Minireview

# Receptor accessory folding helper enzymes: the functional role of peptidyl prolyl *cis*/*trans* isomerases

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**Abstract Receptor accessory peptidyl prolyl *cis*/*trans* isomerases (PPIases) of the FKBP and cyclophilin types form receptor heterocomplexes with different stabilities. PPIases have been found to associate with other receptor heterocomplex constituents via either proline-directed active sites or additional domains of the enzymes. The single-domain PPIases FKBP12 and FKBP12.6 are shown to interact with receptor protein kinases and calcium channels at their active sites. In contrast, heterooligomeric nuclear receptors contain multi-domain PPIases like FKBP51, FKBP52 or cyclophilin 40 that directly interact with the chaperone hsp90 via the tetratricopeptide repeat modules of the folding helper enzymes. PPIases play a critical role in the functional arrangement of components within receptor heterocomplexes.** 

*Key words:* Peptidyl prolyl *cis*/*trans* isomerase; Steroid receptor; Calcium channel; Receptor protein kinase

## 1. Introduction

The balance of events to regulate receptor protein-mediated signaling is often maintained by receptor accessory proteins that form heterooligomeric complexes of different thermodynamic stability together with the ligand binding receptor protein. Among the great variety of accessory proteins, one remarkable feature is that the folding helper proteins of the enzyme class of peptidyl prolyl *cis*/*trans* isomerases (PPIases, EC 5.2.1.8) together with chaperones constitute a major part of the receptor complexes (Fig. 1). PPIases are folding helper enzymes with the capability to catalyze the *cis*/*trans* isomerization of prolyl bonds[1] in different folding states of a target protein, and this interconversion affects the spatial arrangement of backbone segments in the protein. PPIase-catalyzed protein conformational changes were shown for the refolding of denatured proteins [1], de novo protein synthesis [2,3] and the formation of biologically active conformations of polypeptides [4,5]. Obviously, PPIases have to be functional in both proline-directed polypeptide binding and catalysis of conformational interconversions of proline-containing polypeptide substrates [6].

The three phylogenetically conserved families of PPIases involved in catalysis of protein folding are the cyclophilins (Cyps), the FK506 binding proteins (FKBPs) and the parvulins [7]. The PPIase activity of Cyps is inhibited by the immunosuppressant drug cyclosporin A (CsA), whereas FKBPs are inhibited by FK506 and rapamycin. Thus, Cyps and FKBPs have occasionally been termed immunophilins. A major part of the bioactivity of CsA and FK506 is thought to be mediated by calcineurin inhibition as the consequence of a 'gain of function' mechanism, and the inhibitory potency of the respective PPIase/drug complexes. Generally, it appears that the gain of function properties of the PPIase/drug complexes do not explain drug effects on heterooligomeric receptor signaling. The PPIase families comprise members of single-domain prototypes as well as much larger proteins with additional N- or C-terminal domains with a variety of functions. These include tetratricopeptide repeats (TPR), WW domains, calmodulin binding sites, DNA binding sites, RNA binding sites, and dimerization modules.

PPIases have been found to associate with other proteins either by virtue of their complementary domain interfaces or at their proline-directed active sites. The above mentioned enzyme inhibitors and their derivatives can be used to distinguish between active site-mediated interactions and associations directed by peripheral protein regions. However, the multiplicity of the members of the PPIase families in mammalian cell types may mask the loss of function through functional substitution by another [8]. The single-domain PPIase FKBP12 and FKBP12.6 active sites interact with receptor protein kinases and calcium channels. The receptor proteins themselves are probably substrates for the PPIases. In con-

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*Abbreviations:* AhR, arylhydrocarbon receptor; CsA, cyclosporin A; Cyp, cyclophilin; EGF, epidermal growth factor; FKBP, FK506 binding protein; GR, glucocorticoid receptor; PPIase, peptidyl prolyl *cis*/*trans* isomerase; PR, progesterone receptor; RyR, ryanodine receptor; TC, terminal cisternae; TβR, TGF-β receptor; TGF-β, transforming growth factor-β; TPR, tetratricopeptide repeat

[1] The imide peptide bond preceding proline is termed a prolyl bond, and prolyl isomerization is used to denote the *cis*/*trans* isomerization of this peptide bond.


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trast, the heterooligomeric steroid receptors and the arylhydrocarbon receptor (AhR) contain multi-domain PPIases in direct interaction with the chaperone hsp90 via the TPR modules of the folding helper enzymes [9]. A comparison of the characteristics of receptor/PPIase functional interactions can shed light on the role of structural reorganization of the receptor protein during ligand-induced signal transfer processes.

## 2. Interaction of single-domain PPIases with receptors

The endoplasmic reticulum and its tissue-specific derivatives such as the sarcoplasmic reticulum, the main $Ca^{2+}$ storage compartment, contain two different families of $Ca^{2+}$ release channels, the ryanodine receptors (RyR) and the inositol 1,4,5-trisphosphate receptors ($JP_3R$).

The RyR consists of four identical 565 kDa subunits, there are three isoforms of RyR. RyR1 is the primary isoform in skeletal muscles, RyR2 is found in the cardiac sarcoplasmic reticulum, RyR3 is found in the brain. Purified RyR1 from terminal cisternae (TC) of sarcoplasmic reticulum of rabbit skeletal muscle was found to associate tightly with the prototype of the FKBP family of PPIases, the cytoplasmic FKBP12 [10,11]. The 4:1 molar ratio of FKBP12/tetrameric RyR complex indicates the stoichiometric binding of one FKBP12 molecule to one RyR subunit [11]. The heterocomplex dissociation by titration with FK506 reveals that the interaction involves the active site of FKBP12. FKBP12.6, which differs from FKBP12 in 16 of 107 amino acid residues, can also bind to RyR1. In cardiac muscle sarcoplasmic reticulum it is specifically associated with RyR2 [12], selective binding is caused by Gln31, Asn32 and Phe59 of FKBP12.6 [13].

RyR channels from FKBP12-deficient TC vesicles have greater open probabilities and longer mean open times compared to complete TC [14]. FKBP12-depleted RyR channels opened frequently to substate levels of $\sim$0.25, $\sim$0.5, and $\sim$0.75 of the maximum conductance [15]. Functional differences between RyR1 and RyR3 such as skeletal type excitation–contraction-coupled gating behaviors and subconductance levels are thought to arise from FKBP12 binding [16]. FKBP12 was proposed to mediate interactions between RyR channels because addition of external FKBP12 to RyR in planar bilayers induces coupled gating [17]. Dissociation of FKBP12 from TC by competition with FK520, a non-immunosuppressive structural analogue of FK506, increases the $Ca^{2+}$ leak rate. Interestingly, substitution by the FKBP12 variants F36Y, W59H and F99Y, which exhibit reduced PPIase activity, for wild type FKBP12 on the RyR heterocomplex cannot abolish the wild type FKBP12 effects on the $Ca^{2+}$ flux of TC vesicles. These results suggest that either PPIase activity is unnecessary or the residual enzyme activity of the FKBP12 variants suffices for the FKBP-mediated modulation of the calcium release channel [18]. In contrast, Marks [19] reported that the FKBP12 F36Y variant is still able to bind, but not to modulate RyR channeling pointing to a crucial role of the unperturbed enzyme activity of FKBP12. Reviewing these experiments under standardized conditions might provide new insights into this discrepancy.

A still unspecified FKBP is required as a binding partner of RyR during fertilization of *Phallusia mammillata* ascidian oocytes [20]. FKBP12-deficient mice have normal skeletal muscle but severe dilated cardiomyopathy and ventricular septal defects [21]. The functional properties of RyR1 as well as of cardiac RyR2 regarding greater open probability and subconductance states are typical of FKBP12 shortage. The cardiac defects of FKBP12-deficient mice may demonstrate the inability of FKBP12.6 to functionally replace FKBP12 in RyR signaling. In a canine model of pacing-induced heart failure, the ratio of FKBP12.6 per RyR2 is significantly decreased. A conformational change resulted for RyR2 chains concomitant with abnormal $Ca^{2+}$ leak of the channel [22]. Protein kinase A-mediated hyperphosphorylation of RyR in failing human hearts induces dissociation of FKBP12.6 from RyR thus resulting in defective channel function [23]. Biological data as well as in vitro experiments reveal the importance of the interaction between the receptor and FKBP12 or FKBP12.6 in RyR channel function although the mechanism of FKBP function and the role of PPIase activity of these enzymes in the heterocomplex are still unsolved problems.

FKBP12 was also found to be a constituent of $IP_3R$, the other major calcium release channel of the endoplasmic reticulum [24]. FK506 and rapamycin physically disrupt the heterocomplex, indicating that the active site of FKBP12 is involved in binding. The $IP_3R$ sequence region exhibiting the FKBP12 active site affinity mapped within the central modulatory portion, and the leucyl prolyl moiety of the $^{1396}$CNSLLPLDDIV$^{1406}$ amino acid stretch represents the active portion [25]. Surprisingly, the gain of function model of calcineurin inhibition of the FK506/FKBP12 complex was mimicked by the FKBP12/$IP_3R$ (1349–1460 segment) complex because calcineurin was found to be an interaction partner of this complex in a derived yeast two-hybrid system. However, no calcineurin inhibition was obtained with the FKBP12/$IP_3R$ (1349–1460 segment) complex. Nevertheless, interactions of FKBP12 with both major calcium release channels of the endoplasmic reticulum have been shown, which implies that these interactions play a critical role in calcium signaling.

Apparently, this kind of stable heterocomplex does not form during the functional interaction of FKBP with receptor protein kinases, another receptor type influenced by PPIases. The heterodimeric transforming growth factor-β (TGF-β) receptor consists of the serine/threonine kinase subunits type I (TβR-I) and type II (TβR-II). Ligand binding enables TβR-II to recruit and activate TβR-I by phosphorylation of the serine residues in the glycine/serine-rich domain (GS domain) of the juxtamembrane region of TβR-I. FKBP12 binds to the cytoplasmic domain of unactivated TβR-I in vitro and in vivo. The Leu193-Pro194 moiety in the juxtamembrane region of TβR-I, which localizes in close proximity to the activating phosphorylation sites in the GS domain, is required for the interaction. This was shown by the site-directed variants of TβR-I: P194A and L193A/P194A, as well as P194K and L193G. These variants cannot interact with FKBP12 [26–28]. On the other hand, the interaction of FKBP12 with TβR-I does involve the active site of the PPIase. A concentration of 1 μM FK506 prevents association of the two proteins [26]. The crystal structure of the binary complex of an unphosphorylated TβR-I fragment containing both the GS region and the catalytic domain and FKBP12 has been determined. FKBP12 binds to the GS region of the receptor, capping the TβR-II phosphorylation sites and further stabilizing the inactive conformation of TβR-I [29].

Overexpression of a myristylated FKBP12 in TGF-β-responsive mink lung epithelial cells inhibits two separate TGF-β-dependent signal pathways, as shown by the reduction

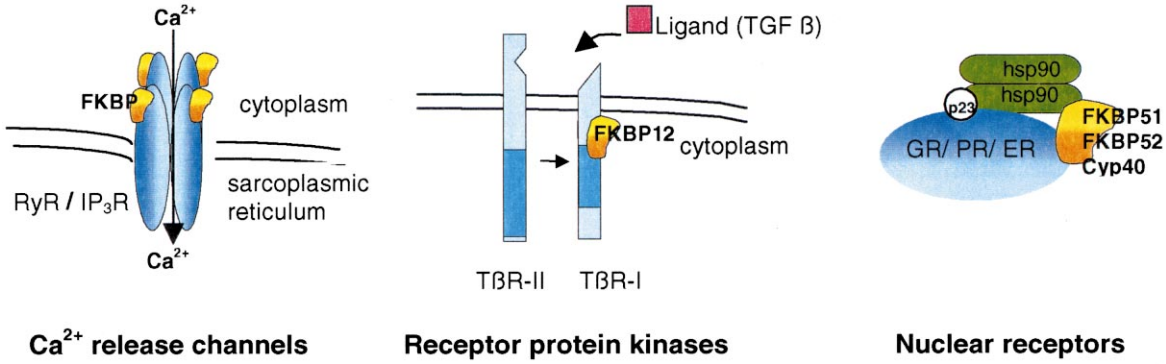


Fig. 1. PPIases as components of receptor heterocomplexes of (A) $Ca^{2+}$ release channels, (B) receptor protein kinases, (C) nuclear receptors.

of TGF-β-induced 3TP-Luc luciferase expression and the increase of expression of a cyclin A promoter-driven luciferase [30]. FKBP12 binding to TβR-I inhibits the receptor activation in the absence or in the presence of low amounts of TGF-β, the absence of FKBP12 causes activation of TGF-β receptor (TβR) signaling even in the presence of threshold doses of TGF-β. Loss of FKBP12 binding properties of the TβR-I variants L193G and P194K accompanies hypersensitivity to activation by basal level interactions with TβR-II [31]. Thus, FKBP12 may serve as a protective agent against leaky signaling resulting from the intrinsic affinity of the TβR subunits by preventing the activation of TβR-I in the absence of ligand.

Both embryonic fibroblasts and thymocytes deficient in FKBP12 do not show any differences from wild type cells with respect to TGF-β-mediated signaling [32]. FKBP12-deficient mice do not phenocopy any TβR or ligand knockout as well as the overexpressor mouse model [21]. Considering the multiplicity of members of FKBP in mammalian cells [8], functional replacement of FKBP12 provides a convincing explanation for this result. FKBP12 apparently functions as a negative regulator of TβR endocytosis. Rapamycin specifically enhances internalization of a chimeric receptor system containing the ligand binding domain of granulocyte-macrophage colony-stimulating factor receptor fused to TGF-β receptor transmembrane and cytoplasmic domains [33]. This might indicate that FKBP12 plays a broader role in TβR signaling than simply inhibiting receptor activation by preventing TβR-I phosphorylation.

As exemplified by the epidermal growth factor (EGF) receptor, FKBP12 is inhibitory to the autophosphorylation of a receptor tyrosine kinase [34]. In many aspects, the effect resembles the inhibition of serine/threonine phosphorylation of TβR-I, but now extends to tyrosine phosphorylation.

FK506 and rapamycin enhance phosphorylation of EGF receptor in a comparable manner in EGF receptor-rich A431 fibroblasts leading to a decrease in cell growth. In response to FKBP12, but not to Cyp18, autophosphorylation of the EGF receptor is reversibly and completely inhibited at 20 μM FKBP12 in microsomal receptor preparations. Other PPIases of the FKBP family such as *Legionella pneumophila* FKBP25, *Escherichia coli* FKBP22 and SlyD are also able to suppress in vitro autophosphorylation of EGF receptor, but to different degrees. The FKBP variants D37L and F99Y, which show reduced PPIase activity but have restored binding capability, do not affect EGF receptor phosphorylation indicating the importance of PPIase activity in the functional interaction [44]. Crosslinking experiments reveal direct binding to EGF receptor dimers by both FKBP12 and FKBP12 D37L. Pulse chase experiments have shown that dephosphorylation of the EGF receptor by intrinsic protein tyrosine phosphatases is not influenced by FKBP12. Similar to TβR-I phosphorylation, the presence of a high concentration of FKBP12 prevents leaky receptor autophosphorylation (C. Schiene-Fischer, unpublished data). The role of FKBP12 in receptor protein kinase heterocomplexes and the impact of these complexes on intracellular signaling are emerging as an interesting component of the conformational regulation of intracellular signaling mechanism.

A common feature of the single domain FKBP/receptor heterocomplexes regarding receptor protein kinases and calcium release channels is that the PPIase inhibits signaling in a manner dependent on enzyme catalysis. Obviously, FKBP12 alone cannot shift the putative native-state prolyl isomer ratio of the receptor, which might cause altered receptor activity, unless the FKBP12 concentration exceeds that of the receptor. However, the receptor being trapped in a high-energy, high-activity state would also provide a hypothesis for the FKBP12-catalyzed inactivation to occur. Coupling of energy-driven reactions as receptor phosphorylation or dephosphorylation opens a way to produce transient high-energy prolyl bond conformations that can be released in response to FKBP12. In fact, single-domain FKBP-mediated receptor activity was shown to be strongly dependent on the receptor phosphorylation status, as shown for TβR and RyR.

## 3. Multi-domain PPIases in heterocomplexes of nuclear receptors

Unactivated steroid receptor heterocomplexes contain multi-domain PPIases of the Cyp and FKBP families that are composed of functional sequence motifs in addition to the catalytic core (Fig. 2). For these heterocomplexes, three fundamental assembly stages can be identified on the basis of their unique protein compositions as demonstrated for progesterone receptor (PR) (Fig. 3). Mature complex formation requires the recruitment of PPIases. One of the PPIases, either FKBP51, FKBP52 or Cyp40 [35–37], is bound to hsp90 via their TPR motifs. These are degenerative sequences of 34 amino acids found to be frequently involved in protein–protein interactions. The PPIases differ in their preferences for distinct steroid receptor heterocomplexes. The PR heterocomplex preferentially assembles with FKBP51 despite its low

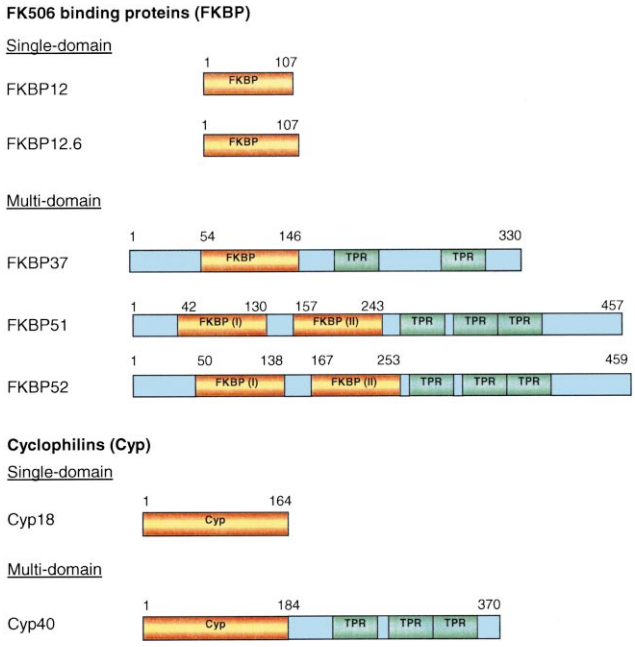


Fig. 2. Schematic representation of the structural organization of PPIases found to be associated with receptors in comparison to the single-domain prototypes of the respective PPIase family.

concentration of 20 nM compared to 100 nM FKBP52 and 200 nM Cyp40 in reticulocyte lysate. The C-terminal region of the PPIases, which along with the TPR motifs contributes to hsp90 binding, also includes the specificity determinants for discrimination between the different steroid receptor heterocomplexes [38,39]. Competition experiments with C-terminally truncated hsp90 variants revealed a single TPR recognition site encompassing a segment between residues 600 and 724 [40,41]. This 124-residue segment, which contains the essential $^{729}$EEVD$^{732}$ sequence motif, overlaps with the hsp90 dimerization domain and is important for hsp90 binding to the steroid receptor protein as well. Binding of steroid hormone to the receptor protein prevents reinitiation of the heterocomplex assembly cycle. The steroid receptor migrates to the nucleus and the heterocomplex components dissociate. The steroid receptor protein is now able to form homodimers, which act as transcription factors that bind to DNA at the hormone response elements in close proximity to targeted genes. The heterocomplex form of steroid receptors keeps unactivated steroid receptors silenced.

Other nuclear receptor proteins found to be associated with PPIases do not homodimerize in the nuclear, transcriptionally active form. After ligand activation the ecdysone receptor of *Manduca sexta* forms heterodimers with the ultraspiracle protein resulting in the control of insect metamorphosis. The FKBP46 that complements the unactivated receptor heterocomplex is made up of a helical DNA binding domain and a C-terminal FKBP-like segment but contains no TPR motif [42,43]. Unliganded cytosolic AhR also exists as a heterocomplex. After ligand activation, AhR migrates to the nucleus and heterodimerizes with Arnt (AhR nuclear translocator protein). The TPR domain PPIase FKBP37 (also termed AIP, ARA9 or XAP2) specifically associates with hsp90 in the unliganded AhR heterocomplex. Overexpression of FKBP37 results in an increase of AhR-mediated transcriptional activity in Hepa1 cells after ligand activation [44,45]. The increasing variety of nuclear receptor heterocomplexes that have been shown to contain PPIases suggests the general importance of these folding helper enzymes in nuclear receptor signaling.

Human FKBP52 (also termed hsp56, p52, FKBP59 or HBI) consists of three globular domains that could be expressed separately in *E. coli* [35] (Fig. 2). Recombinant FKBP52 exhibits PPIase activity mainly due to the N-terminal domain that is inhibited by FK506 efficiently ($K_i = 10$ nM) [46]. Casein kinase II-catalyzed phosphorylation of Thr143, which is situated in the hinge region between the two FKBP homologous domains, controls hsp90 binding properties of FKBP52 [47]. The phosphorylated form of FKBP52 cannot maintain hsp90 binding. The main fraction of FKBP52 is located in the nucleus, the cytoskeleton of interphase cells assembles a minor fraction [48]. Interestingly, it is FKBP52 that was found to mediate the neuroregenerative properties of FKBP inhibitors, as was shown by the inhibition of FK506-induced neurite outgrowth of SHSY5Y cells by monoclonal anti-FKBP52 antibody [49]. However, the mechanism of neuroregeneration and neuroprotection by PPIase inhibitors is still unknown. Human FKBP51 (FKBP54, p54, FF1 antigen) was discovered in the PR heterocomplex [36,38]. It exhibits 55% overall identity to human FKBP52. Like FKBP52, it has a domain structure characterized by two FKBP domains and three TPR motifs (Fig. 2). Additionally, there exists a series of smaller human FKBPs: FKBP36, FKBP37 and FKBP38, all of which lack the second FKBP-like domain of FKBP52. PPIase activity and FK506 binding have not yet been detected for FKBP37, which is associated with unliganded AhR [50]. The common gene deletion region of the developmental disorder Williams syndrome encompasses the human gene *fkb6* that encodes the widely expressed FKBP36 [51]. Despite the presence of the two basic amino acid residues Lys97 and Arg101 shown to be important for PP5 binding via TPR to hsp90 [52] in the TPR motifs of FKBP36 and FKBP38, no association of these proteins to steroid receptor heterocomplexes has been found to date. Cyp40 (PPID, CypD) was discovered in the unactivated bovine estrogen receptor heterocomplex [53]. The N-terminal part of this protein is highly homologous to human Cyp18 and exhibits CsA-inhibitable PPIase activity (Fig. 2). The multitude of multi-domain PPIases found in nuclear receptor heterocomplexes and the existence of preferences of some PPIases to certain heterocomplexes suggest that there is a functional diversity of these enzymes in nuclear receptor signaling.

In fact, the PPIases are not required for glucocorticoid receptor (GR)/hsp90 heterocomplex assembly and proper folding of the hormone binding domain in reticulocyte lysate [54]. Nevertheless, a considerable influence of FKBP51 on hormone binding was recently shown. The high level of FKBP51 in squirrel monkey causes low-affinity hormone binding of GR in this primate [55]. Coexpression of the human GR protein and either squirrel monkey FKBP51 or human FKBP51 in COS-7 cells increases the $EC_{50}$ of GR transactivation by dexamethasone 17-fold and 3-fold, respectively. This FKBP-mediated inhibition of ligand binding to GR, which can be abolished by FK506 and rapamycin, suggests a PPIase-controlled hormone sensitivity of the receptor [56]. As indicated from the colocalization of FKBP52 and the GR protein in the nucleus during microtubule association, there may be an FKBP52-mediated linkage of the GR heterocomplex to the movement machinery of receptor trafficking be-

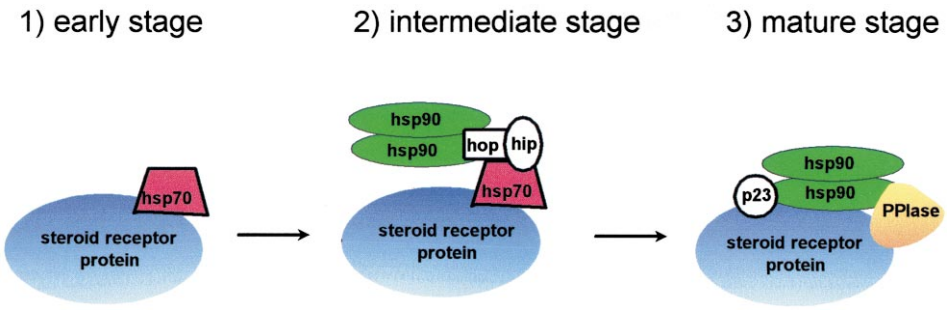


Fig. 3. Schematic representation of the three fundamental assembly stages of steroid receptor heterocomplexes. (1) The early complex is characterized by binding of hsp70 to the receptor protein with the assistance of DnaJ. (2) In the intermediate state, hip (hsp interacting protein) binds to hsp70 and hop (hsp organizing protein) connects the ADP-bound chaperones hsp90 and hsp70. An alternative model of the early steps of heterocomplex formation established for glucocorticoid receptor (GR) includes the preassembly of hsp90, hsp70 and hop to the 'foldosome', followed by receptor binding. It is hypothesized that partial unfolding of the receptor protein is required for the formation of the steroid binding pocket [45]. The hsp90–hop–hsp70 complex is sufficient to generate the high-affinity steroid binding conformation [48]. (3) In both models, formation of a direct contact between ATP-bound hsp90 dimer and the receptor protein and attachment of p23 characterize heterocomplex maturation. One of the PPIases FKBP52, FKBP51 or Cyp40 is bound to the complex.

tween cytosol and nucleus [48,57]. In addition, the first PPIase domain of FKBP52 binds to the microtubule-associated motor protein dynein [58]. A common property of PPIases bound to steroid receptor heterocomplexes beside PPIase activity is their chaperone activity, which may mediate direct binding to non-native receptor proteins [59].

Generally, cellular effects of CsA, FK506 and rapamycin have been used to probe the active site engagement of PPIases in steroid hormone response. Both CsA and FK506 potentiate the dexamethasone-induced expression of a CAT reporter gene in mouse fibroblasts [60,61], but accumulation of dexamethasone in the cytosol was thought to be the cause of the effect [62,63]. At variance with this hypothesis, hormone response showed a similar increase in in vitro experiments that did not suffer from effects of the PPIase inhibitors on accumulation and transport processes [64]. The diversity of the drug binding proteins, the lack of specific inhibitors, and the multiplicity of the signaling pathways affected make it impossible to draw general conclusions from these data [65].

The widespread existence of multi-domain PPIases in nuclear receptor heterocomplexes addresses the general question of the importance of domains complementing the PPIase core. For Cyp40, FKBP51, FKBP52 and FKBP37 the additional TPR is the only common additional motif. The only known functional consequence of TPR is avidly sequestering hsp90. For thermodynamic reasons this association would render the PPIase catalysis more efficient since intermolecular catalysis is converted to an intramolecular event active in the steroid receptor complex. Thus hsp90 and the PPIase domains may act in concert on the receptor protein to keep it in a functional folding state.

## 4. Conclusions

Members of the two major enzyme families of PPIases, the FKBPs and Cyps, have been shown to interact functionally with three different types of cellular receptors as nuclear receptors including steroid receptors and AhR, calcium release channels, as well as receptor protein kinases. More examples can be expected to exist. These enzymes are directed to the multiplicity of different folding states of proteins in cells. Obviously, several reports have already shown that the biological function of native, globular proteins is separated by a distinct prolyl bond isomerization [4,5], but comparable information is still lacking in heterooligomeric complex and membrane receptor signaling. Bringing together the recent advances in the elucidation of the mechanism underlying the conformational control of protein phosphorylation/dephosphorylation [66,4] and the effect of PPIases on receptor phosphorylation has provided a better understanding of the essential steps of the PPIase-mediated receptor downregulation. Thus, FKBPs are likely to accelerate slow receptor protein kinase deactivation rather than cause depletion of the active conformation of the substrate chain. Despite alterations of the *cis*/*trans* isomer ratio found for the Michaelis complexes of PPIases, these enzymes cannot shift this ratio under steady-state conditions. However, in the case of PPIase–receptor interactions, PPIase is usually present in stoichiometric excess. Therefore, it is difficult to distinguish the role of PPIases in catalytic events from their possible function as stoichiometric agents. Because prolyl isomerizations may occur on the time scale of ligand binding, or even more slowly, the subsequent conformational change of a polypeptide chain may become rate-limiting for signaling and, thus, sensitive to PPIases.

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