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Multiprotein Complexes of Retinitis Pigmentosa GTPase Regulator (RPGR), a Ciliary Protein Mutated in X-Linked Retinitis Pigmentosa (XLRP)

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Abstract

Mutations in Retinitis Pigmentosa GTPase Regulator (RPGR) are a frequent cause of X-linked Retinitis Pigmentosa (XLRP). The *RPGR* gene undergoes extensive alternative splicing and encodes for distinct protein isoforms in the retina. Extensive studies using isoform-specific antibodies and mouse mutants have revealed that RPGR predominantly localizes to the transition zone to primary cilia and associates with selected ciliary and microtubule-associated assemblies in photoreceptors. In this chapter, we have summarized recent advances on understanding the role of RPGR in photoreceptor protein trafficking. We also provide new evidence that suggests the existence of discrete RPGR multiprotein complexes in photoreceptors. Piecing together the RPGR-interactome in different subcellular compartments should provide critical insights into the role of alternative RPGR isoforms in associated orphan and syndromic retinal degenerative diseases.

13.1 X-Linked RP (XLRP)

XLRP is a relatively severe form of retinal degeneration, accounting for 10–20% of all RP (Bird 1975; Fishman 1988). Most affected males exhibit early-onset visual symptoms with night-blindness in the first decade and rapid progression towards blindness by age 40 (Bird 1975; Fishman et al. 1978). Heterozygous carrier females can show electroretinographic (ERG) abnormalities and tapetal reflex (Fishman et al. 1986). Some XLRP patients have abnormal sperm phenotype (Hunter et al. 1988) or hearing defects (Iannaccone et al. 2004; Zito et al. 2003). To date, six genetic loci have been mapped: *RP2*, *RP3*, *RP6*, *RP23*, *RP24* and *RP34* (Fujita et al. 1996; Gieser et al. 1998; Hardcastle et al. 2000; McGuire et al. 1995; Melamud et al. 2006; Wright et al. 1991). The genes for two major forms of XLRP, *RP2* [Schwahn et al. 1998] and *RP3* [*RPGR* (Meindl et al. 1996; Roepman et al. 1996)], have been cloned.

Mutations in *RP2* account for approximately 10% of XLRP (Breuer et al. 2002; Hardcastle et al. 1999; Mears et al. 1999; Sharon et al. 2003). The *RP2* gene encodes a putative protein

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Retinitis Pigmentosa (RP: MIM 268000) is a leading cause of inherited blindness in developed countries. RP refers to a group of debilitating neurodegenerative diseases with clinically heterogeneous findings, which include bone spicule like pigmentary deposits in the retina, progressive loss of peripheral vision and eventually deterioration of central vision due to cone loss (Bird 1987; Fishman et al. 1988; Heckenlively et al. 1988; Sullivan and Daiger 1996). Over 30 RP genes have been identified so far (<http://www.sph.uth.tmc.edu/Retnet>) (Hartong et al. 2006). No effective approach exists for the management or treatment of RP.

of 350 amino acids (Chapple et al. 2000; Schwahn et al. 1998). The crystal structure of the RP2 protein reveals an amino-terminal β -helix that is structurally and functionally homologous to the tubulin-specific chaperone, cofactor C (TBCC); most disease-causing missense mutations are present in this domain (Bartolini et al. 2002; Grayson et al. 2002; Kuhnel et al. 2006). RP2 interacts with ADP-ribosylation factor-like 3 (ARL3) (Kuhnel et al. 2006), a microtubule-associated small GTP-binding protein (Kahn et al. 2005) that localizes to the sensory cilium of photoreceptors (Grayson et al. 2002). However, the precise role of RP2 in photoreceptors has not been delineated.

13.2 Retinitis Pigmentosa GTPase Regulator (RPGR)

Mutations in the *RPGR* gene account for over 70% of XLRP and as much as 25% of simplex RP males (Breuer et al. 2002; Shu et al. 2007). Initial analysis of a ubiquitously-expressed *RPGR*^{Ex1-19} transcript (derived from exons 1–19; 815 amino acids) identified mutations in only 10–20% of XLRP patients and families (Buraczynska et al. 1997; Fujita et al. 1997; Meindl et al. 1996; Roepman et al. 1996; Sharon et al. 2000). The discovery of an alternative transcript with a purine-rich terminal exon ORF15, which included a part of the original intron 15 (called *RPGR*^{ORF15}) revealed additional mutations in almost 50% of individuals with XLRP (Breuer et al. 2002; Sharon et al. 2003; Vervoort et al. 2000). Mutations in *RPGR*^{ORF15} have also been identified in patients with cone-rod dystrophy, atrophic macular degeneration, and Coat's-like exudative vasculopathy (Ayyagari et al. 2002; Demirci et al. 2006; Demirci et al. 2002; Sharon et al. 2003; Yang et al. 2002). Some individuals with *RPGR* mutations are reported to show a syndromic phenotype that may include respiratory tract infections, hearing loss, and primary cilia dyskinesia (Iannaccone et al. 2004; Koenekoop et al. 2003; Moore et al. 2006; van Dorp et al. 1992; Zito et al. 2003). In addition, patients with mutations in *RPGR* exons 2–14 appear to display a more severe clinical phenotype than those with exon ORF15 mutations (Sharon et al. 2003). However, further genotype-phenotype studies are needed to elucidate the clinical heterogeneity associated with *RPGR* mutations.

13.3 RPGR Isoforms in the Retina

The N-terminal region of RPGR contains tandem repeats (termed RCC1-like domain; RLD) homologous to RCC1, which is a guanine nucleotide exchange factor (GEF) for Ran-GTPase that is involved in nucleo-cytoplasmic transport (Meindl et al. 1996; Renault et al. 2001). Complex splicing patterns are reported for *RPGR* though the physiological relevance of these transcripts is unclear (Hong and Li 2002; Kirschner et al. 1999; Vervoort et al. 2000; Yan et al. 1998). Multiple immunoreactive bands are observed using isoform-specific RPGR antibodies (Chang et al. 2006; He et al. 2008; Hong and Li 2002; Khanna et al. 2005; Mavlyutov et al. 2002; Otto et al. 2005; Shu et al. 2005; Yan et al. 1998).

Several different groups have reported the localization of RPGR in the retina. Initially RPGR was shown to localize to the photoreceptor cilium independent of the species tested (Hong et al. 2003); however, another study demonstrated species-specific differences in RPGR localization (Mavlyutov et al. 2002). By immunogold labeling, we demonstrated the *RPGR*^{ORF15} protein in the transition zone and basal bodies of both mouse and human photoreceptor cilia though some additional labeling was detected in the inner and outer segments (Khanna et al. 2005; Shu et al. 2005). In proliferating cells, centrioles were labeled with anti-RPGR antibodies (He et al. 2008; Shu et al. 2005). It should be noted that primary cilia arise from mother centrioles in post-mitotic cells (Pedersen et al. 2008). Distinct localization of *RPGR*^{ORF15} isoforms may reflect their relative abundance in distinct subcellular compartments of photoreceptors.

13.4 Animal Models of RPGR

A knockout (ko) mouse with deletion of exons 4–6 of *Rpgr* was reported to show late-onset cone-rod degeneration (Hong et al. 2000); however, this *Rpgr*-ko mouse is not a complete null and expresses some specific RPGR^{ORF15} isoforms that localize to the transition zone of photoreceptor cilia (Khanna et al. 2005). Interestingly, ectopic expression of an ORF15 variant could partially rescue the *Rpgr*-ko phenotype (Hong et al. 2005), or behave as a dominant gain-of-function variant resulting in rapid disease progression (Hong et al. 2004). Attempts to generate a complete *Rpgr* null mutation in mouse have not been successful. Notably, frameshift mutations in RPGR^{ORF15} have been identified in two naturally-occurring canine mutants; the XLPRA2 dog exhibits relatively rapid photoreceptor degeneration and severe ERG abnormalities, whereas the XLPRA1 mutant shows a milder phenotype (Beltran et al. 2006; Zhang et al. 2002). Aberrant behavior of the two mutant ORF15 proteins in cultured cells may reflect the phenotypic differences in the two canine models (Zhang et al. 2002).

13.5 Sensory Cilia

Photoreceptor outer segment (OS) membrane discs and inner segment (IS) are linked by a sensory cilium (CC), which is a modified primary cilium (Young 1968). Ciliogenesis involves an evolutionarily conserved process, called Intraflagellar Transport (IFT) (Rosenbaum et al. 1999). In photoreceptors, components of the IFT complex and cargo proteins are synthesized in the IS, docked at the basal body, and transported distally by the anterograde heterotrimeric motor, Kinesin-II (Besharse et al. 2003). The IFT components are believed to be replenished by their transport back to the basal body by a presumptive retrograde motor cytoplasmic dynein 1b/2 (Besharse et al. 2003).

Vertebrate photoreceptors are highly metabolically active; approximately 10% of OS disks are turned over each day (Bok and Young 1972; Young 1968). It is estimated that ~2000 opsin molecules are transported to the OS per minute in an adult human retina (Besharse 1986; Williams 2002). The opsin molecules are synthesized in the IS and are targeted to the basal body, where they are fused with the ciliary membrane and trafficked distally to the OS (Deretic et al. 2005). Hence, it is not surprising that perturbations in ciliary transport of opsins or OS biogenesis are associated with severe retinal degeneration and blindness (Insinna and Besharse 2008).

13.6 Retinal Degeneration Caused by Mutations in Ciliary Proteins

IFT-mediated transport of rhodopsin and other signaling proteins is critical for photoreceptor survival and function. Conditional *Kif3a*^{-/-} mice and *Tg737^{orp}*, a hypomorphic allele of IFT88, result in opsin accumulation in the IS (Marszalek et al. 2000; Pazour et al. 2002). A number of retinal disease proteins CEP290/NPHP6, RPGRIP1 and RP1 are required for cilia-dependent OS transport, generation or maintenance (Chang et al. 2006; Liu et al. 2004; Zhao et al. 2003). Several pleiotropic disorders, such as Senior-Loken Syndrome, Joubert Syndrome, and Bardet-Biedl Syndrome, are also caused by mutations in ciliary proteins and share retinopathy as a common phenotype (Badano et al. 2006).

13.7 Macromolecular Complexes of RPGR^{ORF15}

RPGR exists in macromolecular complexes with other proteins in photoreceptors. Two proteins were initially identified by yeast two-hybrid analysis using the RPGR-RLD as bait: RPGRIP1, which is localized to the sensory cilium and mutated in retinopathy patients (Boylan and Wright 2000; Dryja et al. 2001; Hong et al. 2001); and delta subunit of rod cyclic guanosine monophosphate phosphodiesterase (PDE6D), a prenyl-binding protein

involved in the retrieval of PDE from rod outer segment membranes by interacting with Rab13 (Linari et al. 1999; Zhang et al. 2004). Two chromosome-associated proteins SMC1 and SMC3 (Hirano 2006; Khanna et al. 2005; Liu et al. 2007) and two ciliary disease-associated proteins, NPHP5 (Otto et al. 2005) and CEP290/NPHP6 (Chang et al. 2006; Sayer et al. 2006) are also reported to be a part of the RPGR^{ORF15} macromolecular complexes. In addition, RPGR can associate with Tg737/Polaris/IFT88 (Davenport and Yoder 2005; Pazour et al. 2002) and several microtubule transport proteins (Khanna et al. 2005).

13.8 Dissection of RPGR^{ORF15} Complexes

We hypothesize that RPGR isoforms are partitioned in distinct multiprotein complexes in photoreceptors. To evaluate this hypothesis, we have carried out sequential immunodepletion experiments. We initially used antibodies against two of the RPGR partners, CEP290/NPHP6 and SMC1, in order to immunodeplete RPGR that is part of these complexes from the retinal ciliary extract preparation. The remaining supernatant was subjected to immunoprecipitation (IP) with the anti-RPGR^{ORF15} antibody, followed by immunoblotting to test for the presence or absence of remaining RPGR^{ORF15}-interacting proteins (Fig. 13.1a). Even after immunodepletion of CEP290 from the retinal ciliary fraction (Fig. 13.1b), RPGR was still associated with IFT88, KIF3A, and γ -tubulin, but not with SMC1 and SMC3 (Fig. 13.1c). This data suggests that RPGR's complex with CEP290, SMC1, and SMC3 is distinct from that with IFT88, KIF3A, and γ -tubulin. On the other hand, after SMC1 immunodepletion, RPGR antibody could immunoprecipitate only a fraction of CEP290 from retinal ciliary extract. Similar results were obtained with SMC3 (data not shown).

These observations indicate that RPGR exists in at least three distinct complexes: first with IFT88, KIF3A, and γ -tubulin; second with CEP290, SMC1, and SMC3 and; third with CEP290 and probably other ciliary proteins (Fig. 13.2). Future detailed analysis of these and additional complexes should assist in dissecting the RPGR function in photoreceptors.

13.9 Conclusion

Despite extensive investigations, the underlying mechanism of ciliary transport-associated photoreceptor dysfunction is poorly understood at this stage. We suggest that RPGR-defect could occur at multiple stages: (a) cargo loading onto the vesicles; (b) vesicular trafficking towards the basal body, (c) docking of the cargo-laden vesicles at the basal body, (d) selection of cargo and transfer to the IFT complex, or (e) anterograde transport towards the distal OS. We reckon that the different RPGR complexes may participate in some or all of these transport processes. Given the importance of these pathways in photoreceptor development and survival, mutations in RPGR may disrupt its interactome thereby leading to retinal degeneration.

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Fig. 13.1.

(a) Schematic representation of the strategy to dissect distinct RPGR-containing multiprotein complexes in photoreceptor cilia. IP: Immunoprecipitation; Ab: antibody, (b) Protein extract (~150 μ g) was subjected to immunoprecipitation using indicated antibodies. The precipitated beads (Pellet) and supernatant (Sup) were analyzed by SDS-PAGE and immunoblotting (IB) using same antibodies. No signal in the supernatant indicates sufficient immunodepletion of the respective proteins from the extract. (c) and (d) CEP290 – or SMC1 – immunodepleted (ID) extract was subjected to IP using RPGR antibody or rabbit IgG

(immunoglobulin) followed by SDS-PAGE and immunoblotting (IB) using indicated antibodies

Fig. 13.2.

Schematic representation of the putative distinct RPGR complexes that can exist in photoreceptors. Proteins *A* and *B* represent as yet unidentified molecular partners that can be part of such complexes