Interaction of retinitis pigmentosa GTPase regulator (RPGR) with RAB8A GTPase: implications for cilia dysfunction and photoreceptor degeneration

Carlos A. Murga-Zamalloa1, Stephen J. Atkins1, Johan Peranen2, Anand Swaroop3 and Hemant Khanna1,∗,†

1Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI 48105, USA, 2Institute of Biotechnology, University of Helsinki, Helsinki, Finland and 3Neurobiology-Neurodegeneration & Repair Laboratory (N-NRL), National Eye Institute, National Institutes of Health, Bethesda, MD 20892, USA

Received March 31, 2010; Revised June 4, 2010; Accepted June 29, 2010

Defects in biogenesis or function(s) of primary cilia are associated with numerous inherited disorders (called ciliopathies) that may include retinal degeneration phenotype. The cilia-expressed gene RPGR (retinitis pigmentosa GTPase regulator) is mutated in patients with X-linked retinitis pigmentosa (XLRP) and encodes multiple protein isoforms with a common N-terminal domain homologous to regulator of chromosome condensation 1 (RCC1), a guanine nucleotide exchange factor (GEF) for Ran GTPase. RPGR interacts with several ciliopathy proteins, such as RPGRIP1L and CEP290; however, its physiological role in cilia-associated functions has not been delineated. Here, we report that RPGR interacts with the small GTPase RAB8A, which participates in cilia biogenesis and maintenance. We show that RPGR primarily associates with the GDP-bound form of RAB8A and stimulates GDP/GTP nucleotide exchange. Disease-causing mutations in RPGR diminish its interaction with RAB8A and reduce the GEF activity. Depletion of RPGR in hTERT-RPE1 cells interferes with ciliary localization of RAB8A and results in shorter primary cilia. Our data suggest that RPGR modulates intracellular localization and function of RAB8A. We propose that perturbation of RPGR–RAB8A interaction, at least in part, underlies the pathogenesis of photoreceptor degeneration in XLRP caused by RPGR mutations.

INTRODUCTION

Primary cilia are microtubule-based membranous extensions that carry out distinct and specialized functions including regulation of cellular homeostasis, signal transduction, cell polarity and protein trafficking (1–6). Cilia are generated from basal bodies that originate from the mother centriole (7–9) by a conserved mechanism called intraflagellar transport (IFT) (7,10,11). Owing to their involvement in diverse cellular processes, defects in cilia assembly or function may result in multisystemic and/or neonatally lethal disorders (also called ciliopathies), such as Bardet–Biedl syndrome (BBS), Joubert syndrome and Meckel–Gruber syndrome (12–14).

Syndromic ciliopathy phenotypes frequently include degeneration of retinal photoreceptors, probably because of the presence of a unique primary cilium that is modified to produce outer segment (OS) membrane discs for capturing light quanta (15–17). As ~10% of the photoreceptor discs are renewed daily (18–21), photoreceptors have high demands for the synthesis and transport of opsins and other phototransduction proteins from inner segments (the site of protein synthesis) to OS discs; any perturbation in cilia assembly or function can therefore lead to dysfunction or death of photoreceptors (22–26).

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous progressive neurodegenerative disease that involves retinal photoreceptors (27). X-linked forms of RP (XLRP) are among the most severe and account for 10–15% of inherited retinal degeneration (28–31). Though multiple genetic loci exist, mutations in the RPGR (RP GTPase regulator) gene account for >70% of XLRP and ~20% of

∗To whom correspondence should be addressed at: 381 Plantation St, Biotech 5, Suite 250, Worcester, MA 01605, USA. Tel: +508-856-8991; Fax: +508-856-1552; Email: hemant.khanna@umassmed.edu
†Present address: Department of Ophthalmology, University of Massachusetts Medical School, Worcester, MA 01605.

© The Author 2010. Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.5), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
RP cases with no family history (30,32). XLRP patients with RPGR mutations show extensive phenotypic variations, with some exhibiting even atrophic macular degeneration, respiratory tract infections and primary cilia dyskinesia (33–37). Multiple isoforms of RPGR are expressed in the retina (38,39). The two major isoforms are RPGR1–19 (exons 1–19) and RPGRORF15 (exons 1–15 + a part of intron 15 ORF15). Mutations in ORF15 isoform are found for 60–80% of XLRP (30,32,40).

RPGR is predominantly a cilia-centrosomal protein (41–43) that interacts with many ciliary proteins, such as RPGRIP1, IQCB1 (NPHP5), CEP290 (NPHP6) and RPGRIP1L (NPHP8) (22,44–46). Mutations in proteins that cause syndromic ciliopathies with retinal degeneration are shown to alter their interaction with RPGR (22,45). Though RPGR is suggested to regulate cilia function and facilitate the trafficking of proteins along the photoreceptor cilium (42,47–49), how its altered localization and/or function lead to photoreceptor degeneration has not been elucidated.

RPGR isoforms share a common N-terminal domain, called RLD (RCC1-like domain), which is homologous to regulator of chromosome condensation (RCC1) (50,51). RCC1 is a guanine nucleotide exchange factor (GEF) for Ran GTPase and regulates nucleocytoplasmic trafficking (52). It contains seven-bladed propeller repeats, a structural motif also found in p532, an exchange factor for RAB and ADP-ribosylation factor (ARF) proteins (53). The RLD of RPGR is shown to interact with delta subunit of rod cyclic cGMP phosphodiesterase in vitro (54), which in turn associates with ARF-like 3 GTPase (55). Based on these observations, RPGR is proposed to have a guanine nucleotide exchange function.

Recent studies have revealed an important role for the small GTPase RAB8A in cilia assembly and function (56). Moreover, RAB8A regulates the trafficking of rhodopsin in photoreceptors (57). We therefore hypothesized that RPGR functions as a GEF for RAB8A and RPGR–RAB8A association may facilitate ciliary trafficking. Here, we demonstrate a physiologically relevant interaction of RPGR with RAB8A and suggest a possible mechanism of photoreceptor degeneration associated with RPGR mutations.

**RESULTS**

**RPGR interacts with RAB8A**

To test whether RPGR associates with RAB8A in mammalian retina, we first performed co-immunoprecipitation (co-IP) experiments using bovine retinal extract. We observed that either anti-RAB8A antibody or anti-RPGR antibody could specifically pull-down RPGR isoforms or RAB8A, respectively, from the retinal extracts. We also detected higher association of RPGR–RAB8A association in the presence of GDP (Fig. 1A). No association was detected when normal immunoglobulin (IgG) or pre-immune serum were used for IP. We then used RPGR1–15, which spans the RLD, as a bait and RAB8A as a prey in yeast two-hybrid analysis and found that RPGR1–15 could directly interact with RAB8A (Fig. 1B). As a negative control, Laminin and T-antigen did not show an interaction in this assay. To further examine the physical interaction of RPGR and RAB8A in vitro, we used purified GST-RPGR1–15 (Fig. 1C) and 35S-labeled wild-type (WT) and mutant RAB8A proteins. RPGR preferentially associated with the GDP-locked form of RAB8A (T22N mutant) (Fig. 1D), and lesser interaction was detected with RAB8A-GTP (Q67L mutant) or WT RAB8A. No association was observed with GDP- or GTP-locked mutants of RAB11, another GTPase involved in intracellular trafficking (Fig. 1E). Using ciliated Madin Darby canine kidney (MDCK) cells, we found that endogenous RPGR and RAB8A co-localized at the primary cilia of epithelial cells (Fig. 1F).

**RPGR mutations alter its interaction with RAB8A**

We then investigated the effect of disease-causing mutations in RPGR-RLD on its interaction with RAB8A. In a GST pull-down assay, the RPGR-H98Q mutant protein exhibited almost 5-fold less association with 35S-RAB8A-T22N compared with WT-RPGR. The G60V, G173R, T99N, F130C and G215V mutations in RPGR did not seem to affect the interaction with RAB8A (Fig. 2A and B).

**RPGR can function as GEF for RAB8A**

We then tested whether RPGR can potentiate the GTPase activity of RAB8A. Using a previously described fluorescence-based exchange assay (58,59), we measured the effect of RPGR on the degree of incorporation of fluorescent GTP into RAB8A. As a control, we utilized Rab8, which is an established GEF for RAB8A (60). Consistent with previous observations, the intrinsic activity of RAB8A (as observed by the amount of bound fluorescent analog of GTP [N-methylanthraniloyl]-GTP (MANT-GTP)] was low (Fig. 3A); however, a 5-fold increase was observed in bound GTP in the presence of purified RPGR (Fig. 3A; Table 1), as was the case when RPGR was replaced by Rab8. In another set of control experiments, RPGR did not show a significant GEF activity for CDC42 GTPase, whereas DBS (an established GEF for CDC42) exhibited ~22-fold exchange activity (Table 2).

**Disease-causing mutations in RPGR affect its GEF function**

Consistent with its diminished association with RAB8A, we found that RPGR-H98Q mutation exhibits ~30% decrease in the GEF activity for RAB8A compared with WT-RPGR (Fig. 3B). The RPGR-F130C mutant exhibited ~50% decrease in GEF activity though it did not display a significant decrease in its interaction with RAB8A (Fig. 2B). As RPGR-F130C mutation is reported to abolish its interaction with RPGRIP1 (61), our results indicate that, in photoreceptors, the conformation of RPGR or its interaction with RPGRIP1 may also modulate RPGR’s RAB8A-GEF activity.

**Knockdown of RPGR in hTERT-RPE1 cells results in shorter cilium and mislocalization of RAB8A**

To further evaluate the role of RPGR in regulating RAB8A, we performed shRNA-mediated suppression of RPGR in...
Figure 1. Interaction of RPGR with RAB8A. (A) Bovine retinal extract was prepared in the presence of the non-hydrolyzable GDP substrate and subjected to immunoprecipitation (IP) using anti-RAB8A, anti-RPGR antibodies or normal IgG and pre-immune (Pre-Imm) serum. Twenty percent of the protein used for IP was loaded in input lane. Vertical line represents different RPGR isoforms expressed in bovine retina, as described earlier (38,42). (B) A known-bait and known-prey yeast two-hybrid analysis. Upper panel represents the growth of yeast transformed with bait (RPGR1–15) and prey (RAB8A) constructs. Interaction between p53 (bait) and T antigen (T-Ag; prey) was used as a positive control, whereas laminin (LAM) and T-Ag association served as a negative control in this experiment. The lower panel represents β-galactosidase (blue) test of the growth assay shown in the upper panel. (C) Coomassie blue staining of the gel showing the purified WT GST-RPGR1–15 or GST proteins utilized in GST pulldown assay. Molecular mass markers are in kilo Daltons (kDa). (D and E) GST pulldown assay: 10 μg of purified GST-RPGR1–15 fusion protein or GST alone was incubated with 35S-labeled in vitro-translated RAB8A (C) or RAB11 (D) (or with indicated mutants). The proteins were collected by binding to Glutathione-Sepharose™ beads and analyzed by SDS–PAGE followed by autoradiography. Lanes are indicated. (F) Ciliated MDCK cells were stained with antibodies to RPGR (purple) and RAB8A (green). Immunostained cells were analyzed with OLYMPUS FV 500. White arrow in 'Merge' image shows co-localization of RPGR and RAB8A at the cilium. Nuclei (N) are stained with Hoechst (blue).
hTERT-RPE1 cells. Transfection of RPGR-shRNA resulted in 
\(~80\%\) reduction of the endogenous RPGR protein compared 
with cells transfected with a scrambled shRNA construct 
(Fig. 4A). The RPGR isoforms at \(~200\) kDa were partially 
deleated, whereas 110 kDa isofrom was undetectable in 
RPGR-shRNA-expressing cells. Interestingly, RPGR-
shRNA-treated cells did have primary cilia, though in 65–
70\% of the cells the cilium was shorter (1.5–3.9 
\(\mu\)m) (Fig. 4B) than in the control cells (expressing scrambled 
shRNA) (Fig. 4C and D). These observations are consistent 
with our recent study showing shorter cilia in Kupffer’s ves-
icles upon morpholino-mediated knockdown of \(rpgr\) in zebra-
fish embryos (49).

As appropriate localization of RAB8A is required for cilia 
biogenesis and elongation, we examined whether depletion 
of RPGR might affect RAB8A localization. We observed 
that RAB8A is not localized at the cilium in 90\% of

---

**Table 1. Activity of RPGR and Rabin8 over RAB8A**

|                  | RAB8A intrinsic | RAB8A + Rabin8 | RAB8A + RPGR |
|------------------|-----------------|---------------|--------------|
| Exchange activity| 0.66            | 4.41          | 3.51         |
| Fold change      | 6.64            | 5.31          |              |

Relative fluorescence recording of incorporated MANT-GTP (\(\lambda_{ex}=360\) nm 
and \(\lambda_{em}=440\) nm) onto His-RAB8A, when incubated with RPGR or RABIN8. 
Intrinsic rate was calculated as the MANT-GTP incorporation into RAB8A 
without the incubation of the GEF. Fold change is calculated as the ratio of 
exchange rate for each GEF over the intrinsic activity of RAB8A as a function 
of time.

**Table 2. Activity of RPGR and DBS over CDC42**

|                  | CDC42 intrinsic | CDC42 + RPGR | CDC42 + DBS |
|------------------|-----------------|--------------|-------------|
| Exchange activity| 149.62          | 192.757      | 2580        |
| Fold change      | 0.8             | 22.5         | 22.5        |

Relative fluorescence recording of incorporated MANT-GTP (\(\lambda_{ex}=360\) nm 
and \(\lambda_{em}=440\) nm) onto CDC42 incubated with RPGR or hDBS, a Dbl-family 
GEF for CDC42. Fold change is calculated as the ratio of exchange rate for 
each GEF over the intrinsic activity of RAB8A as a function of time.
Figure 4. RPGR regulates ciliary localization of RAB8A. (A) Generation of RPGR-knockdown cells: lysates from stable transfected hTERT-RPE1 cells with control (scrambled) shRNA or RPGR shRNA were analyzed using anti-RPGR antibody or actin. Arrows represent distinct RPGR isoforms. (B) This panel represents cilia (red; acetylated α-tubulin) formation in control and RPGR-shRNA cells. Nuclei (blue) are stained with Hoechst. (C) Histograms show the distribution of cilia length in RPGR-shRNA-expressing cells. (D) There is increase in the number of cells with shorter cilia (1.5–3.9 μm) when treated with RPGR-shRNA compared with controls. Distribution of cilia length was calculated from three independent experiments with at least 150 cells analyzed in each experiment. Error bars represent standard deviation. (E) Knockdown of RPGR interferes with ciliary localization of RAB8A (green) to primary cilia (acetylated α-tubulin; red). Yellow in the Merge image indicates RAB8A localization at primary cilia. (F) Histogram showing decrease in number of cells with RAB8A at the cilium. We analyzed a total of 180 cells in each group and the experiment was repeated three times. (G) Knockdown of RPGR does not affect the localization of IFT88/Polaris (purple; left panel) to the cilium (red; Ac. α-tubulin; left panel) or of CEP290 (purple; right panel) to basal bodies (red; γ-tubulin; right panel).
RPGR-shRNA-treated cells compared with scrambled-shRNA-treated cells (Fig. 4E and F) even though RAB8A protein levels were unaltered. RPGR-shRNA treatment did not significantly affect the localization of other RPGR-interacting ciliary and centrosomal proteins tested (CEP290, IFT88/Polaris, PCM1 and Pericentrin) (Fig. 4G; data not shown).

**DISCUSSION**

Ciliary dysfunction and disruption in trafficking of proteins are associated with early-onset severe retinal degeneration and blindness (62). RPGR is frequently involved in the manifestation of inherited severe photoreceptor degeneration. Although RPGR is primarily a ciliary protein and interacts with several ciliary and microtubule-based assemblies (41,42,45), its function and the mechanism of associated pathogenesis are still unclear. Here, we show that RPGR interacts with and regulates the activity of RAB8A, which is a critical GTPase involved in photoreceptor protein trafficking (57). Our results suggest that RPGR facilitates transport of proteins likely by promoting appropriate localization and activity of RAB8A.

RAB8A is involved in polarized trafficking of vesicles from the trans-Golgi network (63–67), and is the only RAB-family protein identified in mammalian cilia (68,69). Recent studies show that macromolecular complexes formed by selected BBS proteins (BBSome) regulate ciliogenesis by mediating the localization of Rabin 8 (56). Disruption of the BBSome compromises RAB8A function and cilia formation. As retinal degeneration is a principal feature of BBS, defects in RAB8A localization or activity may also affect photoreceptor protein trafficking. Given high trafficking demands of photoreceptors due to periodic OS disc shedding and renewal, activation of RAB8A may require RPGR GEF activity in addition to Rabin8. RPGR may therefore play a critical role in promting RAB8A function in photoreceptors. Consistent with this hypothesis, mutations in RPGR that alter its association with RAB8A are predominantly associated with photoreceptor dysfunction and/or degeneration.

Several reports have demonstrated the involvement of extrinsic signals in dynamic regulation of cilia length in mammalian cells (70). Cilia extension has also been characterized in *Chlamydomonas* (71,72) and is believed to be associated with human disease (4,73). It is proposed that the IFT complex, composed of microtubule-based motor assemblies and cargo proteins, are involved in regulating cilia extension. As RPGR associates with IFT88 and the Kinesin-II subunit KIF3A (42) and IFT88 interacts with opsin and other cargo in photoreceptors (74), an interesting hypothesis that remains to be tested is whether RPGR can facilitate protein interactions between cargo and IFT proteins during photoreceptor OS disc renewal. What might be the cargo for RPGR–RAB8A complex(es) in photoreceptors? As RAB8A regulates rhodopsin trafficking in photoreceptors, we tested but did not detect an association of RPGR with rhodopsin in bovine retinal protein complexes (data not shown). We cannot exclude the possibility of dynamic or transient nature of such interactions. It is also possible that opsin is not a cargo protein for RPGR-mediated ciliary transport.

The RPGR H98Q and F130C mutants exhibit compromised interaction with RPGRIP1 (61), suggesting that RPGR–RPGRIP1 interaction may be an important determinant of RPGR activity. As RPGRIP1 appears to link RPGR to the cilium (75), it is possible that RPGR’s effect on RAB8A function may occur in distinct subcellular compartments of photoreceptors and that RPGRIP1 and other interacting proteins may modulate targeting of RPR to such compartments. An indirect support for this hypothesis comes from CEP290, another RPGR-interacting protein (22) that also regulates cilia biogenesis and RAB8A localization (76). However, depletion of RPGR does not alter the localization of CEP290. Hence, there seems to be interplay between discrete protein complexes that function at distinct pathways in ciliary trafficking pathways, which culminate in docking and transport of proteins along the photoreceptor cilium. Identification of such dynamic protein complexes and their coordinated mechanism of action should contribute to better understanding of the dynamic nature of ciliary function in sensory neurons as well as pathomechanism of associated disorders.

**MATERIALS AND METHODS**

**Antibodies and constructs**

Generation and characterization of anti-RPGR<sup>ORF15</sup>, CEP290 and anti-RAB8A antibodies have been described (22,42,60). The γ-tubulin and acetylated α-tubulin antibodies were purchased from Sigma, and pericentrin antibody from Abcam. PCR-based site-directed mutagenesis was performed using QuikChange kit from Stratagene.

**Cell culture, transfection and microscopy**

hTERT-RPE1 and MDCK cells were cultured according to American Type Culture Collection. Cilia growth and staining were performed as described (42). Cells were imaged on Olympus FV-500 or LEICA SP5 confocal microscope. To evaluate cilia length, hTERT-RPE1 cells stably expressing specific RPGR shRNA or scrambled shRNA (as control) were grown to confluence for 24 h followed by serum deprivation. Cells were then fixed with 4% PFA and cilia growth was analyzed by immunostaining with acetylated α-tubulin from (0.25 μm step size) z-stacks taken with LEICA SP6 confocal microscope. Cilia length was measured with LAS AF Lite software.

**Protein–protein interaction**

The protocols for yeast two-hybrid, GST pulldown and co-IP analyses have been described (42,45).

**RAB8A activation assay**

RhoGEF Exchange Assay Biochem kit (Cytoskeleton Inc., Denver, CO, USA) (58,59) was used according to manufacturer’s instructions. The rate of guanine nucleotide exchange was recorded as the increase in *N*-methylanthraniloyl-GTP (MANT-GTP) fluorescence intensity incorporated into the GTPase. Then 0.5 μM RAB8A, 0.5 μM RPGR and 0.32 μM RABIN8 were incubated with the exchange buffer.
Fluorescence intensity was recorded in Flex Station II Fluorometer (Molecular Devices, Sunnydale, CA, USA) at \( \lambda_{\text{ex}} = 360 \text{ nm} \) and \( \lambda_{\text{em}} = 440 \text{ nm} \), for 30 min at 27°C. RAB8A and Rabin 8 were purified from *Escherichia coli*, as described (60).

**Knockdown of RPGR in cells**

Plasmids encoding shRNA against human RPGR (exon 2) were purchased from OpenBiosystems (TGCTGTGACGTT GACGACCCATTCCGCTGTGTTTATAGTAAGGCC ACAGATGTATAAACACAGCACCAGATCGGTGCTA CTGCGCTCGGA). The shRNA plasmids were packaged into lentiviral particles and transfected into hTERT-RPE1 cells using standard protocols. Knockdown of RPGR expression was tested by immunoblotting.

**ACKNOWLEDGEMENTS**

We thank Toby Hurd for valuable discussions.

**Conflict of Interest statement.** None declared.

**FUNDING**

This work is supported by NEI intramural funds and grants from the National Institutes of Health (RO1-EY007961), Midwest Eye Banks and Transplantation Center, Rare Disease Initiative at the University of Michigan and The Foundation Fighting Blindness. This work also utilized the services of Michigan Diabetes Research and Training Center (NIH5P60 DK20572) and Vision Core Facilities (EY07003). Funding to pay the Open Access Charge was provided by Midwest Eye Banks and Transplantation Center.

**REFERENCES**

1. Scholey, J.M. and Anderson, K.V. (2006) Intraflagellar transport and cilium-based signaling. *Cell*, 125, 439–442.
2. Singla, V. and Reiter, J.F. (2006) The primary cilium as the cell’s antenna: signaling at a sensory organelle. *Science*, 313, 629–633.
3. Dawe, H.R., Farr, H. and Gull, K. (2007) Centriole/basal body morphogenesis and migration during ciliogenesis in animal cells. *J. Cell. Sci.*, 120, 7–15.
4. Gerdes, J.M., Davis, E.E. and Katsanis, N. (2009) The vertebrate primary cilium in development, homeostasis, and disease. *Cell*, 137, 32–45.
5. Lancaster, M.A. and Gleeson, J.G. (2009) The primary cilium as a cellular signaling center: lessons from disease. *Curr. Opin. Genet. Dev.*, 19, 220–229.
6. Yoder, B.K. (2006) More than just the postal service: novel roles for IFT proteins in signal transduction. *Dev. Cell.*, 10, 541–542.
7. Dossey, S. (2001) Re-evaluating centrosome function. *Nat. Rev. Mol. Cell. Biol.*, 2, 688–698.
8. Nigg, E.A. and Raft, J.W. (2009) Centrioles, centrosomes, and cilia in health and disease. *Cell*, 139, 663–678.
9. Sorokin, S. (1962) Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell. Biol.*, 15, 363–377.
10. Pazour, G.J. and Witman, G.B. (2003) The vertebrate primary cilium is a sensory organelle. *Curr. Opin. Cell. Biol.*, 15, 105–110.
11. Rosenbaum, J. (2002) Intraflagellar transport. *Curr. Biol.*, 12, R125.
12. Badano, J.L., Mitsuma, N., Beales, P.L. and Katsanis, N. (2006) The ciliopathies: an emerging class of human genetic disorders. *Annu. Rev. Genomics Hum. Genet.*, 7, 125–148.
13. Hildebrandt, F., Attanasio, M. and Otto, E. (2009) Nephronphthisis: disease mechanisms of a ciliopathy. *J. Am. Soc. Nephrol.*, 20, 23–35.
14. Myktykin, K. and Sheffield, V.C. (2004) Establishing a connection between cilia and Bardet–Biedl Syndrome. *Trends Mol. Med.*, 10, 106–109.
15. Besharse, J.C. (1986) The Retina: A Model for Cell Biological Studies Part I. Academic, New York.
16. Besharse, J.C., Baker, S.A., Luby-Phelps, K. and Pazour, G.J. (2003) Photoreceptor intersegmental transport and retinal degeneration: a conserved pathway common to motile and sensory cilia. *Adv. Exp. Med. Biol.*, 533, 157–164.
17. Kennedy, B. and Malicki, J. (2009) What drives cell morphogenesis: a look inside the vertebrate photoreceptor. *Dev. Dyn.*, 238, 2115–2138.
18. Besharse, J.C. and Hollyfield, J.G. (1976) Renewal of normal and degenerating photoreceptor outer segments in the Ozark cave salmonander. *Exp. Zool.*, 198, 287–302.
19. Besharse, J.C., Hollyfield, J.G. and Rayborn, M.E. (1977) Photoreceptor outer segments: accelerated membrane renewal in rods after exposure to light. *Science*, 196, 536–538.
20. LaVail, M.M. (1973) Kinetics of rod outer segment renewal in the developing mouse retina. *J. Cell. Biol.*, 58, 650–661.
21. Young, R.W. and Droz, B. (1968) The renewal of protein in rod retina and cones. *J. Cell. Biol.*, 39, 169–184.
22. Chang, B., Khana, H., Hawes, N., Jimeno, D., He, S., Lillo, L., Paraparam, S.K., Cheng, H., Scott, A., Hurd, R.E. et al. (2006) In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RPGR and results in early-onset retinal degeneration in the rd16 mouse. *Hum. Mol. Genet.*, 15, 1847–1857.
23. Deretic, D., Williams, A.H., Ransom, N., Morel, V., Hargrave, P.A. and Arendt, A. (2005) Rhodopsin C terminus, the site of mutations causing retinal disease, regulates trafficking by binding to ADP-ribosylation factor 4 (ARF4). *Proc. Natl Acad. Sci. USA*, 102, 3301–3306.
24. Krock, B.L. and Perkins, B.D. (2008) The intraflagellar transport protein IFT57 is required for cilia maintenance and regulates IFT-particle-kinases-II dissociation in vertebrate photoreceptors. *J. Cell. Sci.*, 121, 1907–1915.
25. Marszalek, J.R., Liu, X., Roberts, E.A., Chui, D., Marti, J.D., Williams, D.S. and Goldstein, L.S. (2000) Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors. *Cell*, 102, 175–187.
26. Pazour, G.J., Baker, S.A., Deane, J.A., Cole, D.G., Dickert, B.L., Rosenbaum, J.L., Witman, G.B. and Besharse, J.C. (2002) The intraflagellar transport protein transport protein, IFT88, is essential for vertebrate photoreceptor assembly and maintenance. *J. Cell. Biol.*, 157, 103–113.
27. Heckenlively, J.R., Yoser, S.L., Friedman, L.H. and Oversier, J.J. (1988) Clinical findings and common symptoms in retinitis pigmentosa. *Am. J. Ophthalmol.*, 105, 504–511.
28. Bird, A.C. (1975) X-linked retinitis pigmentosa. *Br. J. Ophthalmol.*, 59, 177–199.
29. Fishman, G.A. (1978) Retinitis pigmentosa. Genetic percentages. *Arch. Ophthalmol.*, 96, 822–826.
30. Shu, X., Black, G.C., Rice, J.M., Hart-Holden, N., Jones, A., O’Grady, A., Ramsden, S. and Wright, A.F. (2007) RPGR mutation analysis and disease: an update. *Hum. Mutat.*, 28, 322–328.
31. Fishman, G.A., Farber, M.D. and Derlacki, D.J. (1988) X-linked retinitis pigmentosa. Profile of clinical findings. *Arch. Ophthalmol.*, 106, 369–375.
32. Breuer, D.K., Yashar, B.M., Filippova, E., Hiriyanaw, S., Lyons, R.H., Mears, A.J., Asaye, B., Acar, C., Vervoort, R., Wright, A.F. et al. (2002) A comprehensive mutation analysis of RP2 and RPGR in a North American cohort of families with X-linked retinitis pigmentosa. *Am. J. Hum. Genet.*, 70, 1545–1554.
33. Iannaccone, A., Breuer, D.K., Wang, X.F., Kuo, S.F., Normando, E.M., Filippova, E., Baldis, A., Hiriyanaw, S., MacDonald, C.B., Baldi, F. et al. (2003) Clinical and immunohistochemical evidence for an X linked retinitis pigmentosa syndrome with recurrent infections and hearing loss in association with an RPGR mutation. *J. Med. Genet.*, 40, e118.
34. Moore, A., Escudier, E., Roger, G., Tamalet, A., Pelosse, B., Marlin, S., Clement, A., Geremek, M., Delaisi, B., Bridoux, A.M. et al. (2006) RPGR is mutated in patients with a complex X linked phenotype combining primary ciliary dyskinesia and retinitis pigmentosa. *J. Med. Genet.*, 43, 326–332.
35. van Dorp, D.B., Wright, A.F., Carothers, A.D. and Bleeker-Wagemakers, E.M. (1992) A family with RP3 type of X-linked retinitis pigmentosa: an association with ciliary abnormalities. *Hum. Genet.*, 88, 331–334.
36. Ayayagi, R., Demirci, F.Y., Liu, J., Bingham, E.L., Stringham, H., Kakuk, L.E., Boehnke, M., Gorin, M.B., Richards, J.E. and Steving, P.A. (2002) X-linked recessive atrophic macular degeneration from RPGR mutation. *Genomics*, 80, 166–171.
50. Meindl, A., Dry, K., Herrmann, K., Manson, F., Ciccodicola, A., Edgar, A., Arl, H., Loeys, B., Khanna, H., Hellemans, J., Sudbrak, R., Fan, S., Muerb, H., Hong, D.H., Yue, G., Adamian, M. and Li, T. (2001) Retinitis pigmentosa GTPase regulator (RPGR) protein isoforms in mammalian retina: insights into X-linked retinitis pigmentosa and associated ciliopathies. *Vision Res.*, 41, 366–376.

51. Linari, M., Ueffing, M., Manson, F., Wright, A., Meitinger, T. and Becker, A. (2000) A retinitis pigmentosa GTPase regulator (RPGR)-deficient protein isoform that is disrupted in a patient with X-linked retinitis pigmentosa. *Hum. Mol. Genet.*, 9, 1571–1578.

52. Renault, L., Kuhlmann, J., Henkel, A. and Wittinghofer, A. (2001) Structural basis for guanine nucleotide exchange on Ran by the regulator RCC1. *Nature*, 410, 377–400.

53. Meindl, A., Dry, K., Herrmann, K., Manson, F., Ciccodicola, A., Wright, A.F. and Li, T. (2003) RPGR isoforms in mammalian retina: insights into X-linked retinitis pigmentosa. *Human Molecular Genetics*, 20(18), 3580–3587.

54. Akimoto, M., Wright, A.F., Margolis, B., Williams, D.S. and Li, T. (2000) A retinitis pigmentosa GTPase regulator (RPGR)-deficient protein isoform that is disrupted in a patient with X-linked retinitis pigmentosa 3: homology with the guanine-nucleotide-exchange factor RCC1. *Nature*, 409, 993–1003.

55. Linari, M., Hanzal-Bayer, M. and Becker, J. (1999) The delta subunit of rod specific cyclic GMP phosphodiesterase, PDE delta, interacts with the Arf-like protein Arfl in a GTP specific manner. *FEBS Lett.*, 458, 55–59.

56. Akimoto, M., Wright, A.F., Margolis, B., Williams, D.S. and Li, T. (2000) A retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. *Hum. Mol. Genet.*, 9, 2095–2105.

57. Engel, B.D., Ludington, W.B. and Marshall, W.F. (2001) Intraflagellar transport particle size scales inversely with flagellar length: revisiting the balance-point length control model. *J. Cell Biol.*, 150, 81–89.

58. Satir, P. and Christensen, S.T. (2007) Overview of structure and function of mammalian cilium. *Ann. Rev. Physiol.*, 69, 377–400.

59. Bhowmick, R., Li, M., Sun, J., Baker, S.A., Insinna, C. and Besharse, J.C. (2005) The Rab8 GTPase regulates apical protein localization in intestinal cells. *Nature*, 448, 366–369.

60. Besschetnova, T.Y., Kolpakova-Hart, E., Guan, Y., Zhou, J., Olsen, B.R. and Simons, K. (2007) The Rab8 GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. *Hum. Mol. Genet.*, 9, 3580–3587.

61. Li, S., Wang, Q., Wang, Y., Chen, X. and Wang, Z. (2009) PLC-gammal and Rac1 regulate EGF-induced cytokeratin remodeling and cell migration. *Mol. Endocrinol.*, 23, 901–913.

62. Bock, J.B., Matern, H.T., Peden, A.A. and Scheller, R.H. (2001) A genomic perspective on membrane compartment organization. *Nature*, 409, 839–841.

63. Engel, B.D., Ludington, W.B. and Marshall, W.F. (2009) Intraflagellar transport particle size scales inversely with flagellar length: revisiting the balance-point length control model. *J. Cell Biol.*, 187, 81–89.

64. Bleeker-Wagemakers, L.M., Bergen, A.A., Post, J., Beck, A., Reinhardt, R., Ropers, H.H. and Berger, W. (1999) Positional cloning of the gene for X-linked retinitis pigmentosa 3: homology with the guanine-nucleotide-exchange factor RCC1. *Hum. Mol. Genet.*, 8, 1035–1041.

65. Renaud, L., Kuhlmann, J., Henkel, A. and Wittinghofer, A. (2001) Structural basis for guanine nucleotide exchange on Ran by the regulator of chromosome condensation (RCC1). *Cell*, 105, 245–255.

66. Rosa, J.L. and Barbadic, M. (1997) A giant protein that stimulates guanine nucleotide exchange on ARF1 and Rab proteins forms a cytosolic ternary complex with clathrin and Hsp70. *Oncogene*, 15, 1–6.

67. Linari, M., Ueffing, M., Manson, F., Wright, A., Meitinger, T. and Becker, J. (1999) The delta subunit of rod specific cyclic GMP phosphodiesterase, PDE delta, interacts with the Arf-like protein Arfl in a GTP specific manner. *FEBS Lett.*, 458, 55–59.

68. Besschetnova, T.Y., Kolpakova-Hart, E., Guan, Y., Zhou, J., Olsen, B.R. and Shah, J.V. Identification of signaling pathways regulating primary cilium length and flow-mediated adaptation. *Curr. Biol.*, 20, 182–187.

69. Rosenbaum, J.L., Moulder, J.E. and Ringo, D.L. (1969) Flagellar elongation and shortening in Chlamydomonas. The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. *J. Cell Biol.*, 41, 600–619.

70. Cesare, C., Khanna, H., Peranen, J., Saurao, A., Malhotra, V. and Dynchak, D.C. (2008) A Rab8 GTPase regulates primary cilia formation through its interaction with CEP290, a protein deficient in human ciliary disease. *Dev. Cell.*, 15, 187–197.