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Ciliary Transition Zone (TZ) Proteins RPGR and CEP290: Role in Photoreceptor Cilia and Degenerative Diseases

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Abstract

Introduction—Primary cilia are microtubule-based extensions of the plasma membrane in nearly all cell types. In vertebrate photoreceptors, the sensory cilium develops as outer segment (OS) that contains the photopigment Rhodopsin and other proteins necessary for phototransduction. The distinct composition of proteins and lipids in the OS membrane is maintained by the selective barrier located at the border between the basal body and the ciliary compartment, called the Transition Zone (TZ).

Areas covered—In this review, we will discuss the identification and function of two ciliary TZ proteins, RPGR (retinitis pigmentosa GTPase regulator) and CEP290. Mutations in these proteins account for a majority of retinopathies due to ciliary dysfunction. We will also discuss the potential of such information in designing therapeutic approaches to treat cilia-dependent photoreceptor degenerative diseases.

Expert opinion—RPGR and CEP290 perform overlapping yet distinct functions in regulating trafficking of cargo via the TZ of photoreceptors. While RPGR modulates the trafficking by acting as a GEF for the small GTPase RAB8A, CEP290 may be involved in maintaining the polarized distribution of proteins in the OS by modulating intracellular levels of selected proteins involved in inhibiting OS formation.

Keywords

Ciliopathies; cilia; retina; retinitis pigmentosa; retinal degeneration; Leber congenital amaurosis; Ciliary trafficking; connecting cilium; transition zone

1. Introduction

Cilia are microtubule based microscopic extensions of the plasma membrane present in almost all cell types and are involved in various developmental processes, including signal transduction, left right axis determination, sensory perception and adult homeostasis [1-3]. Consequently, defects in cilia development or function result in diverse developmental and degenerative disorders, collectively termed “ciliopathies”. These include a wide range of disorders, such as Nephronophthisis (NPHP), Retinitis Pigmentosa (RP), Bardet Biedl Syndrome (BBS), Joubert Syndrome (JBTS) and Meckel Gruber Syndrome (MKS) [4].

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Cilia consist of a microtubule-based ciliary axoneme, which is assembled from the basal body or mother centriole. During ciliogenesis, the mother centriole docks at the apical plasma membrane and extends a microtubule-based structure called axoneme [5]. Vesicular trafficking is required for the transport of membrane proteins and lipids to the ciliary base. Once at the base, the proteins have to pass through a narrow transition zone (TZ), to enter the axoneme. The TZ therefore acts as a docking site and selection point for the proteins destined for the distal cilium [6]. The cargo proteins are transported from the TZ to the distal tip of the cilium by the action of macromolecular protein complexes by an evolutionary conserved transport process, called Intraflagellar Transport (IFT) [7]. The IFT was first described in *Chlamydomonas* and has now been identified in almost all ciliary cell types [8, 9].

2. Photoreception and Cilia

Vision, a process of detecting and perceiving light, is initiated in the neural retina. The retina is part of the central nervous system and acts as a hub to receive light and initiate the visual transduction cascade that is mediated by five major neuronal cell types and one type of cilia. The major neurons are photoreceptors, bipolar cells, horizontal cells and ganglion cells. These cell types are arranged in the form of a laminated structure, with photoreceptors in the outer retina [10]. Light passes through inner retinal layers to reach the photoreceptors where it isomerizes the chromophore in the visual pigment and initiates a signal transduction cascade accompanied by changes in the membrane potential. Such information is transmitted to the next order of inner neurons, subsequently reaching the ganglion cells and then to the brain via the optic nerve.

Photoreceptors (PR's) account for 65–70 % of all cell types in the retina. PR's are highly polarized neurons, with a distinct inner segment (IS) and an outer segment (OS). The IS contains the cellular machinery of protein synthesis and trafficking while the OS is the hub of the selected set of proteins that are involved in visual transduction. The OS is arranged in the form of membranous discs arranged in a coin-stack like fashion. In photoreceptors, the OS is the sensory cilium, which concentrates more than 10^9 molecules of the light-sensory G-protein coupled receptor rhodopsin in stacked membranous discs [11]. In addition, there is enrichment of other visual cascade proteins, such as beta and delta subunits of phosphodiesterase (PDEα and PDEβ respectively) [12, 13], cyclic nucleotide gated (CNG) channel [14] and membrane Guanylate cyclases (GC) [15], in OS. The photoreceptors have to synthesize all the OS membrane proteins in the IS because no biosynthetic machinery is present in the cilia. These proteins must pass through a narrow bridge-like structure of the cilium, called the connecting cilium (CC; also called transition zone; TZ), between the BB and the OS [16, 17] (Figure 1). A property that makes the photoreceptor cilia unique among all ciliated cells is its ability to undergo shedding at the tips and renewal of at the base. The tips of the OS undergo periodic shedding due to phagocytosis by the juxtaposed retinal pigment epithelial (RPE) cells [11, 18-20]. The photoreceptors constantly shed their distal discs (~10% of discs are shed each day) [11, 20-23] and must transport approximately 2000 opsin molecules per second through the CC to renew those discs [11]. Photoreceptors also exhibit massive light-dependent translocation of soluble moieties of the phototransduction cascade including transducin and arrestin, likely by diffusion [24-27]. Nonetheless, all of the intersegmental transport must occur through the TZ.

3. Membrane trafficking to PR cilia: role of small GTPases

PR's are specialized and polarized neurons. The polar structure and distribution of membrane proteins in PR's is essential for their development and function. The polarity is achieved by targeted trafficking of the vesicles from the Golgi apparatus to the base of cilia. IFT proteins, particularly IFT20 localizes to the Golgi and seems to be involved in the

trafficking of membrane proteins to OS [28, 29]. In addition, IFT20 interacts with IFT54, which in turn associates with the small GTPase RAB8A [30].

Small GTPases are critical regulators of membrane trafficking. There are numerous members of GTPase families. Among these, small GTPases of Arf/Arl and Rab subfamilies have been implicated in regulating cilia assembly and function [31, 32]. These GTPases function by assisting in hydrolysis of GTP. The Rab family of GTPase is the largest family with at least 38 functionally distinct moieties. The function of GTPases relies on two states: active or GTP- bound state, and inactive or GDP-bound form. The active or inactive state also influences the localization of the GTPases in membrane versus cytosol. Such dynamic conformations are further regulated by accessory factors, including guanine nucleotide exchange factors (GEF's); GTPase activating protein (GAP's). During cargo trafficking, Rab GTPases are targeted to the cargo membrane, and activated by GEF's. The activated GTPase subsequently facilitates vesicle docking to the acceptor membrane. Multiple GTPases include RAB6, RAB11, and RAB8A are involved in the trafficking to the cilium, in association with the exocyst complex [33, 34]. Recent studies have shown that the potential handover mechanisms may exist between RAB11 and RAB8 at the base of the cilium [35]. RAB8A is involved in regulating ciliogenesis and photoreceptor OS formation and function [36, 37]. It has been shown that expression of the dominant negative mutant of RAB8A results in the accumulation of the rhodopsin containing vesicles at the base of the OS in frog photoreceptors [36].

4. Ciliary OS of PR's

The region of the cilium between the BB and the axoneme in the OS is called connecting cilium (CC). It is essentially the only link between the IS and OS and acts as a conduit for trafficking of proteins [38, 39]. As previously described, the CC is analogous to the TZ of other eukaryotic cilia. The TZ consists of doublet microtubules, which are anchored to the PM by Y–shaped cross-linkers [38]. Although these structures were described more than 40 years ago, their molecular composition is still not completely understood. Several proteins mutated in human ciliary diseases are localized to the TZ. These include NPHP proteins, MKS modules, and RP-related proteins, RPGR, and CEP290/NPHP6 [4, 40-44]. The TZ, owing to the Y–links and transition fibers, appears to form a gate like structure, and is involved in selective trafficking of proteins into the cilia. In this review article, we will focus specifically on recent studies that have provided new knowledge on the function of ciliary TZ proteins RPGR and CEP290/NPHP6 mutated in retinal degeneration and their role in regulating protein trafficking via the TZ of photoreceptors.

5. RPGR

The *RPGR* gene was cloned in 1996 as a causative gene for X-linked RP [45, 46]. Patients with *RPGR* mutations exhibit early-onset visual defects starting in the second decade of life [45-48]. There are multiple isoforms of RPGR [49] but two major subtypes are: the constitutive isoform (CI) and a retina-enriched ORF15-isoform. The CI consists of 19 exons and encodes a protein of 815 amino acids. Mutations in this isoform account for 10-15% of XLRP cases and are located within exons 1-15. No mutation has to date been identified in exons 16-19. On the other hand, the retina-enriched ORF15-isoform terminates in intron 15. The terminal exon of this isoform is termed ORF15 (exon 15+part of intron 15) and consists of purine-rich repeats (GAAAGGAA…..). The corresponding unusual amino-acid sequence exhibits highly acidic domain with glutamic acid and glycine-rich repeats (Glu/Gly repeats: EEEGEGE repeat in mouse, EEEGEGEGE repeat in human). A majority (50-60%) of RPGR mutations are found in exon ORF15 and are mostly large deletions or duplications

that result in premature truncation of the protein [45, 46, 50-52]. Hence, this exon was termed a mutational hotspot.

The primary structure analysis of the RPGR protein revealed interesting clues to its potential function in photoreceptors. The amino-terminal domain of RPGR, encoded by exons 2-11 is highly homologous to RCC1 (regulator of chromosome condensation 1; RCC1-like domain; RLD), which is a GEF for Ran GTPases and is involved in regulating nucleo-cytoplasmic trafficking of proteins. It was based on this homology that the RPGR gene was given the name of a GTPase regulator [45, 46, 53]. The discovery of the alternative ORF15-containing isoform shifted the focus of research on RPGR to this unusual transcript. The primary structure of this isoform consists of the RLD and the acidic ORF15-encoded domain. Owing to highly acidic nature of this domain, it has been an uphill task to delineate the function of the ORF15 domain. Nonetheless, remarkable studies of Tiansen Li and colleagues provided first clues to a possible role of this isoform in photoreceptors [54]. They showed that ORF15 acts as an exon splicing enhancer and undergoes additional splicing in cells to produce multiple spliced transcripts of RPGR-ORF15. All these transcripts exhibited in-frame deletions of parts of ORF15 while retaining the carboxyl terminal sequence terminating in –LELK.

A major breakthrough in understanding the role of RPGR in photoreceptors came from the studies on its intracellular localization in photoreceptors. Studies from the labs of Tiansen Li and Paulo Ferreira showed that RPGR localizes to the TZ of photoreceptor and other cilia [54, 55]. If such localization is species dependent is still a debatable issue. The ciliary and centrosomal localization of RPGR was further validated by multiple investigators and by using heterologous systems, including cultured hTERT-RPE cells, Madin Darby Canine Kidney cells and rodent kidneys [43, 56-59]. Although RPGR is widely expressed, mutations in RPGR predominantly affect photoreceptor function, with few documented cases of hearing loss, sino-respiratory infections and sperm dysfunction [60-63]. Such information prompted functional analysis of different isoforms of RPGR. Isoform-specific antibodies against RPGR were used to investigate potential differences in the function or localization of the different RPGR isoforms. An interesting observation was that RPGR protein isoforms seem to be considerably less in number as compared to the RNA isoforms, indicating that all RNA isoforms may not be translated into proteins and that some of them may act to stabilize the translation of RPGR and perform additional as yet unknown regulatory functions in photoreceptors [64]. Another possible scenario is that the distinct isoforms of RPGR may be translated in a tissue-specific manner to meet with the demand of ciliary function. As photoreceptors rely immensely on regulated protein trafficking, a majority of RPGR isoforms expressed in photoreceptors participates in orchestrating polarized transport of proteins to the OS. However, in other cell types, RPGR isoforms may play a relatively less crucial role as the demand of ciliary transport is not as high as in photoreceptors and other compensatory moieties could act to stabilize ciliary function in the absence of RPGR.

All the extensive studies described above beg the question: What is the precise function of RPGR? In the next section, we will go over the studies that have provided insights into the function of RPGR.

5.1. Identification of RPGR-interacting proteins

Initial studies identified delta subunit of cGMP phosphodiesterase (PDEdelta) as an interacting partner of RPGR [65]. Notably, PDEdelta is involved in the extraction of prenylated proteins (such as Rab proteins) from the membrane and subsequent transport of membrane proteins; hence RPGR was proposed to regulate protein transport and localization in photoreceptors. Supporting this hypothesis, the carboxyl-terminal sequence of the CI of RPGR, -CTIL, forms a conserved isoprenylation sequence (CAAX box). The CI of mouse

RPGR was shown to localize to the Golgi and mutagenesis of the CTIL sequence resulted in mislocalization of RPGR in transiently transfected cultured cells [66].

Different groups while working independently, identified a coiled-coil domain containing novel interacting partner of RPGR, which was termed RPGR-interacting protein 1 (RPGRIP1) [67-69]. The RPGRIP protein localizes to the TZ of photoreceptors, binds directly to the ciliary microtubules and is required for the recruitment to or retention of RPGR at the cilium [69]. Further studies showed that RPGRIP1 also localizes to centrosomes and basal bodies along with RPGR [59]. A breakthrough study revealed that the *RPGRIP1* gene is mutated in patients with Leber congenital amaurosis (LCA) [70]. LCA is a severe early-onset childhood blindness disorder that is caused by defective development or maturation of photoreceptors [71]. As RPGR is not detected at the cilium in *Rpgrip1*-null retinas, these studies pointed towards a potentially significant role of RPGR in the cilium and suggested that localization of RPGR at the cilium is critical for photoreceptor function. Further insights into a ciliary role of RPGR came from subsequent co-immunoprecipitation and mass spectrometry studies [42, 43]. These studies not only identified RPGR as part of multiprotein complexes of ciliary and microtubule-based assemblies, it also revealed its association with the IFT complex. Moreover, RPGR was found to directly interact with novel ciliary proteins Structural Maintenance of Chromosomes (SMC) proteins, SMC1 and SMC3. Although a direct correlation between SMC proteins and ciliary function still needs to be established, the fact that these proteins contain large coiled coil domains and other ciliary proteins also contain SMC-like coiled-coil domains, it is intriguing to speculate the SMC-like sequences may act as signature moieties to target to the cilium.

5.2. RPGR interacts with RAB8A

Although RPGR's homology to the GEF RCC1 was identified back in 1996; it was not until 2010 that we obtained first direct evidence for an interaction of RPGR with small GTPases. It was found that RPGR, particularly the RLD, interacts with RAB8A [57]. This interaction is stabilized when RAB8A is present in a GDP-locked manner. Given that GEFs typically interact with GDP-bound GTPases and that RPGR has homology to a GEF, the next obvious step was to interrogate a potential activity of RPGR as a GEF for RAB8A. Concomitantly, the authors further demonstrated that RPGR could indeed act as a GEF and catalyze the conversion of RAB8A-GDP to RAB8A-GTP.

5.3. Role of RPGR in cilia

In the year 2000, Tiansen Li and colleagues reported the generation of *Rpgr*-knock out (*Rpgr*-ko) mouse [72]. The authors targeted exons 4-6 encoding part of the RLD of RPGR and replaced them with a Neomycin cassette. Phenotypic analysis revealed retinal dysfunction and degeneration starting at around 6 months of age. This phenotype was associated by a mild mislocalization of cone opsins and decreased content of rhodopsin in the OS of photoreceptors. Another breakthrough in understanding RPGR-related disease came from the identification and characterization of two canine models of RPGR mutation, named X-linked Progressive Retinal Atrophy (XLPRA) 1 and XLPRA2 [73]. These mutant dogs carried distinct microdeletions in exon ORF15 of RPGR and displayed disparate phenotypes: XLPRA1 exhibits a deletion of carboxyl-terminal 230 residues and is associated with a delayed onset and slowly progressing mild but post-developmental photoreceptor dysfunction and degeneration. On the other hand, the mutation in XLPRA2 causes a frameshift and results in the addition of 34 residues and premature truncation of the RPGR protein. This mutant exhibits a relatively early onset, severe and fast progressing disease. Both canine and mouse models of RPGR represent the disease spectrum of XLRP observed in patients with mutations in the *RPGR* gene. These models offer an excellent platform to study RPGR-associated disease progression and pathogenesis and design treatment

strategies. In fact, an exciting study has demonstrated that expressing the RPGR-ORF15 protein in the retina can ameliorate retinal degeneration in the canine models of RPGR [74]. Nonetheless, the mechanism of associated photoreceptor degeneration was still unclear; such information is a prerequisite to development of rational therapeutic modalities for such a clinically heterogeneous disease.

A clue to the mechanism of action of RPGR was obtained when it was found that knockdown of RPGR results in shorter cilia but spares their development and generation [56]. In zebrafish embryos, silencing of *rpgr* culminates in shorter cilia of Kupffer's vesicles (KV), which are transient structures that form during zebrafish development and regulate body axis asymmetry. Notably, no effect on the development of retina and photoreceptors was detected in a majority of embryos. In another study, Alan Wright and colleagues showed that depletion of *rpgr* in zebrafish could result in abnormal retinal development [75]. Although such a phenotype is expected based on its role in ciliary functions, it does not correlate with the phenotype observed in patients or in larger animal models of RPGR mutation. Studies using RPGR patient samples have revealed no effect on the development of photoreceptors [76]. In early life, a majority of RPGR patients seems to have a close to normal vision and exhibit deterioration with progression in age. If the defect in RPGR results in a post-developmental phenotype and is associated with a slow but progressive effect on cilia function, it is conceivable that the insult is exacerbated with age and hence, a severe phenotype is exhibited at a later age in these patients and in animal models.

6. CEP290/NPHP6

CEP290, also termed NPHP6 is one of the twelve ciliary genes mutated in Nephronophthisis-associated ciliopathies [77]. During investigations on identifying new ciliary disease genes, groups of Friedhelm Hildebrandt and colleagues and of Joseph Gleeson and colleagues independently identified a cilia-centrosomal protein of 290 kDa (CEP290) as a causative gene for developmental ciliopathies JBTS and MKS [78, 79]. These patients exhibited cerebellar vermis aplasia, retinal coloboma and several other developmental and life-threatening anomalies. Another exciting study identified a naturally occurring mouse mutant called *rd16* (retinal degeneration 16), which carries an in-frame deletion in the *Cep290* gene [40]. The domain deleted in the *rd16* mouse is termed deleted in rd16 domain (DRD) [80]. Contrary to the human phenotype, the *rd16* mouse revealed no major extra-retinal anomalies, other than mild olfactory dysfunction [81]. However, the retinal phenotype of the *rd16* mouse revealed interesting observations. The *rd16* retina exhibits early onset photoreceptor degeneration with initial signs of rod and cone photoreceptor dysfunction starting as early as postnatal day (P) 18. This group went on to show that the CEP90 mutation in the *rd16* mouse is in fact a hypomorphic allele; residual levels of the deleted CEP290 protein could be detected in the mutant retina. Using molecular cell biological approaches, it was found that CEP290 localizes predominantly to the TZ of photoreceptors and interacts with several ciliary transport proteins in photoreceptors. The *rd16* retina exhibited mislocalization of the OS proteins, rhodopsin, arrestin and transducin, implicating a transport defect due to CEP290 mutation. Moreover, CEP290 was found to associate with RPGR; the deleted variant of CEP290 showed increased association with RPGR and concomitant mislocalization of RPGR in the *rd16* retina.

Further analysis of the retinal phenotype of patients with CEP290 mutations and that of the *rd16* mouse prompted Koenekoop, den Hollander and colleagues to investigate the involvement of CEP290 mutations in early onset retinal degenerative diseases [82]. A pioneering study from this group reported that CEP290 mutations are found in 22-25% of patients with Leber congenital amaurosis (LCA), a childhood blindness disorder due to defective photoreceptor development or maturation. The most frequent CEP290 mutation

was a splice site defect that resulted in aberrant splicing of the CEP290 gene in patient lymphocytes and production of a truncated transcript [82]. Notably, these authors also detected the expression of the full-length CEP290 transcript in those lymphocytes, albeit at very low levels. Some CEP290 patients also exhibited olfactory deficits. These and additional such observations led to a hypothesis that hypomorphic CEP290 mutations may be associated with a tissue-restricted phenotype, with majority of the defect in sensory neurons. However, a loss of function mutation in CEP290 results in severe developmental disorders, including JBTS, and MKS. Detailed analysis of the genotype-phenotype correlation studies is required to test this hypothesis. With the recent development of a knockout mouse of *Cep290*, it will be interesting to investigate the overlapping and distinct features between this and the *rd16* mouse [83]. Such studies promise to reveal insights into progression and pathogenesis of a tissue-restricted versus systemic effects of CEP290 mutations.

6.1. Functional analysis of CEP290

Studies on silencing of CEP290 in cultured cells and in zebrafish have revealed an important role of CEP290 in regulating cilia assembly and vertebrate ciliary development. In cultured cells, knockdown of CEP290 results in defective ciliogenesis, which is accompanied by mislocalization of RAB8A [84, 85]. It was also found that for CEP290 to mediate cilia assembly, another centrosomal protein CP110 should be removed from the basal bodies. As CEP290 interacts with CP110, it is hypothesized that CEP290 functions to remove CP110 during cilia formation and entry of RAB8A. Commensurate with this, CP110 acts as a negative regulator of ciliogenesis [86].

A recent study proposed a model of CEP290 function in photoreceptors and suggests that CEP290 is involved in targeting inhibitors of ciliogenesis to degradation. Using co-immunoprecipitation and tandem mass spectrometry analysis of proteins precipitated using anti-CEP290 antibody the authors identified proteins that are differentially associated with CEP290 in the WT but no *rd16* retinas [80]. This analysis revealed an association of CEP290 with a novel ciliary protein RKIP (Raf1 Kinase Inhibitory Protein). The RKIP protein was identified predominantly at the TZ and the apical inner segment of photoreceptors. Interestingly, the *rd16* retina as well as *cep290*-knockdown zebrafish embryos and cultured hTERT-RPE1 cells exhibited a more than 2-fold increase in the intracellular levels of RKIP. This accumulation was not due to upregulated transcription or translation of RKIP, and pointed towards increased stabilization of the RKIP protein. The authors went on to show that RKIP interacts directly with RAB8A and that accumulation of RKIP sequesters RAB8A from cilia and results in defective cilia formation in cultured cells and zebrafish embryos.

7. Ciliary Protein Interactomes and Retinopathies

Ciliopathies are almost always associated with multi-systemic phenotypes although the phenotype varies with tissue type. If the basic components of ciliary assembly and function are conserved, it is perplexing to observe variations in the degree of severity in distinct cell types due to ciliary dysfunction. In order to understand this dilemma, studies are being conducted to interrogate how multiple ciliary proteins are assembled in distinct protein complexes in a tissue-specific manner.

Recent years have seen an explosion of information about the protein components of the cilia and their involvement in regulating protein trafficking inside the cilia [32, 39, 87-90]. Pioneering work by Peter Jackson and colleagues described a multiprotein complex called BBSome, which consists of a subset of BBS proteins that regulate cilia formation and maturation [37]. Later studies identified distinct complexes of NPHP proteins that are proposed to function in concert to regulate the docking and entry of proteins inside the cilium [91, 92]. Such studies have provided novel clues to the mechanism of cilia formation

and function and to understanding the mode of ciliary dysfunction when assembly of the complexes is perturbed. One such clue is that multiple protein complexes act in concert at the TZ to regulate protein sorting and entry into the cilia.

7.1. Photoreceptor ciliary Interactomes

After the groundbreaking work of Eric Pierce and colleagues on reporting components of the photoreceptor sensory cilium proteome [39], it has become clear that a majority of retinal disease proteins localize to the cilia in photoreceptors. Moreover, this database is widely used to cross-reference any new retinal disease genes or novel interacting partners of known ciliary proteins for their potential localization and function at the cilia. However, a study on the existence of distinct multiprotein complexes of ciliary proteins in photoreceptors had not been performed. Initial clues to the existence of such complexes came from co-immunoprecipitation experiments in which RPGR and CEP290/NPHP6 were found to associate with overlapping as well as distinct set of ciliary and transport proteins in mammalian retina [40, 43] (Figure 2). More recently, it was found that RPGR and CEP290 indeed exist in multiple distinct protein complexes; however, the individual components of such complexes can be overlapping as well as distinct for each complex [93, 94]. Using serial immunodepletion experiments, it was reported that in bovine retina, RPGR exists in at least two distinct complexes with selected NPHP proteins. Some of the interactions of the NPHP proteins with RPGR were direct while others were found to be indirect associations.

8. Expert Opinion

RPGR and CEP290 are two of the commonly mutated ciliary proteins in retinal ciliopathies. Both these proteins associate with each other; however, mutations are found in a wide spectrum of disorders, ranging from delayed onset post-developmental photoreceptor degeneration (RPGR) to childhood onset blindness disorder, LCA (CEP290). A major hurdle in developing treatment strategies for these diseases is the considerable phenotypic heterogeneity observed in patients. Such heterogeneity is not only restricted to the severity and onset of retinal degeneration but is also observed in multiple tissue types. The phenotypes observed in patients and in experimental models point to the existence of a wide range of effects of mutations on the function of RPGR and CEP290. Some severe mutations seem to have a loss of function effect on the protein while others behave as a hypomorphic variation resulting in reduced function. Developing assay systems to test such effects is a prerequisite to understanding the mechanisms of heterogenic disease presentation.

With the identification of RPGR as a GEF for RAB8A and its effect on cilia extension [56, 57], it is now possible to test the pathogenic potential of disease-causing mutations in RPGR. Additional studies on the role of the GEF activity of RPGR specifically in protein trafficking in photoreceptors will assist in developing novel assay systems to test the pathogenicity of RPGR mutations and classification into loss, gain or hypomorphic effect on protein function. As ORF15 is a hotspot of mutations in RPGR, future studies should be designed to delineate the role of this domain in RPGR. Initial studies have shown that cilia-related functions of RPGR can be carried out in the absence of ORF15; however, such studies were performed in cultured cell systems and should be carefully extrapolated to in vivo scenarios. A promising study to design therapies for RPGR-XLRP was recently reported wherein a construct encoding the full-length RPGR-ORF15 protein was delivered to the photoreceptors of canine models of RPGR ORF15 mutations using adeno-associated viral (AAV)-vector based system [74]. If such studies can be designed for mutations in the amino-terminus of RPGR awaits further investigations.

Examination of the function of CEP290 in photoreceptors revealed interesting observations that overlap with proposed functions of other ciliary proteins. Previously, BBS proteins,

specifically BBS4, were shown to associate with the protein degradation machinery to regulate ciliary signaling cascades during vertebrate development [95]. However, a direct correlation with the degenerative diseases associated with BBS remains to be delineated. Notably, the recent report that CEP290 is involved in regulating intracellular levels of another ciliary protein RKIP in photoreceptors and that abrogation of such function results in photoreceptor degeneration offers clues to the mechanisms of disease progression in retinopathies due to CEP290 mutations [80]. Additional studies, including the mechanism by which CEP290 alters the intracellular levels of RKIP and identification of additional proteins that act as negative regulators of cilia formation (such as CP110) will provide new knowledge of the mode of regulation of ciliary development and function in photoreceptors. These studies will also reveal novel pathway intermediates, such as the protein degradation machinery or inhibitors of ciliogenesis that can be targeted to develop therapeutic strategies for CEP290 and associated disorders. In order to develop a gene therapeutic approach for treating CEP290-associated disease will require a detailed characterization of the effect of mutations on its function. This is particularly important in case of CEP290 because mutations in CEP290 are associated with wide-range of retinal and extra-retinal disorders. If a gene augmentation strategy were to be utilized to treat CEP290-LCA, a crucial hurdle is the limitation of the size of the cDNA that can be packaged into a viral vector. Given the long (~8 kb) cDNA of CEP290, it is going to be an uphill task to generate modified viral vectors to accommodate such long cDNAs. Another strategy would be to identify critical but smaller regions of CEP290 that can ameliorate tissue-specific phenotypes. Two such approaches have been reported that utilized an amino-terminal domain of CEP290 and another using the DRD of CEP290 [80, 96]. Both these studies have reported potentially significant effect on rescuing *cep290*-associated retinal degeneration in zebrafish. However, additional studies, including their likely toxic effects and their usage in a larger animal model need to be performed before developing a therapeutic paradigm using such approach. Another negative effect of such an approach could arise from the fact that majority of CEP290-LCA patients carry a splice site defect and exhibit expression of the wild-type CEP290 transcript, albeit at low levels. A potential interaction between the wild-type protein and the smaller overexpressed domain may result in deleterious effect on photoreceptor function. Another approach is to utilize antisense oligonucleotides to correct the splice site defect. Such studies were successfully utilized to correct a splice site defect in the RPGR gene [97].

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Abbreviations

RPGR	retinitis pigmentosa GTPase regulator
LCA	Leber congenital amaurosis
RKIP	Raf1 Kinase Inhibitory Protein
OS	outer segment
IS	inner segment
CC	connecting cilium
TZ	transition zone
BB	basal body

Article Highlights Box

1. Targeted trafficking of protein from photoreceptor inner segment to sensory outer segment is critical for its development and function.
2. The transition zone of photoreceptors, which is a narrow bridge-like structure between the IS and the OS, regulates the docking and selection of cargo destined for the OS.
3. Recent studies have identified macromolecular complexes of ciliary proteins that function at the TZ to regulate its structure and function. This review focuses on understanding the function of two such proteins, RPGR and CEP290.
4. While RPGR mutations result in X-linked RP, mutations in CEP290 are associated with relatively early onset retinal degeneration called Leber congenital amaurosis.
5. Studies on understanding the function of RPGR and CEP290 have revealed novel clues to their mode of action in photoreceptors and mechanism of associated disease.

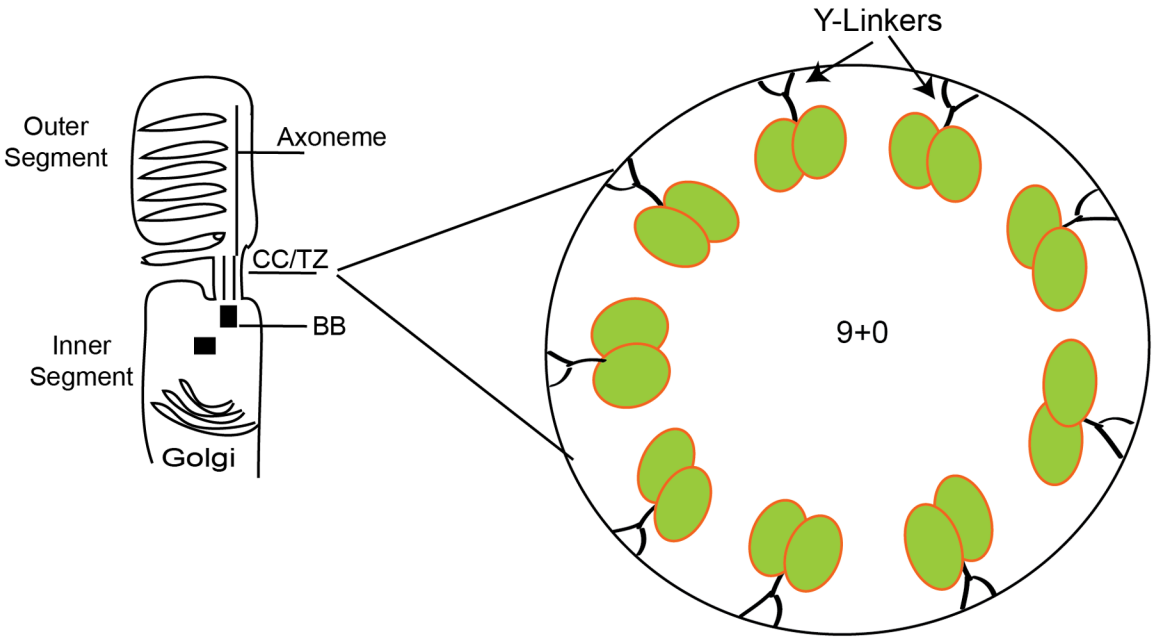

Figure 1. Schematic representation of a rod photoreceptor

The polarized nature of the photoreceptors is represented by an elaborate generation of a distinct membrane component called the outer segment. This compartment contains membranous discs and is generated from the basal body (BB) in the apical inner segment. The region between the axoneme and the BB is called the connecting cilium (CC) or transition zone (TZ). A detailed schematic representation of the CC/TZ of photoreceptors shows the presence of Y-linkers that connect the ciliary microtubules to the plasma membrane. This region acts as a gate to selectively regulate trafficking of proteins to the OS.

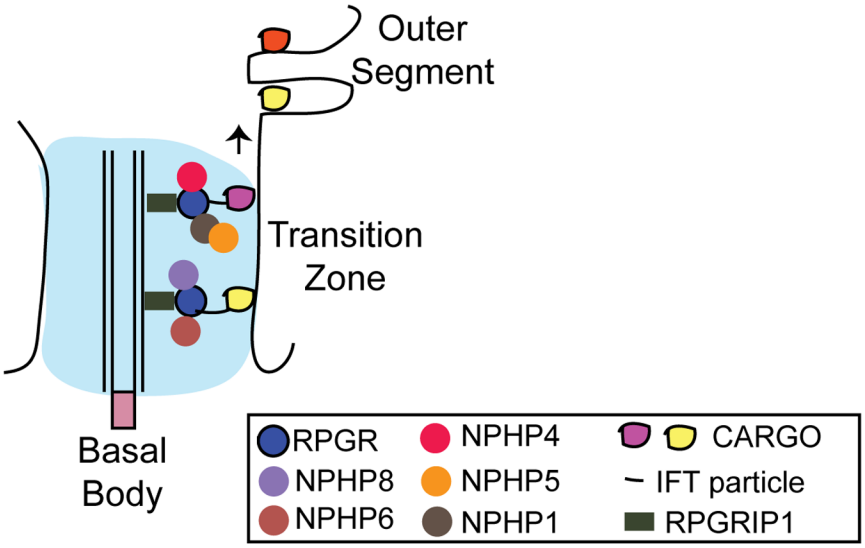

Figure 2. Model of involvement of discrete multiprotein complexes of RPGR and CEP290/ NPHP6 in photoreceptor cilia

The transition zone (TZ) of photoreceptors is largely composed of several protein complexes involved in degenerative disease. They form distinct complexes that may mediate sorting and trafficking of specific cargo to the OS. RPGRIP1 anchors RPGR at the TZ. The cargo is transported via the IFT particle.