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# Photoreceptor Sensory Cilium and Associated Disorders

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Additional information is available at the end of the chapter

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## 1. Introduction

The primary cilium is a microtubule-based extension of the plasma membrane, which is present in almost all cell types. Ciliary microtubules extend from a basal body (or mother centriole), which docks at the apical membrane. Elegant studies have been carried out to determine the mechanism that regulates the docking of the mother centriole at the membrane for cilia formation. Cilia function as antennae of the cell to detect chemical and physical changes of the microenvironment [1-5]. Owing to their near-ubiquitous nature, cilia are involved in diverse cellular functions, such as patterning of left-right asymmetry (nodal cilia), limb development, bone morphogenesis, and neurosensory functions (mechanosensation, olfaction, and photoreception). Cilia are also implicated in several developmental cascades, such as Wnt signaling, sonic hedgehog signaling, and platelet derived growth factor receptor signaling pathways. Such functions of cilia are brought about by the ability of the ciliary membrane to concentrate a specific subset of membrane proteins in the ciliary compartment as compared to the rest of the cell membrane [6-8].

Cilia are generated by an elaborate process of formation of multiple protein complexes and molecular motor dependent transport of membrane cargo from the proximal to the distal tip, thereby extending the microtubule-based axoneme and the ciliary membrane. Such transport, called Intraflagellar Transport, was initially identified in green alga *Chlamydomonas reinhardtii* and is composed of more than 20 IFT subunits arranged in two distinct complexes, IFT-A and IFT-B [9-10]. They interact with motors and transport cargo along axoneme [11]. Microtubules are polarized with a plus end (growing tip), and a minus end (at the proximal end of cilia). The anterograde motor Kinesin (heterotrimeric Kinesin-2 or homodimeric Kif17) mobilizes proteins to the distal (plus) end while cytoplasmic dynein 2 carries cargos to the proximal end of cilia [12-15]. Similarly, IFT-A and IFT-B play complementary roles in ciliary transport. The complex B, contributing to anterograde transport, is indispensable for the ciliogenesis and

maintenance. In contrast, complex A, involved in the retrograde transport, does not play essential role in ciliary assembly [11]. Defects in IFT disturb the ciliogenesis or ciliary maintenance. Even slight defects in the composition of the ciliary membrane or in the generation and/or maturation of cilia result in developmental and degenerative disorders in humans, such as Bardet-Biedl Syndrome (BBS), Joubert Syndrome (JBTS), Meckel-Gruber Syndrome (MKS), Senior-Løken Syndrome (SLSN), Usher Syndrome (USH), renal cystic diseases, and photoreceptor degeneration and blindness [6-7, 16-18].

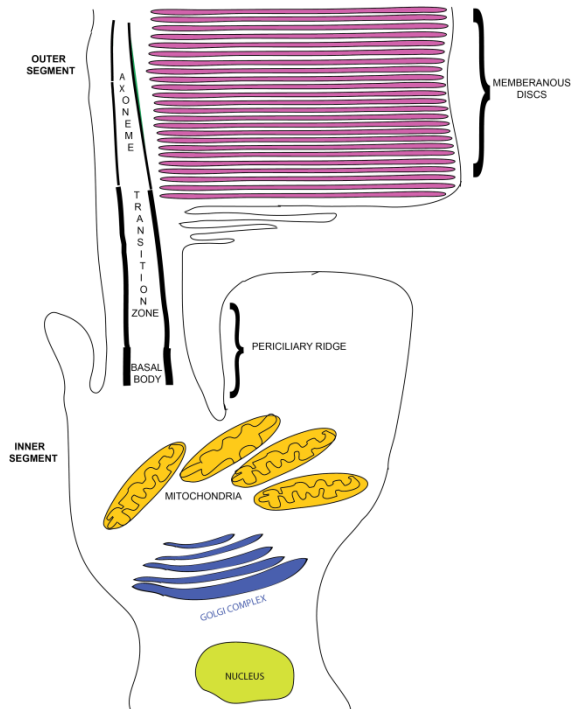
## 2. Photoreceptor sensory cilium and its components

In photoreceptors (rods and cones), cilia are highly specialized and modified into a very distinct part of the cell, which consists multiple membranous discs and initiates phototransduction cascade in response to light. The details of the phototransduction cascade in photoreceptors have been elegantly described elsewhere and will not be covered in this chapter.

There are three major compartments that compose the sensory cilia of photoreceptor: the outer segment (OS), transition zone (TZ) and basal body (Figure 1). Like other primary cilia, photoreceptor cilia are 9+0 microtubule-based structures that are nucleated from the basal body. The mother centriole consists of triplet microtubules and recruits proteins and initiates axoneme assembly. The region adjacent to the basal body is TZ (also called connecting cilium; CC), and consists of doublet microtubules [19-20]. These microtubules are linked to the plasma membrane via transition fibers and Y-linkers, the two distinct structures of TZ [21]. TZ, is a narrow conduit between OS and IS [22]. It is estimated to be 200~500 nm long and 170 nm in diameter. TZ carries out critical transport function by acting as a gate between the IS and the OS. The sensory OS of photoreceptors is enriched in membrane proteins, such as rhodopsin, the cyclic nucleotide gated (CNG) channel, membrane guanylyl cyclases, and peripherin-2 [22-25]. Moreover, the TZ is the only link between the two segments and all proteins need to be transported via this narrow bridge-like structure to the OS. Hence, the TZ serves as a bottleneck as well as a track to generate and maintain the sensory cilium. Several proteins, most of which are associated with human retinal degenerative diseases, are enriched at the TZ of photoreceptors. These include RPGR (retinitis pigmentosa GTPase regulator), CEP290, and Nephrocystin-1 (NPHP1). The microtubules then extend in the form of axoneme. Depending upon the species and cell-type examined, the axoneme can extend to half or full length of the OS. The axoneme is recognized by the fact that it consists of singlet microtubules. Not much is known about the specific function of the axoneme. However, functional analysis of RP1 (retinitis pigmentosa 1) protein that localizes specifically to the axoneme of photoreceptors indicated that it might be involved in stabilizing the OS discs. The membranous discs arranged in a perpendicular orientation to the axoneme and axoneme is believed to prove a structural support to the OS discs.

In addition to maintaining a specific composition of the OS, the photoreceptors also undergo massive protein trafficking. In fact, photoreceptors are most active neurons in the human body and have high-energy demands. This is due to the fact that photoreceptors shed their

distal discs at a high rate. It is estimated that 10% of the distal tips of the OS is shed every day by undergoing phagocytosis by the overlying retinal pigmented epithelium (RPE) cells [26]. As no protein synthesis occurs in the OS, all components necessary for the renewal of OS discs are synthesized in the IS and transported to the OS at a very high rate. Approximately 2000 opsins transported to the OS per second in a normal human photoreceptor. Even slight disturbances in the synthesis and transport of proteins to the OS results in photoreceptor degeneration and blindness.



**Figure 1.** Schematic representation of a rod photoreceptor cell. The membranous discs in the outer segment are enclosed in the plasma membrane. The photoreceptors are rich in mitochondria, which are concentrated around the apical inner segment

### 3. Docking of cargo and selection at the TZ of photoreceptors

Even though the OS proteins can be targeted to the cilia, they are first docked at the basal body or adjacent membrane. Multiple models have been proposed for the site of docking of the cargo vesicles [27]. These propose docking directly at the basal body, docking at the lateral plasma membrane and then movement of vesicles in the plasma membrane towards to the ciliary compartment, or docking at a privileged domain of the apical plasma membrane. In vertebrate photoreceptors, such a privileged domain was identified as periciliary ridge. Opsin-laden vesicles were identified at this privileged region as well as

transiently in the TZ or CC of photoreceptors [25]. More recently, several ciliary disease proteins mutated in Usher Syndrome, were identified at the periciliary ridge and are thought to make a connecting link between the apical plasma membrane and the ciliary membrane [28-29]. If such a domain plays a direct role in cargo docking awaits further investigations, specifically geared towards ascertaining the composition of this microdomain.

After gaining access to the periciliary ridge, the cargo is transported into the TZ, which acts as a 'check post'. Due to its elegant meshwork-like structure with Y-shaped linkers that connect the axonemal microtubules to the plasma membrane, its composition of this structure has been the subject of many recent studies. Remarkable studies identified a network of multiprotein complexes of ciliary disease proteins that are found at the TZ and act as diffusion barrier to limit the trafficking of membrane cargo into the ciliary compartment [22, 30-33]. These proteins include RPGR, RPGR-interacting protein 1 (RPGRIP1) [34-35], CEP290/NPHP6 [36-37], MKS-associated proteins and other JBTS and NPHP-associated proteins [6, 38]. Interestingly, these proteins exist in discrete multiprotein complexes at the TZ. A direct role of TZ proteins in acting as a barrier was established when Witman and colleagues showed that mutation in *Chlamydomonas* CEP290 causes accumulation of non-ciliary membrane proteins to enter cilia and vice versa [39]. However, such a function of CEP290 in photoreceptors still needs to be investigated.

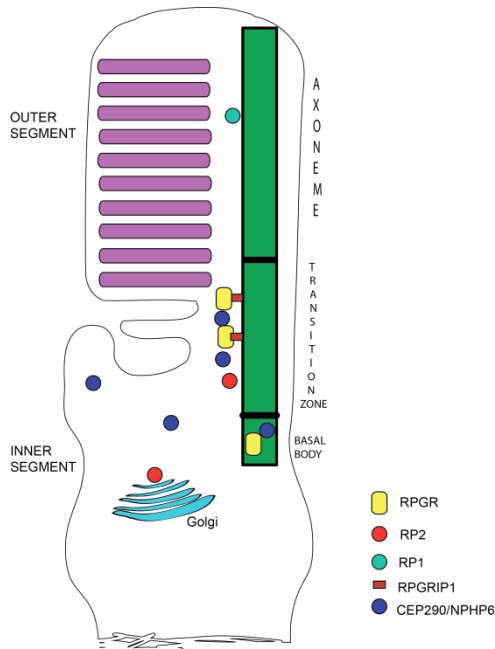
#### 4. Ciliary disorders of retina (retinal ciliopathies)

As the OS of photoreceptors is a sensory cilium, the degenerative diseases that affect the formation or function of the OS can be categorized as a ciliary disorder. However, for simplicity, we will discuss only those cilia-dependent retinopathies that occur due to defects in ciliary TZ proteins and result in defective trafficking of proteins to the OS. Inactivation of the IFT in conditional *Kif3a*<sup>-/-</sup> mice and *Tg737<sup>orpk</sup>*, a hypomorphic allele of IFT88, results in opsin accumulation in the IS [40-41]. Mutations in rhodopsin that affect its trafficking to OS are associated with degenerative blindness disorders of the retina [42-47]. Moreover, ablation of IFT subunit IFT20, which localizes to Golgi and cilia, also results in entrapment of opsins in the IS [48]. Ciliary proteins RP1 and RPGRIP1, mutations in which result in RP/LCA are required for cilia-dependent OS generation [35, 49-50]. Pleiotropic disorders, such as Senior-Loken Syndrome, Joubert Syndrome, and Bardet-Biedl Syndrome, are also caused by mutations in ciliary proteins and share retinal degeneration as a common phenotype [51-53] (Table 1). In this chapter, we will specifically discuss RPGR and RP2, which are mutated in X-linked forms of retinopathies and CEP290, which is a frequent cause of Leber congenital amaurosis (LCA), a childhood blindness disorder (Figure 2).

##### 4.1. Non-syndromic retinal ciliopathies

*Retinitis Pigmentosa (RP)*. RP, detected in 1:3000 people worldwide, is a group of severe blindness disorders that is caused by progressive loss of rod and cone photoreceptors. It is inherited in autosomal recessive, autosomal dominant as well as X-linked manner. Patients exhibit symptoms of night blindness and loss of peripheral vision (due to rod death) in the

first two decades of life, which is followed by complete blindness due to loss of cone photoreceptors [54-55]. Loss of cones can either be due to the fact that the causative gene is also expressed in cone photoreceptors or due to starvation or loss of availability of trophic factors secreted from the rods (majority cell type in photoreceptor layer; 95-97%) if the mutation is in a rod-specific gene [56-57].



**Figure 2.** Schematic representation of the localization of the ciliary proteins being discussed in this chapter. As shown, RPGR localizes to the transition zone and basal body and RP1 is concentrated at the distal axoneme, which extends into the outer segment. RPGRIP1 tethers RPGR at the transition zone. RP2 is detected at the Golgi as well as transition zone in photoreceptors. CEP290/NPHP6 is detected at the transition zone, basal body, as well as in the cytosol.

Some forms of RP are caused by defects in genes that encode for ciliary proteins. These include RPGR, RP1, RP2, and TOPORS [50, 56-60]. RP1 and TOPORS are two ciliary proteins mutated in adRP. However, they localize to distinct ciliary compartments: RP1 localizes to the axoneme whereas TOPORS is concentrated in the basal body and transition zone of photoreceptors (Figure 2). The RPGR and RP2 genes are mutated in X-linked forms of RP and together account for more than 90% of XLRP cases [61-64]. Among these, RPGR mutations are found in 70-80% of XLRP and more than 25% of simplex RP males with no family history. On the other hand, RP2 mutations account for 6-10% of XLRP cases. There is considerable clinical heterogeneity among cases of XLRP, which has affected the ability to differentiate between RPGR and RP2 patients in the clinic. This has prompted investigations into genotype-phenotype correlation studies. Such studies are relatively well documented for RPGR patients owing to their majority occurrence as compared to RP2 mutations [65-67].

Nonetheless, recently, a comprehensive analysis of a large group of RP2 patients revealed interesting observations: a majority of RP2 patients seem to exhibit an early involvement of the macula (the central region of the retina) [68].

*RPGR*: The *RPGR* gene consists of 19 exons and encodes for multiple alternatively spliced transcripts. There are two major transcripts: *RPGR*<sup>1-19</sup> and *RPGR*<sup>ORF15</sup>. As the name suggests, the *RPGR*<sup>1-19</sup> isoform consists of exons 1-19 whereas *RPGR*<sup>ORF15</sup> isoform consists of exons 1-15 and terminates in intron 15. Both these isoforms therefore, contain a common amino-terminal part comprising of exons 1-15. A part of this region, encoded by exons 2-11 contains a domain of the protein that is homologous to RCC1 (regulator of chromosome condensation 1), a guanine nucleotide exchange factor (GEF) for small GTPases involved in nucleocytoplasmic trafficking of proteins. This domain of *RPGR* is termed RCC1-like domain (RLD). The carboxyl-terminal region is distinct between these two isoforms. While the *RPGR*<sup>1-19</sup> isoform possesses an isoprenylation motif at the extreme carboxyl-terminus, the *RPGR*<sup>ORF15</sup> isoform encodes for an unusual stretch of Glutamic acid and Glycine rich (Glu/Gly rich) domain (Figure 3). At DNA level, the terminal exon of this isoform contains purine-rich repeats [60, 62, 64, 69]. Ablation of the *Rpgr* gene in mice affects opsin trafficking and results in photoreceptor degeneration, starting at around 6 months of age [70]. Similar phenotype was detected in two naturally occurring canine models of *RPGR* mutation, although the severity of disease was different in the two mutants [71]. First direct correlation of a function of *RPGR* in cilia was obtained when it was shown that *RPGR* localizes predominantly to the TZ of photoreceptors and interacts with other ciliary and transport proteins [72-73]. More recently, it was found that silencing of *rpgr* in zebrafish embryos results in shorter cilia and developmental anomalies, reminiscent of ciliary dysfunction [74-75]. These findings indicate that *RPGR* is involved in regulating the trafficking of proteins at the TZ. Mechanistic insights into *RPGR* function were obtained when it was shown that *RPGR* possesses enzymatic activity. *RPGR* acts as a GEF for the small GTPase RAB8A, which is involved in cilia formation and maturation. As a GEF, *RPGR* catalyzes the conversion of the inactive, GDP-bound RAB8A to active GTP-RAB8A to facilitate the trafficking of cargo vesicles [76]. The precise function of *RPGR* as a GEF in photoreceptors still needs to be delineated.

*RP2*: The *RP2* gene is composed of 5 exons and encodes a protein of 350 amino acids. The structure of *RP2* reveals two major domains: an amino-terminal domain homologous to tubulin binding cofactor C (TBCC) homology domain and a carboxyl-terminal nucleoside diphosphate kinase domain [77-79] (Figure 3). The purified *RP2* protein possesses GTPase activating protein (GAP) activity towards the small GTPase ARL3 (ADP Ribosylation Factor-Like protein 3). As a GAP, *RP2* assists in the conversion of GTP-bound ARL3 to ARL3-GDP [80]. Although some human mutations affect this association or activity, the precise role of *RP2* as a GAP in photoreceptors is still not clear. The amino terminus of *RP2* is palmitoylated and myristoylated and hence, may associate with cell membrane. In fact, *RP2* has been found to associate with the plasma membrane of cells and of photoreceptors [81]. In addition, *RP2* is also present at the basal body of primary cilia and undergoes trafficking into the cilia like IFT [59]. *RP2* interacts with ciliary protein polycystin-2 and assists in the trafficking of polycystin-2 to the cilia. Recent studies have shown that ciliary localization of *RP2* is regulated by

importins, proteins involved in nucleocytoplasmic trafficking [82]. These data suggest a potential role of such machinery in regulating protein import into the cilia. Silencing of RP2 in cells results in the swelling of the distal tip of the cilium but spares the rate of trafficking of the IFT machinery. Further investigation revealed that RP2 is involved in the secretion of polycystin-2 from ciliary tip to the external microenvironment. One possible scenario is that RP2 may not be directly involved in the secretion rather assists in the trafficking and delivery of an accessory cargo that is required for the secretion of polysystin-2 and other such proteins from the ciliary tip. In photoreceptors, RP2 also localizes to the basal body, TZ as well as Golgi. Silencing of RP2 was also shown to fragment Golgi and may affect Golgi to cilia trafficking in cells [89]. The *in vivo* effect of ablation of RP2 in photoreceptors will provide critical clues to its involvement in cilia formation, function and protein trafficking.

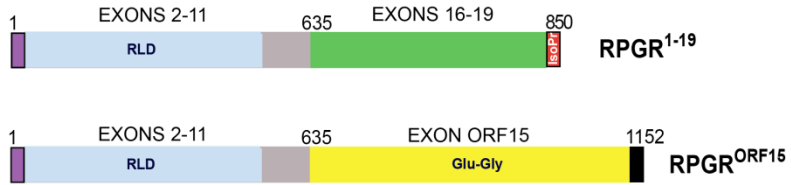
*Leber congenital amaurosis (LCA)*. LCA is considered the most severe form of retinal degenerative disease that occurs in the childhood or early adulthood, with an incidence of 1 in 30,000 births worldwide. Defective retina exhibits perturbations in the initial development of photoreceptors [83]. Like RP, LCA also exhibits considerable genetic and clinical heterogeneity. To date, mutations in 18 genes have been identified to cause LCA (RetNet, <http://www.sph.uth.tmc.edu/Retnet>). Of these, four genes, CEP290, RPGR-interacting protein 1 (RPGRIP1), LCA5 or lebercilin and Tubby-like protein 1, encode for ciliary proteins. We will discuss CEP290 and RPGRIP1 below.

*CEP290*. Mutations in the cilia-centrosomal protein CEP290 are frequently observed in LCA, with an incidence of 22-25% cases [37]. The CEP290 gene consists of 55 exons and encodes a protein of 2,479 amino acids (Figure 3). The CEP290 is a multidomain protein and consists of several coiled-coil domains. Involvement of CEP290 in early onset retinal degeneration was determined when a naturally occurring mouse model called *rd16* (retinal degeneration 16) was identified to carry an in frame deletion in the *Cep290* gene. The *rd16* mouse exhibits early onset severe retinal degeneration, characteristic of LCA in humans, and is accompanied by partial mislocalization of RPGR to the IS. The domain of CEP290 that is deleted in the *rd16* mouse is termed DRD (deleted in *rd16* domain) [84]. The deletion renders the CEP290 protein prone to degradation; however, expression of truncated CEP290 protein can be detected in the retina and other tissues in the *rd16* mouse [36]. CEP290 localizes predominantly to the CC/TZ of photoreceptors and interacts with selected ciliary and transport assemblies, including retinal disease proteins Retinitis Pigmentosa GTPase Regulator (RPGR) and RPGR-interacting protein (RPGRIP1), which are mutated in RP and LCA, respectively [36].

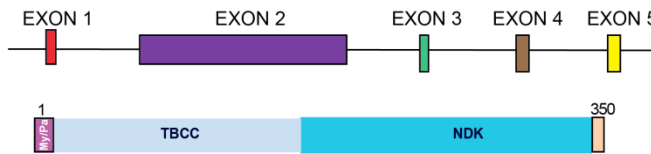
In cell culture studies, CEP290 has been shown to regulate cilia assembly program by modulating the localization of RAB8A and Pericentriolar Material 1 (PCM1) [85-86]. Additionally, studies using *Chlamydomonas* CEP290 indicated that it is involved in the stabilization of the diffusion barrier formed by the Y-linkers [39]. It was recently demonstrated that CEP290 interacts with a novel ciliary protein RKIP (Raf-1 Kinase Inhibitory Protein) and modulates its intracellular protein levels. Silencing of *cep290* in zebrafish or mutation in the *rd16* retina results in aberrant accumulation of RKIP; high levels of RKIP subsequently result in mislocalization of RAB8A [84]. Moreover, CEP290 interacts with BBS6; relative dosage of the two proteins seems to be critical in modulating the

formation of OS, cochlear cilia, and olfactory cilia [87]. These studies further demonstrated the diverse roles of CEP290 in modulating the formation, maturation, and function of cilia.

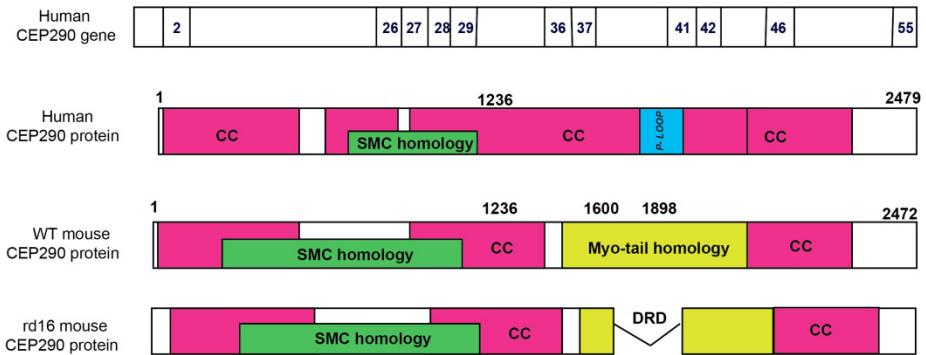
**RPGR**



**RP2**



**CEP290**



**Figure 3.** Schematic representation of the primary structure of RPGR, RP2, and CEP290. The two major isoforms of RPGR: RPGR<sup>1-19</sup> and RPGR<sup>ORF15</sup> are depicted. The RCC1-like Domain (RLD) is encoded by exons 2-11 of RPGR. The RPGR<sup>1-19</sup> isoform possesses a carboxyl terminal isoprenylation (IsoPr) site. The RP2 protein consists of amino terminal myristoylation /palmitoylation (My/Pa) site, tubulin binding cofactor C (TBCC) domain and a nucleoside diphosphate kinase (NDK) domain. The CEP290 protein is a multidomain molecule. Both human and mouse CEP290 protein are shown. In rd16 mouse, there is a deletion in the myosin-tail homology domain of the CEP290 protein. SMC: Structural Maintenance of Chromosomes; CC: coiled coil.



*RPGRIP1*. RPGRIP1 is a ciliary protein that associates directly with the TZ microtubules. Mutations in RPGRIP1 have been identified in a small percentage of LCA cases. In mice, ablation of the *Rpgrip1* gene results in defective OS development and early onset retinal degeneration. RPGRIP1 was identified as an interacting partner of RPGR in photoreceptors. Like *rd16* retina, the *Rpgrip1*<sup>-/-</sup> mouse retina exhibits mislocalization of RPGR to the IS of photoreceptors and its absence from the TZ. These studies indicate that RPGRIP1 tethers RPGR to the TZ. In addition to RPGR, RPGRIP1 also directly interacts with NPHP4; disease-causing mutations in both these proteins perturb this interaction [35, 88].

**Syndromic Ciliopathies.** In addition to non-syndromic retinal ciliopathies described above, photoreceptor degeneration is a common feature in multiple syndromic ciliopathies, such as Senior-Løken Syndrome (cystic kidneys and retinopathy), Joubert Syndrome (cerebellar vermis hypoplasia, cystic kidneys, and retinal coloboma) and Bardet-Biedl Syndrome (BBS; obesity, mental retardation, polydactyly and retinal degeneration) [6]. Interestingly, some of the proteins described above are also mutated in syndromic ciliopathies and/or associate with other ciliopathy proteins in the cilia. For example, some RPGR patients exhibit extra-retinal phenotypes, such as hearing defects, respiratory infections, sperm dysfunction, and primary cilia dyskinesia. CEP290, on the other hand, is also mutated in syndromic ciliopathies JBTS, MKS, and BBS.

*Joubert Syndrome (JBTS).* JBTS is an autosomal recessive disorder characterized by cerebellar vermis hypoplasia and retinal coloboma. A characteristic clinical feature of JBTS is the appearance of ‘molar tooth sign’, which represents a malformation of midbrain-hindbrain junction. Mutations in several ciliary proteins, such as CEP290/NPHP6, NPHP3, RPGRIP1L/NPHP8, AHI1, MKS3, and NPHP1 are associated with JBTS.

*Meckel-Gruber Syndrome (MKS).* MKS is characterized by embryonic lethality as a result of malformation or malfunction of multiple organs during development. Some characteristic clinical features include microphthalmia (small eye), renal dysplasia, polydactyly, and situs inversus. Interestingly, some of the genes that are mutated in JBTS are also associated with MKS. These include CEP290/NPHP6, RPGRIP1L/NPHP8, MKS1, MKS3, CC2D2A, and TMEM216. It has now been demonstrated that the type of mutation, location of the mutation and the relative combination of the different alleles can determine the outcome of the disorder.

*Senior-Løken and Bardet-Biedl Syndromes.* Senior-Løken Syndrome (SLSN) is characterized by renal cystic disease Nephronophthisis (NPHP) and retinal degeneration. Mutations in NPHP5 (or nephroretinin) are associated with SLSN; 100% of NPHP5 patients exhibit retinal degeneration. It was demonstrated that NPHP5 localizes to the cilia and interacts with RPGR in the retina. The retinal phenotype is partly attributed to the perturbed interaction of NPHP5 with RPGR in photoreceptors. Bardet-Biedl Syndrome (BBS), on the other hand, involves retinal degeneration, cystic renal disease, cognitive impairment, obesity, infertility and polydactyly as some of the main features. To date, mutations in 16 genes, all of which encode for ciliary proteins have been identified in BBS. These include BBS1-BBS12, MKS1, CEP290, SDCCAG8, and SEPT7.

In addition to the above-mentioned syndromic ciliopathies, there are several other disorders that have been elegantly described elsewhere and are not discussed in this chapter. All these disorders result from defective ciliary development or function. As cilia are involved in regulating numerous signaling cascades, including Wnt signaling, planar cell polarity, hedgehog signaling and cell cycle control, defects in these pathways have also been implicated as a cause of associated disorders. The involvement of signaling cascades in photoreceptor ciliary development and function is not completely understood.

CILIA DEPENDENT RETNOPATHIES					
SYNDROMIC FORMS			NON-SYNDROMIC FORMS		
DISEASE NAME	GENE INVOLVED	PHENOTYPE	DISEASE NAME	GENE INVOLVED	PHENOTYPE
USHER SYNDROME	USH1, USH1B, USH1C, USH1D, Podh15, PDZ	Retinal Degeneration And Hearing disability	RETINITIS PIGMENTOSA (RP)	RPGR, RP1, RP2, TOPORS	RETINAL DEGENERATION
SENIOR LOKEN SYNDROME	NPHP1-5, RPGR	Retinal Degeneration and Nephronophthisis (NPHP)	LEBER CONGENITAL AMAUROSIS (LCA)	RPGRIP1, CEP290	RETINAL DEGENERATION
JOUBERT SYNDROME	NPHP1, NPHP3, NPHP8/RPGRIP1L, NPHP6/CEP290, RPGRIP1L, AHI1, MKS3	Retinal degeneration, Mental Retardation, Renal disease, Liver disease			
BARDET BIEDL SYNDROME	BBS1-12, MKS1, NPHP6/CEP290, SDCCAG8, SEPT7	Retinal Degeneration, Obesity, Polydactyly, Hypogonitalism, Mental retardation and Renal Abnormalities			
MECKEL GRUBER SYNDROME	MKS1, MKS3, NPHP3, NPHP6/CEP290, NPHP8/RPGRIP1L, CC2D2A, TMEM216	Retinal Degeneration, Kidney disease, Physical Deformities, Respiratory, infections			

**Table 1.** This table depicts selected diseases classified as cilia dependent retinopathies, including non-syndromic as well as syndromic forms. Notably, retinal degeneration is a commonly occurring phenotype in all these disorders.

## 5. Conclusion

As a number of retinal ciliopathy proteins have now been identified the TZ of photoreceptors, the next step is now to delineate the mechanism by which these proteins modulate the function of the TZ and regulate photoreceptor OS development and function. The existence of discrete multiprotein complexes at the TZ indicates that these complexes are involved in the selection and trafficking of specific cargo moieties to the OS. Mutations in the constituent proteins may impair the function of some of the complexes and trafficking

of cognate cargo while other complexes may function normally to extend the life of the photoreceptor. However, if the ciliary protein mutated in disease were involved in the trafficking of proteins regulating the development of OS discs, such as rhodopsin, one would expect a severe and early onset retinal degeneration. It has been shown that RPGR, NPHP proteins and BBS proteins (BBSome) exist in multiprotein complexes and regulate ciliary trafficking.

Some of the TZ proteins possess enzymatic activity. As discussed above, RPGR is a GEF for RAB8A while RP2 is a GAP for ARL3. Such activity of these proteins may impart specificity to the cargo vesicle docking and fusion to the ciliary membrane for crossing the TZ barrier. Moreover, modulating the activity of these proteins may provide insights into developing therapeutic paradigms for associated disorders. It should however, be noted that some TZ proteins are also present in other subcellular compartments of the cell. For example, some RPGR isoforms are detected in the basal body and Golgi; RP2 localizes to Golgi and CEP290 localizes to the cytosol and basal body, in addition to the TZ. These observations beg the question: Are these proteins also involved in extraciliary functions or are these proteins participate in alternative pathways to ultimately regulate cilia dependent cascades. It was recently shown that CEP290 interacts with RKIP, which is involved in modulating MAP Kinase signaling cascades. In addition, CEP290 modulates intracellular levels of RKIP and likely controls its degradation. Hence, CEP290's involvement in intracellular signaling and in protein degradation pathways may be linked to cilia formation or function. However, further investigations are necessary to establish such links and to further delineate the roles of TZ proteins in regulating protein trafficking and photoreceptor OS development and function.

## Author details

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