CHAPTER 10

PROTEIN NETWORKS AND COMPLEXES IN PHOTORECEPTOR CILIA

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Table of Contents

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Abstract: Vertebrate photoreceptor cells are ciliated sensory cells specialized for single photon detection. The photoreceptor outer segment corresponds to the ciliary shaft of a prototypic cilium. In the outer segment compartment, the ciliary membrane is highly modified into membranous disks which are enveloped by the plasma membrane in rod cells. At these outer segment disks, the visual transduction cascade – a prototypical G-protein coupled receptor transduction pathway is arranged. The light sensitive outer segments are linked by the socalled connecting cilium with the inner segment, the photoreceptor compartment which contains all organelles necessary for cell metabolism. The connecting cilium correlates with the transition zone, the short junction between the basal body and the axoneme of a prototypic cilium. The connecting cilium and the calycal processes, including the periciliary ridge complex, as well as the basal body complex are in close functional association with each other. In the latter ciliary compartments, the export and import from/into the outer segment of the photoreceptor cell are controlled and regulated. In all subciliary compartments, proteins are arranged in functional multiprotein complexes. In the outer segment, signaling components are arranged into complexes which provide specificity and speed for the signaling and serve in adaptation. Centrin-G-protein complexes may regulate the light driven translocation of the visual G-protein transducin through the connecting cilium. Intraflagellar transport (IFT) complexes may serve in intersegmental exchange of molecules. The import/export of molecules is thought to be regulated by proteins arranged in networks at the basal body complex. Proteins of the interactome related to the human Usher syndrome are localized in the connecting cilium and may participate in the ciliary transport, but are also arranged at interfaces between the inner segment and the connecting cilium where they probably control the cargo handover between the transport systems of the inner segment and these of the cilium. Furthermore, USH protein complexes may further provide mechanical stabilization to membrane specializations of the calycal processes and the connecting cilium. The protein complex in which the retinitis pigmentosa GTPase regulator (RPGR) participates in the ciliary compartments also plays a key role in the function and maintenance of photoreceptor cells. It further associates through the presumed scaffolding protein RPGRIP1 with the nephrocystin protein network. Although many of these proteins have been also found in prototypic cilia or primary cilia, the arrangements of the proteins in complexes can be specific for vertebrate photoreceptor cells. Defects of proteins in these complexes lead to photoreceptor cell death and retinal degeneration, underlying syndromic and non-syndromic blindness.

1. INTRODUCTION

The visual process is initiated by the detection of a light signal by photoreceptor cells in the outer retina of the vertebrate eye. Photoreceptor cells absorb light (photons) and convert it into an electric neuronal signal. Vertebrate cone and rod photoreceptor cells are highly specialized, polarized neurons, which consist of morphologically and functionally distinct cellular compartments (Figure 1). The light sensitive photoreceptor outer segment is linked with an inner segment by a small intracellular bridge, the so-called connecting cilium, through which all intracellular intersegmental exchanges occur (Besharse and Horst 1990). The inner segment compartment contains all organelles typical for the metabolism of a eukaryotic cell. It continues into the perikaryon and the synaptic region where electrical signals are transmitted from photoreceptor cells to horizontal and bipolar cells of the inner neuronal retina.

In both types of photoreceptor cells, the outer segment contains all components of the visual transduction cascade which is one of the best studied examples of

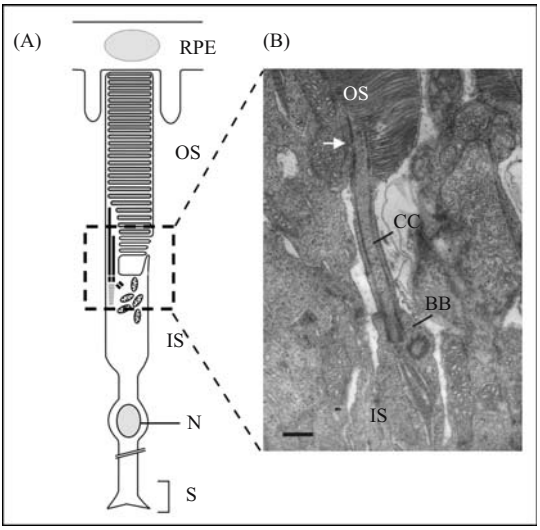

Figure 1. Structure of a ciliated photoreceptor cell in vertebrates. (A) Scheme of a rod photoreceptor cell. (B) Transmission electronmicroscopy image of a part of a mouse rod photoreceptor cell. The apical extensions of cells of the retinal pigment epithelium (RPE) evolve the tips of photoreceptors light-sensitive outer segments (OS). The OS is linked via a connecting cilium (CC) to an inner segment (IS) which bears the basal body complex (BB) in its apical region. Synaptic terminals (S) link the photoreceptor cell and the 2nd-order neurons, bipolar and horizontal cells. N = nucleus; in B, arrow point to axonemal microtubules projecting into the OS. Bar in B = 0.2 μm

G-protein mediated signal transduction cascades (Pugh and Lamb 2000; Okada et al. 2001). In rods, the cascade is arranged separate from the plasma membrane at hundreds of stacked membrane disks. Photoexcitation of the visual pigment rhodopsin (Rh*) activates a heterotrimeric G-protein (the visual G-protein transducin, composed of an α-subunit bearing the guanine nucleotide binding site and an undissociable βγ-complex) cascade, leading to cyclic GMP (cGMP) hydrolysis in the cytoplasm and closing of cGMP-gated (CNG) channels in the plasma membrane (see details reviewed in: Molday and Kaupp 2000; Kaupp and Seifert, 2002; Arshavsky et al. 2002). For termination of the visual cascade, Rh* is phosphorylated by the rhodopsin kinase which allows subsequent binding of arrestin molecules to P-Rh* inhibiting further R*-transducin interaction. CNG channels-closing leads to a decrease of the free Ca^{2+} concentration and an activation of Ca^{2+}-dependent proteins in the outer segment. In turn these proteins activate guanylate cyclases (GC) and lead to a delayed restoration of the cGMP concentration (Nakatani et al. 2002).

The photoreceptor outer segment membranous discs are continually renewed throughout lifetime. Newly synthesized disk membranes are added at the base of the outer segment by the expansion of the plasma membrane (Steinberg et al. 1980) or by incorporation of vesicular structures into nascent disc membranes (Uskura and Obata 1995), whereas disk packages at the distal outer segment tip are phagocytosed by the cells of the retinal pigment epithelium (Young 1967), which is juxtaposed to

the apical rod photoreceptor outer segment (Figure 1A). The abundant membrane turnover of the photoreceptor outer segment implicates an efficient and massive vectorial transport of all disk components from the site of biogenesis, the ER and Golgi apparatus in the photoreceptor inner segment, to the base of the outer segment, the site of disk neogenesis. In addition to these unidirectional constitutive translocations of outer segment molecules, massive light dependent bidirectional movements of visual signal cascade proteins between the inner and outer segment are in the focus of current research (e.g. Pulvermüller et al. 2002; Sokolov et al. 2002; Gießl et al. 2004; Strissel et al. 2006). The massive reciprocal translocation of arrestin and transducin is thought to contribute to the long range light adaptation of rod photoreceptor cells (Sokolov et al. 2002). In any case, the intracellular exchange of molecules between the inner segment and the outer segment is forced to occur through the connecting cilium of the photoreceptor cell.

2. STRUCTURE AND FUNCTION OF THE PHOTORECEPTOR CILIUM AND THEIR RELATION TO THE PROTOTYPIC CILIA

In general, cilia are widespread finger-like cell appendages. The structure of a prototypic motile cilium is characterized as follows: the ciliary shaft originates from a basal body complex in the apical cytoplasm beneath the plasma membrane (Figure 2A).

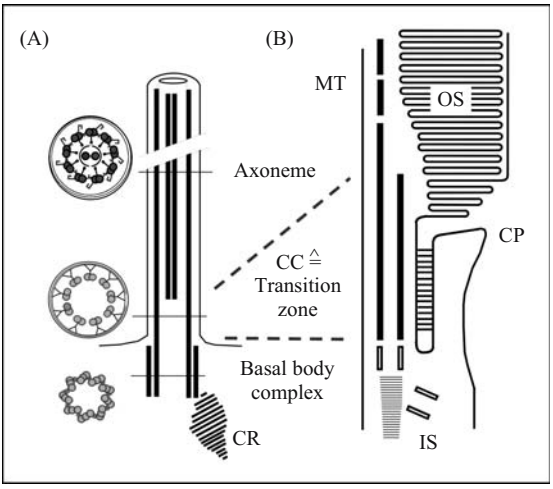

Figure 2. Schematic representations of a prototypic cilium and the photoreceptor cilium in comparison. (A) Scheme of a prototypic cilium, in longitudinal extension and cross sections through subciliary compartments: axoneme ($9 \times 2 + 2$ microtubule arrangement), transition zone ($9 \times 2 + 0$ microtubule arrangement) and centriole ($9 \times 3 + 0$ microtubule arrangement) of the basal body. (B) Scheme of the "ciliary part" of a rod photoreceptor cell. Axonemal microtubules (MT) project into the outer segment (OS). The OS is linked via the connecting cilium (CC) to the inner segment (IS). The CC corresponds to the transition zone of a prototypic cilium. The basal body complex (BB) is localized in the apical region of the IS. The calycal process (CP) of the IS is linked by extracellular fibers with the membrane of the CC.

The centriolar triplet arrangement of microtubules in the basal body is converted in the transition zone ($9 \times 2 + 0$ microtubular array) into the axoneme $9 \times 2 + 2$ (0) microtubular array. Cilia terminate at their tip in an end cap structure where axonemal microtubules are anchored at the apical ciliary membrane. Although, the principle composition of cilia is highly conserved throughout the eukaryote evolution, they show broad diversity in a single multicellular organism. Based on their axonemal microtubule array and their motile attributes, cilia are divided by current definition into four subtypes: (i) motile $9 \times 2 + 2$ cilia (e.g. motile tracheal cilia), (ii) motile $9 \times 2 + 0$ cilia (e.g. monocilia at the embryonic node), (iii) immotile $9 \times 2 + 2$ cilia (e.g. kinocilia in the inner ear), and (iv) immotile $9 \times 2 + 0$ cilia (e.g. renal cilia). Although, we can currently presume that almost all cilia have sensory functions (Singla and Reiter 2006; Scholey and Anderson 2006), animals possess specialized sensory cilia highly tuned for the perception of a single sensory modality, for example vertebrate and invertebrate olfactory cells and the photoreceptor cells of the vertebrate retina. In the latter case, the entire outer segment compartment can be considered as the highly modified distal part of an immotile cilium (Röhlich 1975; Besharse and Horst 1990).

As in prototypic cilia, the arrangement of the ciliary compartments in photoreceptor cells is not only structural but also functional: from the basal body region beneath the highly specialized apical inner segment membrane, long striated ciliary rootlets project through the inner segment into the cell body and can terminate even in the synaptic region terminals (Spira and Milman 1979). Ciliary rootlets are composed of fibers of static rootletin polymers which provide mechanical support for anchoring the basal body complex within the cytoplasm (Yang and Li 2006). The basal body region acts as the major microtubule organizing center (MTOC) in most ciliated cells and vertebrate photoreceptor cells (Troutt et al. 1990; Muresan et al. 1993; Wolfrum and Salisbury 1998). Microtubules nucleate at the MTOC and project with its fast growing plus-end into the cell. But, there is growing evidence that this region also controls the handover of cargos from the minus-end-directed microtubule-based transport through the inner segment transport (or the cell body of ciliated epithelial cells) to the molecular translocation machinery within the cilium (Sung and Tai 2000).

The connecting cilium, often regarded as the photoreceptor cilium, actually correlates with a short part of a prototypic cilium, the so-called transition zone (Röhlich 1975; Besharse and Horst 1990; Schmitt and Wolfrum 2001). In prototypic cilia, the transition zone is the short ciliary segment at the basal body-axoneme junction. It may serve as control gate at which the exchange of ciliary proteins between the cytoplasm and the ciliary compartment is controlled (Fliegauf and Omran 2006). In mammalian photoreceptor cells, the transition zone is extended to the connecting cilium (from $\sim$0.2 μm in a prototypical cilium to $\sim$1 μm in longitudinal extension) and appears to play an important role in photoreceptor organization and function in development and maintenance (Röhlich 1975; Besharse and Horst 1990; Schmitt and Wolfrum 2001). As the transition zone in prototypic cilia, the connecting cilium has a $9 \times 2 + 0$ microtubule configuration and bears a unique transmembrane assemblage: Y-shaped cross-linkers form a stable connection between cell surface glycoconjugates in the ciliary plasma membrane and the underlying microtubule cytoskeleton (Horst et al.

1987, 1990; Besharse and Horst 1990). At the base of the connecting cilium, the apical membrane of the photoreceptor inner segment is specialized as a so-called periciliary ridge complex (Papermaster et al. 1985; Papermaster 2002). Over the extension of the periciliary ridge complex, the inner segment membrane is linked by extracellular fibers with the membrane of the connecting cilium (Figure 2, Besharse and Horst 1990). At this membrane specialization, transport vesicles of the inner segment dock and hand their load (opsin and other outer segment components) over to the ciliary transport machinery (Deretic 2004). Additional specializations of the apical inner segment membrane are calycal processes which are microvilli-like extensions containing a prominent actin cytoskeleton (Pagh-Roehl et al. 1992). In their projection parallel to the outer segment they may support the outer segment against mechanical forces. In photoreceptor cells, the ciliary shaft of a prototypic cilium is extremely modified to the outer segment. In the outer segment of rod cells, thousands of membrane disks are stacked containing the visual signalling cascade (see: Chapter 1 Introduction). The axonemal cytoskeleton of the outer segment looses the stereotypical $9 \times 2 + 0$ arrangement and is reduced to a small number of "axonemal" microtubules which continue from the connecting cilium and project through cytoplasmic compartments of the outer segment, in some species for up to 80% of its length (Kaplan et al. 1987; Liu et al. 2002).

3. PROTEIN COMPLEXES IN PHOTORECEPTOR CILIA AND THEIR FUNCTIONS

Recent proteomic analysis indicated that the cilia of mammalian photoreceptor cells are significantly more complex than other eukaryotic cilium (Liu et al. 2006). Over 1200 different polypeptides have been identified by the quantitative analysis of the proteome of photoreceptor outer segment compared with the proteome of the axoneme/ciliary fraction of mouse photoreceptor cells. This data set contains all previously identified protein components of the photoreceptor cilium (e.g. Schmitt and Wolfrum 2001). An understanding how the identified proteins function in their native environment of the diverse compartments of the photoreceptor cilia will require increased knowledge of their molecular interaction and networking. Insights into the organization and composition of diverse protein complexes may also provide novel information on how functional modules of the cell, recently proposed by Hofmann et al. 2006, are connected.

3.1. Centrin-G-protein Complexes may Regulate Light-dependent G-protein Translocation through the Lumen of the Connecting Cilium

Ca^{2+}-activated centrins form complexes with the visual heterotrimeric G-protein, transducin, in the ciliary apparatus of photoreceptor cells (Pulvermüller et al. 2002; Wolfrum et al. 2002; Gießl et al. 2004a, b, 2006). The visual heterotrimeric G-protein transducin ($G_t$holo) is composed of a un-dissociable $G_t\beta\gamma$-dimer and the $G_t\alpha$-subunit

which acts as the mediator and amplifier of the visual transduction cascade in the outer segment (see above Introduction Chapter 1 and Arshavsky et al. 2002). In rod photoreceptor cells, transducin light dependently shuttles between the inner and the outer segment: in the dark, transducin is localized in the outer segment whereas after light adaptation ~80% of the entire amount of transducin protein is found in the inner segment. This bidirectional intersegmental exchange of transducin through the lumen of connecting cilium is thought to be regulated by the formation of reversal centrin/transducin complexes (Pulvermüller et al. 2002; Wolfrum et al. 2002; Gießl et al. 2004a, b, 2006).

Centrins are members of a highly conserved subfamily of the EF-hand superfamily of Ca^{2+}-binding proteins commonly associated with centrioles of centrosome-related structures (Salisbury 1995; Schiebel and Bornens 1995; Wolfrum et al. 2002; Gießl et al. 2004b). In photoreceptor cells, centrins are also prominent components of the ciliary apparatus where the four centrin isoforms are differentially localized at the basal body and in the lumen of the connecting cilium (Gießl et al. 2004a, 2006). Centrin isoforms 1, 2 and 3 are localized in the lumen of the connecting cilium, centrin isoforms 2 and 3 are also present at the centrioles of the basal body complex whereas centrin 4 is restricted to the basal body. Mammalian centrins are activated by binding of two Ca^{2+} ions to EF-hands III and IV located in the C-terminal half of the molecules (Thompson et al. 2006; Park et al. 2006). In contrast, the two EF-hands in the N-terminal half do not bind, or bind Ca^{2+} ions with significant lower affinity (Park et al. 2006). N-terminus is the most diverse region among mammalian centrins and mediates protein-protein interactions for self assembly or for binding of partner proteins (Park et al 2006; Yang et al. 2006). C-terminal Ca^{2+}-binding probably induces conformational changes in the N-terminus of centrins necessary for oligomerization and protein binding.

Applying a combinative set of biochemical and biophysical protein-protein interaction assays we have demonstrated that all centrin isoforms can interact with the visual heterotrimeric G_t-protein in a Ca^{2+}-dependent manner (Pulvermüller et al. 2002; Wolfrum et al. 2002; Gießl et al. 2004a, b, 2006). All centrin isoforms interact with the $G_t$holo complex, the undissociable $G_t\beta\gamma$-dimer and the isolated $G_t\beta$-subunit, but not with $G_t\alpha$ alone. Nevertheless, centrin isoform 3 has a significant lower affinity to G_t compared to the other three centrin isoforms (Gießl et al. 2004a). Furthermore, centrin 3 interacts as a monomer while the other centrin isoform bind in form of oligomeres to $G_t\beta$ (Gießl et al. 2004a). Recent microtubule binding assays revealed binding of centrins to microtubules which suggests that centrins and their complexes with transducin are anchored to the inner surface of the ciliary microtubules of the photoreceptor connecting cilium (Ph. Trojan and U. Wolfrum unpublished).

In summary, an increase of the ciliary Ca^{2+}-concentration should induce oligomerization of centrin 1 and 2 and binding of these oligomeres to $G_t$holo complexes or to $G_t\beta\gamma$-dimer on their way through the connecting cilium. However, the protein complexes of centrins with G_t are not only regulated by Ca^{2+}, but also by phosphorylation. Recently, we observed that in mammalian retinas, centrin isoforms 1 and 2 are phosphorylated by the casein protein kinase CK2 in a light dependent manner

(Wolfrum et al. 2006; Trojan et al., in prep.). The residues phosphorylated in the dark (amino acids T138 (Cen1) and T137 (Cen2)) are specifically dephosphorylated by protein phosphatase PP2Cβ (Thissen et al. 2006, in prep., Wolfrum et al. 2006; Trojan et al. in prep.). The phosphorylations of centrin 1 and 2 drastically reduce the affinity of both iosforms to G_t. Since both enzymes, CK2 and PP2Cβ, are also found in the ciliary apparatus of photoreceptor cells (Hollander et al. 1999; Thissen et al. 2006; in prep.), it is worst to speculate that they temporarily bind to centrins and/or centrin/G_t-complexes during their enzymatic activity. Moreover, initial blot overlays indicated numerous more centrin binding proteins in the retina which may also contribute to the assembly and regulation of centrin/Gt-complexes in the photoreceptor connecting cilium.

Based on our results there is evidence that the Ca^{2+}-dependent assembly of centrin 1 and 2/G-protein complexes regulates transducin movement through the connecting cilium (Wolfrum et al. 2002; Gießl et al. 2006). Although, the source of the light modulated changes in free Ca^{2+} remains to be solved (Gießl et al. 2006), light-induced Ca^{2+} changes in the connecting cilium should induce binding of the high affine centrin isoforms 1 and 2 to G_t. The assembly into centrin/G_t complexes, together with Ca^{2+}-induced centrin oligomerization may form a barrier for further intersegmental exchanges of transducin (Figure 3) (Wolfrum et al. 2002; Gießl et al. 2006). This mechanism resembles a novel aspect of translocation regulations of signaling proteins

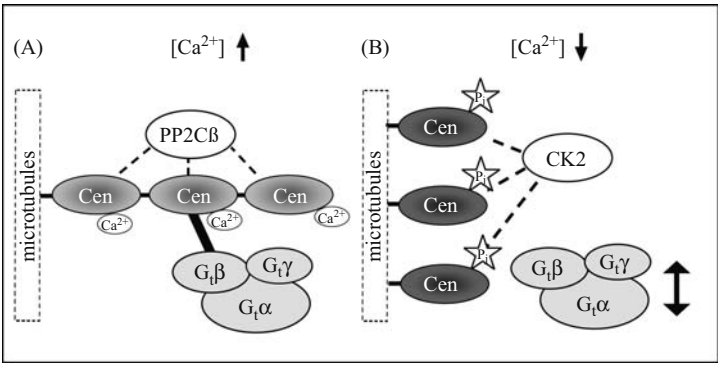

Figure 3. Model for centrin-G-protein complex assembly in the connecting cilium of photoreceptor cell. Schematic representations of a part of the inner lumen of the photoreceptor connecting cilium. Centrins (~ centrin isoforms 1 and 2) (Cen) are physically linked to the inner surface of the microtubule of the connecting cilium(CC). (A) Scenario at high free Ca^{2+} concentrations in CC: Cen are specifically dephosphorylated by protein phosphatase PP2Cβ. Ca^{2+}-binding to Cen induces Cen oligomerization and increases affinity of the G_tβ-subunit of the visual heterotrimeric G-protein transducin (G_ta-G_tβγ). This may result in trapping G-protein molecules in the connecting cilium and G-protein diffusion is inhibited (Barrier hypothesis, Wolfrum et al. 2002). (B) Scenario at low free Ca^{2+} concentrations in CC: Cen are specifically phosphorylated by protein kinase CK2. Cen-P decreases affinity of G-protein to Cen. *Arrow* indicates that free diffusion of G-protein is possible. (for references, please see text)

in sensory cells, as well as a potential link between molecular trafficking and signal transduction in general.

3.2. Intraflagellar Transport Complexes in Mammalian Photoreceptor Cells

Intraflagellar transport (IFT) is an evolutionally conserved mechanism required for the assembly and maintenance of all eukaryotic cilia and flagella. IFT is a bidirectional transport system which moves non-membrane bound particles from basal body out to the tip of the cilium, and then returns them back to the cell body (Piperno and Mead 1997; reviewed in Rosenbaum and Witman 2002). A ~16 S IFT particle fraction, originally described in the green algae *Chlamydomonas* consists of at least 16 proteins that occur in two protein complexes, the complex A composed of four relatively high molecular weight proteins (M_r ~120–150 kDa) and the complex B which contains proteins of mostly lower molecular weight (M_r below 100 kDa) (Piperno and Mead 1997; Cole et al. 1998; reviewed in Rosenbaum and Witman 2002). IFT complexes are assembled near the basal body, moved along the axoneme by the heterotrimeric kinesin-II in an anterograde direction to the ciliary tip and back to the basal body region by the "axonemal" cytoplasmic dynein containing the dch2/1b heavy chain (Kozminski et al. 1995; Pazour et al. 1999; Pedersen et al. 2005). The IFT-system is thought to be associated with the transport of cargos, proteins and ciliary precursors essential for the assembly and maintenance of the axonemal structures, for example, α/β tubulin and axonemal dynein components (Qin et al. 2004). Furthermore, signaling pathways in *Chlamydomonas* gametes are IFT-dependent (Cole et al. 1998; Pan et al. 2005; Pan and Snell 2003; Wang et al. 2006).

The IFT concept was extended by analysis of mutations in IFT-proteins in *C. elegans* and mice (e.g. Orozco et al. 1999; Qin et al. 2001). A hypermorphic mutation in IFT88 leads to both polycystic kidney disease and retinal degeneration due to photoreceptor outer segment abnormalities in mice (Pazour et al. 2000, 2002). The knowledge on the IFT-system in mammalian photoreceptor cells mainly relies on studies by Joe Besharse and colleagues (Pazour et al. 2002; Baker et al. 2003; Besharse et al. 2003a, b). In retinal photoreceptor cells, all IFT proteins were identified so far, for which it has been searched for (Joe Besharse, personal communication). Available data on the IFT protein complex in vertebrate photoreceptor cells are summarized in Figure 4. In photoreceptor cells, IFT20 and IFT57 directly interact through coiled coil domains present in each protein within an IFT complex that also contains IFT88 and IFT52. IFT20 also connect this complex to the heterotrimeric kinesin-II by physical interaction with the kinesin-II motor subunit KIF3B necessary for the anterograde axonemal transport (Baker et al. 2003). Later assembly is regulated by ATP-hydrolysis. A search for IFT88 binding partners revealed direct binding of MRJ (mammalian relative to DnaJ), a molecular chaperone of the DnaJ/Hsp40 family (Li et al. 2004a). Further interaction of MRJ with the retinal guanylate cyclase E (GC-1) suggests that MRJ serves as a cargo linker and GC is one of the cargos for IFT in photoreceptor cells. Mis-localization of opsin and arrestin in mice with a

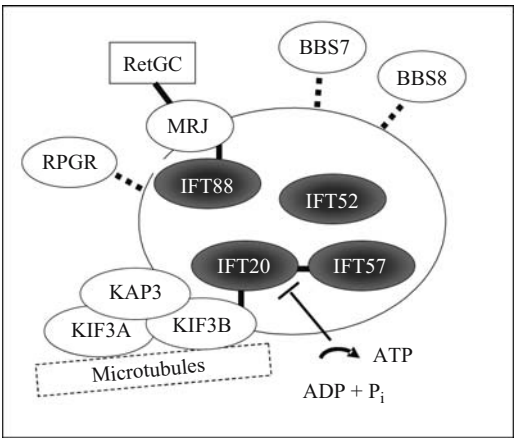

Figure 4. Model of intraflagellar transport (IFT) complex in photoreceptor cells. In the IFT complex found in photoreceptor cells IFT20 binds directly to IFT57 and to the KIF3B subunit of heterotrimeric kinesin-II, composed of KIF3B, KIF3A and KAP3. Latter interaction is reguated by ATP hydrolyses. IFT52 and IFT88 were also identified in photoreceptor IFT complexes. IFT88 interacts directly with the molecular chaperone MRJ which acts as a cargo receptor for photoreceptor specific guanylate cyclase RetGC. Association of BBS7 and BBS8 with mammalian IFT complex has been reported. (for references, please see text)

photoreceptor-specific knockout of the gene for kinesin-II motor subunit KIF3A suggested that opsin and arrestin might also be cargos for kinesin-II driven IFT (Marszalek et al. 2000). However, a direct link of the IFT complexes to these cargos has not been established and alternative motor complexes are described in photoreceptor cilia (see Section 3.3.1.1).

Expression of GFP-IFT proteins in transgenic *Xenopi* and zebrafish demonstrated targeting of IFT proteins to the cilia of photoreceptor cells (Besharse et al. 2003b). Although immunofluorescence data on IFT protein localizations in retinal photoreceptor cells indicate that IFT proteins and associated components are concentrated in the basal body region and in the connecting cilium (Pazour et al. 2000; Baker et al. 2004), they may function as bi-directional transport carriers along the axonemal microtubules in the outer segment (see Chapter 3.4). Knowledge of the molecular and spatial organization of IFT complexes, the regulation of cargo loading and unloading, and motor protein regulation will certainly elucidate the function of the IFT system in photoreceptor cells.

3.3. Protein Complexes of the Photoreceptor Cilium Involved in Human Genetic Disease

A group of inherited retinal degenerations called retinitis pigmentosa (RP) are a common cause of blindness. RP affects 1 out of every 3500 people worldwide (Berson

1993). RP is genetically heterogeneous, with 33 known different gene loci for non-syndromic RP and RP is also one part of several syndromes in which other organs are affected in addition to the retina. In the majority of the retina-related syndromes, ciliary dysfunctions are thought to be the common ground for pathogeneses of these disorders.

3.3.1. Usher syndrome (USH) "interactome" and USH protein complexes in photoreceptor cilia.

Usher syndrome (USH) is the most frequent cause of combined deaf-blindness in man. It is clinically and genetically heterogeneous and at least 11 chromosomal loci are assigned to three clinical USH types, namely *USH1B-G, USH2A,C,D, USH3A* (Gerber et al. 2006; Kremer et al. 2006; Reiners et al. 2006; Ebermann et al. 2007). The proteins encoded by the identified USH genes are members of protein classes with very different functions. Myosin VIIa (USH1B) is a motor protein, harmonin (USH1C), SANS (scaffold protein containing ankyrin repeats and SAM domain) (USH1G), and whirlin (USH2D) are scaffold proteins, cadherin 23 (USH1D) and protocadherin 15 (USH1F) are cell adhesion molecules and USH2A (usherin) and VLGR1 (very large G-coupled protein receptor) (USH2C) are transmembrane proteins with very large extracellular domains. The protein encoded by the UHS3A gene, clarin-1, is a member of the vertebrate-specific clarin family of four-transmembrane-domain proteins.

Molecular analysis of the diverse USH1 and USH2 proteins in the inner ear and retina revealed integration of USH proteins in a USH protein network or "interactome" (reviewed in Reiners et al. 2006; Kremer et al. 2006). In this network, the USH1 and USH2 proteins are thought to be assembled in a multiprotein scaffold, with a central role for the PDZ domain containing protein homologues harmonin and whirlin and the microtubule associated protein SANS (Reiners et al. 2005b, 2006; Kremer et al. 2006; van Wijk et al. 2006; Märker et al. in prep.). Although the colocalization of all USH1 and USH2 proteins at the photoreceptor synapse suggests a synaptic localization of the USH interactome in retinal photoreceptor cells, a subset of USH proteins is also present at the ciliary apparatus of photoreceptor cells indicating a USH network there (Kremer et al. 2006; Reiners et al. 2006).

3.3.1.1. Myosin VIIa (USH1B) molecular motor complex participates in ciliary transport of opsin The USH1B protein myosin VIIa is concentrated at the ciliary membrane of the photoreceptor connecting cilium (Liu et al. 1997; Wolfrum and Schmitt 2000). Immunoelectron microscopic analysis of myosin VIIa deficient shaker-1 mouse retinas showed an accumulation of opsin in the ciliary plasma membrane of photoreceptor cells (Liu et al. 1999; Wolfrum and Schmitt 2000). This excessive concentration of opsin of shaker-1 mice suggests that myosin VIIa is normally responsible for the opsin transport through the connecting cilium. Actin filaments extending beneath the ciliary membrane make myosin VIIa-based transport of opsin within the ciliary plasma membrane feasible (Wolfrum and Schmitt 2000). FERM (4.1-ezrin-redixin-mesoin) domains present as a tandem repeat in the

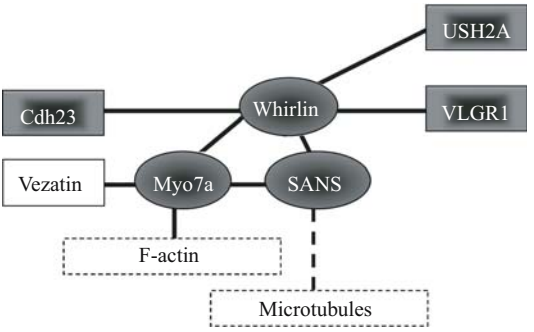

Figure 5. Usher protein complex in photoreceptor cilia and associated compartments. The three USH2 related transmembrane proteins USH2A, and VLGR1b (USH2C) bind via their C-terminal PBM to PDZ1 of whirlin, whereas cadherin 23 (Cdh23) (USH1D) interacts via its C-terminal PBM with PDZ2 of the scaffold protein whirlin (USH2D). Whirlin interacts via PDZ domains with SANS (USH1G). The scaffold protein SANS forms homomers via its central domain. The central domain of SANS is also suitable to interact with the MyTH-FERM domains in the tail of myosin VIIa (Myo7a) (USH1B). In the connecting cilium, myosin VIIa also interacts with the transmembrane protein vezatin. The present USH protein network is directly connected to the actin cytoskeleton through the actin-based molecular motor myosin VIIa dimmers and an association with microtubules is mediated by SANS. Rectangles indicate membrane proteins; dark gray fillings indicate "Usher proteins." (for references, please see text)

myosin VIIa tail has been shown to interact with lipids or proteins of the plasma membrane (Chishti et al. 1998). In the connecting cilium, transmembrane proteins, cadherin 23 (USH1D) and vezatin, are localized (Lillo et al. 2005; Wolfrum, unpublished data) which directly interact with the myosin VIIa FERM2 domain (Küssel-Andermann et al. 2000) and may anchor the molecular motor at the ciliary membrane. However, myosin VIIa is also linked to several other proteins of the USH interactome which were described for the connecting cilium. Myosin VIIa binds to the scaffold protein SANS (USH1G) and its interaction with whirlin provides a link to the USH2 proteins USH2A and VLGR1b (Reiners et al. 2006; van Wijk et al. 2006). In conclusion, these proteins may assemble into a multiprotein complex supporting the transport role of myosin VIIa in the connecting cilium (Figure 5). Thus, this myosin VIIa motor complex is a plausible alternative transport mechanism to the IFT system based on kinesin-II/dynein1b and microtubules described in Chapter 3.2.

3.3.1.2. Usher protein complexes connect the photoreceptor cilium with the apical compartments of the inner segment As described above, several USH proteins are localized in the connecting cilia associated with the membrane. Nevertheless, a prominent assembly of USH proteins is also present in the inner segment of photoreceptor cells associated with the ciliary apparatus. In the calycal processes of the apical region of the inner segment, in the absence of harmonin, the scaffold protein whirlin is localized and interacts via its PDZ domains with the PDZ-binding motifs (PBM) in the cytoplasmic tails of the USH2 proteins USH2A and VLGR1b, as well as

with USH1 proteins protocadherin 15 and SANS (Figure 5) (Märker and Wolfrum, unpublished; Kremer et al. 2006). In mammals, the transmembrane cell adhesion protein protocadherin 15 is concentrated in the plasma membrane of the distal extensions of the calycal process where it faces the newly formed disk membranes of the outer segment base (Reiners et al. 2005a). Here, protocadherin 15 may associate with the photoreceptor specific cadherin prCAD (Rattner et al. 2001, 2004) stabilizing the labile newly formed disks (Reiners et al. 2005a).

In contrast, USH2A, VLGR1b and SANS are associated with the periciliary ridge complex which is thought to be the docking side for cargo loaded post-Golgi vesicles (Papermaster 2002). In mammals, this specialized domain extends over the plasma membrane of the proximal part of the calycal process which is connected via extracellular fibrous links to the plasma membrane of the connecting cilium. Recently, analogous to the fibrous links that connect stereovilli (= stereocilia) in mechanosensory hair cells, these fibers were identified to be composed of the long extracellular parts of cadherin 23 and VLGR1b (McGee et al. 2006). There is evidence that the extracellular domain of USH2A may also participate in the formation of these fibrous links (Liu et al. 2007). While, in the extracellular space between the membranes of the inner segment and the connecting cilium, homo- or/and heteromeric binding of the extracellular domains of USH2A and VLGR1b are developed, their short cytoplasmic tails are anchored by whirlin in the cytoplasm. So far, this USH protein complex at the periciliary ridge is completed by SANS which directly interacts with whirlin and may provide the molecular bridge to microtubules (van Wijk et al. 2006; Adato et al. 2005; Märker et al. in prep.). The present USH protein complex (Figure 5) should provide mechanical support to the membrane junction between the inner segment and the connecting cilium and probably also participate in the control of vesicle docking (reviewed by Deretic 2004) and cargo handover in the region of the periciliary ridge.

Immunoelectron microscopy revealed localization of the molecular components of the periciliary ridge USH protein complex in the region of the basal body of the photoreceptor connecting cilium (van Wijk et al. 2006; Märker et al. in prep.). Here at the ciliary basis, cytoplasmic splice variants of USH2A and VLGR1b together with whirlin and SANS, but also cadherin 23, might be enrolled in the MTOC function of the basal body (microtubule nucleation), but more plausible is a role of the USH protein complex proteins in the control of the import and export of molecules into or from the connecting cilium or they may participate in cargo handover. Unfortunately, little is known about the "cross talk" of the proteins of the USH complex with other proteins and protein complexes of the basal body complex reviewed in the other chapters of the present contribution.

Defects of one component of these USH complexes may cause dysfunction of the entire protein complex and induce sensorineuronal degeneration found as symptoms in USH patients.

3.3.2. The RPGR/RPGRIP1 protein network

3.3.2.1. RPGR and RPGRIP1 The X-linked gene *RPGR* (retinitis pigmentosa GTPase **r**egulator) is mutated in patients with retinitis pigmentosa type 3 (RP3) (Roepman et al. 1996a, b Meindl et al. 1996). All missense mutations in *RPGR* have

been identified in RP3 patients, in the region that encodes the ubiquitously expressed N-terminal 440 amino acids of RPGR. This domain shows significant homology to the regulator of chromosome condensation (RCC1), a guanine nucleotide exchange factor (GEF) for the small GTPase Ran (Roepman et al. 1996b; Meindl et al. 1996). Though as of yet no exchange activity on any GTPase has been described for the RCC1 homologous domain of RPGR, it binds to PDEδ (Linari et al. 1999), and to the C-terminus of RPGR interacting protein 1 (RPGRIP1) (Boylan and Wright 2000; Roepman et al. 2000a, b). The splice variant RPGR^{ORF15}, that is upregulated in rod and cone photoreceptors, harbours a mutational hot-spot (Vervoort et al. 2000), and mutations in this exon can give rise to a variety of retinal phenotypes (reviewed in Ferreira 2005). In addition, some mutations in this exon were found to cause RP in combination with impaired hearing and sinorespiratory infections (Van Dorp et al. 1992; Zito et al. 2003; Iannaccone et al. 2003, 2004) and RP with primary ciliary dyskinesia (PCD) (Moore et al. 2006). The latter conditions indicate a disrupted cilia function, and localization of the RPGR protein to the transitional zone of airway epithelial motile cilia supports this hypothesis (Hong et al. 2003).

In photoreceptors, RPGR localizes to the axoneme and basal bodies of connecting cilia (Hong et al. 2003; Khanna et al. 2005), where it was suggested to be involved in a correct localization of opsins in the outer segments. Localization to other subcellular sites have been described, but the exact nature of that is under debate (reviewed in Ferreira 2005). Multiple proteins were suggested to be associated with the protein complex in which the RPGR^{ORF15} variant participates, including SMC (structural maintenance of chromosomes) proteins 1 and 3, IFT88, KIF3A, p150Glued, and p50-dynamitin (Khanna et al. 2005), and nucleophosmin (Shu et al. 2005). RPGR was shown to be anchored to the connecting cilium by RPGRIP1, which was suggested to be a structural component of the ciliary axoneme based on its resistance to detergent extraction (Hong et al. 2001). Immunoelectron microscopy further indicates RPGRIP1 localization external to the profiles of the microtubule doublets of the connecting cilium. These results could indicate that RPGRIP1 is a component of the microtubule-membrane cross linkers, Y-shaped structures projecting from each microtubule doublet at the junction between the A and B tubules to the adjacent plasma membrane (see Chapter 2, Besharse and Horst 1990). A general role of RPGRIP1 as a scaffold protein at these sites was therefore suggested (Hong et al. 2001).

The gene encoding the RPGR interacting protein 1 (RPGRIP1) harbors mutations that can lead to Leber congenital amaurosis (LCA), a genetically heterogeneous recessive disorder that is regarded to be the earliest and most severe form of all retinal dystrophies (Cremers et al. 2002). Absence of RPGRIP1 in *RPGRIP*$^{-/-}$ mutant mice leads to a reduced ERG amplitude and response sensitivity of both rod and cones, and a defect in outer segment disk formation, indicating a role in disk morphogenesis (Zhao et al. 2003). Similar to RPGR, the exact nature of subcellular localization of RPGRIP1 at other retinal sites is under debate (reviewed in Ferreira 2005). The conserved C2 domain of RPGRIP1, that appears to be encoded by a spliced variant that is expressed in a pan-retinal rather than photoreceptor-restricted

fashion, strongly interacts with nephrocystin-4 (Roepman et al. 2005). Mutations in the gene encoding nephrocystin-4 (*NPHP4*) are associated with nephronophthisis type 4 (NPHP4) and Senior-Løken syndrome (SLSN) (Mollet et al. 2002; Otto et al. 2002).

3.3.2.2. The NPHP connection Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease, and the most frequent monogenic cause for end-stage renal failure in children and young adults (Smith and Graham 1945). SLSN is characterized by nephronophthisis in combination with retinal degeneration, either progressive (retinitis pigmentosa, RP) or congenital (LCA) (Olbrich et al. 2003). Nephrocystin-4 has been localized to kidney primary cilia of renal epithelial cells (Mollet et al. 2005), and interacts with the cilia-localized nephrocystin-1 protein (also called nephrocystin), involved in NPHP1 (Mollet et al. 2002). Furthermore, it is a conserved member of the flagellar apparatus and basal body proteome (Li et al. 2004b). Although nephrocystin-4 shows a panretinal localization, including the connecting cilia (Roepman et al. 2005), the interaction with RPGRIP1 strongly suggests an important functional role of this protein complex in the disease pathology of RP/LCA and NPHP/SLSN. This is emphasized by identification that the ciliary protein nephrocystin-5 (interacting with calmodulin) is involved in SLSN and exists in a complex with RPGR (Otto et al. 2005).

Within the *NPHP* gene family (*NPHP1-6*), there is a variable association with other phenotypes besides renal cyst formation and retinal degeneration, such as congenital oculomotor apraxia (COGAN syndrome) (Mollet et al. 2002), and a complex brain stem malformation and associated brain features (Joubert syndrome (Joubert et al. 1969). Recently, homozygous protein truncating mutations in the gene encoding a new centrosomal protein, CEP-290, were found to be associated with Joubert syndrome (Sayer et al. 2006; Valente et al. 2006), while a specific splice variant of the cognate gene (either homozygous or in combination with a second deleterious mutation on the other allele) is the most frequent cause of LCA known to date (den Hollander et al. 2006). Interestingly, this protein was also found to exist in a complex with RPGR (Chang et al. 2006; Sayer et al. 2006). Nephrocystin-4, inversin (nephrocystin-2) and nephrocystin-3, but not nephrocystin-5 interact with nephrocystin-1, and nephrocystin-3 has been shown to colocalize with inversin in the primary cilia of renal tubular epithelial cells (Otto et al. 2003), similar to what previously was found for the proteins associated with autosomal dominant and recessive polycystic kidney disease, polycystin-1, polycystin-2, polaris, cystin, and polyductin (reviewed in Hildebrandt and Otto 2005). This seems to point towards a unifying pathogenic mechanism, with a central role for the cilia. By combining the recent findings regarding the RPGR-RPGRIP1-nephrocystin-4 interaction with the reports of protein complexes identified by affinity purifications and immunoprecipitations, a putative interaction network arises in the connecting cilium of photoreceptor cells (Figure 6). Future analysis of these components will reveal the actual interplay of the different members of this network, and their subcellular sites of interaction.

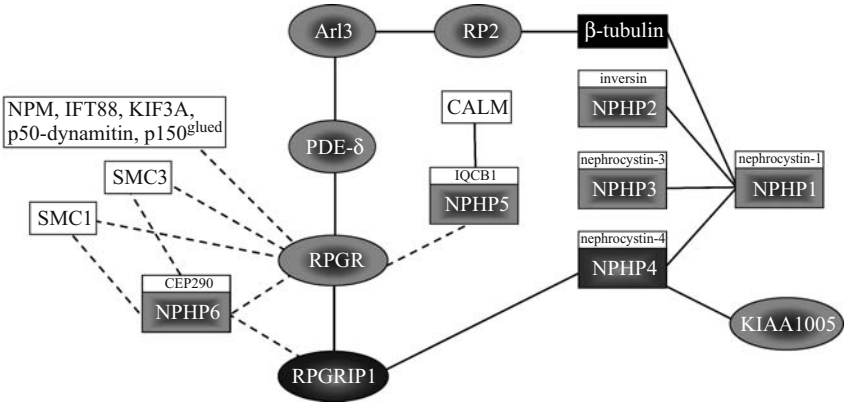

Figure 6. The RPGR-nephrocystin protein network. Putative core oculo-renal protein network in the cilia, interconnected in the retina by the RPGRIP1–nephrocystin-4 interaction. The connections are based on reported protein-protein interactions (for description and references, please see text). Direct interactions are shown by solid lines, interactions that were identified by immunoprecipitations, as part of a protein complex, are shown by dotted lines.

3.3.3. Bardet-Biedl Syndrome Proteins in ciliary protein complexes

The Bardet Biedl Syndrome (BBS) is a rare polygenetic and pleiotropic disorder associated with basal body and ciliary defects (Beales 2005). Patients with this multifaceted disease can suffer from a large number of symptoms, including retinal (rod-cone) degeneration, obesity, cystic kidneys, learning disabilities, hearing loss and anosmia. A full description of the BBS symptoms is shown in several competent overviews (Green et al. 1989; Beales et al. 1999; Moore et al. 2005). In addition to their common role in basal body and ciliary function, the 8 identified BBS proteins are involved in establishing planar cell polarity, modulation of intraflagellar transport (IFT) and lipid homeostasis, and in the regulation of intracellular trafficking and centrosomal functions. While BBS4 may serve as an adaptor protein for IFT and cilia function, BBS7 and BBS8 are certainly associated with IFT complexes and participate in particle assembly (reviewed in Chapter 3.2, Figure 4). These findings point towards a common function of BBS proteins in mediation and regulation of microtubule-based intracellular transport processes, as reviewed in Blacque and Leroux 2006.

3.4. Protein Complexes in the Light Sensitive Outer Segment of Photoreceptor Cells – The Search for the Vertebrate Photoreceptor Transducisomes

The outer segment of vertebrate photoreceptor cells can be considered as the highly modified distal part of an immotile cilium (Röhlich 1975; Besharse and Horst 1990) and contains only a small number of “axonemal” microtubules (see Introduction). The RP1 protein is specifically associated with these microtubules and is required for

correct stacking of disk membranes into organized outer segments (Liu et al. 2002). Mutations in the *RP1* gene are a common cause of dominant retinitis pigmentosa (reviewed in Achenbach et al. 2004). In addition to this microtubule associated protein (MAP), proteins of the visual signal transduction cascade are also associated with the outer segment cytoskeleton (Figure 7). The guanylate cyclase (RetGC-1) was found to bind to actin filaments as well as to microtubules in photoreceptor outer segments (Hallett et al. 1996; Schrem et al. 1999). RetGC was also identified as a cargo of IFT complexes (see Chapter 3.2, Figure 5). Dimers of RetGC form a functional complex with GC-associated proteins GCAPs which regulate the GC enzymatic activity in Ca^{2+}-dependent manner. Peptide affinity chromatographies indicated binding RetGC-1/GCAP to a much bigger protein complex localized at the rim region of outer segment disks (Körschen et al. 1999). In the latter study, glutamic-acid-rich proteins (GARPs) were identified as multivalent proteins which exist as two soluble forms and as a large cytoplasmic tail of the B1-subunit of the

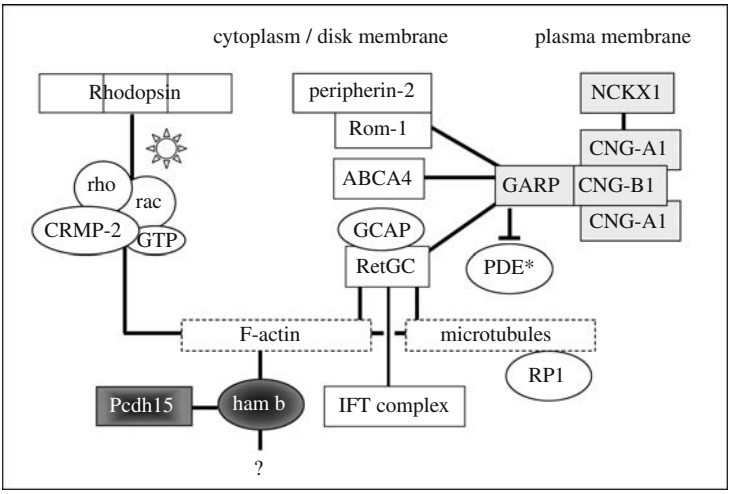

Figure 7. Schematic illustration of protein complexes in the photoreceptor outer segment (OS). The plasma membrane complex composed of the exchanger NCKX1 and the visual CNG channel (mediated by CNG-A1 subunit) interacts through the GARP domain of the CNG-B1 subunit with heteromers of peripherin-2/Rom-1 and may furthermore bind ABCR4, guanylate cyclase RetGC and activated phosphodiesterase (PDE*) at the disk membrane rim. RetGC is regulated by Ca^{2+}-binding GCAPs and serves as cargo for IFT complexes. RetGC also provides an association of disk rim complexes to actin filaments as well as to microtubules. These cytoskeletal elements bridge the latter complex to rhodopsin-associated Rac-GTP-bound protein complexes, recently identified. These multiprotein complexes contain among other components the small G-proteins rho and rac, in the GTP-bound form, and the CRMP-2 protein. The assembly of the rhodopsin-associated complexes is controlled by light ("sun"). The actin-binding scaffold protein harmonin b (hamb) (USH1C splice variant) is exclusively expressed in OS and binds via its PDZ2 domain Pcdh15 (USH1F). The microtubule associated protein RP1 specifically binds to axonemal microtubules in the OS. Rectangles indicate membrane proteins; light grey fillings indicate plasma membrane components, dark gray fillings indicate "Usher proteins." (for references, please see text)

visual cGMP-gated (CNG) channel, and interact with central players of the visual cGMP signaling pathway, RetGC-1 and phosphdiesterase (PDE), and with the ATP-binding cassette receptor (ABCR) (Figure 7). Since GARPs powerfully inhibit PDE activity, the "GARP complex" may constitute an adaptational signaling system, that inactivates active PDE molecules diffused to the disk rim and down regulates the high cGMP turnover under rod saturation conditions during daylight (Körschen et al. 1999). Nevertheless, co-immunoprecipitation experiments demonstrate a GARP containing protein complex of different composition (Poetsch et al. 2001). In the identified arrangement of outer segment membrane proteins, the visual CNG channel form a complex with the Na/Ca-K exchanger (NCKX1) via its A1-subunit in the plasma membrane. Furthermore, the CNG channel interacts through the GARP-domain of the B1-subunit with peripherin-2-ROM-1 oligomers which are localized in the rim of the outer segment disk (Schwarzer et al. 2000; Poetsch et al. 2001; Kang et al. 2003; Molday 2004). These interactions of the visual channel with peripherin-2-ROM-1 may guarantee that a significant portion of the CNG channels is situated next to a disk rim so that it can promptly response to the signaling cascade arranged at the disk membranes. A combination of the two models of the outer segment membrane protein complexes are shown in Figure 7.

The disk rim complexes are most probably associated with the outer segment cytoskeleton (Hallet et al. 1996; Körschen et al. 1999; Kajimura et al. 2000). In invertebrate photoreceptors, the PDZ protein INAD clusters the components of the visual signal transduction cascade into a signal complex associated with the rhabdomeric actin cytoskeleton (Montell 1999). In vertebrates, the scaffold protein harmonin b, a long splice variant of the USH1C gene, exhibits all features of a potent actin-binding protein (Boeda et al. 2002) and was furthermore identified to be exclusively expressed in photoreceptor outer segments (Reiners et al. 2003). Harmonin b might associate with the disk rim complexes through its interaction with the actin cytoskeleton providing its PDZ domains as attractive binding sites for the assembly of further proteins into outer segment protein complexes (Figure 7). The identification of outer segment proteins interacting with the PDZ domains of harmonin will broaden our knowledge on the organization of signal complexes in vertebrate photoreceptor cells.

In conclusion, the assembly of signaling molecules into supramolecular complexes, known as transducisomes, provides specificity, sensitivity and speed in intracellular signaling cascades (Zucker and Ranganathan 1999). A recent study provides evidence for an interdependence of visual perception with signalling networks involved in the organization of proper cellular structure. The Ueffing lab identified several candidates as novel rhodopsin interaction partners (Swiatek-De Lange et al. 2006). These candidates include small GTPases from the Rho and Rab families as well as CRMP-2 (collapsing response mediator protein 2) as a novel small GTPase-binding protein in photoreceptor cells. RhoA and Rac1 regulate protein transport and structural organisation of cells, while the CRMP family is one of main regulators of polarity development in neuronal cells. The identified Rac-GTP-bound protein complexes associate with the cytoskeleton and exhibit light and dark regulated dynamics**.** These complexes interact with light-activated rhodopsin (R*) which might be organized in

form of dimers or higher order oligomers in the disk membrane of photoreceptor outer segments (Fotiadis et al. 2003, 2006). A possible link to the scaffold protein harmonin b is currently investigated. Although, there is good evidence for an involvement of Rac-containing protein complexes in the *de novo* synthesis of membranous disks at the base of the outer segment (Deretic et al. 1995; Deretic 2004), specific subcellular localization of the complexes in the outer segment will provide more information on the function of the light modulated outer segment complex. Nevertheless, these supramolecular complexes may also serve as a link between the transduction module (Hofmann et al. 2006) and the module of signaling networks involved in structural integrity and cell polarity in photoreceptor cells.

4. CONCLUDING REMARKS

Vertebrate photoreceptor cells are ciliated sensory cells modified for single photon detection. In all of the modified subciliary compartments of the photoreceptor cells, proteins are arranged in functional supramolecular complexes. Although, many of these proteins are also found in prototypic cilia or primary cilia, the arrangements of the proteins in complexes can be specific for vertebrate photoreceptor cells. These protein complexes may serve in functional modules specific for photoreceptor cells, for example, for visual signal transduction, adaptation, ciliary signaling, cell polarity and integrity or in specific modules of molecular translocations. The current knowledge on protein complexes in photoreceptor cilia is certainly only the peak of the iceberg and very fragmentary. In particular, the interconnections between the different protein networks and complexes and their side branches seems to be patchy. Future analysis of the molecular interactome in photoreceptor cilia will certainly expand the insights into photoreceptor function/dysfunction, in health and disease.

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