Role of peptidyl-prolyl cis–trans isomerases in infectious diseases and host–pathogen interactions

Gargi Biswas and Rahul Banerjee*
Crystalllography and Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF-Bidhannagar, Kolkata 700 064, India; Homi Bhabha National Institute, Anushaktinagar, Mumbai 400 094, India

Peptidyl-prolyl cis–trans isomerases (PPIases) catalyse the cis–trans isomerization of Cα atoms about the peptide bond preceding a proline residue, thereby regulating a conformational switch which controls a plethora of cellular processes. PPIases play a key role in the survival, reproduction, proliferation and virulence of microbial pathogens vis-à-vis their human host. In addition, human PPIases either aid or retard viral replication and modulate host immune response. The article discusses the structure–function relationships of PPIases in the context of microbial virulence (with an emphasis on viruses), and on targeting PPIases for COVID-19, responsible for untold human sufferings.

Keywords: Host immune response, infectious diseases, pathogenicity, virulence, peptidyl-prolyl cis-trans isomerase.

PEPTIDYL-PROLYL cis–trans isomerases (PPIases) are enzymes found universally in the plant, animal and microbial kingdoms, which catalyse the cis–trans isomerization of peptide bonds preceding a proline residue. In proteins, the trans disposition of Cα atoms about the peptide bond is almost invariably favoured energetically over cis (by 2.6 kcal/mol)\(^1\). However, this preference for trans to cis is substantially reduced in case of proline, due to the cyclization of its side chain in the form of a pyrrolidine ring, thereby reducing the energy difference between the two conformers to only about 0.5 kcal/mol (refs 1, 2). Relative to spontaneous interconversion, PPIases increase by several orders of magnitude the cis–trans transition in such conformers. Initially, PPIases were found to increase the rate of protein folding by facilitating the rearrangement of prolyl isomerization states to their native conformation. However, with time the importance of PPIases has increased enormously. This is due to the recognition that prolyl cis/trans isomerization states (regulated by PPIases) could act as a molecular or conformational switch, leading to the synergistic coordination of several proteins, to accomplish a complex cellular function. Thus, the involvement of PPIases in a plethora of key cellular functions has been observed which includes cell-cycle progression\(^3\)–\(^5\), gene expression\(^6\), signal transduction\(^7\)–\(^9\), immune response\(^10\) and neuronal functions\(^11\). In addition, this class of proteins has been implicated in cancer\(^12\), cardiovascular diseases\(^13\), Alzheimers\(^14\) and in the regulation of microbial infections\(^1\), consequently identifying them as potential drug targets in numerous ailments.

To date, PPIase activity has been observed in four distinct protein folds, namely cyclophilin, FK506 binding protein (FKBP), parvulin and protein Ser/Thr phosphatase 2A (PP2A) activator (PTPA)\(^15\). The cyclophilin fold consists of an eight-stranded β-barrel (consisting of two anti-parallel β-sheets) capped by two helices at either end (Figure 1 a). These helices prevent access to the highly conserved hydrophobic core within the barrel and thus the active site of the enzyme lies on one of the faces of the barrel\(^16\). The FKBP fold on the other hand, consists of a short, centrally located α-helix enwrapped by five anti-parallel β-strands in a right-handed twist\(^17\) (Figure 1 b). The hydrophobic core of FKBP lies at the helix–sheet interface which also accommodates the active site of the enzyme. Cyclophilins and FKBP are jointly referred to as immunophilins, as they are receptors of the immunosuppressive drugs cyclosporine and FK506 respectively, a fact unrelated to their PPIase activity\(^18\). A few of the residues of both the cyclosporine and FK506 binding sites overlap with the native active sites of the respective enzymes, and immunosuppressive action arises as a consequence of cyclophilin–cyclosporine or FKBP–FK506 forming a stable ternary complex with calcineurin (Cn/CaN), a Ca\(^2+\)/calmodulin-dependent serine/threonine protein phosphatase (which dephosphorylates NF-AT, a transcription factor involved in T-cell activation)\(^19\). FKBP are also receptors for the drug rapamycin, the binary complex interacting with mTOR to inhibit T-cell activation\(^20\). Parvulins are somewhat similar to FKBP in that they retain the β-sheet at the centre of the fold. However, replacement of two loops by helices (in parvulin) results in the latter packing on both sides of the four-stranded twisted (half barrel) β-sheet\(^21\) (Figure 1 c), while the enzyme active site lies at the concave surface of the sheet. Despite such structural diversity, the fact that they
have identical enzymatic activity is an interesting problem in the structure–function paradigm, indicative of conserved structural features in their active sites\(^2\). PPIases, however, demonstrate variable recognition and enzymatic activity depending upon the residue preceding proline (P1). For example, human parvulin PIN1 preferentially recognizes phospho-threonine/serine residues at P1, whereas hFKBP12 is specific for either leucine or phenylalanine at the same residue position.

In addition, individual cyclophilin, FKBP and parvulin domains have been found conjoined with other non-PPIase domains such as the WW domain, U-box, EF-hand motifs, TPR, WD40 and RRM to form larger multi-domain proteins, probably targeting or coordinating PPIase function in the context of specific organelles\(^2\), allowing their interactions with multiple partners and conferring chaperone activity.

The scope of this article will encompasses the functions of three significant folds of PPIase (cyclophilins, FKBP\(s\) and parvulins) proteins in the regulation of growth, physiological functions and pathogenicity in case of pathogenic infection-causing bacteria, parasites and fungi. PTPA proteins have not been included here since their function as virulence factors is yet to be characterized. Host PPIases which play a crucial role in the regulation of pathogenic virulence and are considered as drug targets, have also been included here. The article summarizes the available structural information of some essential PPIase proteins and their strategic interaction sites, which might aid in designing efficacious drugs against specific pathogens. One outstanding example in this regard is the design of non-immunosuppressive cyclosporin (CsA) derivatives currently under advanced stage of clinical trial in the treatment of SARS-CoV-2. In short, the compact presentation of structure–function relationships of virulence-associated PPIase proteins in this article might assist in designing new PPIase inhibitors, which can be further administrated in the treatment of a number of pathogen-borne infectious diseases.

**PPIases as virulence factors**

**Pathogenic FKBP proteins**

Virulence factors are generally proteins that promote the colonization, multiplication and propagation of pathogens in a host. In bacteria, two extensively studied PPIases are trigger factor (TF) and SurA, both of which probably play an indirect role in virulence, arising as a consequence of either their chaperone function or involvement in the secretion of virulence factors, such as adhesins. In *Streptococcus pneumoniae*, TF was found to be necessary for the adhesion of bacterial cells to epithelial tissues in the human lung and has been identified as a potential drug target for pneumonial infections\(^2\). TF deletion mutants of *Listeria monocytogenes*, the causative agent for listeriosis, manifest reduced response to heat shock and ethanol exposure although the deletion appeared to have little effect upon bacterial cell growth *in vitro*. However, such mutants exhibit reduced intercellular survival and multiplication *in vivo*, probably significant for bacterial virulence\(^2\). TF deletion mutants in *Streptococcus mutans* were observed to have reduced growth rate coupled to an inability to form biofilms\(^2\). In *Streptococcus pyogenes* TF assisted in the maturation and secretion of specific cysteine proteases\(^2\), while its deletion decreased tolerance to oxidative stress and reduced growth rate\(^2\).

TF is a modular protein consisting of a centrally located FKBP domain flanked on both sides by a predominantly \(\alpha\)-helical C-terminal domain and an N-terminal (\(\alpha\)/\(\beta\)) ribosome-binding domain, capable of chaperone activity (Figure 2a)\(^2\). The crystal structure of TF (from *Escherichia coli*) in complex with the 50S ribosomal subunit (*Haloarcula marismortui*) showed the molecule (with all the three domains in an extended conformation) at the exit of the ribosome tunnel from where nascent polypeptide chains emerge\(^2\). The extended conformation of TF encloses a predominantly hydrophobic space at the exit of the ribosomal tunnel (characteristically called the ‘cradle’),
which facilitates the progress of protein folding unhindered by proteases or aggregation.

The PPIase domain was positioned away from the tunnel exit and thus could access the polypeptide chain only at an advanced stage in folding upon its dissociation from the cradle. The tethering of the peripheral FKBP to the other two cradle-forming domains using an extended double linker also provided structural rationale to the observation that PPIase activity was not necessary for either peptide binding or the chaperone function of TF.

Another FKBP class of proteins that are confirmed virulence factors and therefore validated drug targets are the macrophage infectivity potentiator (MIP) proteins, which are typically localized in the outer membranes of Gram-negative bacteria. The virulence-associated activity of MIP was first identified in Legionella pneumophila, where it was found necessary for the invasion and intracellular replication of the pathogenic bacteria within human alveolar macrophages30. Subsequently, MIP-like proteins were also found in the obligate intracellular pathogen Chlamydia, where its inhibition (by FK506) led to irregularities in inclusion-body formation within the host cell31. In addition, surface-exposed NgMip contributed to the persistence of Neisseria gonorrhoeae within the macrophages32; while NmMip was essential for the survival of Neisseria meningitidis in the blood33. Apart from bacteria, MIP PPlases in protozoan parasites Trypanosoma cruzi and Leishmania infantum facilitate entry of the parasites into mammalian epithelial cells. TcMIP (MIP from T. cruzi) was shown to be involved in host-cell invasion34; and the immunosuppressant FK506 was found to reduce parasitic burden by binding to TcMIP, possibly disrupting the signalling pathway involving Ca2+ (ref. 35). However, till date the maximum information with regard to the role of MIP in pathogenic virulence is in the case of L. pneumophila, where the enzyme contributed to the proliferation of bacteria in the lungs and spleen36. Here, MIP was found to bind collagen IV (a component of the extra cellular matrix: ECM) in the lung tissue, thereby initiating the penetration of bacteria into the alveolar epithelial cells (across the epithelial cell barrier). The PPIase active site appeared to be involved in the host–pathogen interaction as bacterial entry could be inhibited by rapamycin36.

Crystal structures of MIP (from L. pneumophila and T. cruzi) show the enzyme to be constituted of a centrally located FKBP-type PPIase core, flanked on both sides by α-helices (Figure 2b). Two α-helices joined by a six-residue loop (ζ1 and ζ2) are present at the N-terminal of the molecule and are responsible for the formation of biologically active MIP-dimer. The longest helix (ζ3) joins the N-terminal domain (ζ1 and ζ2) with the PPIase domain of the molecule and probably confers structural stability to the molecule, while the N-terminal helices are responsible for the flexibility in the molecule. Since the centrally located PPIase has an FKBP fold, a six-stranded β-sheet wraps around the shortest α-helix (α4), with the active site embedded in a deep hydrophobic pocket bounded by β-strands (3, 4, 6), helix α4 and a loop connecting strands 5, 6 (ref. 37). Generally, MIPs are secreted into the environment and so similar are the proteins from T. cruzi and L. pneumophila that recombinant addition of the enzyme (from T. cruzi) can recover function in a deletion mutant (in L. pneumophila). The rms deviation in Cα atoms between TcMIP and the corresponding LpMIP is only about 1.00 Å, and the most significant difference in their structures is with regard to the relative length of helix α3 (ref. 38).

In various pathogenic fungi, FKBP12 was identified as an important virulence factor and the target for the drugs FK506 or rapamycin, which have been widely used as antifungal agents. In Aspergillus fumigatus, deletion mutational analysis was performed for four FKBP-encoding genes, namely FKBP12-1, FKBP12-2, FKBP12-3 and FKBP12-4, in order to specifically understand the functional roles of these proteins in the fungi. The study showed that ΔFKBP12-1 and ΔFKBP12-4 generally resulted in enhanced sensitivity towards FK506 and reduced growth

---

**Figure 2.** Structure of some important FKBP proteins. a, Escherichia coli trigger factor (PDB ID: 1T11), consisting of three individual domains, viz. FKBP domain (yellow), N-terminal ribosome-binding domain (red) and C-terminal helical domain (green). The linker between FKBP-domain and ribosome-binding domain is also shown (violet). b, Dimeric MIP protein from Legionella pneumophila (PDB ID: 1FD9) showing the FKBP domain and large linker alpha helix (ζ3) which gives flexibility to the whole protein. c, FKBP12, FK506 and calcineurin complex (PDB ID: 6TZ8 from Cryptococcus neoforms). Calcineurin A (CnA) is shown in orange, the calcineurin B (CnB) subunit in green and FKBP12 in yellow. FK506 is shown in sticks representation (blue).
(coupled with growth defects) respectively\textsuperscript{39}. In addition, FK506 treatment modified the localization of FKBP12-1 from the cytoplasm to the hyphal septa of the fungi. FKBP12 deletion mutants in \textit{Beauveria bassiana}, \textit{Candida albicans} and \textit{Cryptococcus neoformans} exhibited increased resistance to antifungal drugs FK506 and rapamycin\textsuperscript{39,40}. The transcriptional analysis had already confirmed that FKBP12 forms ternary complexes with calcineurin (Cn) and FK506 (refs 41, 42). Crystal structures of the Cn–FKBP12–FK506 ternary complexes have been solved for \textit{A. fumigatus} and \textit{C. neoformans}. As has been mentioned previously, Cn is a Ca\textsuperscript{2+}/calmodulin (CaM)-dependent serine, threonine-specific protein phosphatase principally responsible for T-cell activation along with other cellular functions\textsuperscript{42}. Heterodimeric Cn consists of two domains – a catalytic domain (CnA) and a regulatory (CnB) domain which interacts with Ca\textsuperscript{2+}-CaM. Comparison of the individual components of the fungal and human ternary complexes showed a high degree of structural conservation. FKBP12–FK506 binds to an extended hydrophobic groove formed by both subunits of Cn (Figure 2c). However, despite the structural conservation of individual units, critical amino acid differences were observed in the loops corresponding to the human and fungal FKBP\textsubscript{s}, which were exploited to rationally design non-immunosuppressive inhibitors of fungal FKBP12 and Cn\textsuperscript{45}.

**Parvulins of disease-causing bacteria and protozoa**

An extensively studied multidomain protein (which includes a PPIase) is the periplasmic (survival factor A) SurA, which facilitates the folding and assembly of several outer membrane proteins (OMP) in Gram-negative bacteria. SurA-deficient bacteria (as in \textit{Yersinia pseudotuberculosis}) significantly reduce the surface localization of two key adhesins, Ail and OmpA, which hinders the attachment of the parasite to its eukaryotic host cells\textsuperscript{45}. In addition, SurA-deficient strains (in \textit{Pseudomonas aeruginosa} and \textit{Salmonella enteritidis}) exhibit perturbations in their outer membrane structure, permeability and molecular constitution leading to reduced virulence (in a \textit{Galleria mellonella} infection model) and increased sensitivity to a number of antibiotics\textsuperscript{44,45}.

SurA consists of four domains, an \textit{N}-terminal fragment (N) of about 150 amino acids, followed by two PPIase domains (P1, P2) of the parvulin class, and finally ending with a predominantly \textalpha-helical C-terminal domain (C) (Figure 3a). The polypeptide segments N, P1 and C form the densely packed ‘core module’, whereas P2 is tethered to the core by a linker of approximately 30 Å in length\textsuperscript{46}. Completely abolishing all PPIase activity from the molecule by mutagenesis does not appear to hinder the chaperone function carried out by the core module, as mutant cells exhibit wild-type phenotype and intact outer membranes\textsuperscript{46}. Interestingly, the PPIase activity of SurA lies exclusively with P2 whereas P1 mediates molecular recognition and binding, as evident from the crystal structures of (truncated) SurA–peptide complexes\textsuperscript{46}.

The protein homologous to SurA in Gram-positive bacteria is PrsA, of which two isoforms (PrsA1 and PrsA2) have been identified in \textit{Listeria monocytogenes} (\textit{Lm}), a bacterium that resides in the soil but transforms into a pathogen upon mammalian contact. Central to this transition is the secretion of a host of virulence factors such as internalin A, B to initiate host-cell invasion and listeriolysin O (LLO), phospholipases for the lysis of vacuolar membranes, to enable bacterial entry into the host cytosol\textsuperscript{37}. PrsA2, a post-translocation secretion chaperone, is necessary for correct folding and activity of these secreted proteins, in addition to being involved in flagellar-based motility\textsuperscript{46}. Although PrsA1 and PrsA2 share 58\% sequence identity, there is little overlap between their functions and it is only fairly recently that PrsA1 has been implicated in bacterial ethanol resistance\textsuperscript{39}.

\textit{LmPrsA} is a homodimer with each monomer consisting of two distinct domains – a predominantly \textalpha-helical ‘foldase’ domain and a parvulin-type PPIase domain, with considerable flexibility in their relative geometry. Comparison of the PrsA1 crystal structure and a three-dimensional model of PrsA2 identified the ‘hotspots’ in this fold which are probably responsible for transforming PrsA2 into a virulence factor (in contrast to PrsA1). Although there are several random amino acid differences between the surfaces of both proteins, the most concerted difference appears to be in the vicinity of the highly conserved active site of the PPIase domain. Encircling the (PPIase) active site there are eight amino acid substitutions which make the neighbourhood of the PrsA2 active site less charged or polar compared to PrsA1. In other words (in conformity with these substitutions), the PPIase domain in PrsA1 appears highly electronegative in contrast to the relatively uncharged domain in PrsA2. Interestingly, the hydrophobic pocket in the foldase domain

\textbf{Figure 3.} Structures related to the parvulin fold. \textit{a,} SurA protein from \textit{E. coli} (PDB ID:1M5Y) showing its four domains in four different colours, namely N, P1, P2 and C in green, red, yellow and blue respectively. \textit{b,} Ess1 protein from \textit{Candida albicans} (CaEss1), which has a WW domain (red) in addition to the PPIase domain (yellow). The linker domain consists of an alpha helix (green) which is dissimilar to the human PIN1 protein.
which is expected to bind to protein substrates for chaperone activity was found largely conserved in both proteins.49

There is a relative dearth of information with regard to parasitic parvulins with the exception of a few proteins from Trypanosoma, Toxoplasma and Theileria. Depletion of the parvulin 42 gene from Trypanosoma brucei (TbPar42) by RNA interference, reduced both the viability and proliferation rate of parasitic cells.50 Homologs of TbPar42 are found exclusively only in protozoa and thus could be favoured as a drug target. TbPar42 consists of two domains – an N-terminal FHA domain followed by a parvulin-type PPIase domain, which has high structural similarity to human PIN1. FHA domains typically recognize phosphopeptides and play a significant role in DNA damage, replication and cell-cycle progression.51 No catalytic activity was observed for the parvulin domain, in case of phosphorylated peptides (which included p-Thr). NMR studies did not appear to indicate any interaction between the two domains and there is every possibility that the native substrate for this enzyme is yet to be identified.51 It has been hypothesized that TbPar42 could be a scaffold protein that participates in higher-order assemblies, enabling the association of weakly binding proteins. Other parasitic parvulins include the 22 kDa TgMIC5 (Toxoplasma gondii) found in secretory organelles called micronemes, contributing to host cell adhesion and invasion.52 TgMIC5 mimics the function of GPI (glycosyl phosphatidyl inositol)-anchored microneme protein TgSUB1, which processes secreted micronemal (MIC) proteins on the surface of the parasite to enhance their adhesive functions.53 A parvulin from Theileria annulata (TaPIN1) similar to human PIN1, has been found to be secreted into host cells (by the parasites) so as to hijack the host oncogenic signalling pathways. TaPIN1 also interacts with the host ubiquitin ligase FBW7 (which promotes the degradation of oncogenic proteins like c-JUN), thereby causing an elevation of c-JUN levels in the host.54

Parvulin proteins have also been found to be important for survival and virulence for a number of pathogenic fungi. The parvulin CaEss1 from Candida albicans has been found to be essential for the proliferation and survival of the fungal pathogen inside mammalian host cells.55 In addition, the virulence of Ess1 deletion mutants tested on a murine model, demonstrated no disease symptoms in contrast to infection by wild type which resulted in severe cryptococcosis.56 The crystal structure of CaEss1 showed structural similarity to the human PIN1 protein, except for the linker region between PPIase and WW domain (Figure 3 b). In human hPIN1, this flexible domain is devoid of any regular secondary structure. In contrast, the linker domain of CaEss1 consists of a large helix which restricts the mobility of this region and probably suggests a different mode of protein–protein interactions than found in hPIN1 (ref. 55).

Bacterial, protozoan and fungal cyclophilins as virulence factors

Amongst the cyclophilins, two prominent virulence factors are the homologous enzymes PpiA and PpiB located at different cellular sites, as a consequence of specific signal peptides incorporated in their nascent polypeptide chains. In Gram-negative bacteria PpiA is periplasmic, while in Gram-positive bacteria it is associated with (the external side of) the cytoplasmic membrane and is implicated in the transport of secreted proteins.57 In contrast, PpiB is invariably a cytoplasmic protein. Both proteins probably play a synergistic role in modulating cell division, resistance to extracellular stress, chaperone activity associated with virulence factors and regulation of pathogenic virulence. For example, overexpression of PpiA and PpiB from pathogenic Sinorhizobium meliloti in E. coli BL21 cells, increased by several folds bacterial survival under heat and salt stress.58 In Mycobacterium tuberculosis the protein homologous to PpiA was found to be secreted into host cells, due to the presence of an N-terminal signal sequence, conspicuously absent in non-pathogenic strains.59 Overexpression of PpiA and PpiB (from M. tuberculosis) in E. coli cells also conferred fitness to sustain oxidative, hypoxic stress conditions generated by H2O2 and CoCl2 (ref. 59). In addition, both enzymes were found to modulate host immune response, as treatment of THP-1 cells with recombinant PpiA promoted the expression of pro-inflammatory TNF-α and IL-6 cytokines, whereas similar treatment with PpiB inhibited TNF-α and induced IL-10 secretion instead.60 In dental caries causing bacteria, S. mutans, a PpiA-deficient strain was subject to increased phagocytosis by human macrophages,61 thus exposing the crucial role of the bacterial enzyme in antigenic activity. Likewise PpiA/PpiB in Enterococcus faecalis was found to play a significant role in stress response to high NaCl concentration and regulation of virulence in bacterial (E. faecalis) infection of G. mellonella larvae.62

Several crystal structures are currently available for bacterial cyclophilins, of which CypA (from E. coli) is structurally similar to human CypA, though marginally differing in irregular loop regions. Only eight out of the 11 active site residues in bacterial CypA are conserved with respect to humans, though leaving the catalytic function of the bacterial enzyme unaffected.63 Comparison of pathogenic bacterial cyclophilins (including crystal structures of PpiA from M. tuberculosis, cyclophilin A–Azotobacter vinelandii, CypA–Schistosoma mansoni) suggests overall conservation of the protein fold, except for minor variations in the L1 and L4 loop regions.64–66 An especially interesting study compared crystal structures of the complexes (E. coli) CypA bound to Suc–Ala–c–Pro–Ala–pNA (Figure 4 a) and CypB associated with Suc–Ala–trans–Pro–pNA (Figure 4 b). Although the crystals of both complexes were grown under identical conditions,
the relative affinities (of CypA, CypB) were for the cis and trans forms of the peptide respectively\textsuperscript{67,68}. No conformational differences were observed in 13 (of the 14) conserved binding site residues and it appeared that the difference in specificities was probably due to the loops in the neighbourhood of the binding site (extending from strand $\beta_4$ and interconnecting $\beta_3$ to $\beta_5$; Figure 4a and b). These loops condition the orientation of the residue at site P2 (the second residue from the N-terminal of proline in the substrate), which in turn was postulated to determine the difference in specificities (vis-à-vis CypB, CypA) and reaction rates\textsuperscript{67,68}. In \textit{E. coli}, CypA and CypB correspond to PpiA and PpiB, and as has been mentioned previously are localized in the periplasm and cytoplasm respectively.

Cyclophilins from several parasitic protozoa are also known to modulate the host immune system by altering T-cell responses, consequently interfering in the secretions of their associated cytokines which leads to the eventual acceptance of the parasite as 'self' by the host. Such a role in host–pathogen interactions qualifies parasitic cyclophilins as potential vaccine candidates. It may be recalled that immune response by CD4$^+$ T:\textit{H} cells is primarily through secreted cytokines and based on the repertoire of these molecules, T-helper cells can be distinguished into subclasses T:\textit{H}1, T:\textit{H}2, T:\textit{H}3 and T:\textit{H}17. For example, recombinant cyclophilin A of \textit{S. mansoni} (SmCyp), responsible for schistosomiasis, modulated the immune function of bone marrow–derived dendritic cells (DC) by attenuating the DC-mediated CD4$^+$ T-cell activation and concomitant induction of the T:\textit{reg} cell response\textsuperscript{69}. Vaccination with a synthetic peptide derived from SmCyp induced a reduction in parasitic burden and significantly enhanced antibodies against the parasitic antigen in immunized mice\textsuperscript{70}. Such observations appear to identify SmCyp (or SmCyp-derived peptides) as promising vaccine candidates against schistosomiasis. Cyclophilin from another \textit{Schistosoma} species (\textit{Schistosoma japonicum}–SmCyp18) has been shown to favour an IL-4 producing T:\textit{H}2 response \textit{in vivo}, which appeared to promote immunopathological changes such as liver fibrosis\textsuperscript{71}. In case of visceral leishmaniasis (\textit{L. infantum}), the use of recombinant leishmanial cyclophilin (LiCyp1) as an antigen to immunize BALB/c mice led to a significant reduction in parasitic burden in liver and spleen cells. Further, the process also stimulated the circulation of specific CD4$^+$ and CD8$^+$ T-cells at infection sites, promoting the release of relevant effector cytokines and attenuating subsequent leishmanial infection\textsuperscript{72}. The significant reactivity of cyclophilin from \textit{Echinococcus granulosus}, with human IgG and IgE, proves it to be the principal causative agent in allergic cystic echinococcosis\textsuperscript{73}. It is well known that interferon-gamma (IFN-\textgamma) produced during any microbial infection improves the protective immunity of the host. However, continuous production of IFN-\textgamma and its subsequent depletion precede acute-phase neoparosis infection (caused by \textit{Neospora caninum}), probably due to a secretory cyclophilin (NeCyp) from the parasite\textsuperscript{74}. Similarly, cyclophilin of the protozoan parasite \textit{T. gondii} (TgCyp18) secreted through tachyzoites, induces the production of tumour necrosis factor (IL-12) and nitric oxide (NO) by binding to CCR5 (cystine cystine chemokine receptor 5) located on surface macrophages and spleen cells. TgCyp18 also induces IL-6 and IFN-\textgamma in a CCR5-independent manner\textsuperscript{75}. Possibly, as a consequence of these interactions, TgCyp18 enhances the migration of parasites to host macrophages and promotes invasion and proliferation within macrophages, thereby consolidating the survival of the parasite within the host\textsuperscript{76,77}.

A large body of work supports the involvement of cyclophilins in the growth, reproduction, virulence and extracellular stress response of pathogenic fungi. The genome of \textit{C. neoforms}, a pathogenic fungus affecting the human central nervous system, comprises two cyclophilin isofoms, namely Cpa1 and Cpa2, exhibiting diverse functions. ΔCpa1 mutants demonstrated a lower survival rate relative to wild type in infected murine and rabbit cells. Also, in mating assays the double disruption fungal mutants were sterile\textsuperscript{78}. A cyclophilin protein from the opportunistic human pathogen \textit{Lemontospora prolificans} (causing a wide range of diseases in immune-compromised humans) was recognized by human salivary Immunoglobin A (IgA), leading to the identification of this protein as an immunogenic antigen\textsuperscript{79}. Similar binding to cyclophilin \textit{A. fumigatus} as a conidial antigen has identified cyclophilins as a conserved immunogen, which could be used to develop vaccines against this class of pathogenic fungi\textsuperscript{79}.

**Viral interactions of host PPIases**

As is well known, all viruses co-opt the replicative molecular machinery of the host to proliferate as virions,
thereby completing their life cycle. This is accomplished by the interaction between viral and host proteins (also referred to as host cofactors). Several well-studied examples demonstrate the crucial role played by host PPIases in the viral life cycle, either as an essential factor necessary for its replication or as an antiviral agent. Cyclophilin promotes the replication of the human immunodeficiency virus 1 (HIV-1), hepatitis B virus (HBV), hepatitis C virus (HCV) and severe acute respiratory syndrome coronaviruses (SARS-CoVs), while inhibiting influenza and rotaviruses (RV).

HIV-1 belonging to the Retroviridae family consists of a positive-stranded RNA genome. Gaining entry into a host cell, viral pol encoded reverse transcriptase (RT) initiates the incorporation of double-stranded DNA (derived from the viral genome) into the chromosomal DNA of the host. An essential step in the life cycle of the pathogen is the translation of the viral Gag polyprotein (an extended polypeptide consisting of several viral factors) which encodes the information necessary for the assembly and release of virions. As the virions mature, Gag is cleaved into three polypeptide fragments by a (viral) protease to yield the matrix protein (MA – 132 residues – lining the inner surface of the viral membrane), the capsid protein (CA – 230 residues – forming the distinctive core which envelopes the NC/RNA complex at the virion centre) and the nucleocapsid protein (NC – 54 residues – coating the genomic RNA). Thus, these proteins form key components of the fully infective mature virion. Human CypA interacts with the Gag polyprotein by binding to a proline-rich loop (specifically at Gly 89–Pro 90) corresponding to the CA region of Gag, resulting in CypA incorporation into the virions. This is essential for the effective replication of HIV-1, as inhibiting the packaging of CypA within the virion by CsA or Gag-specific mutations leads to a reduction in viral infectivity.

CD147 is the primary signalling receptor for CypA on the surface of human leucocytes and CypA–CD147 interaction could be instrumental in releasing the viral RT complex into the cytoplasm, along with facilitating virion attachment to host cells. Interestingly, cell lines from Old World primates were found to be HIV-1-resistant due to the presence of a Cyp-TRIM5α fusion protein. The cyclophilin domain in the chimerical protein was responsible for directly targeting TRIM5α onto incoming HIV-1 capsids, leading to the arrest of viral proliferation. In humans, TRIM5α is only weakly resistant to HIV-1 probably due to sequence divergence from the homologous protein in primates.

Crystal structure of human CypA complexed with the (151 residue) N-terminal domain of HIV-1 CA revealed in atomic detail, their mutual interaction sites (Figure 5 a). CA151 has an all-α-fold constituted of seven helices, with α-helices 1–4 and 7 packing along their helical axes, while 5, 6 stack on the top of this helical bundle. Residues Ala-88, Gly-89 and Pro-90 within the CypA binding loop of CA151 (from residues 85 to 93) were completely inserted into the CypA active site (Figure 5 b). The peptidyl–prolyl bond corresponding to Pro-90 was found to be in the trans conformation, which along with the presence of glycine at position 89, allowed for deep penetration of the loop into the CypA active site. However, cryo-EM studies of CypA complexed with (supramolecular) tubular CA assemblies, found CypA bridging two CA molecules by means of an additional ‘non-canonical’ binding site involving Pro-29 and Lys-30 (apart from the canonical active site; Figure 5 a). This ability of CypA to bridge two CA molecules might perhaps be structurally relevant in stabilizing the viral capsid.

Studies indicate that similar to HIV-1, CypA also promotes HCV replication by interacting with the viral protein NS5A. This protein consists of three domains and experiments implicate Pro-319 (in the second domain) embedded in a WARP-DYN motif as interacting with CypA. All mutations in NS5A which confer resistance to cyclophilin inhibitors such as CsA are found in the WARP-DYN motif. Additionally, NS5B with RNA polymerase activity and essential for viral RNA replication, forms a replication complex in the endoplasmic reticulum along with host proteins, of which human CypA is an important constituent. Likewise, HBV replication is also facilitated by the human enzymes parvulin 14 and parvulin 17 interacting with viral small surface proteins (SHBs), which engages the immune system of the host. In contrast to all the examples given above, incorporation of CypA within the virion core of influenza virus inhibits its infective cycle. The most abundant protein in case of influenza is the matrix protein M1 which is essential for viral replication, assembly and budding. The antiviral effect of CypA was due to its role in the degradation of M1 via the ubiquitin/proteosome-dependent pathway.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the latest amongst seven previously known viruses belonging to the Coronaviridae family, the latest amongst seven previously known viruses belonging to the Coronaviridae family.
which infects humans. As this article is being written the COVID-19 pandemic (caused by SARS-CoV-2) is ravaging the world in terms of human loss and suffering, with over two lakh deaths in India alone. Four previous corona viruses (HCoV-NL63, HCoV-229E, HCoV-OC43 and HKV1) reported only mild symptoms in immune-competent hosts, while SARS-CoV-1 and MERS-CoV (middle east respiratory syndrome coronavirus) were highly pathogenic with elevated mortality rates (almost 30% for MERS-CoV), though with lower transmissibility. Unfortunately, SARS-CoV-2 combines very high rates of transmission with aggressive pathogenicity. The SARS-CoV-2 genome has 79% and 96% sequence identity with SARS-CoV-1 and bat coronavirus (BatCoVRatG13) respectively. The genome of corona viruses consists of four major structural proteins – the characteristic spike protein (S) which is inserted into the outer membrane, and the membrane (M), envelope (E), the nucleocapsid (N) proteins. The S-glycoproteins are responsible for host recognition by binding to the angiotensin-converting enzyme-2 (ACE2), and the higher transmissibility of SARS-CoV-2 (relative to CoV-1) could be due to the higher affinity of S to ACE2 (ref. 88). Human CypA plays a key role in the replication of the SARS-CoV by its association with the viral N protein. A high-throughput yeast two-hybrid screen (HTY2H) identified the binding of CyPA to the nonstructural Nsp1 in SARS-CoV and abrogation of CyPA through SiRNA hindered viral replication (for HCoV-NL63 in CaCo-2 cells). In addition, the CyPA signalling receptor CD147 was also found to interact with the N protein by surface plasmon resonance and it may be possible that CyPA in tandem with CD147 facilitates viral entry into host cells.

Several experiments have confirmed that CsA inhibits the replication of SARS-CoV-1,2, CoV-229E and CoVNL63 by binding to host cyclophilins. However, the immunosuppressive properties of CsA prejudice its direct use as an antiviral. As has been mentioned previously CsA bound to cyclophilin forms a ternary complex with calcineurin (Cn), thereby curtailing T-cell activation. Crystal structures of the CyPA–CsA–Cn complexes clearly show CsA residues that directly interact with Cn, and several non-immunosuppressive CsA derivatives have been synthesized which could find antimicrobial and antiviral applications. Of these, the most promising appears to be the CsA derivative alisporivir, which inhibits RNA production and replication in SARS-CoV-2. Alisporivir is currently under phase-3 clinical trial
as an antiviral drug\textsuperscript{93,94}. The crystal structures of cyclophilin complexed with CsA\textsuperscript{95} and alisporivir\textsuperscript{96} are available, and the amino acid conformations responsible for abrogating Cn binding (in case of alisporivir) have been determined. Likewise, another CsA derivative SDZ NIM811 selectively inhibits HIV-1 replication in T4 lymphocyte cell lines\textsuperscript{97}. All these derivatives exhibit reduced toxicity and could find extensive use as antivirals in the future.

**Discussion**

The involvement of PPIases in a wide spectrum of important cellular functions lies in their ability to regulate cis/trans prolyl isomerization switches to coordinate complex physiological processes. Although PPIases consist of four structurally distinct superfamilies, conservation of key active-site residues presumably indicates a similar mechanism of action, despite fold differences. Mapping key residues of cyclophilin and FKBP involved in enzymatic function, and drug/protein interaction sites exhibit considerable overlap between residues of the active and the drug binding sites for both proteins (Table 1). Although the active site residues of CypA and FKBP are similar, their respective calcineurin (CaN/Cn) binding sites appear to be divergent. Interestingly, the cyclophilin loop which interacts with calcineurin B (in the CypA–CsA–Cn ternary complex) is also involved in recognizing the HIV-1 Gag protein (Asn71–Thr73). Residues Ala101–Phe113 appears to be of extreme strategic importance for CypA, as it is implicated in every possible interaction of the enzyme. Similarly, in case of FKBP protein, the region Arg42–Phe46 is of extreme importance because it is involved in interactions with FK506, rapamycin as well as CaN.

Host PPIases have also been found to regulate virulence in host–pathogen interactions, significantly for viruses where PPIases are involved in forming multi-protein complexes with viral proteins. These host PPIase interactions with viral proteins have been found to be essential for the survival of the virus within the host and also in its replication. Consequently, cyclophilin inhibitors are being used extensively in antiviral therapy\textsuperscript{80}. Likewise, host parvulins have been found to be a key participant in the life cycle of HBV\textsuperscript{84}. A number of fungal cyclophilins have been found to be important vaccine candidates\textsuperscript{90}. It is expected that in the coming years PPIases (whether from the host or pathogen) will serve as promising therapeutic drug targets and immunogens for vaccine development.


\textsuperscript{4} Gong, Z. et al., Cyclophilin A is overexpressed in hepatocellular carcinoma and is associated with the cell cycle. *Anticancer Res.*, 2017, **37**, 4443–4447.


\textsuperscript{7} Nakatsu, Y. et al., Physiological and pathogenic roles of prolyl isomerase Pin1 in metabolic regulations via multiple signal transduction pathway modulations. *Int. J. Mol. Sci.*, 2016, **17**, 1495.


\textsuperscript{11} Testi, L. et al., Single-nucleotide polymorphism of the FKBP5 gene and childhood maltreatment as predictors of structural changes in brain areas involved in emotional processing in depression. *Neuropsychopharmacology*, 2016, **41**, 487–497.


\textsuperscript{25} Bigot, A., Botton, E., Dubail, I. and Charbit, A., A homolog of Bacillus subtilis trigger factor in Listeria monocytogenes is...
51. Rhee, E. et al., Structural analysis of the 42 kDa parvulin of *trypanosoma brucei*. *Biomolecules*, 2019, 9, 90030093.


77. Ibrahim, H. M., Nishimura, M., Tanaka, S., Awad, W., Furuoaka, H. and Xuan, X., Overproduction of Toxoplasma gondii cyclophilin-18 regulates host cell migration and enhances parasite dissemination in a CCR5-independent manner. *BMC Microbiol.*, 2014, **14**, 76.


84. Saeed, U. et al., Parvulin 14 and parvulin 17 bind to HBx and cccDNA and upregulate hepatitis B virus replication from cccDNA to virion in an HBx-dependent manner. *J. Virol.*, 2019, **93**, e01840–18.


95. Ke, H. et al., Crystal structures of cyclophilin A complexed with cyclosporin A and N-methyl-4-[E(2)-butenyl]-4,4-dimethylthreonynine cyclophilin A. *Structure*, 1994, **2**, 33–44.


ACKNOWLEDGEMENTS. We thank Dr Semanti Ghosh (Saha Institute of Nuclear Physics (SINP), Kolkata) for valuable suggestions and comments. This work is supported by intramural grants from the Department of Atomic Energy (DAE), Government of India (GoI). G.B. acknowledges SINP and DAE, GoI for the award of a Senior Research Fellowship.

Received 8 May 2021; revised accepted 13 July 2021