Peptidylprolyl-isomerases, Protein Folders, or Scaffolders? The Example of FKBP51 and FKBP52

Theo Rein

Peptidylprolyl-isomerases (PPIases) comprise of the protein families of FK506 binding proteins (FKBPs), cyclophilins, and parvulins. Their common feature is their ability to expedite the transition of peptidylprolyl bonds between the cis and the trans conformation. Thus, it seemed highly plausible that PPIase enzymatic activity is crucial for protein folding. However, this has been difficult to prove over the decades since their discovery. In parallel, more and more studies have discovered scaffolding functions of PPIases. This essay discusses the hypothesis that PPIase enzymatic activity might be the consequence of binding to peptidylprolyl protein motifs. The main focus of this paper is the large immunophilins FKBP51 and FKBP52, but other PPIases such as cyclophilin A and Pin1 are also described. From the hypothesis, it follows that the PPIase activity of these proteins might be less relevant, if at all, than the organization of protein complexes through versatile protein binding. Also see the video abstract here https://youtu.be/A33lao0dx5LE.

1. Introduction

Information regarding the 3D conformation of native proteins is fully contained in their amino acid sequence; it comes into effect through protein folding.[1] According to the original concept of protein folding, the native state of thermodynamic stability is reached without further assistance and input of energy.[2] This view had to be revised when it was shown that the folding of several proteins depends on the action of molecular chaperones, some of which use metabolic energy; furthermore, partly folded, misfolded, or even amorphous aggregated conformations of proteins can be states of low free energy.[1–3] Originally, molecular chaperones were defined as mediators of the correct assembly of other proteins ("clients") that are not part of the final functional structures.[4] It has been pointed out that chaperones do not provide specific conformational information, but rather impede incorrect interactions. Thereby, they increase the yield of folding reactions, but not their rate.[1] This has been distinguished from the actions of enzymes that catalyze (i.e., increase the rate of) distinct steps of protein folding. Examples are isomerization of disulfide bonds of proteins destined for secretion,[5] or peptide bond cis–trans isomerization,[6] in particular those preceding proline residues.[7,8] Overall, there is ample evidence for the functional role of molecular chaperones and disulfide bond isomerases in protein folding, contrasting the relatively low number of reports attempting to prove the necessity of proline bond cis–trans isomerization.

2. Getting into Shape: Protein Folding

The exciting advances in understanding cellular protein folding over the last few decades is already documented in several excellent reviews[1,3,9–13] and thus the concepts are only briefly described here. Protein folding is part of the cellular protein homeostasis, often referred to as “proteostasis”. This term denotes the proteome balance in each cell that results from the combined actions of regulated processes such as synthesis, folding, and degradation as well as stochastic processes leading to protein damage and misfolding. Including regulatory proteins, it is estimated that about 2000 proteins control proteostasis in human cells.[15] This is a significant fraction of the overall proteome and thus attests to the pivotal function of maintaining proteostasis. Of these 2000 proteins, 332 form the molecular chaperone network assisting protein folding.[16] In principle, this network chaperones newly synthesized polypeptides as well as proteins that become misfolded during their lifetime. Many chaperones are termed heat shock proteins (Hsps) and function as stress response proteins because their production is induced by various stressors such as heat and other conditions endangering protein integrity.[1,17] The chaperone classes are grouped by sequence homology and named according to the molecular weight of the major representative; this gives rise to the chaperone families of Hsp40s, Hsp60s (also known as chaperonins), Hsp70s, Hsp90s, Hsp100s, and the small Hsps.

Thermodynamically, folding has been described as progression on a rugged free energy landscape where proteins can be kinetically trapped in partly folded, misfolded, or even aggregated states.[3,9,10,18] One of the major mechanisms through which chaperones promote folding is referred to as...
“kinetic partitioning”. Here, broadly functioning chaperones recognize non-native states of proteins by the hydrophobic segments that a wide range of proteins expose when they are not in their native conformation. Through repeated, ATP-dependent cycles of binding and release, they hinder the transition to protein aggregation and favor repartitioning to productive protein folding. Chaperone action may further include changing the energy landscape of protein folding by entropic destabilization of kinetically trapped folding intermediates as well as unfolding activity; these actions allow the energy barrier to be overcome leaving the local energy minima of partial folding, misfolding, or even aggregates. These mechanisms are not mutually exclusive, of course. It appears that which mechanism comes into effect depends on the specific client protein.

The different classes of chaperones often act in a sequential manner. For instance, in eukaryotes, several proteins of various signal transduction pathways are transferred from the Hsp70 folding machinery to the Hsp90-based folding platform. Important examples of these client proteins are steroid hormone receptors and protein kinases. Hsp90 is both stress important examples of these client proteins are steroid hormone receptors and protein kinases. Hsp90 is both stress and highly abundant at basal conditions. Hsp90 associates with the apo-steroid receptors to maintain their conformational state of high affinity to hormone binding. This challenges the original paradigm that chaperones are not part of the final structure of their clients. Some chaperones apparently also are engaged in safe-guarding the assembly of protein complexes.

The vast majority of the studies on PPIases have investigated potential roles in changing the function of already folded proteins. In this way, PPIases are engaged in several pathway activities. It is debated whether this requires scaffolding activity through interaction with several proteins and/or enzymatic PPIase activity. A potential functional role for PPIase activity in protein function comes with at least two requirements: 1) the two isomers of the substrate protein have different functions and 2) the cis/trans equilibrium has not been reached without catalysis (Figure 1).

4. The Role of Peptidylprolyl-Isomerization in Protein Function

The vast majority of the studies on PPIases have investigated potential roles in changing the function of already folded proteins. In this way, PPIases are engaged in several pathway activities. It is debated whether this requires scaffolding activity through interaction with several proteins and/or enzymatic PPIase activity. A potential functional role for PPIase activity in protein function comes with at least two requirements: 1) the two isomers of the substrate protein have different functions and 2) the cis/trans equilibrium has not been reached without catalysis (Figure 1).

4.1. Cyclophilin A

Cyclophilin A (CypA) is the most abundant cytoplasmic cyclophilin. It is another example of a PPIase with clearly established enzymatic activity in vitro, and yet several studies have demonstrated CypA functionality even when this activity is blocked. These studies include evidence that CypA induces leukocyte chemotaxis through direct binding to the ectodomain of the cell surface signaling receptor CD147, independent of its PPIase activity. Conclusion is based on the observation that a PPIase-inactive mutant of CypA exhibits receptor binding and chemotactic activity comparable to wild type CypA. Conversely, mutants that impaired receptor binding of CypA also reduced chemotactic, but not PPIase activity.

The major Ca$^{2+}$ regulating protein Na$^+$–Ca$^{2+}$ exchanger (NCX) is also impacted by CypA. siRNA-mediated knockdown of CypA reduced the level of NCX surface expression and Na$^+$-dependent Ca$^{2+}$ fluxes, without changing the total amount of NCX in the cell. A similar effect has been reported for the treatment with the cyclophilin PPIase inhibitor cyclosporine A. However, the inhibitory effect of cyclosporine A could be alleviated not only by overexpression of wild-type CypA, but also by the PPIase-defective R55A mutant; thus, it was concluded that PPIase activity is not required.

CypA also impairs the early stage of influenza A virus replication in a PPIase-independent manner via direct interaction with the virus matrix protein (M1). This statement was based on the use of the PPIase-defective CypA mutant R55A, which displayed binding to M1 and inhibited viral replication equally efficiently as the wild type CypA. Later, it was shown that CypA regulates ubiquitination and degradation of M1 through the control of protein-protein interaction.

These examples illustrate the widely held view that CypA may act through regulating protein–protein interactions in signal transduction pathways, and the skepticism among several researchers of whether PPIase enzymatic activity is essential in vivo. As another example, the PPIase domain of CypA, but not the activity, turned out to be important for the association with the dynein-dynactin complex through interacting with the dynamin component. Frequently, a functional role of the PPIase activity of CypA is concluded from the inhibition of CypA function by the CypA ligand and the PPIase inhibitor cyclosporine A (see also Table 1). As argued before, however, cyclosporine A
Role of PPIases in protein function. In situations where the two isomers of a substrate protein (S) determined by the cis or trans configurations of a prolyl bond determine two different functional states (e.g., active and inactive), and equilibrium has not been reached yet, the acceleration of the interconversion between the two states will affect protein function. Figure 1.

4.2. The Paradigm of Pin1

Probably the most advanced example for a potential functional role of PPIase activity is Pin1 (protein interacting with NIMA [never in mitosis A]-1). Pin1-like PPIases feature a unique structure of their PPIase pocket conferring specificity for phosphorylated Ser/Thr-Pro bonds, which are not recognized by the other PPIases. Pin1 interacts with diverse proteins and is involved in several pathways and physiological functions. For example, it is essential for the regulation of mitosis. Complementation experiments in yeast also revealed that mutating the ability to isomerize peptidyl–prolyl bonds (G155A, H157A, I159A) abolished the effect of Pin1 in this assay. Pin1 also restores binding of phosphorylated tau to microtubules and promotion of microtubule assembly. Since not only mutants of Pin1 preventing binding to phosphorylated Tau (pTau), but also mutants disrupting its PPIase activity abolish Pin1’s effect on pTau, it was concluded that the PPIase activity is essential for the effect on pTau. Later, it was found that Pin1 facilitates dephosphorylation of Cdc25C and tau proteins by protein phosphatase 2A (PP2A). The conclusion of the mandatory involvement of Pin1’s PPIase activity was derived from the use of mutants. Intriguingly, PP2A only dephosphorylates peptides in the trans conformation, but not in the cis conformation, which represents about 10% in the conformational equilibrium. Thus, PPIase activity could become relevant in situations where the cis/trans ratio is far from equilibrium, in particular with a high proportion of the target protein in the cis conformation. Pin1 has also been portrayed as molecular timer, as outlined in the next section.

4.3. Is peptidyl–prolyl Isomerization Rate-Limiting?

A physiological role of PPIase function appears very plausible for fast physiological processes. Therefore, in addition to the use of PPIase mutants, the regulation of fast, conformation-sensitive processes would support a physiological function of PPIase activity. For instance, structural analysis revealed strong evidence that cis–trans isomerization at a particular proline serves as a gating switch of the 5-hydroxytryptamine type 3 (5-HT3) receptor. Nevertheless, the authors argued that the estimates of the opening rates for this receptor (10–100 s\(^{-1}\)) are within a factor of 10 of the intrinsic cis–trans isomerization rates of peptides, which could be further increased by structural features of the receptor. PPIases could, in principle, expedite this process, but this remains speculative currently.

Another scenario is kinetic partitioning of a PPIase substrate that follows different pathways depending on its conformation (Figure 2). If one of the pathways stabilizes or destabilizes the substrate, thus changing the ratio of cis and trans isomers, the transition between the two conformations may be rate limiting for the degradation of the substrate protein. This could be particularly relevant for Pin1 substrates, where phosphorylation of the preceding threonine or serine changes the balance between the two conformations. A very recent example is the 20 kDa Fanconi anemia (FA)-associated protein (FAAP20).
Table 1. Functions of PPIase proteins with related protein associations and PPIase requirement studies. The list is confined to the examples used in this essay. They were selected to exemplify the efforts in revealing the importance of protein interaction/scaffolding and/or PPIase enzymatic activity for the various functions of the PPIase proteins. Additional functions and protein interactions can be found in recent reviews.[70,83–85,87,110].

| Protein function                  | Protein associations                                                                 | PPIase mutants and ligands                                                                                                                                 |
|----------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Trigger factor                    | Associates with nascent chain peptides[42,47,48]                                      | PPIase point mutants do not abolish TF function.[47,48]                                                                                                                                                      |
| Folding of nascent proteins      | Reagents[42,47,48]                                                                     |                                                                                                                                                                                                           |
| Cyclophilin A                     | Binds to the ectodomain of the cell surface signaling receptor CD147.[16]             | PPIase-inactive point mutant exhibits unimpaired receptor binding and chemotaxis induction.[16]                                                                                                                   |
| Induction of leucocyte chemotaxis |                                                                                                                                                 | Cyclosporine A reduces NCX surface expression. Rescue by wt and PPIase-defective CypA.[15]                                                                                                                   |
| Regulates cell surface expression of the Na+-Ca2+ exchanger | Direct interaction with the virus matrix protein (M1).[17]                              | PPIase-defective mutants display binding to M1 and inhibition of viral replication equal to wt CypA.[15]                                                                                                    |
| Impairs influenza A virus replication |                                                                                                                                                 | Protein complexes also form in the presence of cyclosporine A.[16]                                                                                                                                     |
| May regulate motor protein complex | Associates with Cdc25[18]                                                             | Cyclosporin A enables binding to calcineurin (gain of function).[89]                                                                                                                                   |
| Regulates NFAT pathway            | Interacts with FAAP20[77] (phosphorylation-dependent)                                   | PPIase-defective point mutants of Pin1 exert no effect.[71]                                                                                                                                               |
| Regulates mitosis.[71]            | Binds to tau[72]                                                                       | PPIase-defective point mutant exhibits no effect on tau.[72]                                                                                                                                              |
| Restores binding of tau to microtubules, promotes microtubule assembly[72] | Associates with Cdc25[18]                                                             | Mutation of amino acids critical for PPIase enzymatic activity abolish the effect.[73]                                                                                                                     |
| Facilitates dephospho-rylation of Cdc25 and tau by PP2A[10] | Interacts with FAAP20[77] (phosphorylation-dependent)                                   | Catalytically dead Pin1 mutant does not affect FAAP20 levels.[77]                                                                                                                                     |
| Impacts the stability of FAAP20   |                                                                                                                                                 |                                                                                                                                                                                                           |
| FKBP52                           | Component the receptor chaperone heterocomplex                                        | Several PPIase-defective mutants still impact GR.[104]                                                                                                                                                     |
| Impacts GR and related receptors[95,100,104,114] | Associates with NFkB[106]                                                        | FKS06 and PPIase mutation abrogate the effect on NFkB.[106]                                                                                                                                               |
| Modulates NFkB activity[106]      | Associates with TRPC3[111]                                                             | PPIase-truncated FKBP52, but not FKS06, mimics the effect of FKBP52 knock-down.[111]                                                                                                                     |
| Modulates TRPC3 function and cardiac hypertrophy[111] |                                                                                                                                                 | No tests, antagonizes FKBP51.[124]                                                                                                                                                                       |
| Regulates GSK3β signaling[124]    | Associates with GSK3β, CDK5 and PP2A[124]                                            | Mutating PPIase activity does not impair impact on GR[95,100,101]                                                                                                                                          |
| FKBP51                           | Component the receptor chaperone heterocomplex                                        | Ligands and PPIase mutation affect the NFkB pathway in one study,[18] but not in another.[106]                                                                                                              |
| Impacts GR and related receptors[95,100,104,114] | Associates with NFkB[106]                                                        | PPIase-defective FKBP51 is unable to rescue the effect of FKBP51 knock-down.[114]                                                                                                                     |
| Modulates NFkB activity[106]      | Associates with pathway components[18]                                              | PPIase-defective FKBP51 shows unchanged protein interaction and effect on GSK3β phosphorylation.[124]                                                                                                      |
| Promotes myoblast differentiation[114] |                                                                                                                                                 | Scaffolding of Akt1-PHLPp was concluded using FKBP51 deletion mutants.[135]                                                                                                                                |
| Regulates GSK3β signaling[124]    | Associates with GSK3β, CDK5 and PP2A[124]                                            |                                                                                                                                                                                                           |
| Enhances autophagy[127–129]       | Binds to Akt1, PHLPp, Beclin1, and SKP2[127–129,135]                                 |                                                                                                                                                                                                           |

that was reportedly isomerized by Pin1, thus impacting its stability.[77] Pin1 enhances PP2A-mediated dephosphorylation of FAAP20, thereby diminishing phosphorylation-directed ubiquitination and subsequent degradation of FAAP20. The requirement of the enzymatic PPIase activity was concluded from NMR analysis revealing Pin1-dependent isomerization of FAAP20-derived peptides phosphorylated at serine 48 (preceding proline 49) and from the inability of a catalytically dead Pin1 mutant (K63A/R68A/R69A) to restore the FAAP20 levels reduced by PIN1 deletion.[77] Whether or not peptidyl–prolyl-isomerization actually is the rate-limiting step will be difficult to prove. For similar interesting examples, please refer to these recent publications and reviews.[68,73,78,79]

4.4. Early Studies on the FKBP52: Protein Interaction Matters

The two large immunophilins FKBP51 and FKBP52 originally were not discovered as PPIases, but as components of steroid hormone receptors almost 30 years ago.[80,81] Their PPIase activity in vitro was documented much later.[82] This study also reported chaperone activity for FKBP51 and FKBP52 based on refolding...
Figure 2. Role of PPIases in kinetic partitioning of signaling proteins. Protein modification (here: phosphorylation of a Ser/Thr-Pro motif) can change the equilibrium between the cis and trans conformation (in the depicted example, phosphorylation increases the fraction of cis conformation, and the activation energy for conformational transition). PPIases (Pin1 in this example) may be needed to quickly establish the new equilibrium. Furthermore, the equilibrium could be “disturbed”, when one of the conformations is prone to undergo further processing, such as degradation. PPIase activity may impact overall protein stability (positively or negatively) and thus be of functional relevance in these cases.

and thermal stability assays. Several reviews summarize the knowledge about their structure and function accumulated over the years. Therefore, the general background of these two proteins will be touched on only briefly.

FKBP51 and FKBP52, like the aforementioned cyclosporine binding CypA, are termed “immunophilins” simply for the fact that they bind immune suppressive drugs such as FK506 (tacrolimus) and rapamycin (sirolimus). Binding of these drugs occurs at the domain responsible for PPIase activity and blocks the enzymatic activity. However, other proteins are assigned to this protein family based on sequence homology to the PPIase domain, and not based on PPIase activity or drug binding. Thus, there are several immunophilins that neither bind immune suppressive drugs nor display PPIase activity. Furthermore, the immune-suppressive effect of the drugs only comes about through binding to the smallest members of the immunophilin family, FKBP12 and CypA. Mechanistically, binding of FK506 to FKBP12, or cyclosporine A to CypA, enables the targeted protein to bind to the serine/threonine phosphatase calcineurin by creating a composite surface for interaction. This prevents calcineurin from being activated with the consequence that the transcription factor NFAT (nuclear factor of activated T cells) remains phosphorylated and thus stays in the cytoplasm, hindered from stimulating the expression of genes relevant for immune activation. Thus, while drug binding does inhibit PPIase activity of the immunophilins, the effect relevant for immune suppression is a gain of function phenomenon, that is, the ability to efficiently bind to calcineurin and inhibit this phosphatase. In other words, in this iconic function of immunophilins, evidence suggests that effects through protein-protein interactions are important rather than their PPIase activity.

4.5. The Role of the PPIase Domains of FKBP51 and FKBP52 in Steroid Receptor Function

In general, the functional relevance of PPIase activity for biological function is concluded from experiments employing pharmacological or genetic inhibition of its enzymatic activity. Like with many interventions in biology, it is not always possible to exclude additional (i.e., side) effects beyond inhibiting PPIase enzymatic function. An instructive example is the effect of FKBP52 on the activity of the glucocorticoid receptor (GR). Steroid receptors, such as GR, are functionally regulated by an Hsp90-based chaperone heterocomplex. The discovery of FKBP51 and FKBP52 in steroid receptor complexes raised the question about their role in receptor function in general, in particular the possible requirement of PPIase activity. A landmark study used heterologous expression in Saccharomyces cerevisiae, that lack endogenous FKBP51, FKBP52, and steroid receptors, to demonstrate that FKBP52 potentiates GR-dependent reporter gene activation. The close homologue FKBP51 did not affect GR activity in this yeast model, but counteracted the effect of FKBP52. Using point mutations of FKBP52, the authors further showed that disrupting binding to Hsp90 as well as disrupting PPIase activity abolishes the effect of FKBP52 on GR. To abrogate PPIase function in FKBP2, the double mutant FD67DV was used; the two substituted residues are highly conserved in the FKBP family and were shown to be essential for
PPiase activity in both FKBP51[90] and FKBP12.[97] Furthermore, FK506 also prevented the effect of FKBP52 on GR.[95]

Another study in mammalian cells presented an inhibitory effect of FKBP51 on GR function, consistent with previous reports.[98,99] while FKBP52 displayed no effect.[100] This seeming contradiction to the results in S. cerevisiae can be explained by the different endowment of the two cellular systems: mammalian cells are set up for efficient function of steroid receptors and thus are sensitive for the ectopic expression of inhibitors, while yeast cells lack some of the steroid receptor promoting factors and thus potentiating factors are more readily detectable. Studies in mammalian cells further found that FD67DV mutation of FKBP51 did not alter the inhibitory effect on GR, while the same mutation turned FKBP52 into a potent inhibitor of GR activity, in line with the findings of the yeast study.[95,100,101] Thus, the authors of this study concluded that PPiase activity is important for the function of FKBP52 with respect to GR activity.[100]

FK506 can inhibit target protein function by blocking protein-protein contacts by extruding from its binding pocket.[102,103] Furthermore, the FD67DV mutation may also affect partner binding, possibly through alterations in the PPiase domain conformation.[104] Thus, additional mutants were constructed exchanging critical amino acids in the hydrophobic PPiase pocket. The single amino acid mutations Y57A, F67Y, W90L, and F130Y impaired the PPiase activity of FKBP52.[104] However, they had no effect on the potentiation of GR signaling by FKBP52.[104] Therefore, the prior conclusion that the PPiase activity is relevant for the action of FKBP52 on GR function had to be corrected. The authors confirmed, though, the importance of the PPiase domain and identified as critical feature a surface loop of this domain that overhangs the PPiase active site and that differentiates FKBP52 from FKBP51.[104] The authors further argue that the differences in this surface loop may lead to different modes of protein interaction in particular with the ligand binding domain of GR, as the basis for the differential action of FKBP51 and FKBP52. The relevance of this loop for the conformation of this domain, and thus probably for protein interaction, was confirmed recently.[105] Overall, this example shows again that it is easier to disprove than to prove a role of the enzymatic PPiase activity.[86]

4.6. FKBP51 and FKBP52 Regulate Many Proteins

PPiase activity of FKBP52 is thought relevant for its stimulatory effect on the transcription factor NFκB.[106] This conclusion is based on the two observations: FK506 abrogates the FKBP52 effect and the PPiase mutants F67Y and F130Y produce an overall lower NFκB activation.[106] The inhibitory action of FKBP51 on NFκB clearly was independent of its PPiase activity in this study.[106]

In contrast, a later study on the role of FKBP51 found that FKBP51 promotes NFκB activation in melanoma, by employing both scaffolding and isomerase functions.[107] The necessity of PPiase activity was concluded from experiments using FK506 and other ligands of FKBP51 that were shown to interfere with the stimulatory effect of FKBP51 on NFκB activation. Genetic ablation of FKBP51 PPiase activity was not employed. Overall, the effect of FKBP51 on NFκB appears to depend on the particular experimental system.[87,106–110]

Very recently, PPiase-dependency was reported for the modulation of the transient receptor potential (TRP; C-classical, TRPc) channel TRPC3-dependent Ca2+ signals by FKBP52.[111] However, this was concluded from using a truncation mutant lacking the N-terminal domain of FKBP52.[111] Thus, it remains to be elucidated whether or not the protein association of FKBP52 with TRPC3 requires PPiase enzymatic activity for its functional effect. Intriguingly, FK506 affected protein interactions, but it did not replicate the effect of FKBP52 knock-down; this could be explained by compensatory actions of other FK506 targets.[111]

Glomulin, a regulator of the SCF (Skp1-CUL1-F-box protein) E3 ubiquitin-protein ligase complex,[112] is a recent addition to the list of FKBP51 protein interactors.[113] The interaction turned out to be sensitive to ligands of FKBP51, and the FD67/68DV point mutated FKBP51 binds with substantially reduced affinity to Glomulin. Nevertheless, the authors were careful in speculating about the necessity of PPiase activity for the interaction, since these mutations may alter the domain structure more globally.[113]

Most studies investigating the need for PPiase function of FKBP51 or FKBP52 for a certain activity or target protein fail to elucidate the actual prolyl bond that is isomerized. An example is a study characterizing the promotion of myoblast differentiation by FKBP51.[114] On the one hand, FKBP51 acts via protein-protein interactions by sequestering Cyclin-Dependent Kinase (CDK) 4 within the Hsp90 storage complex and preventing the formation of the cyclin D1-CDK4 complex. On the other hand, FKBP51 isomerizes the peptide bond T172-P173 in a six amino acid model peptide and inhibits phosphorylation of T172.[114] This phosphorylation is an essential step in the activation of CDK4.[115,116] Using rescue experiments transducing WT and PPiase-mutant FKBP51 into myoblasts with an FKBP51 gene knock-out it was further found that only WT FKBP51 is able to reinstate the effect on proliferation and differentiation.[114] Finally, CDK4 knock-down per se did not affect myoblast differentiation, but erased the effect of FKBP51 knock-down. This effect could be rescued by additional transduction of WT CDK4, but not its P173A mutant.[114] Overall, this study is highly suggestive of the relevance of FKBP51’s PPiase involvement in CDK4 activity. Nevertheless, the molecular mechanisms of FKBP51’s action in myoblast differentiation appear quite complex. FKBP51 inhibits CDK4 through determining protein-protein interaction and possibly also peptidylprolyl-isomerization accompanied by interference with CDK4 phosphorylation. However, CDK4 knock-down does not replicate the effect of FKBP51, rather it abolishes it.[114] In addition, it will be difficult to show that T172-P173 is isomerized by FKBP51 in the entire protein, and not just within a short peptide. Furthermore, the impact of PPiase pocket mutations on the mode of interaction of FKBP51 with other proteins remains to be investigated.

4.7. Do Ligands Impact the Conformational Dynamics of PPiase Domains?

Several studies document structural alterations of FKBP51 upon ligand binding. For example, application of multiple short molecular dynamics simulations revealed that ligand binding
to the PPIase pocket produced significant effects on dynamics behavior and conformational changes in distinct regions of FKBP51.\textsuperscript{117} X-ray crystallography and nuclear magnetic resonance (NMR) were used to analyze conformational changes and dynamics of the N-terminal PPIase domains of FKBP51 and FKBP52.\textsuperscript{118,119} Pronounced differences were found between the two immunophilins which underlie their differential interaction with steroid receptors. The authors further argue that the two proteins differentially populate the available conformational space and that selective ligands thus select a particular conformation\textsuperscript{118} rather than operating through induced fit as originally thought.\textsuperscript{120} The validity of this concept has been confirmed recently.\textsuperscript{121} Additionally, conformational dynamics in FKBP domains is relevant for molecular signaling, as outlined elsewhere.\textsuperscript{122}

Overall, these studies on structure and conformational dynamics of PPIase domains illustrate the challenges in firmly establishing the functional relevance of PPIase activity. Ligands inhibiting PPIase activity may disturb protein-protein interactions not only through steric interference, but also through inducing or stabilizing a particular protein conformation incompatible with interaction. Similar effects are likely to be elicited by blocking PPIase activity through amino acid mutation, although this has not been demonstrated in all cases.

4.8. Protein Interactions of FKBP51 and FKBP52

More studies on FKBP51 have been performed and thus more data exist on it than FKBP52. This bias probably originated from the first gene association study in depression that found FKBP51 associated with the response to antidepressant treatment;\textsuperscript{123} this elicited intense interest in analyzing the molecular biology, genetics, epigenetics, and suitability for drug development of this protein worldwide.\textsuperscript{86,110} From a mechanistic point of view, this bias may deserve a second thought, as FKBP51 and FKBP52 frequently act as functional antagonists, despite their close homology.\textsuperscript{83–85} This differential action could be explained, in part, by differences in protein-protein interactions entailing different scaffolding of protein heterocomplexes.

A high-throughput assay combining mass spectrometry and luciferase-based cellular interaction assays revealed a vast interaction network of molecular chaperones.\textsuperscript{28,36} Of the FKBP52, FKBP51 exhibited the most extended and diversified protein interaction network, with pronounced differences to FKBP52.\textsuperscript{16} Among them are kinases, Argonaute proteins, transcription factors, and MCM complex proteins. Of note, at least some of these interactions appear to be independent of Hsp90,\textsuperscript{16} for example the association with GSK3\(\beta\).\textsuperscript{124} The relevance of the PPIase domain for various protein interactions has been documented in several studies.\textsuperscript{87}

Among the recently reported interaction proteins of FKBP52, not listed above or in reviews yet, is Phosphatidylinositol-3-kinase (PI3K).\textsuperscript{125} For FKBP51, it is the PTEN-induced putative kinase 1 (Pink1)\textsuperscript{126} and the E3 ligase S-phase kinase-associated protein 2 (Ske2)\textsuperscript{127} which further links FKBP51 to the ubiquitin-proteasome system and to the previously reported increase of the autophagy protein Beclin1 by FKBP51.\textsuperscript{128,129} The functions of the PPIase proteins selected here are listed in Table 1, along with the related results from analyses assessing protein interaction/scaffolding and requirement of enzymatic PPIase activity.

This plethora of stable protein interactions strongly argues in favor of scaffolding of protein interactions being the more relevant function of FKBP51 and FKBP52, if not the only one. Typically, the interaction of enzymes (here PPIases) with substrates are short-lived, as forming stable complexes contradicts the very nature of enzymes as expeditors of (bio)chemical reactions. As mentioned above, structural analyses revealed several conformations that can be adopted by the FKBP52. This conformational variety might contribute to the diversity of their interactions, as has been argued for scaffolding proteins in general.\textsuperscript{130} Therefore, the development of conformation-specific ligands might enable manipulation of a subset of the protein interactions and thus functions of FKBP51 and FKBP52.

5. PPIase Activity as a Side Effect of Protein Interaction: A Hypothesis

Peptidyl–prolyl bonds typically mark characteristic protein regions, as such they are good candidates for specific protein interactions. Protein interaction has the potential to affect the energy landscape for protein folding of the interacting proteins, in other words the transition from one conformation to another. Thus, the interaction with proline-containing protein domains could facilitate or impede cis–trans isomerization of the peptidyl–prolyl bonds, without being necessary for protein function (Figure 3).

This hypothesis does not contradict the observation that typically one conformation is preferred over the other for binding. For example, using NMR it has been shown that a \(\beta\)-turn motif peptide derived from the estrogen receptor interacts with the catalytic site of FKBP52 in the cis conformation.\textsuperscript{131} In general, the cis conformation is very rare in peptide bonds (less than 0.01%\textsuperscript{132}), but more populated by the bonds preceding proline (5% and more, depending on the sequence context\textsuperscript{67,132}). The fact that FKBP52 is able to catalyze the isomerization of the prolyl bond in the peptide of its receptor interaction motif\textsuperscript{133} does not prove that this catalytic activity is necessary for stable interaction. Conformational selection is a plausible alternative mechanism. It should also be noted that the rate constants determined for the non-catalyzed cis–trans transition are in a broad range between 0.002 s\(^{-1}\) and more than 1000 s\(^{-1}\).\textsuperscript{67,74,76}

Thus, it appears that at least for the establishment of protein-protein interactions the non-catalyzed conversion is fast enough. Among these lines, FKBP52 is able to isomerize prolyl bonds in the human tau protein.\textsuperscript{133} This enzymatic activity, however, is not required for the induction of oligomers in mutant and truncated forms of tau protein by FKBP52, in contrast to molecular interaction through the PPIase domains.\textsuperscript{133}

6. Summary and Perspectives

While there is no doubt that the PPIase domain of FKBP51 and FKBP52 is involved in manifold protein interactions\textsuperscript{84–87} that point to a scaffolding function, the physiological relevance of the PPIase enzymatic activity is not resolved.

It is possible that the enzymatic activity is a “by-product” of protein interaction, and therefore not relevant in most, if not all
Is PPIase activity only a side-product of proline motif interaction? It is hypothesized that proline containing regions present characteristic surfaces for protein interaction. At least in some cases, proteins interacting with these domains change the energy surface for conformational alterations of the target protein (or substrate, S) facilitating the interconversion between the cis and trans configuration ("Folding"). This may not be required for the hypothesized main effect, which is achieved by providing combined interaction surfaces for other proteins engaged in signal transduction ("Scaffolding").

The best evidence is available for Pin1, whose substrates need longer to reach their cis/trans equilibrium than those of the other PPIases. Theoretically, this increases the necessity for facilitating isomerization under conditions far from the equilibrium. In any case, the role of PPIase activity would be in protein function and signal transduction rather than in protein folding per se.

A precondition for the physiological relevance of PPIase activity is that the two conformational isomers of the substrate protein exhibit different functions. While this has been addressed for some substrates of Pin1, this question has received little attention for FKBP51 and FKBP52. Future experiments could make use of artificial proline analogues that impact the cis/trans preference and the energy threshold for their interconversion. In addition, in most cases there is no information or hypothesis about what mechanism may have caused the disturbance of the cis/trans equilibrium which necessitates the action of a PPIase. Where possible, time-resolved experiments would increase the support for the relevance for expediting prolyl-isomerization. Point mutants continue to be very useful for the analyses, even if they cannot provide ultimate proof.

The view of FKBP51 and FKBP52, and possibly other PPIases, as scaffolders is evidenced by numerous interaction studies but also leaves conceptual questions. It is striking, for example, that they are engaged in signal transduction through the interaction with several kinases and phosphatases. Since enzymes typically engage their substrates for a very short time, it appears more plausible that stable complexes may be formed between either enzyme or substrate and the FKBP5s, but not stable ternary complexes. In this sense, the FKBP5s would act as transient scaffolders.

Acknowledgements

The author thanks Jessica Keverne for help with English language editing.

Conflict of Interest

The author declares no conflict of interest.

Keywords

chaperones, FKBP5, FKBP51, FKBP52, Peptidylprolyl isomerases

Received: December 16, 2019
Revised: March 12, 2020
Published online: April 22, 2020

[1] F. U. Hartl, Nature 1996, 381, 571.
[2] C. B. Anfinsen, Science 1973, 181, 223.
[3] Y. E. Kim, M. S. Hipp, A. Bracher, M. Hayer-Hartl, F. U. Hartl, Annu. Rev. Biochem. 2013, 82, 323.
[4] J. Ellis, Nature 1987, 328, 378.
[5] R. B. Freedman, T. R. Hirst, M. F. Tuite, Trends Biochem. Sci. 1994, 19, 331.
[6] C. Schiene-Fischer, J. Habazettl, F. X. Schmid, G. Fischer, Nat. Struct. Biol. 2002, 9, 419.
[7] F. X. Schmid, Annu. Rev. Biophys. Biomol. Struct. 1993, 22, 123.
[8] C. Schiene, G. Fischer, Curr. Opin. Struct. Biol. 2000, 10, 40.
[9] M. S. Hipp, P. Kasturi, F. U. Hartl, Nat. Rev. Mol. Cell Biol. 2019, 20, 421.
[129] N. C. Gassen, J. Hartmann, M. V. Schmidt, T. Rein, *Autophagy* 2015, 11, 578.
[130] D. Garbett, A. Bretscher, *Mol. Biol. Cell* 2014, 25, 2315.
[131] C. Byrne, M. A. Henen, M. Belnou, F.-X. Cantrelle, A. Kamah, H. Qi, J. Giustiniani, B. Chambraud, E.-E. Baulieu, G. Lippens, I. Landrieu, Y. Jacquot, *Biochemistry* 2016, 55, 5366.
[132] M. S. Weiss, A. Jabs, R. Hilgenfeld, *Nat. Struct. Mol. Biol.* 1998, 5, 676.
[133] A. Kamah, F. X. Cantrelle, I. Huvent, J. Giustiniani, K. Guillermeau, C. Byrne, Y. Jacquot, I. Landrieu, E. E. Baulieu, C. Smet, B. Chambraud, G. Lippens, *J. Mol. Biol.* 2016, 428, 1080.
[134] J.-P. Schülke, G. M. Wochnik, I. Lang-Rollin, N. C. Gassen, R. T. Knapp, B. Berning, A. Yassouridis, T. Rein, *PLoS One* 2010, 5, e11717.
[135] H. Pei, L. Li, B. L. Fridley, G. D. Jenkins, K. R. Kalari, W. Lingle, G. Petersen, Z. Lou, L. Wang, *Cancer Cell* 2009, 16, 259.