FKBP12 (A). The six antiparallel β-sheets exhibit +3, +1, -3, +1 side chain topology; the β-sheets are capped with an amphiphilic α-helix. The pipecolinyl ring protrudes into the drug binding site of human FKBP12 (B). The four hydrophobic residues F99, Y26, F46 and W59 are located in β2, β4, β6 strands and the α1 helix respectively (C). The shape of the cavity can accommodate five- and six-membered rings as found in the structures of FK506 (C) and GPI-1046 (D). The four residues are shown in spacefilling mode and labeled; the inhibitors are depicted as stick models.

complex revealed that the pipecolinyl moiety of FK506 (Figure 5B) is deeply buried in a hydrophobic cleft of human FKBP12 (Figure 8B). It was concluded that the pipecolinyl moiety might substitute for prolyl moieties of putative peptide or protein substrates (75). In accordance with this proposal is the finding that in the crystal structure of the GPI-1046/human FKBP12 complex, the five-membered pyrrolidyl moiety of GPI-1046 is located similar to the pipecolinyl function of FK506 (76) (Figure 8C & D). One of the most striking properties of the proposed active site of FKBP is its hydrophobic character. In case of human FKBP12 thirteen residues (Y26, F36, D37, R42, F46, F48, Q53, E54, I56, W59, Y82, H87, and F99) make direct contacts with FK506 (74, 75, 77). Four residues (E54, I56, Y82 and D37) stabilize the drug/enzyme interaction via hydrogen bonds. The residues in direct contact with the pipecolinyl ring have an exclusively aromatic character (Y26, F46, W59, and F99). In contrast to earlier proposals (78) the pipecolinyl-C8 carbonyl bond exhibits a *trans* conformation in its enzyme bound state and does not mimic a twisted amide bond (77). Later it was proposed that the orthogonal conformation of the α-dicarbonyl moiety of FK506 might act as a twisted amide surrogate (74), but the very low activity of catalytic antibodies derived from α-dicarbonyl containing haptens could not provide further evidence (31, 32). To determine how conserved the thirteen drug binding residues are among the FKBP tested for PPIase activity, a pairwise sequence alignment of these enzymes with human FKBP12 was performed and analyzed (Table 5). The degree of conservation is quite low for all

positions. It appears that most of the side chains within the active site are dispensable for FKBP activity. Even *P.horikoshii* PhFKBP29, which has only one active site residue in common with human FKBP12, was reported to be enzymatically active. For some FKBP the low PPIase activity coincides with a low sequence identity in comparison to human FKBP12, namely for *M.jannaschii* MjFKBP26, *M.thermoautotrophicum* FKBP28.3, *M.genitalium* trigger factor, *P.horikoshii* PhFKBP29, *A.thaliana* AtFKBP42, and *Thermococcus sp.* TcFK. On the other hand - the *E.coli* trigger factor - that has only very few active site residues in common with human FKBP12 posses a high catalytic activity. It appears as though the enzymatic activity does not correlate directly with sequence conservation. In contrast, FK506 binding constants provide a better measure of sequence similarity. This might indicate that the residues involved in FK506 binding are not necessarily part of the active site. The active site residues conserved best among 51 FKBP domains aligned with human FKBP12 are Y26, F36, D37, I56, Y82, and F99 (Table 5). Two of these (Y26 and F99) form the hydrophobic cavity thought to accommodate the substrate proline residue. The importance of D37 for the enzymatic activity will be discussed later. From the 15 mutants of human FKBP12 depicted in Table 6 only the D37L, the W59A, the F99Y and the Y26F/Y82F double variant posses less than 10% catalytic activity as compared to the wild type enzyme. The tyrosine mutations do not influence the formation of the Michaelis-Menten complex since the K_M value remained unchanged while k_{cat} is greatly reduced (73). Since a single point mutation Y82L diminished the slight pH dependence observed between pH 8 and 10.5 of human FKBP12, it was concluded that the tyrosine hydroxyl group of Y82 might be acting as a hydrogen bond donor to the prolyl nitrogen and thereby assist the prolyl *cis/trans* isomerization (18, 79). One has to bear in mind that the single Y82L mutation of human FKBP12 still leaves a residual activity of app. 10%, indicating that human FKBP12 stabilizes the transition state by hydrogen bonding only to a limited amount. Further mutations of potential nucleophiles and hydrogen bond donors (S8A, S38A, S67A, S77A, C22A, T75A and T96A) led only to insignificant reduction of the catalytic function (73). Quantitative modification of the cysteine thiols did not impair the catalytic activity (80). Since all nucleophilic residues close to the active site of human FKBP12 are dispensable for activity a side chain assisted covalent mechanism can be been ruled out (73).

Similar to FKBP, a preference for large hydrophobic side chains in the P1 position of oligopeptides was found for the prolyl bond isomerization catalyzed by micelles. Various surfactants have been shown to lower the free activation energy of isomerization by $\Delta\Delta G^* = 1.8$ kcal/mol. In contrast to PPIases, micelles also accelerate the isomerization rate of thioxoproline containing peptides (33). These results proved that only a small part of the catalytic power of FKBP arises from the desolvation of the peptide bond and that the rate enhancement of FKBP depends on a finely tuned spatial and electronic arrangement within the active site. The same conclusions were drawn from the observation that human FKBP12 cannot catalyze the *cis/trans* isomerization of oligopeptides which harbor D-amino acids in position P1, P1´ or P2´ while ground state binding was found to occur (56).

Molecular dynamics and free energy perturbation calculations where the peptides Ac-AAPF-Ame and Ac-LPF-Ame were fitted into the active site of human FKBP12 provided evidence that these peptides adopt a VIa β-turn type conformation. This allows intrachain hydrogen bonding between the backbone nitrogen proton of the phenylalanine residue and the proline imide nitrogen. This interaction is believed to lower the rotational barrier via an "enzyme-induced autocatalysis" mechanism (81, 82). Against such a mechanism stands the observation that an acidification of the respective NH proton in thioxopeptide derivatives does not lead to a substantial lowering of the rotational barrier neither for the free thioxopeptide nor for the FKBP12/thioxopeptide complex. For example for the human FKBP12 catalyzed *cis* to *trans* isomerization of AGP-psi[CS-N]-F-pNA a k_{cat}/K_M value of 67 M^{-1} s^{-1} was estimated which compares to the value of 920 M^{-1} s^{-1} of the oxo congener. In addition to the above mentioned transition state stabilization, examples for ground state destabilization were calculated as well. In agreement with the observed reduced activity of the D37L variant an unfavorable ground state interaction between the carbonyl moiety of the prolyl bond and the D37 side chain was calculated. This interaction changes to a more favorable charge-dipole interaction when the substrate proceeds from the planar ground state to the twisted transition state. The simulation also predicted a catalytically relevant water molecule, which is bound between the carbonyl oxygen of the prolyl bond and the hydroxyl oxygen of Y82 during transition state formation. The computational analysis could not pinpoint the catalytic efficiency to certain side chains or favor one of the proposed enzyme mechanisms (73). It was concluded that a combination of factors like desolvation of the imide bond by the hydrophobic active site, ground state destabilization, preferential transition state binding and enzyme induced autocatalysis contribute to the enzyme activity. The theoretical methods mentioned here all suffer from the same limitations, in particular the lack of FKBP/substrate complex structures to provide valid starting points for the calculations. The methods used rely on the assumption that the conformation of complexed FK506 or rapamycin resembles the substrate conformation in a FKBP bound state. Some doubt is cast on the validity of this assumption by the observation that proline containing peptides quench the fluorescence of W59 located at the bottom of the drug binding site of human FKBP12 differently from FK506 (73).

3.3.3. Parvulins

The first member of the parvulin family of PPIases was identified in *E.coli* in 1994 (5). The substrate specificity of the 92 amino acid long *E.coli* Par10 is similar to the FKBP family of PPIases but the second order rate constant is at least one order of magnitude higher and the activity could not be inhibited by either FK506 or cyclosporin. The differences in the substrate specificity for human Pin1 and *E.coli* Par10 point out that within the parvulin family two subfamilies exist. Whereas the enzymatic activity of all phosphate specific parvulins

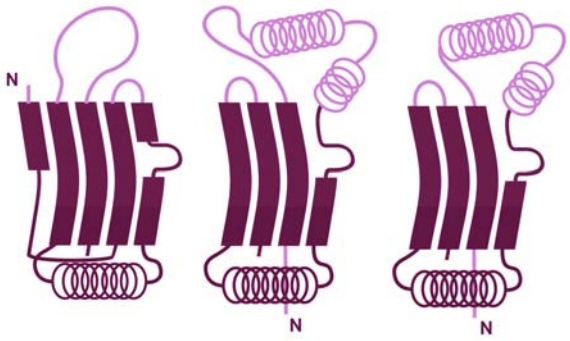

Figure 9. Arrangement of secondary structure elements of human FKBP12 (left), human Pin1 (middle) and human Par14 (right). Conserved secondary structures are in black; grey structures are found to be variable within the individual structures. Bars represent β-strands; wires correspond to α-helices and lines to loops and turns.

reported so far is exceptionally high, the observed k_{cat}/K_M values among the parvulins that show no such preference varies within a range from 10^3 to 10^7 M^{-1} s^{-1} (Table 8). The sequence alignment shows that all phosphate specific parvulins have an identical set of active site residues. On the other hand, *E.coli* Par10 type parvulins show greater sequence variability and both residues which have been proposed to attack the prolyl carbon (C113 and S154 in human Pin1) are not conserved, questioning the described enzymatic mechanism.

A first glimpse of the catalytic mechanism of parvulins was provided by the crystal structure of the human parvulin-like human Pin1 in complex with an Ala-Pro dipeptide (83). Since then, 10 structures of parvulins from human, *A.thaliana* and *E.coli* have been solved (Table 9). The human Pin1 consists of a C-terminally located PPIase domain and an N-terminally located WW-domain, which was found to interact with phosphoserine (pS) or phosphothreonine (pT) containing peptides and proteins. The presence of the WW-domain has no influence on the PPIase activity of the whole enzyme when the standard *in vitro* assay is applied (84). Whereas no sequence homology between FKBP and parvulin exists, a similar general fold can be observed (Figure 9). The PPIase domain of human Pin1 consists of a four stranded anti-parallel β-sheet surrounded by four α-helices (Figure 10A). The active site of human Pin1 is formed by 10 residues (H59, K63, R68, R69, C113, L122, M130, F134, S154 and H157). A basic cluster of side chains (K63, R68 and R69) in the α1/β1 loop region binds a sulfate ion in the crystal structure. The sequestered sulfate is in close proximity to the bound dipeptide and has led to the assumption that human Pin1 possesses a strong preference for negatively charged side chains in position P1 of proline containing substrates. Accordingly it was found that the second order rate constant of Suc-AEPF-pNA is at least two orders of magnitude higher than that of an uncharged peptide like Suc-AQPF-pNA. Later it was observed that substrates with phosphorylated serine or threonine residues in this position were catalyzed most effectively and that the glutamate moiety preceding proline can not activate the full catalytic power of Pin1. Especially the remarkable pH dependence of Pin1 catalyzing the *cis* to *trans* isomerization of Ac-AAS(PO_3H_2)PR-pNA was not found to the same extend using the substrate Suc-AEPF-pNA, pointing out that glutamate can not mimic the phosphorylated serine residue completely (25, 85). The rate enhancement for substrates with phosphorylated side chains vs. their unphosphorylated counterparts is about 1300-fold. Most effective catalysis was observed at pH values where the phosphorylated side chain is in its dianionic form (25). Simultaneous substitution of R68 and R69 with alanine reduced the catalytic efficiency to the level observed with unphosphorylated peptides. The main contribution arises from the positive charge of the R69 side chain, since the R69L variant lowers the enzymatic activity about 50-fold more efficiently than the R68L variant (84). The finding that the α1/β1 sulfate/phosphate binding loop is extended into the solvent when no ligand is bound to the PPIase domain led to the hypothesis that this loop might act as a lid which opens and closes during the catalytic cycle (86). Structural analysis of human Pin1 in solution could not strengthen this induced fit mechanism and the authors concluded that the observed open conformation might be due to the crystallization conditions or crystal contacts (87).

The prolyl moiety of the dipeptide in the crystal structure of human Pin1 is located within a hydrophobic pocket similar to that which is occupied by the pipecolinyl moiety of FK506 in FKBP12/FK506 complexes (Figure 10A). It is formed by residues L122, M130 and F134 (Figure 10C). A minimum of three peptide bonds is essential to enable activation of the catalytic machinery of Pin1. Dipeptides as used in the crystal structure act as weak competitive inhibitors. These results are in agreement with those found for the other two PPIase families. It seems that the extended substrate/enzyme interaction is necessary to stabilize the transition state. Similar to human FKBP12 D-amino acid residues in position P1 are not tolerated in substrates for human Pin1 (88). Close to the dipeptide prolyl bond are the residues H59, C113, S154 and H157. A covalent mechanism was suggested, where the deprotonated side chain of His59 abstracts a proton from the C113 thiol, the resulting thiolate than attacks the carbonyl carbon of the prolyl bond. According to path C (Figure 1) the isomerization barrier of the formed intermediate is greatly reduced and therefore the rate of *cis/trans* isomerization is increased (83). Active site variants C113A and H59A showed a 123-fold and a 17-fold reduced activity respectively towards phosphorylated and unphosphorylated substrates (85). In accordance with this mechanism is the bell-shaped pH dependence of k_{cat}/K_M with apparent pK_a values of 5.6 and 7.5, both pK_a values are consistent with the titration of the active site residues H59 and His157. Further investigations confirmed that the side chain of His59 is responsible for the single dissociation step found below pH 6. No pH dependence was found with substrates containing an Ala-Pro bond and it was concluded that peptides that interact only unspecifically with the active site of human Pin1 are catalyzed via a desolvation mechanism (25).

Although the model of covalent catalysis via a cysteine residue is consistent with a great deal of work, some results argue against it. Juglone (Figure 11A) has been found to covalently modify the active site cysteine of *E.coli* Par10, the reported rate of modification was about 5 times faster than the observed enzyme inactivation. In accordance with the reported CD-spectroscopic data, it was suggested that the juglon-

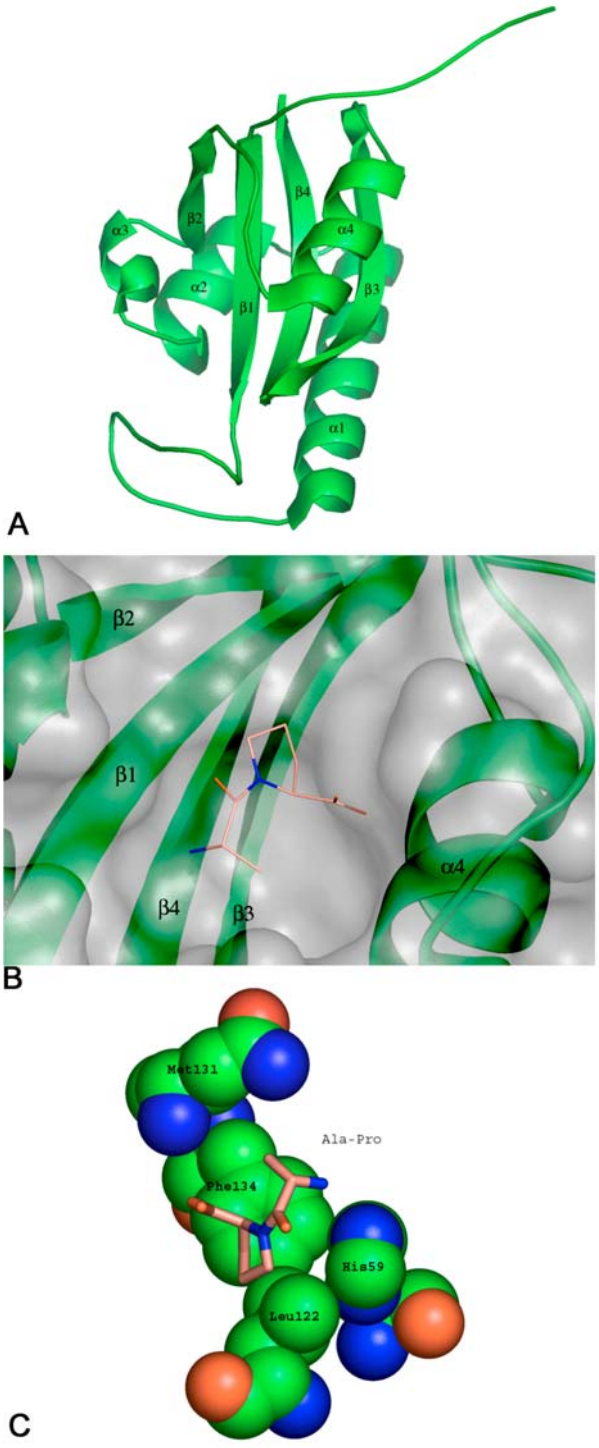

Figure 10. General fold of parvulins shown exemplarily for the PPIase domain of human Pin1 (A). The domain consists of four β-strands (β1 is made up of residues 56 to 63, β2 comprises residues 120 to 124, β3 contains residues 143 to 152 and β4 consists of residues 155 to 164) and four α-helices (α1is made up of residues 81 to 99, α2 comprises residues 103 to 111, α3 harbors residues 114 to 118 and α4 which consists of residues 131 to 140). The prolyl binding pocket is located and shaped similarly to the FKBP (B). The cavity is formed by two β-strands (β1 and β2) and one α-helix (α4). A representation of the prolyl binding pocket of human Pin1 as it was observed in the crystal structure is depicted in panel C. The amino acid residues involved (His59, Leu122, Met131, Phe134) are shown in spacefill and labeled. The bound dipeptide (Ala-Pro) is depicted as a stick model.

Table 8. Parvulins examined for their *in vitro* PPIase activity

Organism	Name as in publication or data bank/ generic name	swissprot	(k_{cat}/K_M) $M^{-1}s^{-1}$	Active site residues as found in a pair wise alignment with human Pin1, +) identical residue, -) residue not present									substrate	Ref.
H. sapiens	Pin1 / Par18	Q13526	$1.9\ 10^7$	H 59	K 63	R 68	R 69	C 113	M 130	F 134	S 154	H 157	AApSPR-pNA [a)]	85
H. sapiens	Par14	Q9Y237	$3.9\ 10^3$	+	+	-	-	D	+	+	F	+	Suc-ARPF-pNA	92
B. subtilis	PrsA / Par33	P24327	$6\ 10^3$	+	A	-	-	D	Q	+	Y	+	Suc-AKPF-pNA	178
E. coli	Par10	P39159	$1.35\ 10^7$	+	+	-	-	+	+	+	F	+	Suc-ALPF-pNA	92
E. coli	SurA / Par47 Domain I	P21202	$1.9\ 10^4$	+	P	P	T	D	L	+	V	+	Suc-ALPF-pNA	92
E. coli	SurA / Par47 Domain II	P21202		+	+	-	-	D	F	+	F	+		
E. coli	PpiD / Par68	P77241	$3.4\ 10^6$	-	Q	-	-	I	I	L	V	L	Suc-AEPF-pNA	179
A. thaliana	AtPar13	Q9SL42	$2.7\ 10^6$	+	+	+	+	+	+	+	+	+	Ac-AApSPF-pNA [a)]	180
D. lanata	DlPar13	Q9LEK8	$1.5\ 10^7$	+	+	+	+	+	+	+	+	+	Ac-ApSPY-pNA [a)]	181
M. domes-tica	MdPar13	Q94G00	$3.1\ 10^6$	+	+	+	+	+	+	+	+	+	Ac-AApSPF-pNA [a)]	180
N. crassa	Ssp1 / Par21	O60045	$6.5\ 10^6$	+	+	+	+	+	+	+	+	+	Ac-AApSPF-pNA [a)]	182
S. cervicea	Ptf1 / Par22	P22696	$1.7\ 10^7$	+	+	+	+	+	+	+	+	+	Ac-ApSPY-pNA [a)]	181
X. laevis	Pin1 / Par18	Q9I9K6	active	+	+	+	+	+	+	+	+	+	no information	183

The activity was measured with the indicated substrate. The reported enzymatic constants were obtained as described in the respective publications. The two parvulin domains of *E.coli* SurA have been aligned individually; the activity shown was determined for the full length protein. The statement "active" means that in the publication PPIase activity assays were performed but no (k_{cat}/K_M) values were provided. The generic name consists of the abbreviation of the respective PPIase family followed by the rounded molecular weight of the full length protein, a) the substrate contains a phosphorylated serine (pS) preceding proline

Table 9. Parvulin structures available in the RCSB Data Bank

Organism	Name as used in publication	Number of structures	PDB identifier
H. sapiens	Pin1	4	1nmv, 1nmw, 1pin, 1f8a
H. sapiens	Par14	2	1eq3, 1fjd
A. thaliana	Pin1At	1	1j6y
E. coli	Par10	2	1jns, 1jnt
E. coli	SurA	1	1m5y

modified E.coli Par10 is destabilized and that the misplacement of catalytic residues leads to partial unfolding and thereby inactivation of the enzyme (89). Interestingly, it was also observed that juglone derived inhibitors (Figure 11B & C) could inhibit human Pin1 as well as human Par14 in a competitive and noncovalent manner (90).

The solution structure of a human Pin1 homolog from A.thaliana (Pin1At) also questioned the participation of an activated cysteine in the catalytic mechanism (91). The protein is highly homologous to human Pin1, but lacks the WW domain, like all plant human Pin1 homologues reported so far. All above mentioned active site residues are present in Pin1At and the general fold reveals a high level of similarity. The substrate binding pocket was mapped using NMR chemical shift perturbation experiments initiated by addition of a phospho-threonine peptide to Pin1At. Whereas it was found that the residues R21, R22, M87 and F91 (corresponding to R68, R69, M130 and F134 in human Pin1) were clearly involved in substrate binding, no or only slight changes in chemical shifts were observed for L79, C70, H12 and H114 (L122, C113, H59 and H157 in human Pin1) upon addition of saturating concentrations of substrate. Structural comparison of the active sites showed that the C70 side chain of Pin1At pointed away from the hydrophobic prolyl binding pocket, making it unlikely to interact with the prolyl bond. The authors suggested that the serine residue S71 (corresponds to S154 in human Pin1), which showed large chemical shift perturbation upon ligand binding, might instead be involved in catalysis.

Human parvulin 14 displays only a weak PPIase activity ($k_{cat}/K_M = 10^3\ M^{-1}\ s^{-1}$) with a preference towards positively charged residues in P1 position (92). This preference is due to a shortened loop region where the positively charged side chains K63, R68 and R69 of the human Pin1 are replaced by a negatively charged area made of two negatively charged side chains D74 and E73 of human Par14 (93). The active site C113 of human Pin1 is exchanged to D74 in human Par14 and S154 is exchanged to F120. Only the two histidine residues in the active center of human Pin1 are conserved in human Par14. It is thought that the negative charges provided by the E73-D74 patch might be necessary to support catalytic assistance of other active site residues or enzyme bound water molecules.

4. PERSPECTIVE

The question asked in the introduction - "Do PPIases facilitate their enzymatic action by utilizing a common catalytic pathway?" is still unanswered, not even the nature of the catalytic pathway of a single PPIase

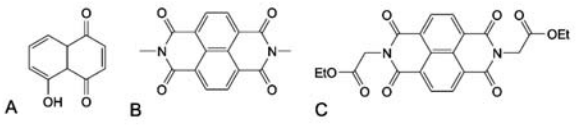

Figure 11. Juglone (A) and tetraoxobenzophenanthrolines (B, C) found to inactivate the PPIase site of parvulins.

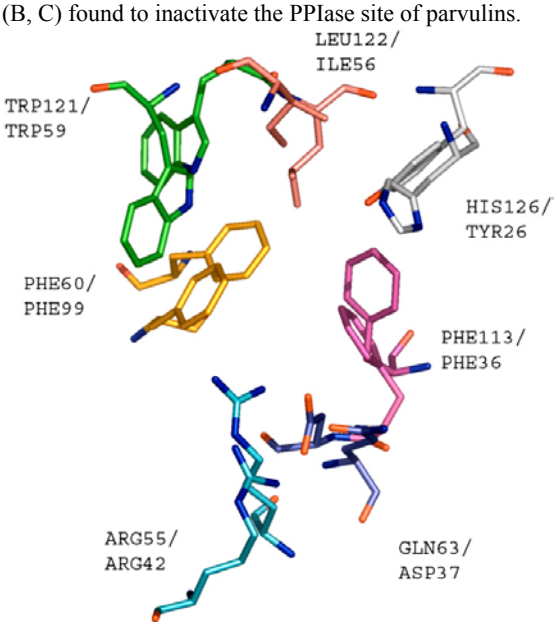

Figure 12. Active site residues of human Cyp18 (R55, F60, Q63, F113, W121 and H126) have been manually aligned with active site residues of human FKBP12 (Y26, D37, R42, I56, W59 and F99). Corresponding residues of the two structures are colored identically and labels are shown adjacent to them.

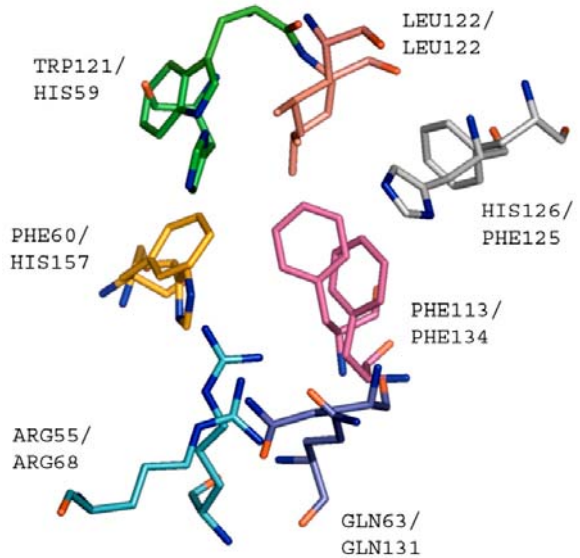

Figure 13. Active site residues of human Cyp18 (R55, F60, Q63, F113, W121 and H126) have been manually aligned with those of human Pin1 (H59, R68, L122, F125, Q131 and F134). Corresponding residues of the two structures are colored identically and labels are shown adjacent to them.

family is yet fully understood. As it seems none of the mechanisms depicted in Figure 1 can explain all of the published data, but some can be ruled out. A direct involvement of a tetrahedral intermediate formed by a nucleophilic amino acid sidechain originating from the PPIase active site seems not to be supported by the data. Furthermore, it has been shown for different members of the three PPIase families that a number of atomic features in the substrate chain are necessary to activate the full catalytic power of these enzymes, therefore mechanisms which do not require specific enzyme/substrate interactions can also be ruled out. On grounds of the existing data and new results obtained by investigating the influence of cosolvents and heavy water on PPIase catalysis (Fanghänel et al., in preparation) we favor a concerted mechanism in which the electron pair is stabilized on the amide nitrogen bond by an H-bond donor of the protein. A simultaneous electrostatic transition state stabilization by an enzyme bound general base-polarized water molecule of the perpendicularly oriented carbonyl group is thought to occur (Figure 1, path D and E). One major handicap to elucidate a common catalytic pathway of PPIases is obviously the lack of structures of FKBP and parvulins in complex with substrates. In addition, the danger posed by cyclophilin/substrate complexes is the potential problem of analyzing dead complexes which do not map to the catalytic pathway. On a molecular level we have only limited knowledge of how these two enzyme families bind to their natural protein ligands. That the three enzyme families might catalyze the prolyl isomerization in a similar way became evident in structural comparisons.

The active site residues of human Cyp18 have been superimposed with some of the known drug binding residues of human FKBP12 (Figure 12). Four of the depicted active site residues of human Cyp18 have identical counterparts in human FKBP12, the other three residues are conservatively exchanged (L122 to I56, H126 to Y26 and Q63 to D37). Interestingly, all residues crucial for the activity of human FKBP12 (Y26, D37, W59 and F99) coincide with active site residues found to be essential (H126, Q63, F60) or at least important (W121) for human Cyp18 catalysis. The pipecolinyl residue of FK506 does not superimpose with the proline residue of the human Cyp18 bound substrate, underlining the doubt that it occupies the active site of human FKBP12. It should also be noted that in the crystal structure of the human FKBP12 in complex with the cytoplasmic domain of the type I TGF-beta receptor the proposed proline binding pocket of human FKBP12 is occupied by a leucine residue of the receptor (94).

When the same human Cyp18 residues are superimposed with the active site of human Pin1 a similar picture is observed (Figure 13). Four residues of human Cyp18 have identical counterparts in the active site of human Pin1 (R55 to R68, F113 to F134, Q63 to Q131 and L122 to L122). Again, among them are three residues necessary for human Cyp18 activity (R55, Q63, F113), and F60 and H126 show a mirror symmetry to the human Pin1 residues H157 and F125. The superimposed active site residues of human Pin1 also include R68 and His59, which

have been shown to be important for efficient human Pin1 catalysis. In contrast to the superimposed human Cyp18 and human FKBP12 structures, the proline residues of the bound ligands of human Cyp18 (Suc-AAPF-pNA) and human Pin1 (Ala-Pro) share a common space.

Despite the lack of a common general fold cyclophilins, FKBP and parvulins have a very similar active site structure. This observation leads us to the assumption that the catalytic pathway utilized by the different PPIase families is closely related.

5. ACKNOWLEDGMENT

This work was supported by the DFG grant SFB 610 and the Fonds der Chemischen Industrie. We thank G. Wille for helpfull discussion.

6. REFERENCES

1. Pauling, L., R. B. Corey and H. R. Branson: The structure of proteins: two hydrogen-bonded helical configurations of the polypeptide chain. *Proc Natl Acad Sci USA* 37, 205-211 (1951)

2. Brandts, J. F., H. R. Halvorson and M. Brennan: Consideration of the Possibility that the slow step in protein denaturation reactions is due to *cis-trans* isomerism of proline residues. *Biochemistry* 14, 4953-63 (1975)

3. Fischer, G., H. Bang and C. Mech: Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides. *Biomed Biochim Acta* 43, 1101-11 (1984)

4. Schiene-Fischer, C., J. Habazettl, F. X. Schmid and G. Fischer: The hsp70 chaperone DnaK is a secondary amide peptide bond *cis-trans* isomerase. *Nat Struct Biol* 9, 419-24 (2002)

5. Rahfeld, J. U., K. P. Rucknagel, B. Schelbert, B. Ludwig, J. Hacker, K. Mann and G. Fischer: 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, 180-4 (1994)

6. Lu, K. P.: Prolyl isomerase Pin1 as a molecular target for cancer diagnostics and therapeutics. *Cancer Cell* 4, 175-80 (2003)

7. Shaw, P. E.: Peptidyl-prolyl isomerases: a new twist to transcription. *EMBO Rep* 3, 521-6 (2002)

8. Harrar, Y., C. Bellini and J. D. Faure: FKBPs: at the crossroads of folding and transduction. *Trends Plant Sci* 6, 426-31 (2001)

9. Lehnart, S. E., F. Huang, S. O. Marx and A. R. Marks: Immunophilins and coupled gating of ryanodine receptors. *Curr Top Med Chem* 3, 1383-91 (2003)

10. Schmid, F. X.: Prolyl isomerases. *Adv Protein Chem* 59, 243-82 (2001)

11. Dugave, C. and L. Demange: *Cis-trans* isomerization of organic molecules and biomolecules: implications and applications. *Chem Rev* 103, 2475-532 (2003)

12. Fischer, G.: Chemical aspects of peptide bond isomerisation. *Chem Soc Rev* 29, 119-127 (2000)

13. Drakenberg, T.: The barrier to internal rotation in amides VI acetamide solvent dependent entropy of activation. *Tetrahedron Lett*, 1743-1746 (1972)

14. Keller, M., C. Sager, P. Dumy, M. Schutkowski, G. Fischer and M. Mutter: Enhancing the proline effect - pseudo-prolines for tailoring c*is/trans* isomerization. *J Am Chem Soc* 120, 2714-2720 (1998)

15. Eberhardt, E. S., S. N. Loh, A. P. Hinck, and R. T. Raines: Solvent effects on the energetics of prolyl peptide bond isomerization. *J Am Chem Soc* 114, 5437-5439 (1992)

16. Gerig, J. T.: The effect of adjacent charges on the kinetics of rotation of the peptide bond. *Biopolymers* 10, 2435-43 (1971)

17. Lindskog, S.: Structure and mechanism of carbonic anhydrase. *Pharmacol Ther* 74, 1-20 (1997)

18. Stein, R. L.: Mechanism of enzymatic and nonenzymatic prolyl *cis-trans* isomerization. *Adv Protein Chem* 44, 1-24 (1993)

19. Harrison, R. K. and R. L. Stein: Mechanistic studies of peptidyl prolyl *cis-trans* isomerase: evidence for catalysis by distortion. *Biochemistry* 29, 1684-9 (1990)

20. Kern, D., M. Schutkowski and T. Drakenberg: Rotational barriers of c*is/trans* isomerization of proline analogues and their catalysis by cyclophilin. *J Am Chem Soc* 119, 8403-8408 (1997)

21. Renner, C., S. Alefelder, J. H. Bae, N. Budisa, R. Huber and L. Moroder: Fluoroprolines as tools for protein design and engineering. *Angew Chem Int Ed Engl* 40, 923-925 (2001)

22. Beausoleil, E., R. Sharma, S. W. Michnick and W. D. Lubell: Alkyl 3-position substituents retard the isomerization of prolyl and hydroxyprolyl amides in water. *J Org Chem* 63, 6572-6578 (1998)

23. Eberhardt, E. S., N. Panasik and R. T. Raines: Inductive effects on the energetics of prolyl Peptide bond isomerization - implications for collagen folding and stability. *J Am Chem Soc* 118, 12261-12266 (1996)

24. Pao, Y. L., M. R. Wormarld, R. A. Dwek and A. C. Lellouch: Effect of serine O-glycosylation on *cis-trans* proline isomerization. *Biochem Biophys Res Commun* 219, 157-62 (1996)

25. Schutkowski, M., A. Bernhardt, X. Z. Zhou, M. Shen, U. Reimer, J. U. Rahfeld, K. P. Lu and G. Fischer: Role of phosphorylation in determining the backbone dynamics of the serine/threonine-proline motif and Pin1 substrate recognition. *Biochemistry* 37, 5566-75 (1998)

26. Schutkowski, M., S. Wollner and G. Fischer: Inhibition of peptidyl-prolyl *cis/trans* isomerase activity by substrate analog structures: thioxo tetrapeptide-4-nitroanilides. *Biochemistry* 34, 13016-26 (1995)

27. Texter, F. L., D. B. Spencer, R. Rosenstein and C. R. Matthews: Intramolecular catalysis of a proline isomerization reaction in the folding of dihydrofolate reductase. *Biochemistry* 31, 5687-91 (1992)

28. Cox, C. and T. Lectka: Intramolecular catalysis of amide isomerization - kinetic consequences of the 5-NH- - N-alpha hydrogen bond in prolyl peptides. *J Am Chem Soc* 120, 10660-10668 (1998)

29. Rabenstein, D. L., T. S. Shi and S. Spain: Intramolecular catalysis of the *cis-trans* isomerization of proline peptide bonds in cyclic disulfide-containing peptides. *J Am Chem Soc* 122, 2401-2402 (2000)

30. Reimer, U., N. elMokdad, M. Schutkowski and G. Fischer: Intramolecular assistance of *cis/trans* isomerization of the histidine-proline moiety. *Biochemistry* 36, 13802-8 (1997)

31. Ylikauhaluoma, J. T., J. A. Ashley, C. H. L. Lo, J. Coakley, P. Wirsching and K. D. Janda: Catalytic Antibodies with Peptidyl-Prolyl *Cis-Trans* Isomerase Activity. *J Am Chem Soc* 118, 5496-5497 (1996)

32. Ma, L., L. C. Hsieh-Wilson and P. G. Schultz: Antibody catalysis of peptidyl-prolyl *cis-trans* isomerization in the folding of RNase T1. *Proc Natl Acad Sci USA* 95, 7251-6 (1998)

33. Kramer, M. L. and G. Fischer: Fkbp-like catalysis of peptidyl-prolyl bond isomerization by micelles and membranes. *Biopolymers* 42, 49-60 (1997)

34. Fischer, G. and T. Aumuller: Regulation of peptide bond *cis/trans* isomerization by enzyme catalysis and its implication in physiological processes. *Rev Physiol Biochem Pharmacol* 148, 105-50 (2003)

35. Zhao, Y., Y. Chen, M. Schutkowski, G. Fischer and H. Ke: Cyclophilin A complexed with a fragment of HIV-1 gag protein: insights into HIV-1 infectious activity. *Structure* 5, 139-46 (1997)

36. Zhao, Y. and H. Ke: Crystal structure implies that cyclophilin predominantly catalyzes the *trans* to *cis* isomerization. *Biochemistry* 35, 7356-61 (1996)

37. Zhao, Y. and H. Ke: Mechanistic implication of crystal structures of the cyclophilin-dipeptide complexes. *Biochemistry* 35, 7362-8 (1996)

38. Kallen, J. and M. D. Walkinshaw: The X-ray structure of a tetrapeptide bound to the active site of human cyclophilin A. *FEBS Lett* 300, 286-90 (1992)

39. Ke, H., D. Mayrose and W. Cao: Crystal structure of cyclophilin A complexed with substrate Ala-Pro suggests a solvent-assisted mechanism of *cis-trans* isomerization. *Proc Natl Acad Sci USA* 90, 3324-8 (1993)

40. Konno, M., M. Ito, T. Hayano and N. Takahashi: The substrate-binding site in *Escherichia coli* cyclophilin A preferably recognizes a *cis*-proline isomer or a highly distorted form of the *trans* isomer. *J Mol Biol* 256, 897-908 (1996)

41. Howard, B. R., F. F. Vajdos, S. Li, W. I. Sundquist and C. P. Hill: Structural insights into the catalytic mechanism of cyclophilin A. *Nat Struct Biol* 10, 475-81 (2003)

42. Wu Sy, S., J. Dornan, G. Kontopidis, P. Taylor and M. D. Walkinshaw: The first direct determination of a ligand binding constant in protein crystals. *Angew Chem Int Ed Engl* 40, 582-586 (2001)

43. Scholz, C., G. Scherer, L. M. Mayr, T. Schindler, G. Fischer and F. X. Schmid: Prolyl isomerases do not catalyze isomerization of non-prolyl peptide bonds. *Biol Chem* 379, 361-5 (1998)

44. Zydowsky, L. D., F. A. Etzkorn, H. Y. Chang, S. B. Ferguson, L. A. Stolz, S. I. Ho and C. T. Walsh: Active site mutants of human cyclophilin A separate peptidyl-prolyl isomerase activity from cyclosporin A binding and calcineurin inhibition. *Protein Sci* 1, 1092-9 (1992)

45. Kallen, J., C. Spitzfaden, M. G. Zurini, G. Wider, H. Widmer, K. Wuthrich and M. D. Walkinshaw: Structure of human cyclophilin and its binding site for cyclosporin A determined by X-ray crystallography and NMR spectroscopy. *Nature* 353, 276-9 (1991)

46. Fischer, G., B. Wittmann-Liebold, K. Lang, T. Kiefhaber and F. X. Schmid: Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. *Nature* 337, 476-8 (1989)

47. Fischer, G., E. Berger and H. Bang: Kinetic beta-deuterium isotope effects suggest a covalent mechanism for the protein folding enzyme peptidylprolyl *cis/trans*-isomerase. *FEBS Lett* 250, 267-70 (1989)

48. Liu, J., M. W. Albers, C. M. Chen, S. L. Schreiber and C. T. Walsh: Cloning, expression, and purification of human cyclophilin in *Escherichia coli* and assessment of the catalytic role of cysteines by site-directed mutagenesis. *Proc Natl Acad Sci USA* 87, 2304-8 (1990)

49. Harrison, R. K. and R. L. Stein: Mechanistic studies of enzymic and nonenzymic prolyl *cis-trans* isomerization. *J Am Chem Soc* 114, 3464-3471 (1992)

50. Kofron, J. L., P. Kuzmic, V. Kishore, E. Colon-

Bonilla and D. H. Rich: Determination of kinetic constants for peptidyl prolyl *cis-trans* isomerases by an improved spectrophotometric assay. *Biochemistry* 30, 6127-34 (1991)

51. Kakalis, L. T. and I. M. Armitage: Solution conformation of a cyclophilin-bound proline isomerase substrate. *Biochemistry* 33, 1495-501 (1994)

52. Kern, D., G. Kern, G. Scherer, G. Fischer and T. Drakenberg: Kinetic analysis of cyclophilin-catalyzed prolyl *cis/trans* isomerization by dynamic NMR spectroscopy. *Biochemistry* 34, 13594-602 (1995)

53. Demange, L. and C. Dugave: Synthesis of phosphinic alanyl-proline surrogates Ala psi(PO_2R-CH)Pro as potential inhibitors of the human cyclophilin hCyp-18. *Tetrahedron Lett* 42, 6295-6297 (2001)

54. Wang, H. C., K. Kim, R. Bakhtiar and J. P. Germanas: Structure-activity studies of ground- and transition-state analogue inhibitors of cyclophilin. *J Med Chem* 44, 2593-600 (2001)

55. Schechter, I. and A. Berger: On the size of the active site in proteases. I. Papain. *Biochem Biophys Res Commun* 27, 157-62 (1967)

56. Schiene, C., U. Reimer, M. Schutkowski and G. Fischer: Mapping the stereospecificity of peptidyl prolyl *cis/trans* isomerases. *FEBS Lett* 432, 202-6 (1998)

57. Hur, S. and T. C. Bruice: The mechanism of *cis-trans* isomerization of prolyl peptides by cyclophilin. *J Am Chem Soc* 124, 7303-13 (2002)

58. Li, G. and Q. Cui: What is so special about Arg 55 in the catalysis of cyclophilin A? insights from hybrid QM/MM simulations. *J Am Chem Soc* 125, 15028-38 (2003)

59. Hsu, V.L, Handschumacher, R.E, Armitage and I.M: Peptidyl prolyl *cis/trans* isomerase activity of cyclophilin studied by one-dimensional 1-H nmr. *J Am Chem Soc* 112, 6745-6747 (1990)

60. Luban, J., K. L. Bossolt, E. K. Franke, G. V. Kalpana and S. P. Goff: Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell* 73, 1067-78 (1993)

61. Gamble, T. R., F. F. Vajdos, S. Yoo, D. K. Worthylake, M. Houseweart, W. I. Sundquist and C. P. Hill: Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* 87, 1285-94 (1996)

62. Colgan, J., H. E. Yuan, E. K. Franke and J. Luban: Binding of the human immunodeficiency virus type 1 Gag polyprotein to cyclophilin A is mediated by the central region of capsid and requires Gag dimerization. *J Virol* 70, 4299-310 (1996)

63. Schutkowski, M., M. Drewello, S. Wollner, M. Jakob, U. Reimer, G. Scherer, A. Schierhorn and G. Fischer: Extended binding sites of cyclophilin as revealed by the interaction with HIV-1 Gag polyprotein derived oligopeptides. *FEBS Lett* 394, 289-94 (1996)

64. Bosco, D. A., E. Z. Eisenmesser, S. Pochapsky, W. I. Sundquist and D. Kern: Catalysis of *cis/trans* isomerization in native HIV-1 capsid by human cyclophilin A. *Proc Natl Acad Sci USA* 99, 5247-52 (2002)

65. Eisenmesser, E. Z., D. A. Bosco, M. Akke and D. Kern: Enzyme dynamics during catalysis. *Science* 295, 1520-3 (2002)

66. Chambraud, B., N. Rouviere-Fourmy, C. Radanyi, K. Hsiao, D. A. Peattie, D. J. Livingston and E. E. Baulieu: Overexpression of p59-HBI (FKBP59), full length and domains, and characterization of PPIase activity. *Biochem Biophys Res Commun* 196, 160-6 (1993)

67. Barent, R. L., S. C. Nair, D. C. Carr, Y. Ruan, R. A. Rimerman, J. Fulton, Y. Zhang and D. F. Smith: Analysis of FKBP51/FKBP52 chimeras and mutants for Hsp90 binding and association with progesterone receptor complexes. *Mol Endocrinol* 12, 342-54 (1998)

68. Callebaut, I., J. M. Renoir, M. C. Lebeau, N. Massol, A. Burny, E. E. Baulieu and J. P. Mornon: An immunophilin that binds M(r) 90,000 heat shock protein: main structural features of a mammalian p59 protein. *Proc Natl Acad Sci USA* 89, 6270-4 (1992)

69. Kamphausen, T., J. Fanghanel, D. Neumann, B. Schulz and J. U. Rahfeld: Characterization of *Arabidopsis thaliana* AtFKBP42 that is membrane-bound and interacts with Hsp90. *Plant J* 32, 263-76 (2002)

70. Iida, T., M. Furutani, F. Nishida and T. Maruyama: FKBP-type peptidyl-prolyl *cis-trans* isomerase from a sulfur-dependent hyperthermophilic archaeon, *Thermococcus sp.* KS-1. *Gene* 222, 249-55 (1998)

71. Ideno, A. and T. Maruyama: Expression of long- and short-type FK506 binding proteins in hyperthermophilic archaea. *Gene* 292, 57-63 (2002)

72. Ideno, A., T. Yoshida, M. Furutani and T. Maruyama: The 28.3 kDa FK506 binding protein from a thermophilic archaeum, *Methanobacterium thermoautotrophicum*, protects the denaturation of proteins *in vitro*. *Eur J Biochem* 267, 3139-49 (2000)

73. Park, S. T., R. A. Aldape, O. Futer, M. T. DeCenzo and D. J. Livingston: PPIase catalysis by human FK506-binding protein proceeds through a conformational twist mechanism. *J Biol Chem* 267, 3316-24 (1992)

74. Albers, M. W., C. T. Walsh and S. L. Schreiber: Substrate specificity for human rotamase FKBP: a view of FK506 and rapamycin as leucine-(twisted amide)- proline mimic. *J Org Chem* 55, 4984-4986-4986 (1990)

75. Van Duyne, G. D., R. F. Standaert, P. A. Karplus, S. L. Schreiber and J. Clardy: Atomic structures of the human immunophilin FKBP-12 complexes with FK506 and rapamycin. *J Mol Biol* 229, 105-24 (1993)

76. Sich, C., S. Improta, D. J. Cowley, C. Guenet, J. P. Merly, M. Teufel and V. Saudek: Solution structure of a neurotrophic ligand bound to FKBP12 and its effects on protein dynamics. *Eur J Biochem* 267, 5342-55 (2000)

77. Van Duyne, G. D., R. F. Standaert, P. A. Karplus, S. L. Schreiber and J. Clardy: Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. *Science* 252, 839-42 (1991)

78. Rosen, M. K., R. F. Standaert, A. Galat, M. Nakatsuka and S. L. Schreiber: Inhibition of FKBP rotamase activity by immunosuppressant FK506: twisted amide surrogate. *Science* 248, 863-6 (1990)

79. Bossard, M. J., D. J. Bergsma, M. Brandt, G. P. Livi, W. K. Eng, R. K. Johnson and M. A. Levy: Catalytic and ligand binding properties of the FK506 binding protein FKBP12: effects of the single amino acid substitution of Tyr82 to Leu. *Biochem J* 297 (Pt 2), 365-72 (1994)

80. Tocci, M. J., D. A. Matkovich, K. A. Collier, P. Kwok, F. Dumont, S. Lin, S. Degudicibus, J. J. Siekierka, J. Chin and N. I. Hutchinson: The immunosuppressant FK506 selectively inhibits expression of early T cell activation genes. *J Immunol* 143, 718-26 (1989)

81. Orozco, M., J. Tirado-Rives and W. L. Jorgensen: Mechanism for the rotamase activity of FK506 binding protein from molecular dynamics simulations. *Biochemistry* 32, 12864-74 (1993)

82. Fischer, S., S. Michnick and M. Karplus: A mechanism for rotamase catalysis by the FK506 binding protein (FKBP). *Biochemistry* 32, 13830-7 (1993)

83. Ranganathan, R., K. P. Lu, T. Hunter and J. P. Noel: Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell* 89, 875-86 (1997)

84. Rippmann, J. F., S. Hobbie, C. Daiber, B. Guilliard, M. Bauer, J. Birk, H. Nar, P. Garin-Chesa, W. J. Rettig and A. Schnapp: Phosphorylation-dependent proline isomerization catalyzed by Pin1 is essential for tumor cell survival and entry into mitosis. *Cell Growth Differ* 11, 409-16 (2000)

85. Yaffe, M. B., M. Schutkowski, M. Shen, X. Z. Zhou, P. T. Stukenberg, J. U. Rahfeld, J. Xu, J. Kuang, M. W. Kirschner, G. Fischer, L. C. Cantley and K. P. Lu: Sequence-specific and phosphorylation-dependent proline isomerization: a potential mitotic regulatory mechanism. *Science* 278, 1957-60 (1997)

86. Verdecia, M. A., M. E. Bowman, K. P. Lu, T. Hunter and J. P. Noel: Structural basis for phosphoserine-proline recognition by group IV WW domains. *Nat Struct Biol* 7, 639-43 (2000)

87. Bayer, E., S. Goettsch, J. W. Mueller, B. Griewel, E. Guiberman, L. M. Mayr and P. Bayer: Structural analysis of the mitotic regulator hPin1 in solution: insights into domain architecture and substrate binding. *J Biol Chem* 278, 26183-93 (2003)

88. Zhang, Y., S. Fussel, U. Reimer, M. Schutkowski and G. Fischer: Substrate-based design of reversible Pin1 inhibitors. *Biochemistry* 41, 11868-77 (2002)

89. Hennig, L., C. Christner, M. Kipping, B. Schelbert, K. P. Rucknagel, S. Grabley, G. Kullertz and G. Fischer: Selective inactivation of parvulin-like peptidyl-prolyl *cis/trans* isomerases by juglone. *Biochemistry* 37, 5953-60 (1998)

90. Uchida, T., M. Takamiya, M. Takahashi, H. Miyashita, H. Ikeda, T. Terada, Y. Matsuo, M. Shirouzu, S. Yokoyama, F. Fujimori and T. Hunter: Pin1 and Par14 peptidyl prolyl isomerase inhibitors block cell proliferation. *Chem Biol* 10, 15-24 (2003)

91. Landrieu, I., J. M. Wieruszeski, R. Wintjens, D. Inze and G. Lippens: Solution structure of the single-domain prolyl cis/trans isomerase PIN1At from *Arabidopsis thaliana*. *J Mol Biol* 320, 321-32 (2002)

92. Uchida, T., F. Fujimori, T. Tradler, G. Fischer and J. U. Rahfeld: Identification and characterization of a 14 kDa human protein as a novel parvulin-like peptidyl prolyl *cis/trans* isomerase. *FEBS Lett* 446, 278-82 (1999)

93. Sekerina, E., J. U. Rahfeld, J. Muller, J. Fanghanel, C. Rascher, G. Fischer and P. Bayer: NMR solution structure of hPar14 reveals similarity to the peptidyl prolyl *cis/trans* isomerase domain of the mitotic regulator hPin1 but indicates a different functionality of the protein. *J Mol Biol* 301, 1003-17 (2000)

94. Huse, M., Y. G. Chen, J. Massague and J. Kuriyan: Crystal structure of the cytoplasmic domain of the type I TGF beta receptor in complex with FKBP12. *Cell* 96, 425-36 (1999)

95. Joseph, J. D., J. Heitman and A. R. Means: Molecular cloning and characterization of *Aspergillus nidulans* cyclophilin B. *Fungal Genet Biol* 27, 55-66 (1999)

96. Derkx, P. M. and S. M. Madrid: The *Aspergillus niger* cypA gene encodes a cyclophilin that mediates sensitivity to the immunosuppressant cyclosporin A. *Mol Genet Genomics* 266, 527-36 (2001)

97. Derkx, P. M. and S. M. Madrid: The foldase CYPB is a component of the secretory pathway of *Aspergillus niger* and contains the endoplasmic reticulum retention signal HEEL. *Mol Genet Genomics* 266, 537-45 (2001)

98. Grebe, M., J. Gadea, T. Steinmann, M. Kientz, J. U. Rahfeld, K. Salchert, C. Koncz and G. Jurgens: A conserved domain of the arabidopsis GNOM protein

mediates subunit interaction and cyclophilin 5 binding. *Plant Cell* 12, 343-56 (2000)

99. Lippuner, V., I. T. Chou, S. V. Scott, W. F. Ettinger, S. M. Theg and C. S. Gasser: Cloning and characterization of chloroplast and cytosolic forms of cyclophilin from *Arabidopsis thaliana*. *J Biol Chem* 269, 7863-8 (1994)

100. Page, A. P., D. Landry, G. G. Wilson and C. K. Carlow: Molecular characterization of a cyclosporin A-insensitive cyclophilin from the parasitic nematode *Brugia malayi*. *Biochemistry* 34, 11545-50 (1995)

101. Ma, D., X. Hong, N. Raghavan, A. L. Scott, J. S. McCarthy, T. B. Nutman, S. A. Williams and C. K. Carlow: A cyclosporin A-sensitive small molecular weight cyclophilin of filarial parasites. *Mol Biochem Parasitol* 79, 235-41 (1996)

102. Achenbach, T. V., S. F. Gothel and M. A. Marahiel: Histidine 109 in peptidyl-prolyl *cis-trans* isomerase of *Bacillus subtilis* plays an important role in catalysis and in cyclosporin A binding. *FEMS Microbiol Lett* 154, 139-44 (1997)

103. Bose, S., M. Mucke and R. B. Freedman: The characterization of a cyclophilin-type peptidyl prolyl *cis-trans*-isomerase from the endoplasmic-reticulum lumen. *Biochem J* 300 (Pt 3), 871-5 (1994)

104. Koser, P. L., G. P. Livi, M. A. Levy, M. Rosenberg and D. J. Bergsma: A *Candida albicans* homolog of a human cyclophilin gene encodes a peptidyl-prolyl *cis-trans* isomerase. *Gene* 96, 189-95 (1990)

105. Page, A. P., K. MacNiven and M. O. Hengartner: Cloning and biochemical characterization of the cyclophilin homologues from the free-living nematode *Caenorhabditis elegans*. *Biochem J* 317, 179-85 (1996)

106. Ma, D., L. S. Nelson, K. LeCoz, C. Poole and C. K. Carlow: A novel cyclophilin from parasitic and free-living nematodes with a unique substrate- and drug-binding domain. *J Biol Chem* 277, 14925-32 (2002)

107. Skruzny, M., M. Ambrozkova, I. Fukova, K. Martinkova, A. Blahuskova, L. Hamplova, F. Puta and P. Folk: Cyclophilins of a novel subfamily interact with SNW/SKIP coregulator in *Dictyostelium discoideum* and *Schizosaccharomyces pombe*. *Biochim Biophys Acta* 1521, 146-51 (2001)

108. Cavarec, L., T. Kamphausen, B. Dubourg, I. Callebaut, F. Lemeunier, D. Metivier, J. Feunteun, G. Fischer and N. Modjtahedi: Identification and characterization of Moca-cyp. A *Drosophila melanogaster* nuclear cyclophilin. *J Biol Chem* 277, 41171-82 (2002)

109. Hong, X., D. Ma and C. K. Carlow: Cloning, expression and characterization of a new filarial cyclophilin. *Mol Biochem Parasitol* 91, 353-8 (1998)

110. Compton, L. A., J. M. Davis, J. R. Macdonald and H. P. Bachinger: Structural and functional characterization of *Escherichia coli* peptidyl-prolyl *cis-trans* isomerases. *Eur J Biochem* 206, 927-34 (1992)

111. Ostoa-Saloma, P., J. Cesar Carrero, P. Petrossian, P. Herion, A. Landa and J. Pedro Laclette: Cloning, characterization and functional expression of a cyclophilin of *Entamoeba histolytica*. *Mol Biochem Parasitol* 107, 219-25 (2000)

112. Nagashima, K., S. Mitsuhashi, K. Kamino and T. Maruyama: Cyclosporin A sensitive peptidyl-prolyl *cis-trans* isomerase in a halophilic archaeum, *Halobacterium cutirubrum*. *Biochem Biophys Res Commun* 198, 466-72 (1994)

113. Rinfret, A., C. Collins, R. Menard and S. K. Anderson: The N-terminal cyclophilin-homologous domain of a 150-kilodalton tumor recognition molecule exhibits both peptidylprolyl *cis-trans*-isomerase and chaperone activities. *Biochemistry* 33, 1668-73 (1994)

114. Kieffer, L. J., T. Thalhammer and R. E. Handschumacher: Isolation and characterization of a 40-kDa cyclophilin-related protein. *J Biol Chem* 267, 5503-7 (1992)

115. Teigelkamp, S., T. Achsel, C. Mundt, S. F. Gothel, U. Cronshagen, W. S. Lane, M. Marahiel and R. Luhrmann: The 20kD protein of human [U4/U6.U5] tri-snRNPs is a novel cyclophilin that forms a complex with the U4/U6-specific 60kD and 90kD proteins. *Rna* 4, 127-41 (1998)

116. Janowski, B., S. Wollner, M. Schutkowski and G. Fischer: A protease-free assay for peptidyl prolyl *cis/trans* isomerases using standard peptide substrates. *Anal Biochem* 252, 299-307 (1997)

117. Bergsma, D. J., C. Eder, M. Gross, H. Kersten, D. Sylvester, E. Appelbaum, D. Cusimano, G. P. Livi, M. M. McLaughlin, K. Kasyan and: The cyclophilin multigene family of peptidyl-prolyl isomerases. Characterization of three separate human isoforms. *J Biol Chem* 266, 23204-14 (1991)

118. Gasser, C. S., D. A. Gunning, K. A. Budelier and S. M. Brown: Structure and expression of cytosolic cyclophilin/peptidyl-prolyl *cis-trans* isomerase of higher plants and production of active tomato cyclophilin in *Escherichia coli*. *Proc Natl Acad Sci USA* 87, 9519-23 (1990)

119. Rascher, C., A. Pahl, A. Pecht, K. Brune, W. Solbach and H. Bang: *Leishmania major* parasites express cyclophilin isoforms with an unusual interaction with calcineurin. *Biochem J* 334, 659-67 (1998)

120. Schmidt, B., T. Tradler, J. U. Rahfeld, B. Ludwig, B. Jain, K. Mann, K. P. Rucknagel, B. Janowski, A. Schierhorn, G. Kullertz, J. Hacker and G. Fischer: A

cyclophilin-like peptidyl-prolyl *cis/trans* isomerase from *Legionella pneumophila*--characterization, molecular cloning and overexpression. *Mol Microbiol* 21, 1147-60 (1996)

121. Schonbrunner, E. R., S. Mayer, M. Tropschug, G. Fischer, N. Takahashi and F. X. Schmid: Catalysis of protein folding by cyclophilins from different species. *J Biol Chem* 266, 3630-5 (1991)

122. Faou, P. and M. Tropschug: A novel binding protein for a member of CyP40-type Cyclophilins: *N.crassa* CyPBP37, a growth and thiamine regulated protein homolog to yeast Thi4p. *J Mol Biol* 333, 831-44 (2003)

123. Chen, H., X. L. Li and L. G. Ljungdahl: A cyclophilin from the polycentric anaerobic rumen fungus *Orpinomyces sp. strain* PC-2 is highly homologous to vertebrate cyclophilin B. *Proc Natl Acad Sci USA* 92, 2587-91 (1995)

124. Reddy, G. R.: Cloning and characterization of a *Plasmodium falciparum* cyclophilin gene that is stage-specifically expressed. *Mol Biochem Parasitol* 73, 111-21 (1995)

125. Hirtzlin, J., P. M. Farber, R. M. Franklin and A. Bell: Molecular and biochemical characterization of a *Plasmodium falciparum* cyclophilin containing a cleavable signal sequence. *Eur J Biochem* 232, 765-72 (1995)

126. Mortillaro, M. J. and R. Berezney: Matrin CYP, an SR-rich cyclophilin that associates with the nuclear matrix and splicing factors. *J Biol Chem* 273, 8183-92 (1998)

127. Connern, C. P. and A. P. Halestrap: Purification and N-terminal sequencing of peptidyl-prolyl *cis-trans*-isomerase from rat liver mitochondrial matrix reveals the existence of a distinct mitochondrial cyclophilin. *Biochem J* 284, 381-5 (1992)

128. Cardenas, M. E., E. Lim and J. Heitman: Mutations that perturb cyclophilin A ligand binding pocket confer cyclosporin A resistance in *Saccharomyces cerevisiae*. *J Biol Chem* 270, 20997-1002 (1995)

129. Scholz, C., P. Maier, K. Dolinski, J. Heitman and F. X. Schmid: R73A and H144Q mutants of the yeast mitochondrial cyclophilin Cpr3 exhibit a low prolyl isomerase activity in both peptide and protein-folding assays. *FEBS Lett* 443, 367-9 (1999)

130. Warth, R., P. A. Briand and D. Picard: Functional analysis of the yeast 40 kDa cyclophilin Cyp40 and its role for viability and steroid receptor regulation. *Biol Chem* 378, 381-91 (1997)

131. Mayr, C., K. Richter, H. Lilie and J. Buchner: Cpr6 and Cpr7, two closely related Hsp90-associated immunophilins from *Saccharomyces cerevisiae*, differ in their functional properties. *J Biol Chem* 275, 34140-6 (2000)

132. Bugli, F., A. Khattab, E. Vigneti, R. Butler, D. Cioli and M. Q. Klinkert: Expression cloning and biochemical characterizations of recombinant cyclophilin proteins from *Schistosoma mansoni*. *Protein Expr Purif* 12, 340-6 (1998)

133. Kiang, D., N. E. El Ghazalie, A. M. Medhat, M. Abdel-Fattah, A. M. Karim and P. T. LoVerde: Identification and characterization of *Schistosoma mansoni* p17.7, a cyclophilin. *Mol Biochem Parasitol* 76, 73-82 (1996)

134. Pemberton, T. J., S. L. Rulten and J. E. Kay: Identification and characterisation of *Schizosaccharomyces pombe* cyclophilin 3, a cyclosporin A insensitive orthologue of human USA-CyP. *J Chromatogr B Analyt Technol Biomed Life Sci* 786, 81-91 (2003)

135. Pahl, A., M. Uhlein, H. Bang, W. Schlumbohm and U. Keller: Streptomycetes possess peptidyl-prolyl *cis-trans* isomerases that strongly resemble cyclophilins from eukaryotic organisms. *Mol Microbiol* 6, 3551-8 (1992)

136. Pahl, A., A. Gewies and U. Keller: ScCypB is a novel second cytosolic cyclophilin from *Streptomyces chrysomallus* which is phylogenetically distant from ScCypA. *Microbiology* 143, 117-26 (1997)

137. Bua, J., L. Aslund, N. Pereyra, G. A. Garcia, E. J. Bontempi and A. M. Ruiz: Characterisation of a cyclophilin isoform in *Trypanosoma cruzi*. *FEMS Microbiol Lett* 200, 43-7 (2001)

138. High, K. P., K. A. Joiner and R. E. Handschumacher: Isolation, cDNA sequences, and biochemical characterization of the major cyclosporin-binding proteins of *Toxoplasma gondii*. *J Biol Chem* 269, 9105-12 (1994)

139. Kano, R., Y. Nakamura, S. Watanabe, H. Tsujimoto and A. Hasegawa: Characterization of the cyclophilin of *Trichophyton mentagrophytes*. *Microbiol Immunol* 44, 51-6 (2000)

140. Takaki, Y., T. Muta and S. Iwanaga: A peptidyl-prolyl *cis/trans*-isomerase (cyclophilin G) in regulated secretory granules. *J Biol Chem* 272, 28615-21 (1997)

141. Zocher, R., U. Keller, C. Lee and K. Hoffmann: A seventeen kilodaltons peptidyl-prolyl *cis-trans* isomerase of the cyclosporin-producer *Tolypocladium inflatum* is sensitive to cyclosporin A. *J Antibiot (Tokyo)* 45, 265-8 (1992)

142. Luan, S., W. S. Lane and S. L. Schreiber: pCyP B: a chloroplast-localized, heat shock-responsive cyclophilin from *fava bean*. *Plant Cell* 6, 885-92 (1994)

143. Miele, R., M. Borro, M. L. Mangoni, M. Simmaco and D. Barra: A peptidylprolyl *cis/trans* isomerase from *Xenopus laevis* skin: cloning, biochemical characterization and putative role in the secretion. *Peptides* 24, 1713-21 (2003)

144. Sheldon, P. S. and M. A. Venis: Purification and characterization of cytosolic and microsomal cyclophilins from maize (*Zea mays*). *Biochem J* 315, 965-70 (1996)

145. Fanghanel, J. and G. Fischer: Thermodynamic characterization of the interaction of human cyclophilin 18 with cyclosporin A. *Biophys Chem* 100, 351-66 (2003)

146. Zarnt, T., K. Lang, H. Burtscher and G. Fischer: Time-dependent inhibition of peptidylprolyl *cis-trans*-isomerases by FK506 is probably due to *cis-trans* isomerization of the inhibitor's imide bond. *Biochem J* 305, 159-64 (1995)

147. Lam, E., M. M. Martin, A. P. Timerman, C. Sabers, S. Fleischer, T. Lukas, R. T. Abraham, S. J. O'Keefe, E. A. O'Neill and G. J. Wiederrecht: A novel FK506 binding protein can mediate the immunosuppressive effects of FK506 and is associated with the cardiac ryanodine receptor. *J Biol Chem* 270, 26511-22 (1995)

148. Sewell, T. J., E. Lam, M. M. Martin, J. Leszyk, J. Weidner, J. Calaycay, P. Griffin, H. Williams, S. Hung and J. Cryan: Inhibition of calcineurin by a novel FK-506-binding protein. *J Biol Chem* 269, 21094-102 (1994)

149. Futer, O., M. T. DeCenzo, R. A. Aldape and D. J. Livingston: FK506 binding protein mutational analysis. Defining the surface residue contributions to stability of the calcineurin co-complex. *J Biol Chem* 270, 18935-40 (1995)

150. Jin, Y. J., S. J. Burakoff and B. E. Bierer: Molecular cloning of a 25-kDa high affinity rapamycin binding protein, FKBP25. *J Biol Chem* 267, 10942-5 (1992)

151. Ma, D. and C. K. Carlow: Molecular characterization of FKBP13 from filarial parasites. *Mol Biochem Parasitol* 99, 263-7 (1999)

152. Galat, A., W. S. Lane, R. F. Standaert and S. L. Schreiber: A rapamycin-selective 25-kDa immunophilin. *Biochemistry* 31, 2427-34 (1992)

153. Mo, Y. Y., N. P. Cianciotto and L. P. Mallavia: Molecular cloning of a *Coxiella burnetii* gene encoding a macrophage infectivity potentiator (Mip) analogue. *Microbiology* 141, 2861-71 (1995)

154. Lundemose, A. G., J. E. Kay and J. H. Pearce: *Chlamydia trachomatis* Mip-like protein has peptidyl-prolyl *cis/trans* isomerase activity that is inhibited by FK506 and rapamycin and is implicated in initiation of chlamydial infection. *Mol Microbiol* 7, 777-83 (1993)

155. Tradler, T., G. Stoller, K. P. Rucknagel, A. Schierhorn, J. U. Rahfeld and G. Fischer: Comparative mutational analysis of peptidyl prolyl *cis/trans* isomerases: active sites of *Escherichia coli* trigger factor and human FKBP12. *FEBS Lett* 407, 184-90 (1997)

156. Rahfeld, J. U., K. P. Rucknagel, G. Stoller, S. M. Horne, A. Schierhorn, K. D. Young and G. Fischer: Isolation and amino acid sequence of a new 22-kDa FKBP-like peptidyl-prolyl *cis/trans*-isomerase of *Escherichia coli*. Similarity to Mip-like proteins of pathogenic bacteria. *J Biol Chem* 271, 22130-8 (1996)

157. Hottenrott, S., T. Schumann, A. Pluckthun, G. Fischer and J. U. Rahfeld: The *Escherichia coli* SlyD is a metal ion-regulated peptidyl-prolyl *cis/trans*-isomerase. *J Biol Chem* 272, 15697-701 (1997)

158. Iida, T., T. Iwabuchi, A. Ideno, S. Suzuki and T. Maruyama: FK506-binding protein-type peptidyl-prolyl *cis-trans* isomerase from a halophilic archaeum, *Halobacterium cutirubrum*. *Gene* 256, 319-26 (2000)

159. Bang, H., A. Pecht, G. Raddatz, T. Scior, W. Solbach, K. Brune and A. Pahl: Prolyl isomerases in a minimal cell. Catalysis of protein folding by trigger factor from *Mycoplasma genitalium*. *Eur J Biochem* 267, 3270-80 (2000)

160. Furutani, M., T. Iida, S. Yamano, K. Kamino and T. Maruyama: Biochemical and genetic characterization of an FK506-sensitive peptidyl prolyl cis-trans isomerase from a thermophilic archaeon, *Methanococcus thermolithotrophicus*. *J Bacteriol* 180, 388-94 (1998)

161. Solscheid, B. and M. Tropschug: A novel type of FKBP in the secretory pathway of *Neurospora crassa*. *FEBS Lett* 480, 118-22 (2000)

162. Tropschug, M., E. Wachter, S. Mayer, E. R. Schonbrunner and F. X. Schmid: Isolation and sequence of an FK506-binding protein from *N. crassa* which catalyses protein folding. *Nature* 346, 674-7 (1990)

163. Sampson, B. A. and E. C. Gotschlich: *Neisseria meningitidis* encodes an FK506-inhibitble rotamase. *Proc Natl Acad Sci USA* 89, 1164-8 (1992)

164. Ideno, A., M. Furutani, Y. Iba, Y. Kurosawa and T. Maruyama: FK506 binding protein from the hyperthermophilic archaeon *Pyrococcus horikoshii* suppresses the aggregation of proteins in *Escherichia coli*. *Appl Environ Microbiol* 68, 464-9 (2002)

165. Nielsen, J. B., F. Foor, J. J. Siekierka, M. J. Hsu, N. Ramadan, N. Morin, A. Shafiee, A. M. Dahl, L. Brizuela and G. Chrebet: Yeast FKBP-13 is a membrane-associated FK506-binding protein encoded by the nonessential gene FKB2. *Proc Natl Acad Sci USA* 89, 7471-5 (1992)

166. Manning-Krieg, U. C., R. Henriquez, F. Cammas, P. Graff, S. Gaveriaux and N. R. Movva: Purification of FKBP-70, a novel immunophilin from *Saccharomyces cerevisiae*, and cloning of its structural gene, FPR3. *FEBS Lett* 352, 98-103 (1994)

167. Alnemri, E. S., T. Fernandes-Alnemri, K. Pomerenke, N. M. Robertson, K. Dudley, G. C. DuBois and G. Litwack: FKBP46, a novel Sf9 insect cell nuclear immunophilin that forms a protein-kinase complex. *J Biol Chem* 269, 30828-34 (1994)

168. Luan, S., J. Kudla, W. Gruissem and S. L. Schreiber: Molecular characterization of a FKBP-type immunophilin from higher plants. *Proc Natl Acad Sci USA* 93, 6964-9 (1996)

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

170. Peattie, D. A., M. W. Harding, M. A. Fleming, M. T. DeCenzo, J. A. Lippke, D. J. Livingston and M. Benasutti: Expression and characterization of human FKBP52, an immunophilin that associates with the 90-kDa heat shock protein and is a component of steroid receptor complexes. *Proc Natl Acad Sci USA* 89, 10974-8 (1992)

171. Sinars, C. R., J. Cheung-Flynn, R. A. Rimerman, J. G. Scammell, D. F. Smith and J. Clardy: Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes. *Proc Natl Acad Sci USA* 100, 868-73 (2003)

172. Carol, R. L., A. Breimann, N. Erel, P. Vittorioso and C. Bellini: PASTICCINO1 (*At*FKBP70) is a nuclear-localised immunophilin required during *Arabidopsis thaliana* embryogenesis. *Plant Science* 161, 527-535 (2001)

173. Shadidy, M., X. Caubit, R. Olsen, O. M. Seternes, U. Moens and S. Krauss: Biochemical analysis of mouse FKBP60, a novel member of the FKPB family. *Biochim Biophys Acta* 1446, 295-307 (1999)

174. Coss, M. C., D. Winterstein, R. C. Sowder, 2nd and S. L. Simek: Molecular cloning, DNA sequence analysis, and biochemical characterization of a novel 65-kDa FK506-binding protein (FKBP65). *J Biol Chem* 270, 29336-41 (1995)

175. Marquis-Omer, D., G. Sanyal, D. B. Volkin, A. I. Marcy, H. K. Chan, J. A. Ryan and C. R. Middaugh: Stabilization of the FK506 binding protein by ligand binding. *Biochem Biophys Res Commun* 179, 741-8 (1991)

176. DeCenzo, M. T., S. T. Park, B. P. Jarrett, R. A. Aldape, O. Futer, M. A. Murcko and D. J. Livingston: FK506-binding protein mutational analysis: defining the active-site residue contributions to catalysis and the stability of ligand complexes. *Protein Eng* 9, 173-80 (1996)

177. Yang, W., L. W. Rozamus, S. Narula, C. T. Rollins, R. Yuan, L. J. Andrade, M. K. Ram, T. B. Phillips, M. R. van Schravendijk, D. Dalgarno, T. Clackson and D. A. Holt: Investigating protein-ligand interactions with a mutant FKBP possessing a designed specificity pocket. *J Med Chem* 43, 1135-42 (2000)

178. Vitikainen, M., I. Lappalainen, R. Seppala, H. Antelmann, H. Boer, S. Taira, H. Savilahti, M. Hecker, M. Vihinen, M. Sarvas and V. P. Kontinen: Structure-function analysis of PrsA reveals roles for the parvulin-like and flanking N- and C-terminal domains in protein folding and secretion in *Bacillus subtilis*. *J Biol Chem* (2004)

179. Dartigalongue, C. and S. Raina: A new heat-shock gene, ppiD, encodes a peptidyl-prolyl isomerase required for folding of outer membrane proteins in *Escherichia coli*. *Embo J* 17, 3968-80 (1998)

180. Yao, J. L., O. Kops, P. J. Lu and K. P. Lu: Functional conservation of phosphorylation-specific prolyl isomerases in plants. *J Biol Chem* 276, 13517-23 (2001)

181. Metzner, M., G. Stoller, K. P. Rucknagel, K. P. Lu, G. Fischer, M. Luckner and G. Kullertz: Functional replacement of the essential ESS1 in yeast by the plant parvulin DlPar13. *J Biol Chem* 276, 13524-9 (2001)

182. Kops, O., C. Eckerskorn, S. Hottenrott, G. Fischer, H. Mi and M. Tropschug: Ssp1, a site-specific parvulin homolog from *Neurospora crassa* active in protein folding. *J Biol Chem* 273, 31971-6 (1998)

183. Winkler, K. E., K. I. Swenson, S. Kornbluth and A. R. Means: Requirement of the prolyl isomerase Pin1 for the replication checkpoint. *Science* 287, 1644-7 (2000)

Abbreviations: Ac, acetyl; amc, amidomethylcoumarin; Suc, succinyl; pNA, 4-nitroanilide; ame, methylamide; Ome, methylester; DMA, N,N-dimethylacetamide; Me, methyl; CsA, cyclosporin A, PPIase, peptidyl prolyl *cis*/*trans* isomerase; FKBP, FK506 binding protein; KSIE, kinetic solvent isotope effect.

Key Words: Cyclophilin, FKBP, Parvulin, Enzyme mechanism, Review

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