[Frontiers in Bioscience 5, d821-836, September 1, 2000]

ARCHAEAL PEPTIDYL PROLYL *CIS-TRANS* ISOMERASES (PPIases)

Tadashi Maruyama [1] and Masahiro Furutani [2]

[1] Marine Biotechnology Institute, Kamaishi Laboratories, 3-75-1 Heita, Kamaishi-shi, Iwate 026-0001, Japan, [2] Minase Research Institute, Sekisui Chemical Co. Ltd., 2-1, Hyakuyama, Shimamoto-cho, Mishima-gun, Osaka 618-8589, Japan

TABLE OF CONTENTS

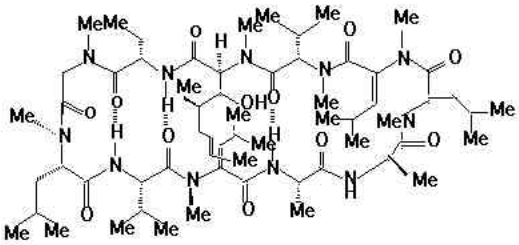
1. ABSTRACT

PPIases are ubiquitous in living organisms. While 3 families of PPIases, cyclophilin (CyP), FK506 binding protein (FKBP) and parvulin (Pvn), have been studied in detail in Eukarya and Bacteria (eubacteria), little is known about archaeal PPIases. Among 2 cyclophilins found in Archaea, only *Halobacterium* cyclophilin (HcCyP19) has been characterized. It is a cyclosporin A (CsA) sensitive CyP with a MW of 19.4kDa. The PPIase activity and CsA sensitivity of this CyP is higher at higher salt concentration in the medium. No parvulin or its homolog has been found in Archaea. Two types of FKBPs, 26-30kDa long type and 17-18 kDa short type FKBP, have been found in Archaea. While the N-terminal regions of these 2 type FKBPs are similar to each other, the long type archaeal FKBP has an additional ca. 100 amino-acid sequence at its C-terminal region. In comparison with human HsFKBP12, the N-terminal region of the archaeal FKBP has 2 insertion sequences in the regions corresponding to Bulge and Flap of HsFKBP12. A short type archaeal FKBP from *Methanococcus thermolithotrophicus* has been shown to have not only a PPIase activity but also a chperone like activity, which includes protein refolding and aggregation suppressing activities with regard to protein folding intermediates. Mutational analysis revealed that this chaperone-like activity was independent of the PPIase activity, and that the insertion sequence in the region corresponding to the Flap seemed to be important.

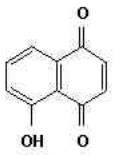
2. OVERVIEW OF PPIase

2.1. History of PPIase discovery

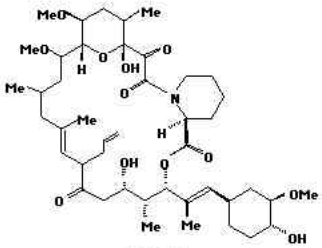
Among peptide bonds in the protein, rotation of prolyl imide bond is the slowest and is thought to be the rate-limiting step in the folding process of proteins. An enzyme which catalyses this slow rotation was found and called peptidyl prolyl *cis-trans* isomerase (PPIase) (1). In 1989, porcine kidney PPIase was purified and its gene was cloned. It was shown to have the same amino-acid sequence with that of bovine cyclophilin (CyP), a target protein for an immunosuppressant, cyclosporin A (CsA) (2, 3). Soon after this discovery, a target protein for FK506 (FKBP: FK506 binding protein), a macrolide immunosuppressant, was also shown to be a PPIase, while amino-acid sequence homology between them was low (4). Another macrolide immunosuppressant, rapamycin (Rap) also binds to FKBP (5). In 1994, the third family of PPIase, parvulin (Pvn), was discovered in *Escherichia coli* (6). This PPIase has low homology to CyPs and FKBPs, and insensitive to either of CsA or FK506. The PPIase is also referred to as rotamase or protein foldase. Because CyPs and FKBPs bind the corresponding immunosuppressants, they are also called immunophilins. Various PPIases in these 3 families have been reported and characterized. Usually more than one gene in each of these families are found in eukaryotic and prokaryotic cells. Even a parasitic bacterium, *Mycoplasma genitalium*, with the smallest known genome of 0.58Mbp has a gene for a FKBP, trigger



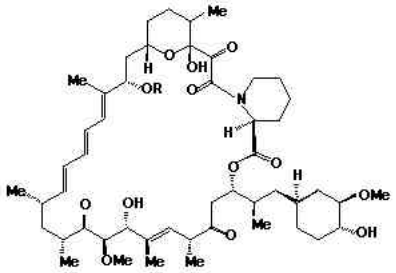
Cyclosporin A



Juglone



FK506



Rapamycin

Figure 1. Chemical structures of PPIase inhibitors.

factor (7). These observations suggest that PPIase plays an essential role in the living cell. However, mutational analysis has revealed that in yeast all of the CyP and FKBP genes are dispensable, indicating that they are not essential for the growth (8). Function of PPIase is still enigmatic. While PPIases have been studied in Eukarya and Bacteria, little is known about PPIases in Archaea. Many members of Archaea live in extreme environments with high temperature, high salt concentration and/or extremely anaerobic conditions. Studies on archaeal PPIases may show the new function of PPIase in the life in extreme environments. Since a variety of PPIases have been reported with different names, it is useful to name them with species name, type of PPIase and molecular weight in kDa. For example, *E. coli* SlyD is called EcFKBP20 (*E. coli* FKBP with molecular weight of 20kDa).

2.2. Three families of PPIase

Three PPIase families have been reported. Cyclophilins (CyP) are PPIases sensitive to CsA (figure 1) or homologs to the prototype CyP, mammalian CyP18. The smallest human CyP is HsCyP18 with molecular weight of 18kDa (9) and the largest one is a giant nucleopore protein, HsCyP350 (Nup358 or nucleoporin), with molecular weight of 350kDa (10). Despite of the wide variety of their molecular weights, most of them have only one PPIase domain. Most of the small CyPs in Eukarya have high binding affinity to CsA; *i.e.*, IC_{50} of human CyP18 against CsA is approximately 6 nM (11). Bacterial CyPs have lower affinities for CsA; 2 CyPs from *E. coli* are insensitive to CsA (12) but *Bacillus subtilis* BsCyP17 is moderately sensitive (IC_{50}=120 nM) (13). Crystallographic analysis has revealed that human HsCyP18 has 8 antiparalell alpha-sheets with 2 short alpha-helices forming a beta-barrel structure (14). 3D structure of human cylcophilin A (HsCyP18) is available in PDB (http://pdb.protein.osaka-u.ac.jp/pdb/cgi/resultBrowser.cgi) with ID of 2CPL. Cyclophilins have been found in multicellular eukaryotes (animals and plants), unicellular eukaryotes, and bacteria except a parasitic bacterium with the smallest known genome, *M. genitalium* (7). Some viruses, such as HIV, contain the CyP of host cell in their virion which is important for starting reproduction after infection to the target cells (15). Large CyPs, which are involved in the interaction with various cellular components have modular structures to execute their function. Human HsCyP40, which is a component of heterocomplex of steroid receptor and hsp90, has three units of TPR (tetratrico-pepetide-repeat) domain and calmodulin binding motif at its C-terminus (16). The TPR is a degenerate 34 amino-acid motif involved in various protein-protein interactions and found in the three domains of living organisms, Eukarya, Bacteria and Archaea (17). The giant human HsCyP358 have a Leu rich region, eight Zn finger domains and RanBP (Ras related nuclear protein binding protein) homologous domains in addition to a cyclophilin domain (10).

PPIases with high binding affinity for FK506 and their homologous proteins are called FK506 binding protein (FKBP). The prototype of this group is human FKBP12 (HsFKBP12) (18). Three dimensional structure of HsFKBP12 has been resolved by X-ray crystallography (19) and NMR (20), and is available at PDB (http://pdb.protein.osaka-u.ac.jp/pdb/cgi/resultBrowser.cgi) with ID of 2FKE. It has five antiparallel beta-strands wrapping around a short alpha-helix. Like large CyPs, large FKBPs have modular structures but with multi-FKBP domains. HsFKB52 is a component of a heterocomplex of hsp90-steroid hormone receptor and has two FKBP-, three TPR- and a calmodulin binding-domains (21). An *E. coli* FKBP, Sly D (EcFKBP20), which is required for the sensitivity to a phage, phiX174, is inhibited by Ni^{2+} and is composed of two domains, a N-terminal FKBP domain and a His rich C-terminal domain (22).

Trigger factor (EcFKBP48) is a member of bacterial FKBPs but has low homology to other FKBPs without detectable binding affinity to FK506 or Rapamycin (23). It binds to the 50S ribosomal subunit and is thought to participate in folding of nascent proteins. Trigger factor has high PPIase activity and is composed of three domains, middle FKBP domain and flanking N- and C-terminal domains. While the truncated middle FKBP domain has high PPIase activity for short peptide substrates, both N- and C-terminal domains are required for high PPIase activity for protein substrates (24). Although the trigger factor itself is not essential for survival of *E. coli*, its depletion in the DnaK lacking mutant results in massive aggregation of cytosolic proteins (25). DnaK and trigger factor probably cooperate to promote proper folding of a variety of proteins in *E. coli*. Genes for the trigger factor or its homolog has been found in the genomes of two hyperthermophilic bacteria, *Aquifex aeolicus* (26) and *Thermotoga maritima* (27).

The third family of PPIase is the Parvulin (Pvn: from Parvulus, very small in Latin) family. The prototype of this family was found as the smallest *E. coli* PPIase with no significant homology with the other two families of PPIases (6). Parvulins have no affinity to the immunosuppressant, FK506 or CsA. Larger parvulins have been reported in Bacteria and Eukarya. SurA (EcPvn47) is a periplasmic *E. coli* parvulin with two parvulin domains which is thought to be involved in the folding of the outer membrane protein, porin (28). Eukaryotic members of this family, human Pin1 (HsPvn18) and yeast ESS1 (ScPvn19) are essential for cell cycle progression (29). Pin1/ESS1 and related larger parvulins have a WW domain, which contains 38-40 amino-acid residues in a triple-stranded antiparallel beta-sheet with two highly conserved tryptophans and functions as a phospho-Ser and phospho-Thr binding module in protein-protein interaction of the signal transduction (30). Recently a specific irreversible inhibitor, Juglone, has been reported for this family (31) (figure 1). Recent genome sequence analyses have revealed that hyperthermophilic bacteria, *Thermotoga maritima* and *Aquifex aeolicus* have two parvulin homologs in each of their genomes (http://www.ncbi.nlm.nih.gov/cgi-bin/COG/palog?BS_prsA).

2.3. Mechanism of immunosuppression by cyclosporin A, FK506 and rapamycin

Inhibition of PPIase activity is not important in the immunosuppressive action of CsA or FK506. Instead, the ligand-PPIase complexes act as inhibitors for signal transduction pathways in the immunological reactions. CsA-cyclophilin complex or FK506-FKBP complex binds and inhibits a protein phosphatase, calcineulin which is involved in the signal transduction to produce a T-cell growth factor, interleukin (IL)-2 (for review, see 32).

Interestingly, rapamycin (Rap) which also binds to FKBP mediates immunosuppression by a different mechanism. The Rap-FKBP complex binds to a protein kinase, TOR (Target of Rapamycin) in yeast or mTOR (mammalian TOR) (for review, see 33). This protein regulates the activity of p70S6 kinase, which is a key enzyme involved in cell-cycle signal transductions. Consequently, the Rap-FKBP complex inhibit IL-2 stimulated growth of T-cells.

2.4. Assay methods for PPIase activity

A chymotrypsin (or trypsin) coupled assay method for PPIase activity was first invented by Fischer *et al.* in 1984 and is widely used (1, 34). Chymotrypsin cleaves only the *trans*-form of the Xaa-Pro bond amino acid (where Xaa is the preceding amino acid) of a small model peptide such as N-succinyl-Ala-Xaa-Pro-Phe-*p*-nitroanilide. In aqueous solution, 90% of Xaa-Pro bond of this molecule is in *trans*-conformation. After addition of excess amount of α-chymotrypsin, the *trans* form of Xaa-Pro bond is cleaved instantaneously. The hydrolysis rate of the remaining 10% Xaa-Pro bond is limited by its *cis* to *trans* isomerization. Thus the *cis-trans* isomerization rate of the model peptide is measured by the release of *p*-nitroanilide by absorption spectroscopy.

A chymotrypsin-free spectrophotometric assay was developed by Janowski (35). In the mixture of trifluoroethanol and LiCl, the percentage of *cis* conformation of the Xaa-Pro bond of a small peptide N-succinyl-Ala-Xaa-Pro-Phe-*p*-nitroanilide increases to 70%. After a solvent jump from this solution to aqueous buffer, *cis-trans* isomerization

A chymotrypsin-free spectrophotometric assay was developed by Janowski (35). In the mixture of trifluoroethanol and LiCl, the percentage of *cis* conformation of the Xaa-Pro bond of a small peptide N-succinyl-Ala-Xaa-Pro-Phe-*p*-nitroanilide increases to 70%. After a solvent jump from this solution to aqueous buffer, *cis-trans* isomerization occurs. *Cis-trans* isomerization is measured by the small difference in absorbance between the *cis* and *trans* forms of the prolyl imide bond in the model peptide. This method is useful to detect the PPIase activity of yeast mitochondrial cyclophilin, Cpr3 (ScCyp20), whose rotamase activity cannot be measured by the conventional chymotrypsin coupled assay (36). NMR spectroscopy is also employed to directly measure the interconversion rate of *cis* and *trans* forms of peptides (37). If the conventional method does not work in some conditions, the other methods might help.

To study the PPIase activity in proteins, ribonuclease T1 (RNaseT1) is a suitable model, because it is completely refoldable and its refolding is rate-limited by prolyl isomerization (38). RNaseT1 is a small peptide of 104 amino acids containing two disulfide bonds (Cys2-Cys10 and Cys6-Cys103), and two *cis* (Tyr38-Pro39 and Ser54-Pro55) peptidyl prolyl bonds. The refolding of 8M urea-denatured RNaseT1 proceeds in a biphasic fashion because of these two *trans* to *cis* prolyl isomerizations. Mücke and Schmid (39, 40) improved this model protein by reduction of its two disulfide bonds and subsequent carboxymethylation. The resulting reduced and carboxymethylated (RCM)-RNaseT1 is unfolded in 0.1M Tris/HCl (pH8.0), and complete refolding is induced by the addition of 1-2 M NaCl. The RCM form of the S54G/P55N variant of RNaseT1 is a suitable substrate for studying PPIase activity in protein folding. Only a single *trans-cis*

isomerization of Pro39 is involved in its folding. This folding system does not require denaturants, such as guanidine hydrochloride or urea which often affect the activities of PPIases even at low concentration.

2.5. PPIase in *in vivo* protein folding

Although a yeast mutant lacking all the cyclophilins and FKBPs has been shown to be viable (8), there has been ample evidence showing that PPIase is involved in protein folding *in vivo*. Cyclosporin A partially inhibits collagen triple-helix formation in chick embryo and human fibroblasts (41). A fruit fly mutant lacking the eye-specific ninaA (DmCyP26) gene has a defect in vision with reduced amount of rhodopsin (42). This cyclophilin was shown to form a complex with a rhodopsin, Rh1, *in vivo* (43). When the *Drosophila* ninaA gene was introduced into an insect cell line Sf9 and expressed, folding of a foreign protein expressed by recombinant baculovirus was improved (44). In *S. cerevisiae*, accumulation of unfolded protein in the endoplasmic reticulum (ER) induced upregulation of FKBP13 (45). A periplasmic parvulin of *E. coli*, Sur A (EcPvn47), was shown to be involved in the folding and assembly of outer membrane protein, porin (28).

In protein folding in living cells, PPIase may collaborate with other chaperones. PPIase is reported to enhance oxidative folding of RNasT1 by protein disulfide isomerase (46). In mouse fibroblast ER and Golgi, CyPB (MmCyP20) and Hsp47 form a complex with a newly synthesized procollagen I and are involved in its folding (47). In *E. coli* cells, trigger factor (EcFKBP48) cooperate with DnaK in nascent protein folding (25).

2.6. PPIase in *in vitro* protein folding

PPIases increase the refolding rate of RNase T1 and its reduced and carboxymethylated derivative RCM-RNaseT1, which are completely refoldable (24, 46, 48).

In addition to this PPIase activity, some PPIases have been suggested to have a chaperone-like activity which prevents aggregation of protein folding intermediates and increases the yield of refolded protein. Porcine kidney CyP (SsCyP18) and NK-TR (HsCyP150) have chaperone-like activity in addition to the PPIase activity (49, 50). Human CyP (HsCyP18), porcine SsCyP18 and human HsFKBP12 were reported to accelerate the speed of refolding of human carbonic anhydrase II but did not prevent its aggregation during refolding (51). Human HsCyP18, HsFKBP12 and bovine serum albumin (BSA) were reported to increase the yield of refolding of antibody Fab fragment *in vitro* even in the presence of the inhibitor CsA (52). This suggested that this effect might be a result of nonspecific protein-protein interaction because they were insensitive to the inhibitor and because BSA also showed the similar effect. Human HsFKBP52, on the other hand, protects porcine heart citrate synthase from thermal aggregation in PPIase independent fashion (53). These somewhat contradictory results indicate that the chaperone-like activity of PPIase is still in question and remains to be studied in more detail.

2.7. Other functions of PPIases

At least one PPIase is found in any one of organisms so far studied. Large variety has been found in the PPIases so far reported. They are probably participated in various biological processes. Some of their possible functions are shown in table 1.

3. STRUCTURE AND FUNCTION OF ARCHAEAL PPIASES

3.1.PPIases in Archaea

The domain Archaea includes microorganisms living under extreme conditions, hyperthermophiles, thermophiles, halophiles and methanogens. There is little available information of archaeal PPIases. A cyclophilin has been found only in a halophilic archaeum, *Halobacterium cutirubrum* (66, 67) and in the genome sequence of *Methanobacterium themoautotrophicum* (68). No cyclophilin gene or its homolog has been found in complete genome sequences of hyperthermophilic archaea, *Methanococcus jannaschii* (69), *Archaeoglobus fulgidus* (70), *Pyrococcus horikoshii* (71) and *Aeropyrum pernix* (72), but one or two FKBPs. Hyperthermophilic bacteria, *Thermotoga maritima* and *Aquifex aeolicus* which are phylogenetically closest to archaea, have a trigger factor and two parvulin homologs (26, 27). However, the gene for trigger factor or its homolog has not been found in archaeal genomes (68-72). Likewise, no gene for parvulin homolog has been found in reported archaeal genome sequences.

Several molecular chaperones, GroE, DnaK, DnaJ, and GrpE cooperate in the protein folding in the bacterial cytosol (73, 74). HSP70 (DnaK homolog) and HSP60 (GroEL homolog) cooperate in the protein folding in mitochondria (75). The genes encoding group II chaperonin (GroEL homolog) are found in all of the archaeal genomes studied. While genes encoding DnaK, DnaJ or GrpE, have been found in mesophilic (76) and some thermophilic archaea, such as *Methanobacterium thermoautotrophicum* (68), they have not been found in the genomes of hyperthermophilic archaea (69-72). Interestingly, the archaea which have Cyp gene also have a gene for HSP70 (67, 68, 76).

3.2. Structure of archaeal PPIases

3.2.1. Cyclophilins in archaea

A cyclophilin (CyP) was purified from a halophilic archaeum, *Halobacterium cutirubrum*. This cyclophilin (HcCyP19) was sensitive to cyclosporin A with the IC_{50} value of 15nM in the presence of 2.9M KCl (66). While this is the only archaeal CyP characterized so far, a gene encoding a CyP-like protein has been found in the genome of a thermophilic archaeum, *M. thermoautotrophicum* (68) (table 2). The alignment of amino-acid sequences of *H. cutirubrum* and *M. thermoautotrophicum* CyPs with those of other eukaryotic and bacterial CyPs is shown in figure 2. The amino-acid sequence of *H. cutirubrum* CyP is 52.9% identical to that of MbtCyP15 and 40-45% to those of eukaryotes and *Bacillus subtilis* with high cyclosporin A-sensitivity, but 27% identical to those of cyclosporin A-insensitive CyPs from *Escherichia coli* (67). HcCyP19 was reported to have a unique 23-amino-acids insertion sequence that showed no

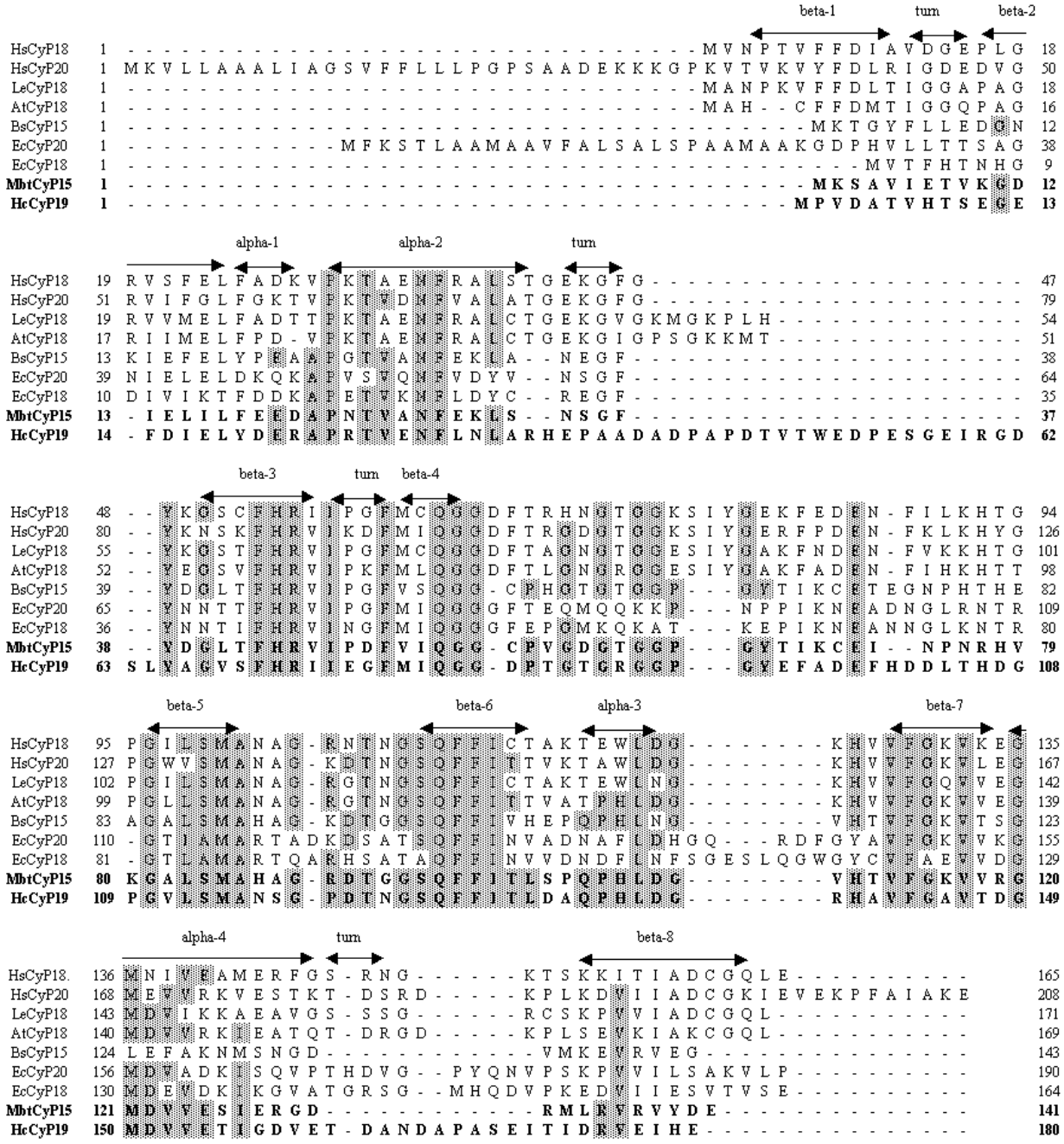


Figure 2. Alignment of archaeal CyP amino acid sequences with those of other bacterial and eukaryotic CyPs. Secondary structure reported for HsCyP18 is shown above the aligned sequences. alpha, alpha helix; beta, beta helix: tau, turn. Amino-acid residues common in archaeal CyPs are shadowed. Archaeal CyPs are shown in bold. Source of CyPs are; HsCyP18 (human); HsCyP20 (human); LeCyP18 (Tomato); AtCyP18 (*Arabidopsis thaliana*); BsCyP15 (*B. subtilis*); EcCyP20 (*E. coli*); EcCyP18 (*E. coli*); MbtCyP15 (*M. thermoatutotrophicum*); HcCyP19 (*H. cutirubrum*)

Table 1. Possible functions of PPIases

Function	PPIase	Cells/Organisms	References
Cell cycle progression	Pin1/ESS1 (HsPar18/ScPar19)	Eukaryotic cells	29
Restoration of Alzheimer's disease associated phophorylated tau to interact with microtubules	Pin 1 (HsPar18)	Human	54
Association with hormone receptors	FKBP52/CyP40	Mammals	55
Apoptosis	Cyclophilins A-C, D	Eukaryotic cells	56, 57
Mitochondrial membrane permeabilization to calcium ion	Cyclophilin D	Eukaryotic cells	57
Calcium ion release from sarcoplasmic reticulum	FKBP12	Mammals	58
Nuclease activity	Cyclophilins A-C	Eukaryotic cells	56
Nucleopore formation and protein transport to nucleus	RanBP2, Nup358	Mammals	10, 59
Protein synthesis and folding of nascent proteins	trigger factor	Bacteria	23, 25
Virulence factor in pathogenic bacteria	MIP[a]	Pathogenic bacteria	60, 61
Possible involvement in parasitism	Cyclophilins	Parasites	62
Virus particle formation and virulence in virus	Cyclophilin A	Virus	63
Nerve regeneration	FKBP52	Mamamls	64
Protection against oxidative stress	Cyclophilin A	Mammalian cells	65
Vision (correct folding of rhodopsin)	NinaA (DmCyP26)	Fruit fly	42

[a], macrophage infectivity potentiator

Table 2. Cyclophilins in Archaea

CyPs	Sources	AA[a]	MW(kDa)[b]	PI[b]	IC50	References
HcCyP19	*Halobacterium cutirubrum*	180	19.4	3.9	15 nM (at 2.9 M KCl), 140 nM (at 1.4 M KCl)	66, 67
MbtCyP15	*Methanobacterium thermoautotrophcum*	141	15.4	5.4	ND[c]	68

[a], amino acid residues; b, calculated using Genetyx-Mac v. 8.0 (Software Development Co.); c, not determined

homology to other CyPs from bacteria and eukaryotes (67). However, this insertion sequence is not found in *Methabacterium thermoautotraphicum* CyP (MbtCyP15). It may be involved in halotolerance of *H. cutirubrum* CyP. While amino-acid sequence homology between MbtCyP15 and HcCyP19 is higher than those between

HcCyP19 and other CyPs, further investigation about archaeal cylophilins are necessary to determine whether there are distinctive feature of archaeal cyclophilins.

3.2.2. FKBPs in archaea

The short-type and the long-type FKBPs have been reported from Archaea. The molecular masses are approximately 17-18 and 26-30 kDa, respectively (68-72, 77, 78) (table 3). While both a short-type and a long-type archaeal FKBP genes have been found in the genome of *Methanococcus jannaschii* (69), only one FKBP gene encoding a long-type FKBP was found in the genomes of *M. thermoautotrophicum* (68), *A. fulgidus* (70), *P. horikoshii* (71), and *Aeropyrum pernix* (72). We isolated genes encoding a short-type FKBP from *Methanococcus thermolithotrophicus* (77) and *Thermococcus* sp. KS-1 (78). It is not clear whether they have the long-type FKBP, or not. The alignment of the amino-acid sequences of archaeal FKBPs with those of eukaryotic and bacterial FKBPs are shown in figure 3. The alignment shows that the archaeal short-type FKBPs has a FKBP domain, and the archaeal long-type FKBP has a FKBP domain with a 100-amino-acids surplus region at the C-terminus. The secondary structures of human FKBP 12 kDa (HsFKBP12) are arranged in the order: (N-terminus)-beta-1-beta-4- beta-5-alpha- beta-2- beta-3-(C-terminus). Between beta-2 and beta-3, a surface loop called “flap” exists, and in the middle of beta-5, an intervening sequence called “bulge” splits beta-5 into two (79). The alignment analysis revealed that the archaeal FKBP domain (short-type FKBP) has a 13-amino acids insertion sequence in the bulge region of hFKBP12, and a 44-amino acids insertion sequence in flap region of hFKBP12. *E. coli* slyD (EcFKBP20) also has insertion sequence homologous to the archaeal 44 amino acids-insertion in the flap region (figure 3). Figure 4 shows the 3-D structure of HsFKBP12 with positions of the insertions.

Two consensus amino-acid sequence motifs for FKBP are found in PROSITE database (http://www.expasy.ch/prosite/). In the amino-acid sequences of long-type archaeal FKBPs, and the 2 short-type FKBPs, TcFKBP18 and MjFKBP18, one of these motifs, [LIVMFY]-X(2)-[GA]-X(3,4)-[LIVMF]-X(2)-[LIVMFHK]-X(2)-G-X(4)-[LIVMF]-X(3)-[PSGAQ]-X (2)-[AG]-[FY]-G (accession no. PS00454) is found, whereas the other motif, [LIVMC]-X-[YF]-X-[GVL]-X(1,2)-[LET]-X(2)-G-X(3)-[DE]-[STAEQK]-[STAN] (accession no. PS00453) is not. They are not found in *Escherichia coli* trigger factor (ecTIG: EcFKBP48) but both of them are found in a short type archaeal FKBP, MtFKBP17 (MTFK), *E. coli* SlyD (EcFKBP20), and HsFKBP12.

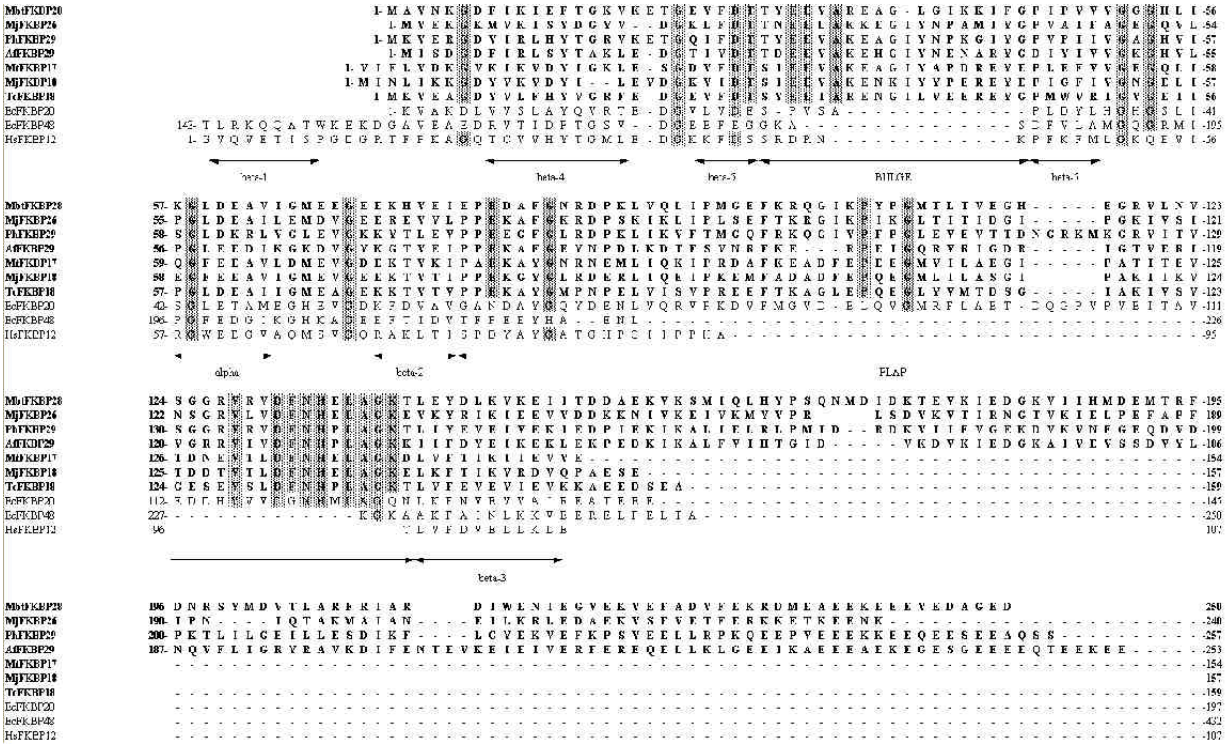


Figure 3. Alignment of archaeal FKBP amino acid sequences with those of other bacterial and eukaryotic FKBPs. Amino-acid residues common in archaeal FKBPs are shadowed. Archael FKBPs are shown in bold. Amino-acid residues common in archaeal FKBPs are shadowed. For the abbreviations for archaeal FKBPs, see table 3. EcFKBP20, *E. coli* slyD; EcFKBP48, *E. coli* trigger factor; HsFKBP12, human FKBP12. Secondary structure of HsFKBP12 is shown below the alignments.

Table 3. FKBPs from Archaea

FKBPs	Sources	AA[a]	MW(kDa)	PI[b]	IC50	Reference
MtFKBP17 (MTFK)	*Methanococcus thermolithotrophicus*	154	17.2	4.1	250 nM	76
MjFKBP18 (MjFKBP-1)	*Methanococcus jannaschii*	157	17.7	4.3	NR[c]	69
TcFKBP18 (TCFK)	*Thermococcus* sp. KS1	159	17.6	4.2	NR[c]	77
MjFKBP26 (MjFKBP-2)	*Methanococcus jannaschii*	231	25.9	7.5	NR[c]	69
MbtFKBP28 (MbFK)	*Methanobacterium thermoautotrophicum*	250	28.3	4.5	NR[c]	68
AfFKBP29	*Archaeoglobus fulgidus*	253	29.0	4.5	NR[c]	70
ApFKBP30	*Aeropyrum pernix*	268	29.6	4.8	NR[c]	72
PhFKBP29	*Pyrococcus horikoshii*	257	29.0	4.9	NR[c]	71
PfFKBP29	*P. furiosus*	258	29.1	4.9	NR[c]	Website[d]
PaFKBP30	*P. abysii*	266	30.0	5.0	NR[c]	CAB49667[e]

[a], amino acid residues; [b], calculated by using Genetyx v. 8.0; [c], not reported; [d], http://combdna.umbi.umd.edu/bags.html; [e], accession number in GenBank, EMBL and DDBJ

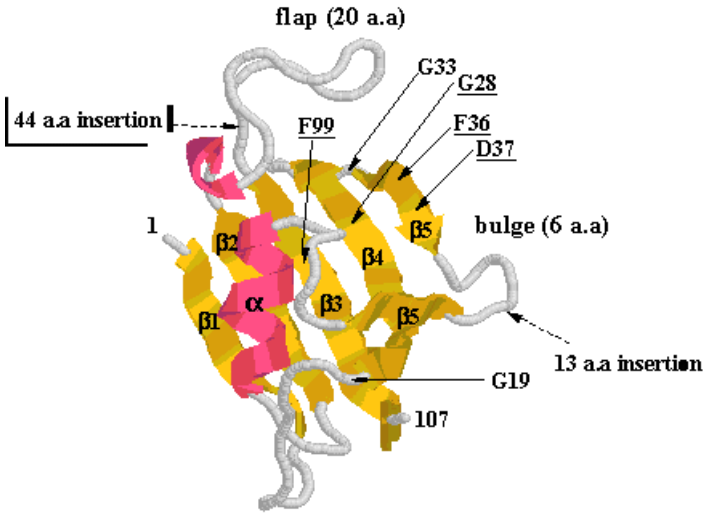


Figure 4. Three dimensional structure of human HsFKBP12 showing the positions of insertion sequences found in MtFKBP17. Positions of amino-acid substitutions in MtFKBP17 mutants are also shown. FK-dB is a deletion mutant lacking the insertion sequence in the region of bulge. FK-dF is the deletion mutant lacking the insertion sequence in the flap region and FK-dBF is the deletion mutant lacking both of these insertion sequences (84).

Table 4. Catalytic efficiencies of PPIases against N-suc-Ala-Xaa-Pro-Phe-p-nitroanilide (Kcat/Km ($mM\text{-}1s^{-1}$)[a]

Xaa	FKBPs					CyPs	
	Methanococcus MtFKBP17	*Thermococcus TcFKBP18*	*Methanobacterium MbtFKBP28*	*Escherichia trigger factor*	*Bovine BtFKBP*	*Escherichia EcCyP18*	*Bovine BtCyP*
Leu	350	350	0.74	430	640	23400	2700
Ala	200	290	0.4	160	50	67400	3180
Leu/Ala[b]	1.75	1.21	1.85	2.69	12.8	0.35	0.71
Reference	76	77	82	23	79	80	79

[a], PPIase activities were measured by the chymotrypsin-coupled assay at 10 or 15°C [b], Ratio of Kcat/Km for N-suc-Ala-Leu-Pro-Phe-pNA to that for N-suc-Ala-Ala-Pro-Phe-Phe-pNA

3.3. PPIase activity of archaeal FKBPs

The PPIase activities of archaeal FKBPs and other PPIases against tetrapeptide susbtrate are shown in table 4. The catalytic efficiencies (k_{cat}/K_m) for N-succinyl-Ala-Leu-Pro-Phe-pNA of MTFK (MtFKBP17) and that of TcFK (TcFKBP18) are 350 $mM^{-1}S^{-1}$ (77, 78). These values are similar to those of *E. coli* trigger factor (430 $mM^{-1}S^{-1}$) (23) and bovine FKBP (640 $mM^{-1}S^{-1}$) (80), and significantly lower than those of *E. coli* CyP18 (23400 $mM^{-1}S^{-1}$) (81) and bovine CyP (2700 $mM^{-1}S^{-1}$) (80). The PPIase activities of MtFKBP17 and TcFKBP18 are inhibited by FK506 with IC_{50} values of 250 nM and 7microM, respectively (77, 78).

A long-type archaeal FKBP, MbtFKBP28 (MbFK) from *M. thermoautotrophicum*, was shown to have a weak PPIase activity (table 4), which was insensitive to FK506 (82). Another long-type FKBP, AfFKBP29 from a hyperthermophilic sulfate reducer, *A. fulgidus*, also exhibited little PPIase activity (Ideno et al. unpublished data). One of two consensus FKBP motifs in PROSITE database (see section II-2) is not found in the protein sequence of MbtFKBP28. The substitution of F99 with Y in human HsFKBP12 (83), and the corresponding substitutions in *E. coli* trigger factor (EcFKBP48) (83), and in MtFKBP17 (84), significantly reduce their PPIase activities. In the protein sequences of MbtFKBP28 and other archaeal long-type FKBPs, the residues corresponding to F99 in HsFKBP12 is substituted with Y (figure 2). This may be the cause of low PPIase activity in MbtFKBP28.

Generally, cyclophilins prefer N-suc-Ala-Ala-Pro-Phe-pNA to N-suc-Ala-Leu-Pro-Phe-pNA, while the reverse is true for FKBPs (table 4). The Leu /Ala values, the ratio of k_{cat}/K_m for N-suc-Ala-Leu-Pro-Phe-pNA versus that for N-suc-Ala-Ala-Pro-Phe-pNA, of thermophilic archaeal FKBPs are 1.2 to 1.85, respectively, while those of other FKBPs are 2.69 to 22.0 (table 4). This may indicate that archaeal FKBPs have low substrate specificy.

At higher temperature, it is expected that the spontaneous rotation rate of peptidyl-prolyl imide bond increases. With increasing temperature (15-35°C), the difference between the rate constant of spontaneous peptidyl-prolyl isomerization of tetrapeptide and that of the MtFKBP17-catalyzed isomerization became smaller (77) (figure 5). This suggests that PPIase activity at least for small peptides is less important at higher temperature. Most of hyperthermophilic or thermophilic archaea have no

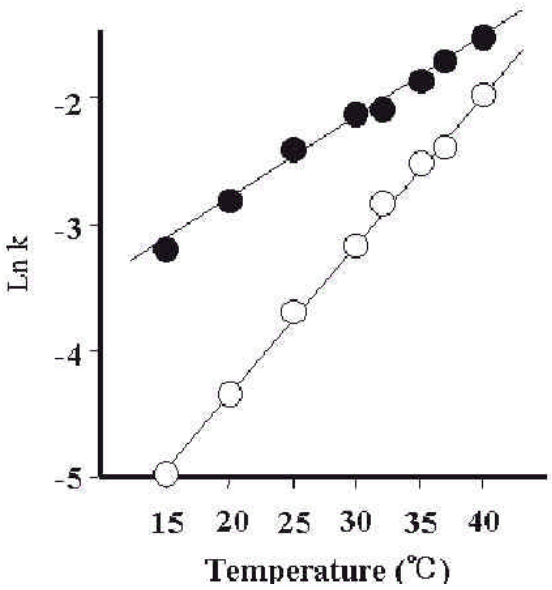


Figure 5. Effect of temperature on the first-order rate constant (K) of *cis-trans* isomerization measured using tetrapeptide substrate and chymotrypsin coupled assay. Open circles, absence of PPIase; closed circles, presence of MTFK (MtFKBP17) (76).

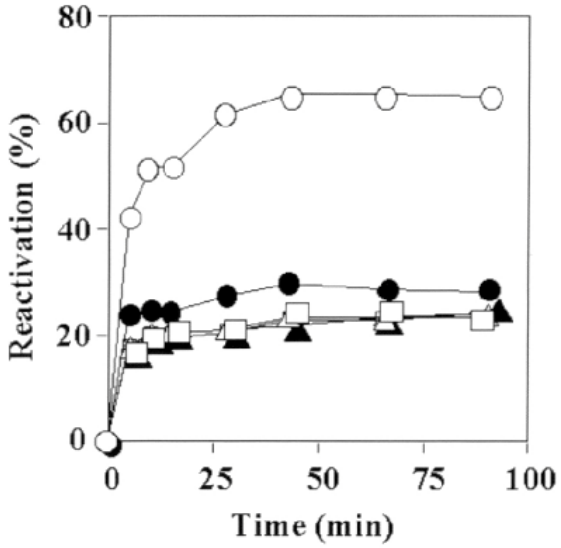


Figure 6. Effect of PPIases on the refolding of denatured rhodanese. The 6 M guanidine hydrochloride-denatured hodanese was diluted 60 fold at time 0 min. with the refolding buffer (10 mM DTT, 50 mM Na-thiosulfate, and 50 mM K-phosphate (pH 7.8) containing PPIase at 25°C. Open aquares, absence of PPIase; open circles, 5 microM recombinant MtFKBP17; closed circles, 5 microM recombinant MtFKBP17 with 20 microM FK506; closed triangles, microM recombinant human HsFKBP12; open triangles, 5 microM bovine BtCyP18 (84).

cyclophilin but FKBP of which PPIase activities are lower than those of other PPIases from mesophiles at low temperature (table 4). These archael FKBPs may have higher activity at higher temperature and/or may have some other functions in addition to the PPIase activity.

3.4. Chaperone-like activity of a short-type archaeal FKBP

Two different model substrate proteins, RNase-T1 (ribonuclease T1) and rhodanese, have been used to study the protein folding activity of a short type archaeal FKBP (84). RNase-T1 is completely refoldable and has two *cis* peptidyl-prolyl bonds (Tyr38-Pro39 and Ser54-Pro55) (38). On the other hand, folding intermediates of rhodanese tend to aggregate and thus rhodanese is convenient to assess the activity of chaperones by measuring their capacity to prevent aggregation of folding intermediates and to increase the yield of properly folded rhodanese. *Escherichia coli* GroE binds to folding intermediates of rhodanese and releases them in an ATP-dependent fashion (85, 86).

MtFKBP17 accelerates the refolding of 8M urea-denatured RNase-T1 in a dose-dependent fashion. This PPIase activity of MtFKBP17 is completely inhibited by FK506 (84). MtFKBP17 protects aggregation of folding intermediates and elevated the final recovery of rhodanese refolding in dose-dependent fashion (figure 6) (84). This is called chaperone-like activity of MtFKBP17 and is partially inhibited by FK506. Neither hFKBP12 nor bovine cyclophilin 18kDa (bCyP18) exhibits chaperone-like activity in rhodanese refolding. The k_{cat}/K_m values (PPIase activity) of HsFKBP12, BtCyP18, and the recombinant MtFKBP17 for N-suc-Ala-Leu-Pro-Phe-pNA are 2.7 (87), 0.64 -2.2 (80, 87), and 0.96 – 1.0 (84) micro $M^{-1}S^{-1}$, respectively. These raise the question whether the PPIase activity of MtFKBP17 contributes the chaperone-like activity or not.

3.5. Deletion analysis of the short-type FKBP from *M. thermolithotrophicus*

The role of the insertion sequences of a short type archaeal FKBP, MtFKBP17, in protein folding has been analyzed by making deletion mutants (84) (figure 4). Catalytic efficiency (k_{cat}/K_m) of PPIase activity of the mutant with deletion of the bulge insertion (FK-dB), or both bulge and flap insertions (FK-dBF), was dramatically reduced to 0.4 and 0.6% as compared with the wild type MtFKBP17 (FK-W), respectively. The PPIase activity of the mutant lacking the flap insertion (FK-dF) was undetectable. The far-UV circular dichroism (CD) spectral analysis revealed that the both bulge and flap insertions are important for a proper conformation of MtFKBP17 (84). While the secondary structure of FK-dB was shown to be changed, 62% of chaperone-like activity of the wild type remained (table 5). On the other hand, FK-dF that has an almost intact secondary structure exhibited little chaperone-like and undetectable PPIase activities. This suggests that the flap insertion sequence is important for the chaperone-like activity of MtFKBP17, and that the contribution of PPIase activity to chaperone-like activity is low.

Table 5. Deletion analysis of MTFK (MtFKBP17)

MTFKs	PPIase activity[a]	Chaperone-like activity[b]
	kcat/Km($\mu M^{-1}S^{-1}$)	% of rhodanese activity[c]
Wild type (FK-W)	0.96 (100%)[d]	64.2+/-2.8 (100%)[d]
FK-W + FK506	0 (0%)[d]	30.0+/-0.7 (16%)[d]
none[e]	0 (0%)[d]	14.1+/-0.4 (0%)[d]
FK-dB	0.004 (0.4%)[d]	45.3+/-1.7 (62%)[d]
FK-dF	undetectable[f] (0%)[d]	14.5+/-0.4 (0.8%)[d]
FK-dBF	0.006 (0.6%)[d]	15.4+/-0.7 (2.6%)[d]

[a], PPIase activity was assayed at 25 °C by the chymotrypsin-coupled assay in 50mM K-phosphate buffer (pH7.5).[b], For chaperone activity, the denatured rhodanese (37.8 microM) was 60-fold diluted in the presence of 5.0 microM mutants, or FK-W in 50mM K-phosphate buffer (pH7.8) containing 10mM DTT and 50mM Na thiosulfate at 35°C. [c], Recovered rhodanese activity after 60min refolding in the presence of FKBPs. Means +/- S.D. (n=3) [d], percentage in parenthesis indicates relative yield of refolded rodanese to that by wild type (FK-W). [e], Spontaneous refolding of rhodanese without PPIase, [f], No PPIase activity was detected with 5 microM mutant MtFKBP17.

Table 6. PPIase and chaperone-like activities of amino-acid substitution mutants of MtFKBP17 (MTFK) from *M. thermolithotrophicus*

MTFKs	PPIase activity	Chaperone-like activity[b]
	[k_{cat}/K_m ($mM^{-1}S^{-1}$)][a]	[% of rhodanese activity][c]
rMtFKBP17[d]	1.0 (100%)[e]	66.7 +/- 3.3 (100%)[e]
FK-dB	0.005 (0.5%)[e]	49.3 +/- 3.4 (65%)[e]
F141Y	0.072 (7.2%)[e]	20.6 +/- 1.6 (8.3%)[e]
F21Y/D22V	0.013 (1.3%)[e]	67.4 +/- 4.1 (101%)[e]
G4R/F21Y/D22V	0.009 (1.0%)[e]	62.7 +/- 3.3 (92%)[e]
G13R/F21Y/D22V	0.006 (0.6%)[e]	32.4 +/- 2.6 (32%)[e]
G18R/F21Y/D22V	0.014 (1.4%)[e]	66.8 +/- 2.5 (100%)[e]
None[f]	n.m.[g]	16.4 +/- 1.5 (0%)[e]

[a], PPIase activity was assayed at 25°C by chmotrypsin-coupled assay in 50mM K-phosphate buffer (pH7.5). [b], For chaperone activity, the denatured rhodanese (37.8microM) was diluted 60-fold in the presence of 5.0microM mutants or wild type recombinant MTFK in 50mM K-phosphate buffer (pH7.8) containing 10mM DTT and 50mM Na thiosulfate at 35°C. [c], Recovered rhodanese activity after 60min refolding in the presence of FKBPs. Mean +/- S.D. (n=3). [d], recombinant wild type MtFKBP17. [e], percentage in the parenthesis indicates relative folding yield to that by wild type (rMtFKBP17). [f], Spontaneous refolding of rhodanese without PPIase. [g], Not measured.

3.6. Is chaperone activity of MtFKBP17 independent of PPIase activity ?

The contribution of MtFKBP17 PPIase activity to chaperone-like activity has been estimated by analyzing amino-acid substitution mutants with reduced PPIase activities (figure 4) (84). The CD spectra of the substitution mutants, F21Y/D22V, G4R/F21Y/D22V, G18R/F21Y/D22V, and F141Y, indicated that the secondary structure was intact in these mutants (84). While the double mutant, F21Y/D22V exhibited 1.3% of the PPIase activity of wild-type (FK-W), it exhibited chaperone-like activity comparable to that of FK-W (table 6) (84). This indicates that F21 and D22 are important for PPIase activity, but not for chaperone-like activity. The triple mutants, both G4R/F21Y/D22V and G18R/F21Y/D22V also exhibited little PPIase activity, but their chaperone-like activities remained almost intact. While PPIase activity of F21Y/D22V was lower than that of F141Y which has a dramatically reduced chaperone-like activity, it showed intact chaperone activity. The amino-acid residue F141 is probably important not only for PPIase but also for chaperone-like activity. These mutation analyses indicate that chaperone-like activity of MtFKBP17 is independent of PPIase activity. The three dimensional (3-D) structure of MtFKBP17 is currently being determined to understand the mechanism underlying its chaperone-like activity (88) .

3.7. The long-type archaeal FKBP

The 28 kDa archaeal long-type FKBP has a FKBP-domain similar to archaeal 17 kDa short-type FKBP, and an additional peptide with 100 amino-acid residues at the C-terminal region. The PPIase activity of MbtFKBP28 was not sensitive to FK506 and was much lower than those of other FKBPs reported (table 4) (82). While A103, E106, and A120 of MtFKBP17 are conserved in other archaeal short-type FKBPs, the corresponding residues are substituted with other amino acids in the long-type FKBPs (82). These substitutions may be the cause for the low PPIase activity of the long type FKBP, MbtFKBP28. However, MbtFKBP28 was recently shown to prevent aggregation of folding intermediates of 8M urea-denatured

rhodanese in a dose dependent-fashion (82). The C-terminal domain of MbtFKBP28 has high content of acidic amino-acid residues (29.0%). The previous studies have revealed that the acidic region of the C-termini of human cyclophilin 40 kDa and HsFKBP52 are important for the interaction with hsp90 (89). The acidic amino acid-rich C-terminal region of MbtFKBP28 may be important for the suppression of protein aggregation (82). Other archaeal long type FKBPs also have acidic amino acid-rich region (figure 3). This suggests that the aggregation-suppressing activity may be a common feature of archaeal long type FKBPs.

4. PERSPECTIVES

In vivo-function of archaeal PPIases is yet enigmatic. In high temperature environments, where hyperthermophiles and thermophiles thrive, PPIase activity may be less important because of high spontaneous rotation rate of the peptidyl-prolyl bond. Besides the PPIase activity, the hyperthermophilic and thermophilic archaeal FKBPs seem to have chaperone-like activity or aggregation-suppression activity that may be more important at high temperature. In addition, hyperthermophilic archaea lack some chaperones like DnaK, DnaJ and GrpE (90) and cyclophilin type PPIase (see the section 3.1). This suggest that these hyperthermophilic archaea do not need these protein folding factors like DnaK and trigger factor, or that the fewer kinds of protein folding factors are multifunctional and fulfil the requirements for the protein folding in the cells of hyperthermophilic archaea, or that they have unknown new protein folding factors. To understand the *in vivo*-function of archaeal PPIases, the following questions are ought to be answered.

In the first place, a question arises whether PPIases, CyP and/or FKBP, are essential for their growth or not. In yeast, all the CyP and FKBP genes except ESS1 (ScPvn19) are dispensable (8, 29). Gene manipulation techniques for hyperthermophilic archaea are required to answer this question, especially considering that for these archaea genetic analysis is not yet fully developed (91).

In *Escherichia coli*, trigger factor (EcFKBP48) is reported to associate with ribosomes (92) and collaborate with DnaK in nascent protein folding (25). Hyperthermophilic archaea lack both the factor and the chaperone in their genomes. It is pertinent to ask whether archaeal FKBP functions as a trigger factor. To answer this question, the *in vitro* translation technique developed for *Sulfolobus solfataricus* (93) may be useful.

A short type archaeal FKBP, MtFKBP17, has chaperone-like activity in addition to the PPIase activity (84). Because little information is available at present, it is not known whether this is a general character of archaeal FKBPs. It is interesting to study the mechanisms underlying the chaperone-like activity of archaeal FKBP. Analysis of the 3-D structure of MBTFKBP17 will contribute to the understanding of the mechanism.

Some enzymes from hyperthermophiles have unique properties that manifest themselves in the face of heat treatment. When *Pyrococcus* glutamate dehydrogenase is expressed in *E. coli* as a recombinant protein, its activity is elevated by heat treatment (94). The heat treatment is thought to affect the monomer structure and facilitate hexamer formation (95). This may indicate that these proteins are in a transient metastable state at lower temperature and require heat energy to take a proper structure. It would be interesting whether chaperones or PPIases are involved in this transition from the intermediate or metastable state to a properly-folded stable enzyme.

Expression of yeast CyP1 (ScCyP17) is heat inducible but that of FKBP1 (ScFKBP12) is not (96). Disruption of the CyP1 gene decreases the survival at high temperature. Expression of the *Vicia fava* (plant) FKBP (VfFKBP13) is also heat inducible (97). On the other hand, *E. coli* trigger factor (EcFKBP48) is a cold-shock protein and is thought to contribute to viability at low temperature (98). It is not yet clear whether the archaeal CyP and/or FKBP are a cold- or heat-shock protein or not.

Many PPIases are known to bind to other proteins to form a heterocomplex. Human FKBP52 (HsFKBP52) and HsCyP40 bind to steroid receptor (16, 21). No WW- or TRP- or Leu rich-domain has been found in archaeal FKBPs or CyPs. However, it is possible that the PPIase bind to other cellular proteins to form a heterocomplex. Cross-linking experiments may answer this question.

Although some archaea, especially hyperthermophilic archaea, lack some chaperones, they have group II chaperonin, small heat shock protein and prefoldin (for review see 76). It is still a question whether archaeal PPIase (CyP or FKBP) collaborate with these protein folding factors in protein folding.

What is the functional differentiation between CyPs and FKBPs in archael cells? Most hyperthermophilic archaea so far studied have only one PPIase (FKBP). However, *M. thermoautotrophicum* have both CyP and FKBP, and *M. jannaschii* have two types of FKBPs. Functional differentiation between these PPIases remains to be elucidated.

5. ACKNOWLEDGEMENTS

A. Ideno is acknowleged for critical discussion on the manuscript. We thank T. Iwabuchi for drawing the chemical structures of PPIase inhibitors. We are grateful to E. Conway de Macario for critical comments on the manuscript. This study was conducted at the Marine Biotechnology Institute of Japan as part of The Basic Knowledge Creation and Development program supported by the New Energy and Industrial Technology Development Organization of Japan.

6. REFERENCES

1. Fischer, G., J.Bang, & C. Mech: Nachweis einer Enzymkatalyse fur die *cis-trans*-isomerisierung der

Peptidbindung in prolinhaltigen Peptiden. *Biomed Biochem Acta* 43, 1001-1111 (1984)

2. Fischer, G., B. Wittmann-Liebold, K. Lang, T. Kiefhaber & F. X. Schmid: Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. *Nature* 337, 476-478 (1989)

3. Takahashi, N., T. Hayano & M. Suzuki: Peptidyl-prolyl *cis-trans* isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337, 473-475 (1989)

4. Harding, M. W., A. Galat, D.E. Uehling & S. L. Schreiber: A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 341,758-760 (1989)

5. Bierer, B. E., P.K. Somers, T.J. Wandless, S. J. Burakoff & S. L. Schreiber: Probing immunosuppressant action with a nonnatural immunophilin ligand. *Science* 250, 556-559 (1990)

6. Rahfeld, J.U., K.P. Rucknagel, B. Schelbert, B. Ludwig, J. Hacker, K. Mann & 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* 352,180-184 (1994)

7. Fraser, C.M., J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, J. L. Fritchman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Uttervack, D. M. Saudek, C. A. Phillips, J. M. Merrick, J. T. Brian, A. Dougherty, K. F. Bott, P-C. Hu, T. S. Jucier, S. N. Peterson, J. O. Smith, C. A. Hutchison III & J. C. Venter: The minimal gene complement of *Mycoplasma genitalium*. *Science* 270, 397-403 (1995)

8. Dolinski, K., S. Muir, M. Cardenas & J. Heitman: All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA*. 94, 13093-13098 (1997)

9. Haendler, B., R. Hofer-Warbinek & E. Hofer: Complementary DNA for human T-cell cyclophilin. *EMBO J* 6, 947-950 (1987)

10. Wu, J., M. J. Matunis, D. Kraemer, G. Blobel & E. Coutavas: Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. *J Biol Chem* 270,14209-14213 (1995)

11. Liu, J., C. M. Chen & C. T. Walsh: Human and *Escherichia coli* cyclophilins: sensitivity to inhibition by the immunosuppressant cyclosporin A correlates with a specific tryptophan residue. *Biochemistry* 30, 2306-2310 (1991)

12. Hayano, T., N.Takahashi, S. Kato, N. Maki & M. Suzuki: Two distinct forms of peptidylprolyl-cis-trans-isomerase are expressed separately in periplasmic and cytoplasmic compartments of *Escherichia coli* cells. *Biochemistry* 30, 3041-3048 (1991)

13. Göthel, S. F., M. Herrler & M. Marahiel: Peptidyl-prolyl cis-trans isomerase of *Bacillus subtilis*: identification of residues involved in cyclosporin A affinity and catalytic efficiency. *Biochemistry* 35, 3636-3640. (1996)

14. Ke, H. M., L. D. Zydowsky, J. Liu & C. J. Walsh: Crystal structure of recombinant human T-cell cyclophilin A at 2.5 Å resolution. *Proc Natl Acad Sci USA* 88, 9483-9787 (1991)

15. Braaten, D., E. K. Franke & J. Luban: Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *J Virol* 70, 3551-3560 (1996)

16. Ratajczak, T., A. Carrello, P. J. Mark, B. J. Warner, R. J. Simpson, R. L. Moritz & A. K. House: The cyclophilin component of the unactivated estrogen receptor contains a tetratricopeptide repeat domain and shares identity with p59 (FKBP59). *J Biol Chem* 268, 13187-13192 (1993)

17. Kyrpides, N. C. & C. R. Woese: Tetratrico-peptide-repeat proteins in the archaeon *Methanococcus jannaschii*. *Trends Biochem Sci* 23, 245-247 (1998)

18. Maki, N., F. Sekiguchi, J. Nishimaki, K. Miwa, T. Hayano, N. Takahashi & M. Suzuki: Complementary DNA encoding the human T-cell FK506-binding protein, a peptidylprolyl *cis-trans* isomerase distinct from cyclophilin. *Proc Natl Acad Sci USA* 87, 5440-5443 (1990)

19. Van Duyne, G. D., R. F. Standaert, P. A. Karplus, S. L. Schreiber & J. Clardy: Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. *Science* 252, 839-842 (1991)

20. Moore, J. M., D. A. Peattie, M. J. Fitzgibbon & J. A. Thomson: Solution structure of the major binding protein for the immunosuppressant FK506. *Nature* 351, 248-250 (1991)

21. Peattie, D. A., M. W. Harding, M. A. Fleming, M. T. DeCenzo, J. A. Lippke, D. J. Livingston & 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, 10975-10978 (1992)

22. Hottenrott, S., T. Schumann, A. Pluckthun, G. Fischer & J. U. Rahfeld: The *Escherichia coli* SlyD is a metal ion-regulated peptidyl-prolyl cis/trans-isomerase. *J Biol Chem* 272, 15697-15701 (1997)

23. Stoller, G., K. P. Rücknagel, K. H. Nierhaus, F. X. Schmid, G. Fischer & J. U. Rahfeld: A ribosome-associated peptidyl-prolyl cis/trans isomerase identified as the trigger factor. *EMBO J* 14, 4939-4948 (1995)

24. Zarnt, T., T. Tradler, G. Stoller, C. Scholz, F. X. Schmid & G. Fischer: Molecular structure of the trigger factor required for high affinity in protein folding. *J Mol Biol* 271, 827-837 (1997).

25. Deuerling, E., A. Schulze-Specking, T. Tomoyasu, A. Mogk & B. Bukau: Trigger factor and DnaK cooperate in folding of newly synthesized proteins. *Nature* 400, 693-696 (1999)

26. Deckert, G., P. V. Warren, T. Gaasterland, W. G. Young, A. L. Lenox, D. E. Graham, R. Overbeek, M. A. Snead, M. Keller, M. Aujay, R. Huber, R. A. Feldman, J. M. Short, G. J. Olson & R. V. Swanson: The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. *Nature* 392, 353-358 (1998)

27. Nelson, K. E., R. A. Clayton, S. R. Gill, M. L. Gwinn, R. J. Dodson, D. H. Haft, E. K. Hickey, J. D. Peterson, W. C. Nelson, K. A. Ketchum, L. McDonald, T. R. Utterback, J. A. Malek, K. D. Linher, M. M. Garrett, A. M. Stewart, M. D. Cotton, M. S. Pratt, C. A. Phillips, D. Richardson, J.Heidelberg, G. G. Sutton, R. D. Fleischmann, O. White, S. L. Salzberg, H. O. Smith, J. C. Venter & C. M. Fraser: Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima. Nature* 399, 323-329 (1999)

28. Rouvière, P. E & C. A. Gross: SurA, a periplasmic protein with peptidyl-prolyl isomerase activity, participates in the assembly of outer membrane porins. *Genes Dev* 10, 3170-3182 (1996)

29. Lu, K. P, S. D. Hanes & T. Hunter: A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature* 380, 544-547 (1996)

30. Lu, P. J., X. Z. Zhou, M. Shen & K. P. Lu: Function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science* 283, 1325-1328 (1999)

31. Hennig, L., C. Christner, M. Kipping, B. Schelbert, K. P. Rucknagel, S. Grabley, G. Kullertz & G. Fischer: Selective inactivation of parvulin-like peptidyl-prolyl *cis/trans* isomerases by juglone. *Biochemistry* 37, 5953-5960 (1998)

32. Schreiber, S. & G. R. Crabtree: The mechanism of action of cyclosporin A and FK506. *Immunology Today* 13, 136-142 (1992)

33. Abraham, R. T. & G. J. Wiederrecht: Immunopharmacology of rapamycin. Annu *Rev Immunol* 14, 483-510 (1996)

34. Harisson, R. K. & R.L. Stein: Mechanistic studies of peptidyl prolyl *cis-trans* isomerase: evidence for catalysis by distortion. *Biochemistry* 29, 1684-1689 (1990)

35. Janowski, B., M. Wöllner, M. Schutkowski & G. Fischer: A protease-free assay for peptidyl prolyl *cis/trans* isomerase using standard peptide substrates. *Anal Biochem* 252, 299-307 (1997)

36. Scholz, C., T. Schindler, K. Dolinski, J. Heitman & F. X. Schmid: Cyclophilin active site mutants have native prolyl isomerase activity with a protein substrate. *FEBS Lett* 414, 69-73 (1997)

37. Kern, D., G. Kern, G. Scherer, G. Fischer & T. Drakenberg: Kinetic analysis of cyclophilin-catalyzed prolyl cis/trans isomerization by dynamic NMR spectroscopy. *Biochemistry* 34, 13594-13602 (1995)

38. Schönbrunner, E.R., S. Mayer, M. Tropschung, G. Fischer, N. Takahashi & F.X. Schmid: Catalysis of protein folding by cyclophilins from different species. *J Biol Chem* 266, 3630-3635 (1991)

39. Mücke, M. & F.X. Schmid: Enzymatic catalysis of prolyl isomerization in an unfolding protein. Biochemistry 31, 7848-7854 (1992)

40. Mücke, M. & F.X. Schmid: Folding mechanism of ribonuclease T1 in the absence of the disulfide bonds. Biochemistry 33, 14608-14619 (1994)

41. Steinmann, F., P. Bruckner & A. Superti-Furga: Cyclosprin A slows collagen triple-helix formation *in vivo*: indirect evidence for a physiologic role of peptidyl-prolyl *cis-trans* isomerase. *J Biol Chem* 266, 1299-1303 (1991)

42. Schneuwly, S., R. D. Shortridge, D. C. Larrivee, T. Ono, M. Ozaki & W. L. Pak: *Drosophila* ninaA gene encodes an eye-spcific cyclophilin (cyclosporine A binding protein). *Proc Natl Acad Sci USA* 86, 5390-5394 (1989)

43. Baker, E. K., N. J. Colley & C. S. Zuker: The cyclophilin homolog NinaA functions as a chaperone, forming a stable complex *in vivo* with its protein target rhodopsin. *EMBO J* 13,4886-4895 (1994)

44. Lenhard, T. & H. Reilander: Engineering the folding pathway of insect cells: generation of a stably transformed insect cell line showing improved folding of a recombinant membrane protein. *Biochem Biophys Res Commun* 238, 823-30 (1997)

45. Partaledis, J. A. & V. Berlin: The FKB2 gene of *Saccharomyces cerevisiae*, encoding the immunosuppressant-binding protein FKBP-13, is regulated in response to accumulation of unfolded proteins in the endoplasmic reticulum. *Proc Natl Acad Sci USA* 90, 5450-5454 (1993)

46. Schönbrunner, E. R. & F. X. Schmid: Peptidyl-prolyl *cis-trans* isomerase improves the efficiency of protein disulfide isomerase as a catalyst of protein folding. *Proc Natl Acad Sci USA* 89, 4510-4513 (1992)

47. Smith, T., L. R. Ferreira, C. Hebert, K. Norris & J. J. Sauk: Hsp47 and cyclophilin B traverse the endoplasmic

reticulum with procollagen into pre-Golgi intermediate vesicles. A role for Hsp47 and cyclophilin B in the export of procollagen from the endoplasmic reticulum. *J Biol Chem* 270, 18323-18328 (1995)

48. Scholz, C., J. Rahfeld, G. Fischer & F.X. Schmid: Catalysis of protein folding by parvulin. *J Mol Biol* 273, 752-762 (1997).

49. Freskgård, P. O., N. Bergenhem, B. H. Jonsson, M. Svensson & U. Carlsson: Isomerase and chaperone-like activity of prolyl isomerase in the folding of carbonic anhydrase. *Science* 258, 466-468 (1992)

50. Rinfret, A., C. Collins, R. Menard & 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, 1668-1673 (1994)

51. Kern, G., D. Kern, F. X. Schmid & G. Fischer: Reassessment of the putative chaperone function of prolyl-*cis/trans*-isomerases. *FEBS Lett* 348, 145-148 (1994)

52. Lilie, H., K. Lang, R. Rudolph & J. Buchner: Prolyl isomerases catalyze antibody folding *in vitro*. *Protein Sci* 2, 1490-1496 (1993)

53. Bose , S., T. Weikl, H. Bügl & J. Buchner: Chaperone function of hsp90-associated proteins. Science 274, 1715-1717 (1996)

54. Lu, P. J., G. Wulf, X. Z. Zhou, P. Davies, K. P. Lu: The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* 399, 784-788 (1999)

55. Pratt, W.B. & D. O. Toft: Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev* 18, 306-360 (1997)

56. Montague, J.W., F. M. Jr. Hughes, J. A. Cidlowski: Native recombinant cyclophilins A, B, and C degrade DNA independently of peptidylprolyl *cis-trans*-isomerase activity. Potential roles of cyclophilins in apoptosis. *J Biol Chem* 272, 6677-6684 (1997)

57. Crompton, M.: The mitochondrial permeability transition pore and its role in cell death. *Biochem J* 341, 233-249 (1999)

58. Marks, A.: Cellular functions of immunophilins. *Physiol Rev* 76, 631-649 (1996)

59. Singh, B. B., H. H. Patel, R. Roepman, D. Schick & P. A. Ferreira: The zinc finger cluster domain of RanBP2 is a specific docking site for the nuclear export factor, exportin-1. *J Biol Chem* 274, 37370-37378 (1999)

60. Fischer, G., H. Bang, B. Ludwig, K. Mann & J. Hacker: Mip protein of *Legionella pneumophila* exhibits peptidyl-prolyl-cis/trans isomerase (PPlase) activity. *Mol Microbiol* 6, 1375-1383 (1992)

61. Lyon, W. R., C. M. Gibson & M. G. Caparon: A role for trigger factor and an rgg-like regulator in the transcription, secretion and processing of the cysteine proteinase of *Streptococcus pyogenes*. *EMBO J* 17, 6263-6275 (1998)

62. Bell, A., H. C. Roberts & L. H. Chappell: The antiparasite effects of cyclosporin A: possible drug targets and clinical applications. *Gen Pharmacol* 27, 963-971 (1996)

63. Aiken, C.: Mechanistic independence of Nef and cyclophilin A enhancement of human immunodeficiency virus type 1 infectivity. *Virology* 248, 139-147 (1998)

64. Gold, B. G.: FK506 and the role of the immunophilin FKBP-52 in nerve regeneration. *Drug Metab Rev* 31, 649-663 (1999)

65. Doyle, V., S. Virji & M. Crompton: Evidence that cyclophilin-A protects cells against oxidative stress. *Biochem J* 341. 127-132 (1999)

66. Nagashima, K., S. Mitsuhashi, K. Kamino & T. Maruyama: Cyclosporin A sensitive A sensitive peptidyl prolyl *cis-trans* isomerase in a halophilic archaeum, *Halobacterium cutirubrum*. *Biochem Biophys Res Comn* 198, 466-472 (1994)

67. Iida, T., M. Furutani, T. Iwabuchi & T. Maruyama: Gene for a cyclophilin–type peptidyl-prolyl *cis-trans* isomerase from a halophilic archaeum, *Halobacterium cutirubrum*. Gene 204**,** 139-144 (1997)

68. Smith, D. R., L. A. Doucette-Stamm, C. Deloughery, H. Lee, J. Dubois, T. Aldredge, R. Bashirzadeh, D. Blakely, R. Cook, K. Gilbert, D. Harrison, L. Hoang, P. Keagle, W. Lumm, B. Pothier, D. Qiu, R. Spadafora, R. Vicaire, Y. Wang, J. Wierzbowski, R. Gibson, N. Jiwani, A. Caruso, D. Bush, H. Safer, D. Patwell, S. Prabhakar, S. Mcdougall, G. Shimer, A. Goyal, S. Pietrokovski, G. M. Church, C. J. Daniels, J-I. Mao, P. Rice, J. Nölling & J. N. Reeve: Complete genome sequence of *Methanobacterium thermoautotrophicum* deltaH: functional analysis and comparative genomics. *J Bacteriol* 179, 7135-55 (1997)

69. Bult, C. J., O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. FitzGerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, B. A. Dougherty, J. F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. F. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodek, J. L. Scott, N. S. M. Geoghagen, J. F. Weidman, J. L. Fuhrmann, D. Nguyen, T. R. Utterback, J. K. Kelley, J. D. Peterson, P. W. Sadow, M. C. Hanna, M. D. Cotton, K. M. Roberts, M. A. Hurst, B. P. Kaine, M. Borodovsky, H-P. Klenk, C. M. Fraser, H. O. Smith, C. R. Woese & J. C. Venter: Complete genome sequence of the methanogenic archaeum, *Methanococcus jannaschii*. *Science* 273, 1058-1073 (1996)

70. Klenk, H. P., R. A. Clayton, J. F. Tomb, O. White, K. E. Nelson, K. A. Ketchum, R. J. Dodson, M. Gwinn, E. K.

Hickey, J. D. Peterson, D. L. Richardson, A. R. Kerlavage, E. Graham, N. C. Kyrpides, R. D. Fleischmann, J. Quackenbush, N. H. Lee, G. G. Sutton, S. Gill, E. F. Kirkness, B. A. Dougherty, K. McKenney, M. D. Adams, B. Loftus, S. Peterson, C. I. Reich, L. K. McNeil, J. H. Badger, A. Glodek, L. Zhou, R. Overbeek, J. D. Gocayne, J. F. Weidman, L. McDonald, T. Utterback, M. D. Cotton, T. Spriggs, P. Artiack, B. P. Kaine, S. M. Sykes, P. W. Sadow, K. P. D'Andrea, C. Bowman, C. Fujii, S. A. Garland, T. M. Mason, G. J. Olsen, C. M. Fraser, H. O. Smith, C. R. Woese & J. C. Venter: The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature* 390, 364-370 (1997)

71. Kawarabayasi, Y., M. Sawada, H. Horikawa, Y. Haikawa, Y. Hino, S. Yamamoto, M. Sekine, S. Baba, H. Kosugi, A. Hosoyama, Y. Nagai, M. Sakai, K. Ogura, R. Otsuka, H. Nakazawa, M. Takamiya, Y. Ohfuku, T. Funahashi, T. Tanaka, Y. Kudoh, J. Yamazaki, N. Kushida, A. Oguchi, K. Aoki, T. Yoshizawa, Y. Nakamura, F. T. Robb, K. Horikoshi, Y. Masuchi, H. Shizuya & H. Kikuchi: Complete sequence and gene organization of the genome of a hyper-thermophilic archaebacterium, *Pyrococcus horikoshii* OT3. *DNA Res* 5, 55-76 (1998)

72. Kawarabayasi, Y., Y. Hino, H. Horikawa, S. Yamazaki, Y. Haikawa, K. Jin-no, M. Takahashi, M. Sekine, S. Baba, A. Ankai, H. Kosugi, A. Hosoyama, S. Fukui, Y. Nagai, K. Nishijima, H. Nakazawa, M. Takamiya, S. Masuda, T. Funahashi, T. Tanaka, Y. Kudoh, J. Yamazaki, N. Kushida, A. Oguchi, K. Aoki, K. Kubota, Y. Nakamura, N. Nomura, Y. Sako & H. Kikuchi: Complete genome sequence of an aerobic hyper-thermophilic crenarchaeon, *Aeropyrum pernix* K1. *DNA Res* 6, 83-101, 145-152 (1999)

73. Szabo, A., T. Langer, H. Schroder, J. Flanagan, B. Bukau & F. U. Hartl: The ATP hydrolysis-dependent reaction cycle of the *Escherichia coli* Hsp70 system DnaK, DnaJ, and GrpE. *Proc Natl Acad Sci USA* 91, 10345-10349 (1994)

74. Langer, T., C. Lu, H. Echols, J. Flanagan, M.K. Hayer & F. U. Hartl: Successive action of DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding. *Nature* 356, 683-689 (1992)

75. Stuart, R.A., D. M. Cyr, E. A. Craig & W. Neupert: Mitochondrial molecular chaperones: their role in protein translocation. *Trends Biochem. Sci.* 19, 87-92 (1994)

76. Macario, A. J., M. Lange, B. K. Ahring, E. Conway de Macario: Stress genes and proteins in the archaea. *Microbiol Mol Biol Rev* 63, 923-967 (1999)

77. Furutani, M, T. Iida, S. Yamano, K. Kamino & T. Maruyama: Biochemical and genetic characterization of an FK506-sensitive peptidyl prolyl *cis-trans* isomerase from a thermophilic archaeon, *Methanococcus thermolithotrophicus. J Bacteriol* 180, 388-394 (1998)

78. Iida, T., M. Furutani, F. Nishida & T. Maruyama: FKBP-type peptidyl *cis-trans* isomerase from a sulfer-dependent hyperthermophilic archaeon, *Thermococcus* sp. KS-1. *Gene* 222, 249-255 (1998)

79. Callebaut, I. & J.-P. Mornon: Trigger factor, one of the *Escherichia coli* chaperone proteins, is an original member of the FKBP family. *FEBS Lett* 374, 211-215 (1995)

80. Harrison, R.K.& R.L. Stein: Substrate specifities of the peptidyl prolyl *cis-trans* isomerase activities of cyclophilin and FK-506 binding protein: evidence for existence of a family of distinct enzymes. *Biochemistry* 29, 3813-3816 (1990)

81. Compton, L. A., J. M. Davis, J. R. Macdonald & H. P. Bächinger: Structural and functional characteriztion of *Escherichia coli* peptidyl-prolyl *cis-trans* isomerases. *Eur J Biochem* 206, 927-934 (1992)

82. Ideno, A., T. Yoshida, M. Furutani & 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, 3139-3149 (2000)

83. Tradler, T., G. Stoller, K.P. Rücknagel, A. Schierhon, J.-U. Rahfeld & G. Fischer: Comparative mutational analysis of peptidyl prolyl *cis/trans* isomerase: active sites of *Escherichia coli* trigger factor and human FKBP12. *FEBS Lett* 407, 184-190 (1997)

84. Furutani, M., A. Ideno, T. Iida & T. Maruyama: FK506 binding protein from a thermophilic archaeon, *Methanococcus thermolithotrophicus* has chaperone-like activity *in vitro*. *Biochemistry* 39, 453-462 (2000)

85. Martin, J., T. Langer, R. Boteva, A. Schramel, A. L. Horwich & F. U. Hartl: Chaperonin-mediated protein folding at the surface of groEL through a 'molten globule'-like intermediate. *Nature* 352, 36-42 (1991)

86. Hayer-Hartl, M. K., J. Martin & F. U. Hartl: Asymmetrical interaction of GroEL and GroES in the ATPase cycle of assisted protein folding. *Science* 269, 836-841 (1995)

87. Fischer, G.: Peptidyl-prolyl *cis/trans* isomerases and their effectors. *Angew Chem Int Ed* 33, 1415-1436 (1994)

88. Suzuki, R., K. Nagata, M. Kawakami, N. Nemoto, M. Furutani, K. Adachi, T. Maruyama & M. Tanokura: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of FKBP from *Methanococcus thermolithotrophicus. J Biomol NMR* (in press) (2000)

89. Ratajczak, T. & A. Carrello: Cyclophilin 40 (CyP-40), mapping of its hsp90 binding domain and evidence that FKBP52 competes with CyP-40 for hsp90 binding. *J Biol Chem* 271, 2961-2965 (1996)

90. Macario, A. J. L. & E. Conway de Macario: The archaeal molecular chaperone machine: peculiarities and paradoxes. *Genetics* 152, 1277-1283 (1999)

91. Noll, K. M. & M. Yargas: Recent advances in genetic analyses of hyperthermophilic Archaea and Bacteria. *Arch Microbiol* 168, 73-80 (1997)

92. Hesterkamp, T., S. Hauser, H. Lutcke & B. Bukau: *Escherichia coli* trigger factor is a prolyl isomerase that associates with nascent polypeptide chains. *Proc Natl Acad Sci USA* 93, 4437-4441 (1996)

93. Condo, I., A. Ciammaruconi, D. Benelli, D. Ruggero & P. Londei: *Cis*-acting signals controlling translational initiation in the thermophilic archaeon *Sulfolobus solfataricus*. *Mol Microbiol* 34, 377-384 (1999)

94. Diruggiero, J. & F. T. Robb: Expression and *in vitro* assembly of recombinant glutamate dehydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus*. *Appl Environ Microbiol* 61, 159-64 (1995)

95. Rahman, R. N., S. Fujiwara, M. Takagi, S. Kanaya & T. Imanaka: Effect of heat treatment on proper oligomeric structure formation of thermostable glutamate dehydrogenase from a hyperthermophilic archaeon. *Biochem Biophys Res Commun* 241, 646-652 (1997)

96. Sykes, K., M. J. Gething & J. Sambrook: Proline isomerases function during heat shock. *Proc Natl Acad Sci USA* 90, 5853-5857 (1993)

97. Luan, S., J. Kudla, W. Gruissem & S. L. Schreiber: Molecular characterization of a FKBP-type immunophilin from higher plants. *Proc Natl Acad Sci USA* 93, 6964-6969 (1996)

98. Kandror, O. & A. L. Goldberg: Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures. *Proc Natl Acad Sci USA* 94, 4978-81 (1997)

Key Words: PPIase, FKBP, cyclophilin, protein folding, chaperone, Archaea, Review

Send correspondence to: Dr Tadashi Maruyama, Marine Biotechnology Institute, Kamaishi Laboratories,3-75-1 Heita, Kamaishi-shi, Iwate 026-0001, Japan, Tel: +81-193-26-5814, Fax:+81-193-26-6584, E-mail: tadashi.maruyama@kamaishi.mbio.co.jp

This manuscript is available on line at:

http://www.bioscience.org/2000/d/maruyama/fulltext.htm