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Review

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.

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 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

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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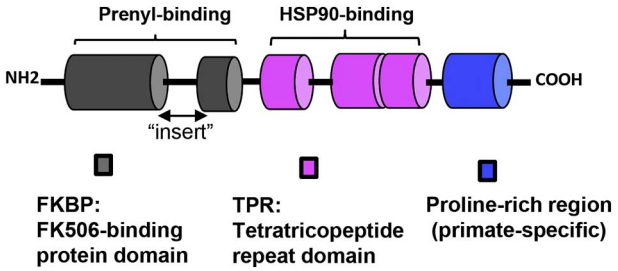

Fig. 1. Domain organization of AIPL1.
Vertebrate AIPL1 proteins contain an FKBP domain and a TPR-domain with three tetratricopeptide repeats. Primate AIPL1 proteins contain an additional proline-rich region. "Insert" indicates the insert region, which links the last two β-strands in the core FKBP domain and distinguishes the FKBP domains of AIPL1 and AIP from classical FKBP domains.

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 down-regulation 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 variants, 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