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Aberrant protein trafficking in retinal degeneration: The initial phase of retinal remodelling

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Abstract

Retinal trafficking proteins are involved in molecular assemblies that govern protein transport, orchestrate cellular events involved in cilia formation, regulate signal transduction, autophagy and endocytic trafficking, all of which if not properly controlled initiate retinal degeneration. Improper function and or trafficking of these proteins and molecular networks they are involved in cause a detrimental cascade of neural retinal remodeling due to cell death, resulting as devastating blinding diseases. A universal finding in retinal degenerative diseases is the profound detection of retinal remodeling, occurring as a phased modification of neural retinal function and structure, which begins at the molecular level. Retinal remodeling instigated by aberrant trafficking of proteins encompasses many forms of retinal degenerations, such as the diverse forms of retinitis pigmentosa (RP) and disorders that resemble RP through mutations in the rhodopsin gene, retinal ciliopathies, and some forms of glaucoma and age-related macular degeneration (AMD). As a large majority of genes associated with these different retinopathies are overlapping, it is imperative to understand their underlying molecular mechanisms. This review will discuss some of the most recent discoveries in vertebrate retinal remodeling and retinal degenerations caused by protein mistrafficking.

1. Introduction

Retinal remodeling induced by protein mistrafficking followed by photoreceptor degeneration is a concept that although has been well established, has reemerged due to technical advances in the field (Marc and Jones 2003, Jones and Marc 2005, Marc, Jones et al. 2007). Retinal remodeling occurs in three known phases (Marc and Jones 2003, Jones and Marc 2005, Jones, Kondo et al. 2012). The first phase is due to an initial insult such as protein mislocalization that elicits cell stress within the photoreceptor or retinal pigmented epithelium (RPE) cells. Although the insult may be subtle, it can induce cascades of regulatory molecular and physiological pathways. These modifications provoke the second phase of retinal remodeling, in which the outer nuclear layer is remodeled due to photoreceptor death, resulting in cell apoptosis. This causes depletion of neuronal classes, leading into the third phase, extreme retinal remodeling, described by modifications of the

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neural retina by cell migration, new neurite and or synapse formation and neuronal death (Marc and Jones 2003, Marc, Jones et al. 2003, Marc, Jones et al. 2007, Jones, Kondo et al. 2012).

Protein trafficking within the photoreceptors must occur efficiently and at a high fidelity to maintain structural maintenance and overall retinal homeostasis. The goal of this review, while non-comprehensive, is to bring together some of the main details of what is known on a molecular level of retinal degenerative diseases and retinal remodeling in regards to improper protein trafficking. It is known that mutations occurring in genes encoding vital trafficking proteins display similar pathophysiologies, yet are associated with categorically different blinding diseases. Understanding at a molecular level what is occurring in these diseased states will provide a better explanation on a larger scale of how these molecular assemblies function and overlap.

1.1 Polarized trafficking occurs in the vertebrate outer retina

Vertebrate photoreceptors are unusual neurons that contain highly modified primary cilium (Horst, Johnson et al. 1990) composed of approximately two thousand of flattened membranous disks which house all of the protein components necessary for phototransduction in the outer segment (OS) region (Figure 1). This is separated from the inner segment (IS) region containing cellular organelles such as mitochondria and Golgi apparatus *via* a transition zone, or connecting cilium (CC). In addition to a structural role, the CC plays a critical functional role as a selective ciliary gate and transport pathway from the IS to the OS (Christensen, Pedersen et al. 2007, Leroux 2007, Insinna and Besharse 2008, Berbari, O'Connor et al. 2009, Emmer, Maric et al. 2010).

The movement of proteins is governed by vesicular interaction with the transition zone complexes, such as the BBSome and the intraflagellar transport (IFT) machinery (Nachury, Loktev et al. 2007). In photoreceptors, proteins of the transition zone interact with IFT complexes to support movement of phototransduction components (Chuang, Vega et al. 2004, Zhao and Malicki 2011). Ciliary targeting sequences such as the VXPX motif ensure a more efficient system of delivery of key signal molecules (Berbari, Johnson et al. 2008, Mazelova, Astuto-Gribble et al. 2009, Hollingsworth and Gross 2013). These sequences also facilitate ciliary trafficking by associating with small GTPases such as ARF4, ASAP1, rab8 and rab11a and other associated proteins (Figure 1, Table) (Deretic, Puleo-Schepke et al. 1996, Deretic, Schmerl et al. 1998, Reish, Boitet et al. 2014).

The RPE is comprised of a polarized monolayer of pigmented cuboidal epithelial cells positioned between the photoreceptors and the fenestrated choroid capillaries. The RPE provide a support system for the neural retina, offering retinoid recycling and processing, vectorial transport of metabolites and nutrients, receptor-mediated engulfment of shed OSs, provide a selective blood-retinal barrier and absorption of stray light (Young 1967, Lehmann, Benedicto et al. 2014, Rizzolo 2014). One RPE cell supports up to 30–50 photoreceptors, providing diverse necessary support functions for the neural retina (Strauss 2005, Sparrow, Hicks et al. 2010, Lehmann, Benedicto et al. 2014). To provide these support functions, the RPE have very long microvilli processes that surround rod photoreceptor outer segments (Figure 1) used for receptor-mediated phagocytosis of rod outer segment (ROS).

Another important function for the RPE is to control the subretinal space fluid levels; apically located Na⁺, K⁺, Cl⁻ co-transporters, CLC2 and other transporters provide fluid homeostasis, without which retinal edema and detachments occur (Hamann 2002). Interestingly, many of these transporters are located in opposite polarity of the cell as in most other transporting epithelial cells (Gundersen, Orłowski et al. 1991, Marmorstein 2001).

2. Mutations occurring in post-Golgi to centrosome trafficking in photoreceptors

2.1 C-terminal rhodopsin mutations

C-terminal mutations in rhodopsin cause some of the earliest onset retinal degenerations in autosomal dominant retinitis pigmentosa (ADRP) patients which is supported to be due to the initial insult of mistrafficked rhodopsin (Hollingsworth and Gross 2012). Rhodopsin mutations have been previously associated with aberrant retinal remodeling due to neurite formation, which has been observed in human RP patients (Li, Kljavin et al. 1995, Tam, Xie et al. 2006). One such rhodopsin mutant found in humans, Q344ter, is missing the VXPX sorting motif due to the early truncation of the protein (Jacobson, Kemp et al. 1994, Sung, Makino et al. 1994, Deretic 2006, Concepcion and Chen 2010, Hollingsworth and Gross 2013). Loss of the ciliary targeting domain results in a loss of interaction with the necessary trafficking machinery for proper rhodopsin trafficking (Deretic, Traverso et al. 2004). Universal findings in *Q344ter* animal models are the severe mislocalization of rhodopsin in the rod IS and outer nuclear layer (ONL), sub-micrometer sized vesicles in the interphotoreceptor space and shortened, disorganized ROS structures (Deretic, Puleo-Schepke et al. 1996, Deretic, Schmerl et al. 1998, Gross, Q. et al. 2008, Mazelova, Astuto-Gribble et al. 2009, Concepcion and Chen 2010, Sandoval, Price et al. 2014).

Ter349Glu rhodopsin manifests as an early rapid retinal degeneration, exhibiting drastic loss of photoreceptor function by early adulthood (Bessant, Khaliq et al. 1999, Hollingsworth and Gross 2013). This mutation is due to an additional 51 amino acids at rhodopsin's C-terminus, which causes the VXPX motif to be occluded. Recently, it has been shown that the mouse model of Ter349Glu exhibits an activated unfolded protein response (UPR), triggering an inflammatory response to the retina and an increase of infiltrating microglial projections (Rana, Shinde et al. 2014). Statistically significant increased expression of the inflammatory marker, cytokine protein interleukin-1 beta (IL-1 β) was observed at 30 days postnatal, when photoreceptor death reached 50 percent. (Rana, Shinde et al. 2014). Although *Ter349Glu* rhodopsin behaves similarly to wild-type rhodopsin, aberrant morphological abnormalities in the ROS disks occur, including disks oriented parallel to the axoneme, disk membrane whirls and loss of properly formed outer segments (Hollingsworth and Gross 2013). Also, homozygote human knock-in *Ter349Glu* mice exhibit extracellular vesicles near the tip of the cilium where the ROS forms, similar to other degenerative phenotypes such as the human rhodopsin-EGFP knock-in (*hrhoG/hrhoG*) mouse. A powerful synthetic model of rhodopsin-mediated retinal degeneration is the human rhodopsin-EGFP knock-in (*hrhoG/hrhoG*) mouse (Chan, Bradley et al. 2004, Wensel, Gross et al. 2005, Gross, Decker et al. 2006). Some of the fundamental findings of the homozygous

rhodopsin-EGFP mutant mice were failed biogenesis of proper disk membrane formation and a disintegration of the distinction between the inner and outer segment boundaries indicating abnormal outer segment formation (Figure 2) (Gross, Decker et al. 2006). Over time these mice undergo thinning of the retinal layer and by 14 weeks postnatal no more than one row of nuclei is observed in the ONL. In contrast, heterozygous rhodopsin-EGFP mice are able to maintain healthy retinas and are able to efficiently transport rhodopsin-EGFP to the outer segment, despite the presence of the fluorescent protein fused adjacently to the VXPX sorting motif of rhodopsin (Deretic, Schmerl et al. 1998, Chan, Bradley et al. 2004, Chuang, Vega et al. 2004, Gross, Decker et al. 2006). This localization could be due to the fusion protein being transported in concert with rhodopsin in these animals. It was also shown in late-stage degeneration within the *hrhoG/hrhoG* mouse that bipolar cells lost group III, type 6 metabotropic (mGluR6) glutamate and ionotropic (iGluR) receptors, whereas amacrine and ganglion cells retained functional expression of the ionotropic (iGluR) receptor which models evidence of rod bipolar cell dendrite switching found in human RP (Marc, Jones et al. 2007).

2.2 Rab11a

Rab11a is one of the small GTPases that has been identified as a major C-terminus dependent rhodopsin binding protein and is part of the targeting complex that recognizes the ciliary targeting motifs of rhodopsin at the trans-Golgi network (Mazelova, Astuto-Gribble et al. 2009, Wang, Morita et al. 2012, Reish, Boitet et al. 2014). A majority of intracellular membrane transport is governed by the small GTPases of the Rab family. These small GTPases that function as molecular switches are responsible for multiple roles, organizing membrane trafficking and directing the delivery of membrane cargo to the proper location of function (Wang and Deretic 2014). Rab GTPases are regulated by guanine-nucleotide-exchange factors (GEFs) and GTPase accelerating proteins (GAPs), which cooperatively control intracellular membrane traffic by promoting them to be active or inactive form respectively (Mizuno-Yamasaki, Rivera-Molina et al. 2012).

Using human cDNA mutants of rab11a, it was shown that the nucleotide binding status of rab11a affects its subcellular localization in *Xenopus laevis* tadpole rods; when constitutively active, GTP-locked mutants concentrated in the IS, versus when constitutively inactive, GDP-locked mutants were concentrated in the ROS (Reish, Boitet et al. 2014). The transgenic expression of the empty nucleotide-binding state of rab11a led to ectopic process formation, shortened outer segments and retinal degeneration after 2 weeks. Upon the expression of miR30-based hairpin directed against rab11a in *X. laevis* retina, it was shown that the depletion of rab11a led to phenotypes imitating the appearance of human rods in post-mortem studies of RP. Although there are numerous studies highlighting the importance of rab11a and other G protein-coupled receptor-promoted trafficking in humans in other cell types and diseases, to date there has yet to be any published data directly relating to retinal rab11a mutations in photoreceptor degeneration or retinal remodeling. A comprehensive review written by Wang and Deretic gives a more in-depth evaluation of rab trafficking (Wang and Deretic 2014).

3. Mutations occurring in the connecting cilium (transition zone)

3.1 Retinitis Pigmentosa GTPase Regulator (RPGR)

Predominately located in the connecting cilium, Retinitis Pigmentosa GTPase regulator (RPGR) is a cilia-centrosomal protein that plays a role in proper photoreceptor polarization and protein trafficking (Figure 1). RPGR participates in IFT and interacts with vital trafficking proteins within photoreceptors, such as RPGRIP1, CEP290, IFT88, NPHP and others, possibly facilitating their function (Roepman and Wolfrum 2007, Murga-Zamalloa, Swaroop et al. 2009, Hosch, Lorenz et al. 2011). (Weleber, Francis et al. 1993, Murga-Zamalloa, Swaroop et al. 2009, Brancati, Dallapiccola et al. 2010). Phenotypes found in RPGR patient case studies range from early onset RP to late stage atrophic AMD with bone spicule pigment clumping as well as a recent discovery of annual loss of sensitivity within the transition zone of the photoreceptors found by frequency-domain optical coherence tomography (Lorenz, Andrassi et al. 2003, Walia, Fishman et al. 2008, Birch, Locke et al. 2015). Although there are numerous isoforms of RPGR, one of the major subtypes is the retina-enriched RPGR^{ORF15} isoform, which causes a premature truncation of the protein and accounts for at least 50% of RPGR mutations (Meindl, Dry et al. 1996, Roepman, van Duijnhoven et al. 1996, Breuer, Yashar et al. 2002, Vervoort and Wright 2002). Mutations occurring in RPGR not only result in RP but other blinding diseases as well such as Leber congenital amaurosis (LCA), Joubert syndrome (JBTS) and Senior-Loken syndrome (SLS), affecting both rod and cone photoreceptors. Within patients phenotypes vary, showing diverse patterns of rod and cone dysfunction and varied disease onset. More detailed information about LCA, JBTS and SLS can be found in these comprehensive reviews (Weleber, Francis et al. 1993, Murga-Zamalloa, Swaroop et al. 2009, Brancati, Dallapiccola et al. 2010).

Recently published data from Swaroop and colleges revealed results from a comprehensive long-term dose efficiency study for gene replacement therapy for X-linked retinitis pigmentosa (XLRP) (Wu, Hiriyantha et al. 2015). Using knockout Rpg mice and AAV8 or AAV9 vectors, eyes treated with a single injection of human or mouse RPGR^{ORF15} vector at an optimal working dose preserved the expression of RPGR^{ORF15}, exhibiting thicker photoreceptor layer, higher electroretinogram amplitude, and more efficient targeting of opsins to outer segments compared to control eyes. They also found that even older mice displayed notable preservation of retinal function and structure, encouraging human RPGR^{ORF15} vector as a possible clinical candidate.

3.2 RPGR Interacting Protein 1 (RPGRIP1)

RPGRIP1 (LCA6) encodes RP GTPase regulator-interacting protein, is a key component of the axoneme of the rod and cone photoreceptor connecting cilium and is involved with disk morphogenesis. RPGRIP1 C-terminus interacts with the N-terminus of RPGR and is believed to anchor RPGR to the photoreceptor ciliary axoneme and to the centrioles (Roepman, Letteboer et al. 2005). RPGRIP1 may also play a role in the multi-protein complex that regulates actin cytoskeleton reorganization during new disk formation, actin-fragmin kinase protein (Roepman, Letteboer et al. 2005). In photoreceptors, the RPGRIP1 interactome was discovered to be comprised of at least three proteins, RPGRIP1 itself,

RGPR and nephrocystin-4/nephroretinin (NPHP4) (Boylan and Wright 2000, Roepman, Schick et al. 2000, Hong, Yue et al. 2001, Roepman, Letteboer et al. 2005). Mutations in RPGRIP1 have been associated with juvenile RP, Leber congenital amaurosis, cone-rod dystrophy as well as some forms of glaucoma (Dryja, Adams et al. 2001, Gerber, Perrault et al. 2001, Hameed, Abid et al. 2003, Hanein, Perrault et al. 2004, Booij, Florijn et al. 2005, Galvin, Fishman et al. 2005, Fernandez-Martinez, Letteboer et al. 2011, Patil, Tserentsoodol et al. 2012).

To date there are several animal models of *Rpgrip1* all of which display variable retinal phenotypes (Zhao, Hong et al. 2003, Mellersh, Boursnell et al. 2006, Lheriteau, Libeau et al. 2009, Won, Gifford et al. 2009). One of the mouse models, *Rpgrip1^{nmf247}*, which has been shown to resemble the clinical expression of LCA found in patients (Won, Gifford et al. 2009), was used in a study to examine the underlying effects of selective loss of RPGRIP1-dependent ciliary targeting on NPHP4, RPGR and SDCCAG8 (Patil, Tserentsoodol et al. 2012). The largest isoform of RPGRIP1, RPGRIP α , determines distinct ciliary localizations of NPHP4 and RPGR and that loss of its expression promotes changes in the subcellular partitioning of NPHP4 and SDCCAG8 to the ER-associated membrane. Photoreceptors in *Rpgrip1^{nmf247}* mice exhibited ruffling of the ciliary membrane throughout the cilium and a decrease of connecting cilium and basal body length by at least 25% (Patil, Tserentsoodol et al. 2012). This and others work support that the RPGRIP1 complex provides a robust amount of physiological and subcellular reinforcement in photoreceptors.

4. Mutations involved in ciliary trafficking

4.1 BBS4

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder in which phenotypes evolve slowly throughout the first decade of life although there is considerable variability (Forsythe and Beales 2013). The primary finding of the disease is retinal dystrophy, mainly rod-cone dystrophy, but there are also reports of cone-rod dystrophy, choroidal dystrophy, with childhood RP being the most common clinical finding occurring in over 90% of the patients (Beales, Elcioglu et al. 1999, Adams, Awadein et al. 2007). Sixteen BBS genes have been identified to date, all of which have been associated to either cilium function or biogenesis. Seven BBS proteins (BBS1, 2, 4, 5, 7, 8 and 9) participate in the formation of a stable protein complex named the BBSome, which was found necessary for cilia formation and is involved in vesicular trafficking to the ciliary membrane (Nachury, Loktev et al. 2007). Whereas, BBS6, 10 and 12 mediate the BBSome assembly by formation of a chaperone complex (Nachury, Loktev et al. 2007, Seo, Baye et al. 2010). Mutations in BBS1 and BBS10 are the two most commonly found in patients, accounting for 20% of the cases. Numerous studies of BBS knock out mice have shown that these animal models in fact emulate the human disease, particularly in regards to retinal degeneration (Mykytyn, Mullins et al. 2004, Nishimura, Fath et al. 2004, Ross, May-Simera et al. 2005, Eichers, Abd-El-Barr et al. 2006, Abd-El-Barr, Sykoudis et al. 2007).

BBS4-induced photoreceptor dystrophy is associated with disruption of intraflagellar transport of proteins and defects in synaptic transmission from the photoreceptors to secondary neurons rather than gross structural defects of the cilia (Abd-El-Barr, Sykoudis et

al. 2007). Bbs4-null mice initially have normal photoreceptor morphology, connecting cilium and basal body microtubule structure but later acquire decreased OS dimensions and increased cell death with age. Transport defects of rhodopsin and cone opsins manifest early in photoreceptor development of Bbs4-null mice are found before indications of cell death, specifically affecting the light-dependent translocation of numerous phototransduction proteins but not certain structural proteins. There are also synaptic transmission defects occurring in photoreceptors to secondary neurons of the visual system. This is a novel role for BBS4 in synaptic transmission which could play an underlying role in the retinal remodeling of this mutation.

4.2 CEP290

CEP290 (otherwise known as LCA10 and NPHP6), encodes the CEP290 multi-domain centrosomal protein which is involved in ciliary assembly and trafficking (Coppieters, Lefever et al. 2010). In the retina, CEP290 has been found to associate with many microtubule-based transport proteins and it is one of the most prevalent gene mutations occurring in multiple blinding diseases such as nephronophthisis (NPHP), BBS, Meckel-Gruber syndrome (MKS), LCA, SLS and JBTS. Some patient phenotypes include retinal coloboma, abnormally reduced ONL with increased ganglion cell layer thickness and early rod cell loss with abnormal IS and OS lamination (Jacobson, Sumaroka et al. 2013). Although the mutations within CEP290 have been identified, clear genotype-phenotype correlations have yet to be established due to the variation of disease onset and phenotypes associated with it (Chang, Khanna et al. 2006, Coppieters, Lefever et al. 2010, Baye, Patrinoastro et al. 2011).

In the field there are multiple animal models of the CEP290 mutation (Chang, Khanna et al. 2006, Baye, Patrinoastro et al. 2011, Narfstrom, Holland Deckman et al. 2011). The mouse model of CEP290, *rd16* (retinal degeneration 16), contains a hypomorphic CEP290 mutation (Chang, Khanna et al. 2006). These mice show rapid degeneration of rod and cone photoreceptors starting at postnatal day 14, appearance of white retinal vessels at 1 month and exhibit major mislocalization of retinal OS proteins, arrestin, rhodopsin and transducin. CEP290 has also been proposed to implicate degradation of inhibitors of ciliogenesis within photoreceptors (Murga-Zamalloa, Ghosh et al. 2011). This is supported by the abnormal accumulation of RKIP, Raf-1 kinase inhibitor, which inhibits cilia formation may play a role as a potential mechanism of photoreceptor death (Chang, Khanna et al. 2006, Murga-Zamalloa, Ghosh et al. 2011).

4.3 INPP5E

MORM syndrome (mental retardation, truncal obesity, retinal dystrophy and micropenis) was initially defined in a consanguineous Northern Pakistani pedigree as a novel autosomal recessive disorder associated with a non-progressive retinal dystrophy (Hampshire, Ayub et al. 2006). These patients experience poor night vision within the first year of life and reduced visual acuity at 3 years of age that remained unchanging. Within this family cohort the mutated gene identified was *INPP5E* (inositol polyphosphate-5-phosphatase) is related to JBTS and associated disorders (Bielas, Silhavy et al. 2009). Phosphoinositide phosphatases

regulate endocytosis, vesicular trafficking, cell proliferation and migration, PI3K/Akt signaling, and apoptosis (Hakim, Bertucci et al. 2012).

Homozygous mutant mice for *INPP5E* led to postnatal death, with eye development that ceased at the optic vesicle stage just before the development of the optic cup (Jacoby, Cox et al. 2009). In order to analyze the effects of *INPP5E* inactivation in adult mice, *Inpp3e^{fllox}* mice were crossed with a *CAGG-Cre-ER^{TM}* transgenic mice expressing a tamoxifen-inducible form of Cre recombinase in several tissues (Hayashi and McMahon 2002, Jacoby, Cox et al. 2009). At 6 months the retinal photoreceptor cell layer in these mice were completely absent. This mutation has been predicted to produce early truncation of the protein due to the absence of the terminal 18 amino acids, causing the loss of the C-terminal CaaX domain (Jacoby, Cox et al. 2009). The CaaX domain is conserved in all mammalian and avian INPP5E proteins and encodes a motif for addition of a farnesyl or geranylgeranyl or lipid group to the cysteine, which leads to proper membrane targeting of the protein (De Smedt, Boom et al. 1996). Ciliary functions of mutations in *INPP5E* reveal the importance of phosphoinositide metabolism within photoreceptor development and maintenance.

4.4 Myosin VIIa

The *MYO7A* gene encodes protein myosin VIIa, one of the most well characterized proteins that cause Usher syndrome, a progressive blinding disease (Weil, Blanchard et al. 1995, Chuang, Hsu et al. 2015). Myosin VIIa is mostly localized in the apical region of RPE cells and also has roles within the connecting cilium and periciliary region, although they are not well defined (Williams and Lopes 2011). It has been shown that a lack of myosin VIIa causes a delayed rate of disk membrane renewal as well as an abnormal accumulation of opsin in the connecting cilium, indicating deficiency of delivery of opsin to the site of disk membrane morphogenesis (Liu, Udovichenko et al. 1999).

Zebrafish animal models have been the most successful with mutant or knocked down genes that correspond to Usher orthologues (Wasfy, Matsui et al. 2014). In the Usher 1B zebrafish model, which express mutant *MYO7A* exhibit similar behavioral phenotypes to the mouse model *shaker1* as well as defective apical localization of RPE melanosomes within the retinas (Ernest, Rauch et al. 2000). Myosin VIIa has been hypothesized to interact with other Usher proteins (Hasson, Heintzelman et al. 1995) and it has been shown to transport both melanosomes and phagosomes in accordance with Rab27a (Futter, Ramalho et al. 2004, Gibbs, Azarian et al. 2004). Eight genes have been identified with Usher's syndrome and there is growing evidence that a unifying feature of the proteins encoded by Usher genes form an integrated protein network involving the cilia and the synapse (Kremer, van Wijk et al. 2006). Therefore, improper function of myosin VIIa compromises various cellular defects that span the neural retina.

5. Mutations associated with disk formation

5.1 Retinitis Pigmentosa 1 (RP1)

Retinitis Pigmentosa 1 (RP1) encodes a retinal photoreceptor specific protein of which mutations cause both dominant and recessive forms of RP. RP1 is a microtubule-associated

protein that forms part of the photoreceptor axoneme and is directly involved in the proper orientation and higher order stacking of outer segment disks in rods and cones (Ferrari, Di Iorio et al. 2011). The N-terminal portion of RP1 mediates its interaction with the axoneme, whereas the c-terminus has been hypothesized to interact with other proteins to assist in the organization of outer segment disks (Gao, Cheon et al. 2002, Liu, Lyubarsky et al. 2003, Liu, Zuo et al. 2004, Liu, Collin et al. 2012).

One mouse model of RP1, *Rp1-Q662X*, is a knock-in mouse model with Q662X nonsense point mutation in exon 4 of human RP1, produces a truncated Rp1 protein. Homozygous *Rp1-Q662X* mice experience grossly abnormal outer segment formation, at postnatal day 10, small packets of enlarged disks have replaced the normal organized stacks and by 1 month the ONL of these animals contain 2–3 rows of nuclei (Liu, Collin et al. 2012). This group performed human genetic studies and experiments using several lines of Rp1 gene targeted and transgenic mice and revealed that expression of wild-type Rp1 protein in homozygous human knock-in *Rp1-Q662x* mice prevented photoreceptor degeneration (Liu, Collin et al. 2012). In regards to gene therapy and blinding diseases, the results from this study suggest that the truncated *Rp1-Q662X* protein does not employ a lethal gain-of-function effect, implying that principle gene augmentation therapy could be beneficial for both recessive and dominant *RP1* patients, but the levels of RP1 protein delivered for therapy must be carefully monitored.

5.2 Meckelin

Meckelin is a ciliary and transmembrane protein associated in disorders such as MKS, BBS, COACH syndrome, Nephronophthisis and JBTS (Collin, Won et al. 2012). Meckelin is encoded by *MKS3/TMEM67* and has been suggested to be critical to cilia function within the retina and other organs (Leitch, Zaghoul et al. 2008, Tammachote, Hommerding et al. 2009, Collin, Won et al. 2012). Studies have shown molecular interactions of MKS3 with MKS1, NPHP4 and others (Dawe, Smith et al. 2007, Valente, Logan et al. 2010, Williams, Masyukova et al. 2010, Garcia-Gonzalo, Corbit et al. 2011, Williams, Li et al. 2011, Roberson, Dowdle et al. 2015).

A mouse model for *MKS3*, *Tmem67^{bpck}*, has similar clinical features to those reported in human patients (Cook, Collin et al. 2009). Although these mice experience mislocalization of phototransduction proteins, ultrastructural analyses reveal morphologically intact connecting cilia but malformed and disoriented OSs (Collin, Won et al. 2012). *Tmem67^{bpck}* homozygous mutant mice undergo the appearance of a granular fundus at postnatal day 21, reduced ONL layer by postnatal day 24 and multiple malformed patches and attenuation of retinal blood vessels by postnatal day 34. These data suggest meckelin may play a role in governing photoreceptor intraciliary transport and outer segment morphogenesis and maintenance (Cook, Collin et al. 2009, Collin, Won et al. 2012).

Discussion

Protein mistrafficking occurring in the retina encompasses a multitude of blinding diseases affecting all of its cell types. One of the overarching goals towards the treatment of blinding diseases is properly identifying and determining the physiological functions of human

retinopathy associated proteins and protein networks. Severe dysfunction in the orchestration of these protein complexes allow for overlapping of diseases. Mutations encoding proteins that gate ciliary function cause major disruption of the ciliary membrane composition. Likewise, those involved in transition zone dysfunction cause an array of retinal phenotypes that may relate to whether or not they directly affect ciliogenesis, in addition to disturbing ciliary membrane configuration.

One hypothesis for the variability in retinal disease onset could be caused by redundancies in these protein networks, for instance if the functionality of a family of proteins are similar, the disease onset will occur later in life. Similarly, if a protein complex is functionally dissimilar, corruption of surrounding protein networks that rely on its proper function can arise. For instance, mutations in BBS2, BBS4 and BBS6 have been identified in patients with Meckel syndrome (MKS) (Karmous-Benailly, Martinovic et al. 2005). Likewise, mutations in MKS1, which usually lead to Meckel syndrome, may be associated with a BBS phenotype and mutations in MKS3 have been recognized in patients with BBS and JBTS (Gerdes, Davis et al. 2009). Therefore, it appears that there is an association in the ciliopathy phenotype reflecting both the specific mutated loci and the total mutational load (Leitch, Zaghoul et al. 2008, Gerdes, Davis et al. 2009).

Animal models have provided substantial progress in further understanding these complex molecular assemblies, offering significant insight into disease pathogenesis. A powerful animal model of RP that contains a photoactivatable rhodopsin tag, allows rhodopsin trafficking to be visualized *in vivo* (Sammons and Gross 2013). This method could be applicable to study mutations encoding other trafficking proteins, providing the ability to monitor their activity to see where initial mislocalization occurs, possibly providing insight for initial retinal remodeling that occur. Likewise, recent studies have been conducted using the photoconvertible fluorescent protein Dendra2 which has allowed visualization of protein trafficking dynamics and localization *in vivo*, providing insight into characterization of proteins in a spatiotemporal manner within individual photoreceptors (Lodowski, Lee et al. 2013, Nemet, Tian et al. 2014, Tian, Lodowski et al. 2014, Lodowski and Imanishi 2015). Many efforts have been made in regards to gene therapy, some of which advancing to human clinical trials. Improving classification and distinction in early detection of retinal remodeling, physicians can better treat and predict potential treatment methods. Based on the current literature it is undecipherable whether or not retinal remodeling elicited by protein mistrafficking is categorically different from other defects such as visual cycle defects. Understanding the underlying mechanisms and characteristics of these proteins on a molecular level will provide further insight for the multitude of devastating blinding diseases and possibly leading to eventual therapeutic treatment.

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- Review of polarized trafficking in vertebrate outer retina
- Includes mutations occurring in post-Golgi to centrosome trafficking in photoreceptors
- Includes mutations involved in ciliary trafficking
- Includes mutations associated with rod disc formation
- Includes non-photoreceptor trafficking mutations

Figure 1.

The vertebrate retina and rod cell highlighting trafficking proteins. Scanning electron micrograph (SEM) of wild-type mouse reveals the precise architecture of the neural retina (left). Schematic of rod cell (middle) highlighting an outer (OS), connecting cilium (CC) and inner segment (IS). Retinal pigmented epithelial (RPE) cells lay over the photoreceptor OS. Schematic of transition zone, BBSome and trans-Golgi network showing the hypothesized order and localization of the trafficking proteins (right) discussed in this review and others. SEM taken by Alecia K. Gross and Ivan Anastassov at the Marine Biological Labs, Fundamental Issues in Vision Research course 2010. *, tips of rod OS.

Figure 2.

Retinal remodeling at 10 day and 4 week-old *hrhoG/hrhoG* mice. Electron micrographs of ultrathin retinal sections comparing wild-type (+/+), (A and B), heterozygote (+/*hrhoG*), (C and D) and homozygous knock-in mice (*hrhoG/hrhoG*), (E and F), knock-in mice generated by segmental replacement (*hrhoG(H)/hrhoG(H)*), (G and H) and homozygous rhodopsin knockout mice (-/-), (I and J). Asterisks (*) indicate visible connecting cilia (A, C, E, and G), whereas diamonds indicate nascent disk stacks in +/+ and +/*hrhoG* mice (A and C). Evident disorganized lamellae are present in *hrhoG/hrhoG* mice by 4 weeks. Pigment granules in the RPE are the dark patches in *hrhoG(H)/hrhoG(H)* and -/- mice (G and I). Reprinted from Vision Research, Volume 46, Issue 27, Alecia K. Gross, Glenn Decker, Fung Chan, Ivette M. Sandoval, John H. Wilson, Theodore G. Wensel, 'Defective development of

photoreceptor membranes in a mouse model of recessive retinal degeneration' 4510–4518,
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Table

Abbreviations of proteins used in text.

Protein abbreviation	Protein full name; alternative names associated with protein
Arf4	ADP-Ribosylation Factor 4
ASAP1	ArfGAP With SH3 Domain, Ankyrin Repeat And PH Domain 1
BBS1	Bardet-Biedl syndrome 1
BBS2	Bardet-Biedl syndrome 2
BBS3	Bardet-Biedl syndrome 3
BBS4	Bardet-Biedl syndrome 4
BBS5	Bardet-Biedl syndrome 5
BBS6	Bardet-Biedl syndrome 6
BBS7	Bardet-Biedl syndrome 7
BBS9	Bardet-Biedl syndrome 9
BBS10	Bardet-Biedl syndrome 10
BBS12	Bardet-Biedl syndrome 12
CEP290	Centrosomal Protein 290kDa; Leber Congenital Amaurosis 10 (LCA10), Nephronophthisis 6 (NPHP6)
MKS1	Meckel Syndrome, Type 1; Bardet-Biedl syndrome 13 (BBS13)
MKS2	Meckel Syndrome, Type 2; Transmembrane Protein 216 (TMEM216), Joubert Syndrome 2 (JBTS2), Cerebello-Oculo-Renal Syndrome 2
MKS3	Meckel Syndrome, Type 3; Transmembrane Protein 67 (TMEM67), Nephronophthisis 11 (NPHP11), Joubert Syndrome 6 (JBTS6)
MKS6	Meckel Syndrome, Type 6; Coiled-Coil And C2 Domain Containing 2A (CC2D2A)
MKSR1	MKS1-Related Protein 1; B9 Domain-Containing Protein 1 (B9D1)
MKSR2	MKS2-Related Protein 2; B9 Domain-Containing Protein 2 (B9D2)
NPHP1	Nephronophthisis 1; Nephrocystin-1, Joubert Syndrome 4 (JBTS4)
NPHP4	Nephronophthisis 4; Nephrocystin-4, Nephroretinin
NPHP5	Nephronophthisis 5; Nephrocystin-5
Rab8	Ras-Associated protein in Brain 8
Rab11	Ras-Associated protein in Brain 11
Rabin8	Rabin8; Rab3A interacting protein (Rab3IP)
RP1	Retinitis Pigmentosa 1
RPGR	Retinitis Pigmentosa GTPase Regulator
RPGRIP1	Retinitis Pigmentosa GTPase Regulator Interacting Protein 1; Leber Congenital Amaurosis 6 (LCA6)
TCTN1	Tectonic Family Member 1; Joubert Syndrome 13 (JBTS13)