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AIPL1: a specialized chaperone for the phototransduction effector

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Abstract

Molecular chaperones play pivotal roles in protein folding, quality control, assembly of multimeric protein complexes, protein trafficking, stress responses, and other essential cellular processes. Retinal photoreceptor rod and cone cells have an unusually high demand for production, quality control, and trafficking of key phototransduction components, and thus, require a robust and specialized chaperone machinery to ensure the fidelity of sensing and transmission of visual signals. Misfolding and/or mistrafficking of photoreceptor proteins are known causes for debilitating blinding diseases. Phosphodiesterase 6, the effector enzyme of the phototransduction cascade, relies on a unique chaperone aryl hydrocarbon receptor (AhR)-interacting protein-like 1 (AIPL1) for its stability and function. The structure of AIPL1 and its relationship with the client remained obscure until recently. This review summarizes important recent advances in understanding the mechanisms underlying normal function of AIPL1 and the protein perturbations caused by pathogenic mutations.

Keywords

retina; photoreceptor; molecular chaperone; AIPL1; phosphodiesterase 6; FKBP domain

1. Introduction

The *AIPL1* gene encoding aryl hydrocarbon receptor (AhR)-interacting protein-like 1 (AIPL1) was originally discovered due to its association with Leber congenital amaurosis (LCA) [1]. LCA is a severe, early-onset, inherited retinopathy that causes blindness in early childhood [2]. Various forms of LCA are currently linked to 25 genes, but the *AIPL1*-related LCA4 is one of the most clinically severe forms [3, 4]. The name AIPL1 reflects its high sequence homology (49% identity) and similar domain organization with the ubiquitously expressed aryl hydrocarbon receptor (AhR)-interacting protein (AIP) [1]. In contrast to AIP, AIPL1 expression is restricted to the retina and the pineal gland [1, 5]. AIP and AIPL1 both contain an N-terminal FK506-binding protein (FKBP) domain and a C-terminal

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tetratricopeptide repeat (TPR)-domain with three tetratricopeptide repeats (Fig. 1) [1]. In addition, in primates, AIPL1 proteins carry at the C-termini a proline-rich region (PRR) of poorly understood function. LCA-linked AIPL1 mutations have been localized in all three domains of AIPL1: FKBP, TPR, and PRR [1, 6–9].

FKBP- and TPR-domains are often found in proteins with chaperone activity, including AIP and FKBP51/52 [10, 11]. AIP is a co-chaperone with HSP90 in the maturation of AhR, whereas FKBP51/52 are co-chaperones of steroid hormone receptors [10, 11]. Thus, the domain organization of AIPL1 may have offered an early hint of its possible function as a chaperone, but the interactions of AIPL1 underlying LCA initially remained elusive. The first tangible evidence on potential relevant partners of AIPL1 resulted from a yeast two-hybrid screen that indicated specific interactions of AIPL1 with farnesylated retinal proteins [12]. However, the breakthrough in identification of the critical AIPL1 partner came from the analyses of the AIPL1 knockout and knockdown mouse models [13, 14]. AIPL1 knockout mice displayed rapid and severe degeneration of rod and cone photoreceptors thus closely recapitulating LCA4 in humans [13]. Biochemical analysis revealed that the absence of AIPL1 in mouse photoreceptors caused a dramatic reduction in protein levels of rod phosphodiesterase 6 (PDE6), a key effector enzyme in the phototransduction cascade [13]. Furthermore, the remaining PDE6 protein was catalytically inactive. The drastic reduction in PDE6 was selective, as the levels of other main photoreceptor proteins remained unaffected [13]. These original findings led to the hypothesis that AIPL1 is a specialized chaperone for PDE6 [13, 14], but it took many years to develop compelling experimental evidence that validates this hypothesis.

2. AIPL1 is a specialized chaperone of PDE6

Cyclic nucleotide phosphodiesterases of the sixth family (PDE6) are the key effectors in the visual transduction cascade in rod and cone photoreceptors [15, 16]. The rod PDE6 catalytic core is a heterodimer of PDE6A and PDE6B subunits, whereas cone PDE6C subunits form a catalytic homodimer [16]. In the dark, activity of the PDE6 catalytic dimers is restrained by two tightly bound inhibitory γ-subunits (Pγ). This allows cGMP to maintain depolarizing “dark” current through a cGMP-gated channel in the photoreceptor plasma membrane. Photoexcitation leads to G-protein (transducin) mediated activation of PDE6 followed by a drop in cytoplasmic cGMP, channel closure, hyperpolarization of the plasma membrane, and propagation of an electrical signal to downstream retinal neurons [15]. Besides being essential to photoreceptor physiology, PDE6 is critical to the health and survival of rods and cones. Loss of function of PDE6 leads to elevation of intracellular cGMP levels, which causes photoreceptor cell death via excessive opening of the cGMP- gated channels in plasma membrane and unrestrained influx of Ca^{2+} [17–22]. The notion of AIPL1 as a chaperone of PDE6 is conceptually very appealing, because it readily explains LCA4 by underlying deficits of PDE6. An important step in understanding the link between AIPL1 and PDE6 was made with the demonstration that in the absence of AIPL1, the synthesis of rod PDE6 subunits proceeds normally, but the holoPDE6 is misassembled leading to rapid degradation of the enzyme [23]. Furthermore, direct association of AIPL1 with the PDE6A subunit in mouse retina extract was revealed using immunoprecipitation with AIPL1-specific antibodies [23]. AIPL1 apparently plays a similar role in assembly and stability of cone

PDE6. Mouse models with selective ablation of AIPL1 in cones also show highly reduced levels of cone PDE6 and cone photoreceptor degeneration, albeit at a slower rate compared to rods lacking the chaperone [24, 25]. Interestingly, in an all-cone mouse model lacking AIPL1, retinal guanylate cyclase-1 (RetGC1), which mediates synthesis of cGMP, is also dramatically reduced [26]. As a result, the levels of cGMP in this model are reduced, possibly accounting for the slower pace of degeneration of cones [26]. Evidence for the direct effect of AIPL1 on stability or trafficking of RetGC1 is lacking, and the downregulation of RetGC1 in cones lacking AIPL1 might be secondary to the deficiency of cone PDE6.

The ultimate proof of the function of AIPL1 as the PDE6 chaperone was recently obtained with successful heterologous expression of cone PDE6C in the presence of AIPL1 [27]. This study demonstrated that AIPL1 is absolutely necessary for the expression of active PDE6 in HEK293T cells. Remarkably, the AIPL1-dependent production of functional PDE6 was markedly elevated in the presence of Pγ, revealing a novel role for the regulatory subunit in the folding/assembly of the enzyme [27]. Interestingly, the choice of the HEK293T cell line contributed to the robustness of the system, as the expression of functional PDE6 in COS7 cells was less efficient [27]. As a unique readout of AIPL1 chaperone function, the heterologous expression of PDE6 opened up unprecedented opportunities for studies of structure-function relationships of AIPL1, and it provided an excellent tool for screening AIPL1 variants for pathogenicity.

3. Heterologous expression system of PDE6 as a tool to screen AIPL1 and PDE6 variants for pathogenicity

A high degree of polymorphism in the *AIPL1* gene has made it difficult to reliably establish disease causation [8, 28]. Previously, when experimental approaches to probe the chaperone activity of AIPL1 were unavailable, the potential pathogenicity of mutant AIPL1 proteins has been evaluated *in silico* [8, 28], using mutation analysis software such as PolyPhen-2, SIFT, and PMut [29–31]. However, various prediction programs do not always agree on whether a specific mutation is benign or pathological, and the robustness of the computational approach is unclear. Recent screens of LCA-linked AIPL1 mutants for the ability to chaperone PDE6 in transfected HEK293T revealed similar proportions of pathogenic and seemingly benign variants [27]. The AIPL1 FKBP domain mutants V71F, W72S, and C89R, and the TPR-domain mutant C239R were confirmed as pathological because they fail to chaperone PDE6C in the absence or presence of Pγ [27]. In contrast, the chaperone ability of AIPL1 FKBP domain mutants R38C, R53W, V96I, T114I, and Y134F did not differ significantly from that of WT AIPL1, suggesting that they are probably benign variants [27]. Thus, some of the AIPL1 variants behaved differently than suggested based on computational analysis. This underscores the significance of the experimental approach to validate the pathogenicity of AIPL1, which is required for the development of future patient-specific therapies. Interestingly, residues corresponding to the benign variants are surface exposed and/or situated in the flexible “insert” region in the core FKBP domain. In contrast, pathogenic mutations V71F, W72S, and C89R involve residues that are embedded in the core FKBP domain [27]. The mechanisms of these mutations have been subsequently

uncovered with the structural analysis of AIPL1-FKBP by X-ray crystallography and NMR [32].

Reciprocally, the system based on co-expression of PDE6C, AIPL1, and Pγ is well-suited to evaluate the pathogenicity and mechanisms of PDE6 mutations underlying retina diseases [33]. Mutations in the *PDE6A* and *PDE6B* genes cause autosomal recessive retinitis pigmentosa (RP), a progressive degeneration of rods leading to blindness [20, 21]. Mutations in *PDE6C* lead to a loss of cone function and cause autosomal recessive achromatopsia [34–36]. The first study of the achromatopsia–linked mutations in the context of the wild-type PDE6C template uncovered two general mechanisms triggering this retina disorder [33]. First, for a group of PDE6C mutations, AIPL1 was unable to fold mutant PDE6 proteins leading to complete catalytic inactivity. For the second group of PDE6C mutations, the key deficiency was the failure of Pγ to serve as a co-chaperone with AIPL1, which led to dramatic reductions in the levels of functional enzyme [33]. Future studies are expected to reveal the mechanisms of rod PDE6 in retina disease.

4. Interaction of the AIPL1 FKBP domain with isoprenyl-lipid modifications of PDE6

Rod PDE6A and PDE6B subunits are farnesylated and geranylgeranylated, respectively [37], whereas cone PDE6C is geranylgeranylated [38]. Farnesyltransferase and geranylgeranyltransferase I catalyze the attachment of C15 farnesyl and C20 geranylgeranyl groups to the cysteine residues of a protein C-terminal “CAAX” –motif, followed by the cleavage of AAX residues and carboxymethylation of the prenylated Cys [39]. The finding that AIPL1 interacts with farnesylated PDE6A [23] raised the question of whether or not this interaction involves direct binding of the farnesyl moiety to the chaperone molecule. Indeed, such a direct interaction was revealed with the use of a Fluorescence Resonance Energy Transfer (FRET) assay from AIPL1 Trp residues to the S-farnesyl-L-cysteine methyl ester (FC) probe modified with a FRET acceptor group [40]. Moreover, the FKBP-domain of AIPL1 bound the FC probe comparably to the full-length protein, whereas no FC-binding was detected for the TPR domain [40]. Is the prenyl-lipid binding critical to the function of AIPL1 as a PDE6 chaperone? A comparison of AILP1 to AIP suggests that it is. AIP did not appreciably bind the farnesylated C-terminal peptide of PDE6A, and it also failed to chaperone PDE6 in the HEK293T heterologous expression system [27]. Furthermore, a confirmed pathogenic FKBP-domain mutant of AIPL1, C89R, had a severe impairment in binding FC [27, 40]. However, the analysis of FC binding to AIPL1 and its LCA-linked mutants raised several important questions. No FKBP domain–containing protein has been previously shown to bind prenyl-lipid moieties [41]. In addition, two pathogenic FKBP-domain mutants of AIPL1, V71F and W72S, retained the ability to bind FC [27, 40]. Thus, the origin of their pathogenicity remained unclear. Also, the ability of AIPL1 to bind the geranylgeranyl moiety of PDE6 has not been clearly established. The requirement of AIPL1 for stability of cone PDE6C indicated that the chaperone may bind the geranylgeranyl group [25]. Further, FC binding to AIPL1 was partially reversed by N-acetyl-S-geranylgeranyl-L-cysteine in competition studies [42]. Yet, conclusive evidence for the interaction of AIPL1 with the geranylgeranyl modification of PDE6 was lacking. The mounting unanswered

questions regarding the interactions of AIPL1 with the PDE6 lipid modifications and the underlying mechanisms of the FKBP-domain mutations associated with LCA necessitated application of structural biology approaches.

5. Atomic structures of the AIPL1 FKBP domain: a novel module for prenyl-lipid binding

Until recently, the absence of structural information on AIPL1 stood in stark contrast to a wealth of structural data developed on many other FKBP-domain containing proteins (FKBPs). FKBPs play essential roles in cellular signaling, protein folding, and transcription [11, 41, 43, 44]. Many FKBPs are peptidylprolylisomerases (PPIases) that catalyze the *cis-trans* conversion of peptidylprolyl bonds [45]. FKBPs, and the family founding member FKBP12, are best known as the mediators of the effects of the immunosuppressive drugs FK506 and rapamycin [41, 46, 47]. The FK506-bound FKBP12 sequesters calcineurin, whereas the rapamycin-bound FKBP12 inhibits the mTOR-mediated immunosuppressive pathway [41, 48]. A classical FKBP fold is comprised of a half-conical six-stranded β-sheet surrounding a short central α-helix [46]. FK506 and rapamycin bind within a conserved hydrophobic cavity between the β-sheet and the α-helix, which also overlaps with the PPIase catalytic site [49]. A short hairpin-like loop linking the βstrands β5 and β6 at the periphery above the cavity is an important element contributing to the ligand-binding by conventional FKBPs [46]. The FKBP domains of AIPL1 and AIP are unusual in several respects, most importantly they neither bind FK506 nor have PPIase activity [50, 51]. In addition, in AIPL1 and AIP the hairpin loop is replaced by longer “insert” regions of equivalent lengths [50]. In the NMR-derived structure of AIP, the insert region overhangs the hydrophobic cavity and serves as a lid [50]. Recently, the crystal structures of the AIPL1 FKBP domain have been solved in the apo form and in complex with isoprenyl moieties revealing the unique features enabling the lipid binding [32]. Similarly to AIP-FKBP, the AIPL1 FKBP structure featured the classical FKBP fold and the insert region overhanging the hydrophobic cavity (Fig. 2A). Two key differences between AIPL1 and AIP became apparent from the analysis of the crystal structures [32]. The first difference is in the conformation of the β4-α1 loop that precedes the central α1-helix (Fig. 2B,C). In AIP, this loop is in the loop-in conformation where it interacts with the insert region [50]. As a result, the insert region and the loop seal the hydrophobic cavity making it inaccessible from the surface. In AIPL1, the β4-α1 loop adopts a markedly distinct “loop-out” conformation that creates two narrow openings into the hydrophobic cavity [32]. The second critical difference is in the position of the side chain of the essential AIPL1 tryptophan residue, W72. The side chains of the corresponding residues in AIP (W73) and FKPB12 (W59) are perpendicular to the axis of α1 and form the base of the cavity [46, 50]. In AIPL1, the side chain of W72 flips out of the cavity to a position parallel to the α1 axis (Fig. 2). The most notable consequence of the “loop-out” conformation of the β4-α1 loop and the “flip-out” of W72 is an accessible and deep hydrophobic pocket sandwiched between the core FKBP fold and the α2 helix of the insert region [32]. This hydrophobic pocket accommodates the farnesyl and geranylgeranyl moieties by repositioning of the side chains of the contributing residues without causing major structural changes in the backbone of AIPL1-FKBP (Fig. 3). The side-chain of Cys89 is buried in the hydrophobic pocket (Fig. 3), suggesting that the

pathogenic C89R mutation destabilizes the ligand-binding site [32]. Both the core FKBP and the α2 residues make extensive contacts with the prenyl ligands. The isoprenyl binding module of AIPL1-FKBP is novel and structurally unrelated to the well-characterized immunoglobulin-like β-sandwich fold of the prenyl binding proteins PDD6D and RhoGDI [52, 53].

6. Conformational dynamics of the AIPL1 FKBP domain in solution

Recent NMR analysis of AIPL1-FKBP conformation in solution revealed very interesting and unusual protein dynamics [32, 54]. The HSQC spectra of the ^{15}N- and 13C-methyl labeled AIPL1-FKBP indicated the presence of two major conformations in the apo protein termed “open” and “closed” states [32]. Interestingly, upon FC-binding, the two conformations converged into a single liganded conformation. It was deduced that the open state is apparently similar to the crystal structure of AIPL1-FKBP, where W72 flips out of the ligand-binding pocket. In the closed state, W72 flips into the ligand-binding pocket such that the position of its side chain resembles that in AIP and classical FKBPs. Paradoxically, the positions of NMR peaks of the closed state of apo AIPL1-FKBP are similar to those in the liganded state, because W72 occupies the binding pocket and mimics the ligand. W72 in the flipped-in closed state is predicted to stabilize AIPL1-FKBP. Thus, protein instability may underlie the pathogenicity of the W72S mutant. Indeed, this mutant has been shown to be thermally unstable at 37°C [32]. The existence of the closed state of AIPL1-FKBP in solution also appears to explain the mechanism of the LCA-associated V71F mutant. The crystal structures of V71F revealed no significant differences with the crystal structures of WT AIPL1-FKBP showing W72 in the “flipped out” position [32]. However, the V71F mutant in solution assumed predominantly the closed conformation, which attenuates ligand binding [32]. While V71F was still capable to transition to the open state and bind prenyl ligands *in vitro*, it has been hypothesized that in the native environment in the presence of all AIPL1-interacting partners the mutant is locked into the closed conformation [32]. Thus, by unmasking protein conformational dynamics underlying AIPL1 malfunction in retina disease, the NMR analysis has proven to be highly synergistic with the X-ray crystallography studies of AIPL1-FKBP [32].

7. Role of the AIPL1 TPR domain

Similarly to AIP, AIPL1 contains a TPR-domain comprised of three TPR-motifs [1, 55]. TPR-motif, a degenerate sequence of 34 amino acid residues with a loose consensus, is found in various proteins, where it forms two anti-parallel α-helices separated by a turn. Pairs of αhelices, typically 3-to-16 motifs, arrange at regular angles and form curved superhelical arrays or TPR-domains, which often serve as sites for protein-protein or protein-ligand interactions [56, 57]. AIP utilizes its TPR-domain to recruit the HSP90 chaperone machinery to promote maturation of the AhR [10]. Similarly to well-known interactions of TPR-domain proteins with HSP90 [58], the AIPL1 TPR-domain binds to the C-terminal signature sequence MEVEED of HSP90 [59]. Several LCA-associated mutations within the TPR-domain of AIPL1 impacted its interaction with HSP90 [59]. Furthermore, pharmacological inhibition of HSP90 reduced the stability of PDE6 in mouse retina [60]. Altogether, these studies strongly implicate the HSP90 chaperone machinery in folding/

assembly of PDE6, and AIPL1 as an obligate specialized co-chaperone, which is recruited to the chaperone-client complex via its FKBP and TPR-domains. Chimeric proteins between AIP and AIPL1 have been developed to probe the role of individual domains by taking advantage of the inability of AIP to chaperone PDE6 [27]. Two chimeras, one containing AIPL1-FKBP linked to the AIP-TPR domain, and the other containing AIP-FKBP linked to the AIPL1 TPR domain, utterly failed to chaperone PDE6C in transfected HEK293T cells suggesting that each domain of AIPL1 plays a unique role in chaperoning PDE6 [27]. The unique role of the AIPL1-FKBP is consistent with its exclusive ability to bind prenyl modifications of PDE6 [27, 40]. However, the basis for the unique role of AIPL1-TPR in PDE6 folding is not known. Since both AIP-TPR and AIPL1-TPR bind the C-terminus of HSP90 [42, 59, 61], the mechanism of AIPL1-TPR is distinct from “generic” binding of HSP90 to TPR domains. One possibility is that AIPL1-TPR allows for its distinct orientation with the AIPL1-FKBP domain that cannot be recapitulated by AIP-TPR. A model of AIPL1 featuring the two independent domains has been generated based on the Small Angle X-ray Scattering (SAXS) profile of the protein [40]. In this model, the relative orientation of AIPL1-FKBP and AIPL1-TPR differs from orientations of the respective domains in FKBP51 and FKBP52 [40]. An updated AIPL1 model that incorporates the crystal structure of AIPL1-FKBP and is consistent with the AIPL1 SAXS profile is shown in figure 4. The relative orientation of domains in AIPL1 might be important for proper positioning of AIPL1 and HSP90 with respect to the PDE6 catalytic dimer during the enzyme assembly. HSP90 functions as a dimer [62, 63], and its two TPR-acceptor sites, in theory, could be simultaneously occupied by two molecules of AIPL1 that would act on the two catalytic subunits of the PDE6 dimer. This hypothetical chaperone-client complex would require a precise alignment of PDE6-AIPL1 and PDE6-HSP90 interaction surfaces.

In addition to the FKBP and TPR domains, AIPL1 proteins in primates carry at the C-termini a proline-rich region (PRR) [1, 51]. PRR is of interest because it harbors several mutations linked to LCA. Since PRR is absent in the majority of vertebrate AIPL1 proteins, it is not required for the folding/assembly of PDE6. PRR mutations may destabilize human AIPL1 or impede proper functions of its FKBP and TPR domains. Recent analysis suggested that PRR assumes an extended conformation and does not interact with the FKBP or TPR domains [42]. The secondary structure and stability of human AIPL1 were not affected by selected LCA-associated PRR variants. Furthermore, tested PRR mutations caused no appreciable changes in human AIPL1 binding to PDE6 and HSP90, raising the possibility that these PRR variants are benign [42].

8. Selectivity of AIPL1 for its client: the role of the Pγ-subunit

The question of AIPL1 selectivity is two-fold: (a) why does AIPL1 selectively chaperone PDE6 in contrast to a highly homologous AIP, and (b) why does AIPL1 not appear to chaperone other prenylated photoreceptor proteins? The answer to the former question is clear from the comparison of the structures of the FKBP-domains of AIPL1 and AIP [50]. The latter question still needs to be resolved. A number of proteins in photoreceptors are prenylated, including rhodopsin-kinase and the transducin γ-subunit [64–66]. Yet, only PDE6 was found to be selectively destabilized in the absence of AIPL1 [13]. Hypothetically, the PDE6 sequences adjacent to the prenylation site may contribute to the specificity of

AIPL1 for its client. Here, it is instructive to compare AIPL1 and PDE6D (Fig. 5). PDE6D is a ubiquitous prenyl-binding protein that facilitates intracellular trafficking of rod PDE6, rhodopsin kinase, and small GTPases of the Ras superfamily [67]. The Ser residue at the -3 position relative to the geranylgeranylated Cys in PDE6C interacts with PDE6D and increases PDE6C affinity for the prenyl-binding protein [68]. High-affinity interaction between PDE6D and PDE6C appears to be essential for the Arl3-dependent cargo release into the photoreceptor cilium [69]. The prenyl-binding pocket of PDE6D is deeper, allowing the geranylgeranylated Cys to enter the pocket and the upstream PDE6C residues to contact PDE6D (Fig. 5B) [68]. In the structure of AIPL1-FKBP:FC, the Cys part of the prenylated Cys is not resolved in the electron density, and it appears that the Cys residue is outside the pocket [32]. Thus, it seems unlikely that PDE6 residues upstream of the prenylated Cys are interacting with AIPL1-FKBP (Fig. 5). We propose a different mechanism for the selectivity of AIPL1 for its client whereby the specificity is provided by additional interaction of AIPL1 with the Pγ subunit, or an AIPL1-binding site at the interface of Pγ with the catalytic subunits of PDE6. Direct interaction of Pγ with AIPL1 has been recently demonstrated [42]. Moreover, AIPL1 was able to bind Pγ associated in a tight complex Pγ-PDE6AB-Pγ and inhibit basal activity of holoPDE6 [42]. Current models of PDE6 suggest that binding of AIPL1 to the prenyl moieties of nascent enzyme would well position AIPL1 for the interaction with Pγ occupying the PDE6 catalytic site (Fig. 6) [70–72]. Thus, Pγ may act as an adaptor that increases the affinity of AIPL1 for PDE6 during maturation of the enzyme. This mechanism would be akin to that whereby Pγ modulates activity of the RGS9 GTPase accelerating protein (GAP) complex towards transducin during the recovery phase of phototransduction [73]. Although Pγ itself is not a GAP for transducin $G\alpha_t$, it potentiates the GAP activity of RGS9 by increasing its affinity for $G\alpha_t$ [73]. Similarly, co-expression of PDE6C with Pγ alone does not yield functional PDE6, but Pγ potentiates the chaperone activity of AIPL1 [27]. Mapping of the interaction sites of AIPL1 with Pγ or the Pγ-PDE6 complexes will help to tease out the role of Pγ as the AIPL1 co-chaperone, and possibly, the selectivity adaptor in the chaperone complex.

9. Concluding remarks

This review outlines significant recent advances in understanding the critical chaperone function of AIPL1 and the relationships with its client PDE6, an effector in the phototransduction cascade. These advances became possible largely through the application of structural approaches such as X-ray crystallography, SAXS, and NMR. The newly determined crystal structures of the AIPL1 FKBP domain uncovered a novel molecular module for PDE6 prenyl lipid binding and will guide forthcoming studies to delineate the mechanisms of pathogenic mutations linked to LCA4 [32]. Moreover, unusual molecular dynamics of AIPL1-FKBP were unmasked by NMR, which appear to be essential to the normal AIPL1 function in healthy retina and underlie the protein malfunction in retinal disease [32]. Future challenges include solving the structure of the full-length AIPL1 and gaining structural insights into the overall molecular chaperone complex for PDE6. This complex, at a minimum includes the catalytic and regulatory Pγ subunits of PDE6, AIPL1, and HSP90. Recent advances in cryo-EM technology make a structure solution of such large macromolecular complexes at high resolution feasible [74, 75]. This promises to give us a

greater understanding of why PDE6 folding and assembly requires a unique specialized chaperone complex and how this complex operates.

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Abbreviations

AIPL1	aryl hydrocarbon receptor-interacting protein-like 1
PDE6	photoreceptor phosphodiesterase-6
AIP	aryl hydrocarbon receptor-interacting protein
LCA	Leber congenital amaurosis
FKBP	FK506-binding proteins
TPR	tetratricopeptide repeat
PDE6D	prenyl-binding protein PDE6δ also known as PrBP/δ

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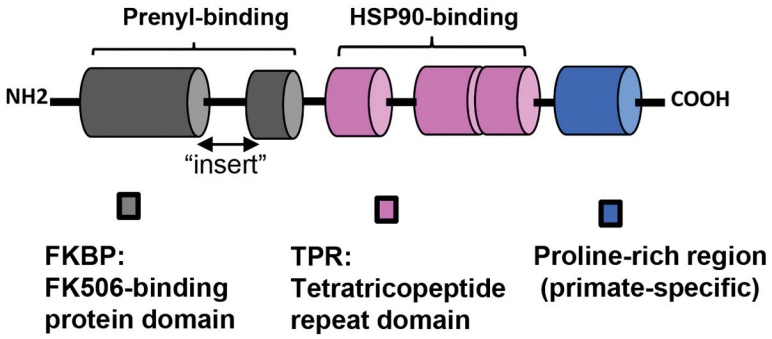

Fig. 1. Domain organization of AIPL1

Vertebrate AIPL1 proteins contain an FKBP domain and a TPR-domain with three tetratricopeptide repeats. Primate AIPL1 proteins contain an additional proline-rich region. "Insert" indicates the insert region, which links the last two β-strands in the core FKBP domain and distinguishes the FKBP domains of AIPL1 and AIP from classical FKBP domains.

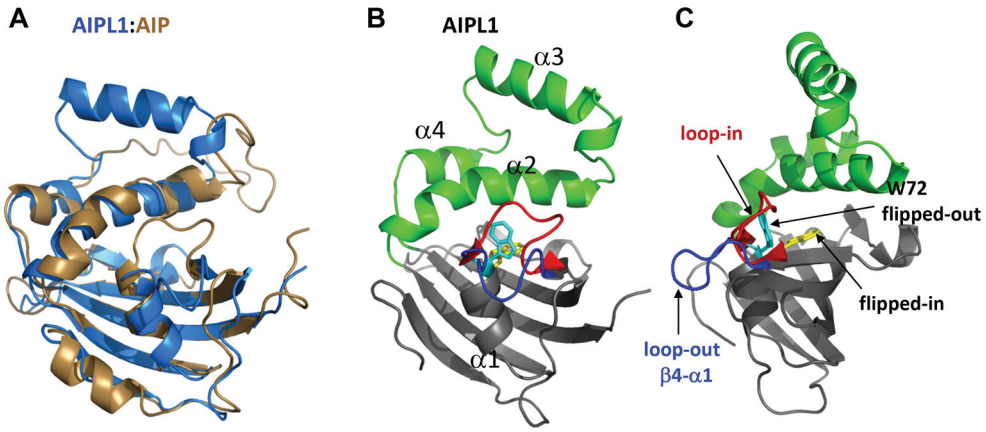

Fig. 2. Crystal structure of apo AIPL1-FKBP

A. Overlay of the crystal structure of apo AIPL1-FKBP (blue) (PDB 5U9A) with the NMR structure of AIP-FKBP (brown) (PDB 2LKN) indicates overall similar folds. **B.** The structure of apo AIPL1-FKBP: highlighted are the structured insert region (green), the β4-α1 loop in the "loop-out" conformation (blue) and the W72 residue in the "flip-out" conformation (cyan). The AIP β4-α1 loop in the "loop-in" conformation (red) and the W73 in the "flip-in" conformation are superimposed onto the structure of AIPL1-FKBP from the NMR structure of AIP (PDB 2LKN). **C**. The view in (B) rotated ~60 degrees around the Y-axis.

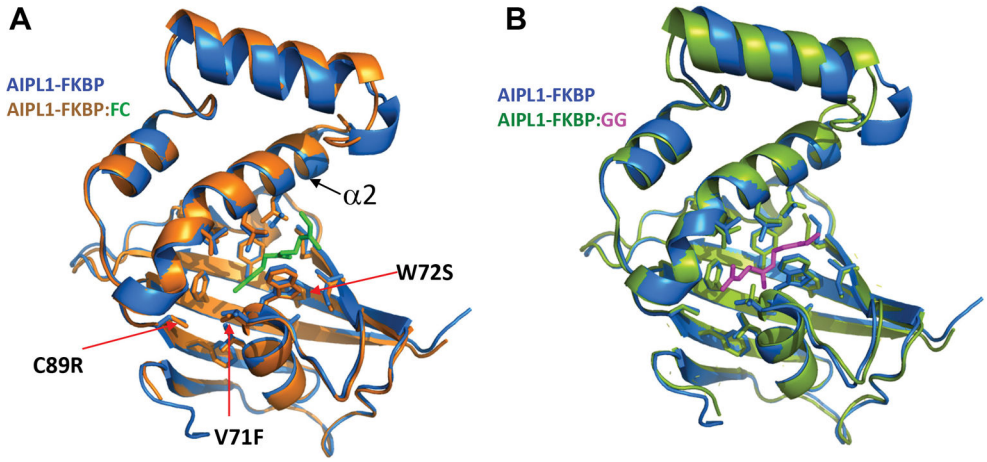

Fig. 3. Crystal structures of AIPL1-FKBP complexed with the farnesyl and geranylgeranyl moieties

Overlays of the apo AIPL1-FKBP structure (blue) (PDB 5U9A) with the crystal structures of AIPL1-FKBP:FC (orange:green) (PDB 5U9I) (**A**) and AIPL1-FKBP:GGpp (green:magenta) (5U9J) (**B**). The hydrophobic pocket sandwiched between the core FKBP fold and the α2 helix (black arrow) of the insert region accommodates the farnesyl and geranylgeranyl moieties by repositioning of the side chains of the residues lining the pocket, whereas the backbone of AIPL1-FKBP remains largely unchanged. Red arrows indicate positions confirmed pathogenic mutations causing LCA.

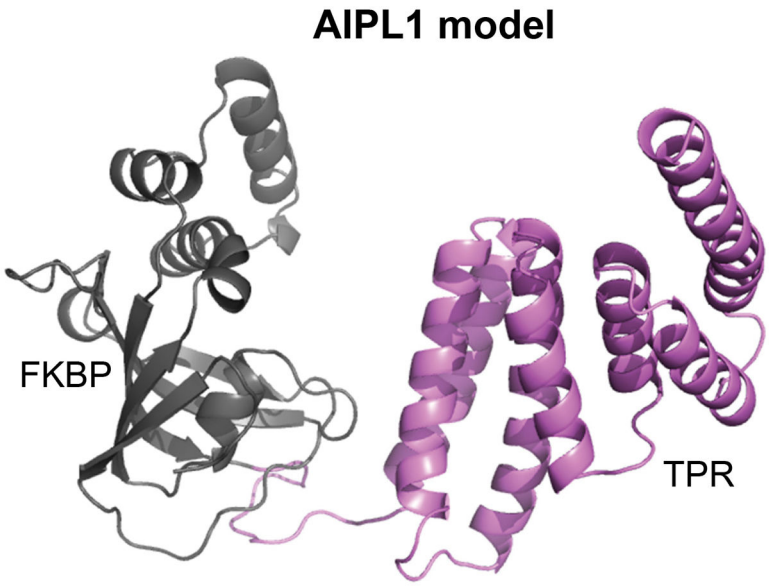

Fig. 4. A model of the full-length mouse AIPL1

A model of mouse AIPL1 based on the previously obtained solution structure [40] and modified to incorporate the crystal structure of apo AIPL1-FKBP (PDB 5U9A).

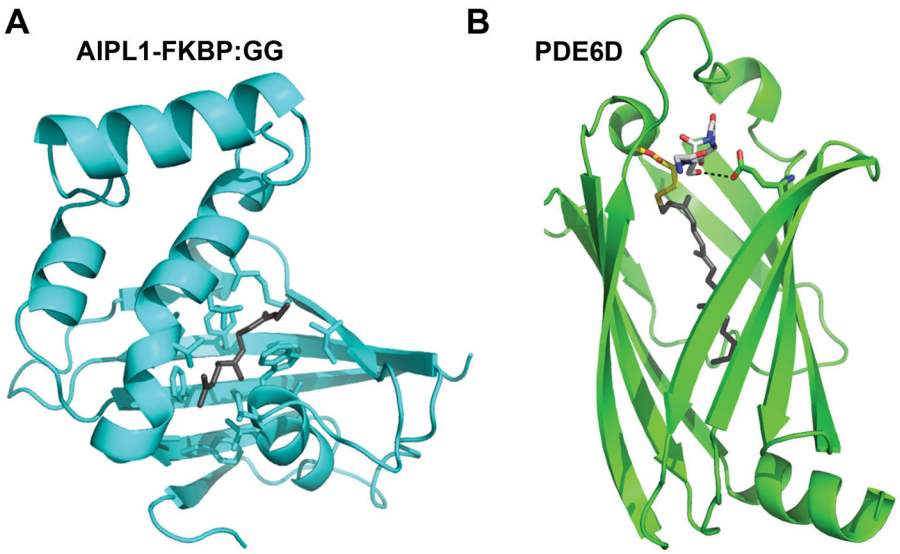

Fig. 5. Isoprenyl ligand binding by AIPL1-FKBP and PDE6D

The isoprenyl binding pocket of AIPL1-FKBP (cyan) (PDB 5U9J; chain B) (**A**) is structurally unrelated to a known immunoglobulin-like β-sandwich prenyl lipid-binding fold of PDE6D (green) (PDB 5E8F) (**B**). The structure of PDE6D complexed with the geranylgeranylated PDE6C peptide indicates the interaction of PDE6D Glu88 with the peptide Ser residue at the -3 position relative to the geranylgeranylated Cys (yellow) [68]. The geranylgeranyl moiety is shown as grey sticks.

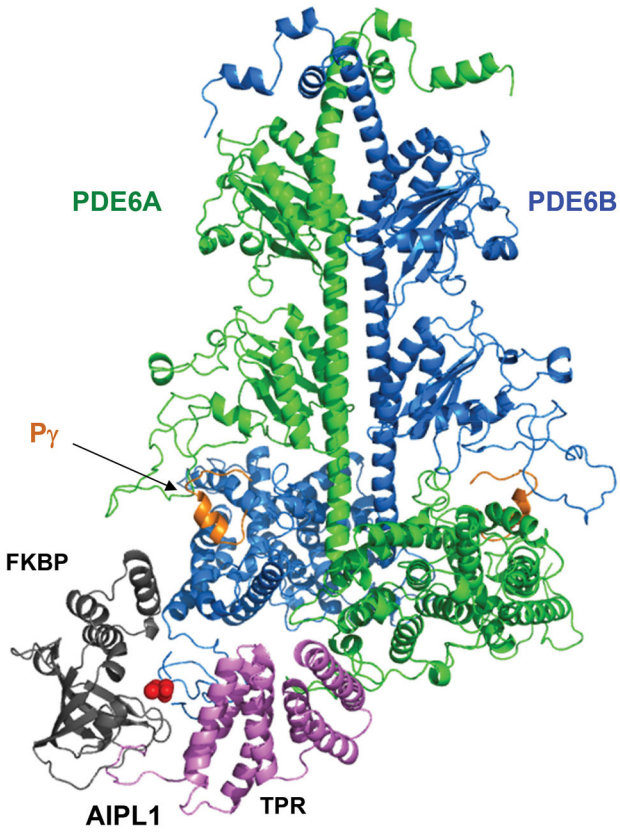

Fig. 6. Hypothetical orientation of AIPL1 and PDE6 in the chaperone-client complex

A model of mouse AIPL1 is positioned against the model of bovine rod PDE6AB (PDE6A – green, PDE6B – blue) [71]. The C-terminal inhibitory fragment of Pγ (orange) is superimposed from the structure of the PDE5/6 catalytic domain (PDB ID 3JWR) [70]. The prenylation site (Cys) is shown as red spheres. Such an orientation of the proteins allows AIPL1 (FKBP – grey, TPR – pink) to bind the C-terminal prenyl modifications and the Pγ subunit of PDE6.