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Intraflagellar Transport and the Sensory Outer Segment of Vertebrate Photoreceptors

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Introduction

Research over the past decade on the role of intraflagellar transport (IFT) in ciliogenesis has led to the realization that cilia throughout the body are sensory antennae that coordinate development and function of many tissues and organs (Rosenbaum and Witman, 2002; Snell et al., 2004; Singla and Reiter, 2006). The sensory role of cilia evolved in early eukaryotes and is seen in simple organisms (Wang et al., 2006; Inglis et al., 2007) as well as in mammals (Michaud and Yoder, 2006; Eggenschwiler and Anderson, 2007). Thus, it is not surprising that one of the best understood sensory signaling systems, the outer segment (OS) of vertebrate rod and cone photoreceptors, depends on IFT for both development and maintenance (Marszalek et al., 2000; Pazour et al., 2002a; Baker et al., 2003; Besharse et al., 2003). The goal of this brief overview is to illustrate how the photoreceptor OS can be viewed as a highly elaborate sensory cilium and model for analysis of IFT. We bring attention to the problem of rod and cone OS morphogenesis as a special case in the rapidly emerging field of ciliogenesis. We also distinguish between general features of ciliogenesis common to all sensory cilia and the unique features relevant to photoreceptors. Finally, we introduce emerging data that suggest the existence of an alternative IFT kinesin motor that is involved in photoreceptor OS formation.

Phototransduction Occurs in a Sensory Cilium

Vertebrate rod and cone photoreceptors are sensory neurons whose function in vision depends on formation of a complex sensory cilium (Figure 1A). The cell body of each photoreceptor extends a short axon, which makes synaptic contact with second order neurons (bipolar and horizontal cells), and a short dendrite, which terminates in a phototransduction organelle called the outer segment (OS). The dendritic region between the OS and nucleus is called the inner segment. Extensive analysis of the photoreceptor sensory OS over several decades has yielded a reasonably clear picture of the key molecular events of phototransduction and deactivation of the photoresponse (Burns and Arshavsky, 2005). At the most fundamental level (Figure 1B) these events depend on the presence of rhodopsin (R) to detect photons, membrane guanylyl cyclase (GC) to produce cGMP (cG), and a cyclic nucleotide gated channel (CNG) to regulate movement of cations. Light absorbed by rhodopsin activates the heterotrimeric G protein, transducin, which in turn activates cGMP phosphodiesterase (PDE) to reduce cGMP levels. In the dark cGMP keeps the CNG channel open, whereas in the light reduced cGMP levels result in channel closing and cell hyperpolarization. Further mechanisms requiring additional OS proteins (not shown in Figure 1) include deactivation of rhodopsin through phosphorylation

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and binding of arrestin, as well as regulation of transducin activity by a regulator of G-protein signaling complex.

The numerous membrane and membrane-associated proteins involved in phototransduction are synthesized in the inner segment and then concentrated in the OS. Some such as arrestin and transducin move dramatically between the segments in response to light (Calvert et al., 2006). This, along with fact that mammalian photoreceptors exhibit turnover of the OS at rates approximating 10% of their length per day (LaVail, 1973), means that mechanisms for trafficking of phototransduction proteins into the OS are highly relevant for both initial development and maintenance of the cell. Although multiple mechanisms including random diffusion and binding of soluble proteins are likely to play a role, intraflagellar transport (IFT) has been identified as an important mechanism (Rosenbaum et al., 1999; Marszalek et al., 2000; Pazour et al., 2002a; Jimeno et al., 2006).

Membrane Proteins are Required for Sensory OS Development

The sensory OS of photoreceptors is similar to that of other sensory cilia, but photoreceptor specific mechanisms are required for the morphogenesis and turnover of the photosensitive disc membranes. The OS arises through transformation of the plasma membrane with the axoneme forming a structural backbone (De Robertis, 1956; Tokuyasu and Yamada, 1959; De Robertis, 1960; Besharse et al., 1985; Knabe and Kuhn, 1997). At the earliest stages, the precursors of disc membranes are formed as a disorderly array of vesicular and tubular structures, but quickly become organized into discs that are oriented perpendicular to the axoneme. Even before disc assembly begins, however, the cilium has a distinct proximal transition zone and a distal domain containing its dominant membrane proteins, rhodopsin (see Figure 2, (Besharse, 1986)) and peripherin-2 (Lee et al., 2006). This finding allows one to conceptually distinguish between ciliary transport mechanisms for membrane proteins, which may be common to many different sensory cilia, and separate mechanisms underlying morphogenesis and alignment of discs. Neither is well understood, but some progress has been made recently with a proposed role for IFT in rhodopsin trafficking (Marszalek et al., 2000; Pazour et al., 2002a; Jimeno et al., 2006).

An important feature of the photoreceptor OS is that at least two of its membrane proteins are required for OS assembly. Disc membranes in rods (see Figure 3) have expanded, flat domains containing two membrane guanylyl cyclases (Dizhoor et al., 1994;Liu et al., 1994;Hallett et al., 1996) along with rhodopsin at a concentration of about 25,000 μm^2 (Calvert et al., 2001) and an edge or rim domain containing tetramers and higher order oligomers of the tetraspanin proteins, peripherin-2 and Rom1 (Molday and Molday, 1987;Arikawa et al., 1992;Loewen and Molday, 2000). The disc rim also contains the ABC transporter, ABCA4 (Illing et al., 1997). Analysis of mutant mice shows that both peripherin-2 (Jansen and Sanyal, 1984;Kedzierski et al., 1998) and rhodopsin (Humphries et al., 1997;Lem et al., 1999) are essential for OS formation. In both cases initial ciliogenesis occurs and a distal membrane expansion similar to that in wildtype cells is formed, but disc assembly and OS elongation fail entirely (Lee et al., 2006;Lee and Flannery, 2007). This suggests that both proteins play important morphogenetic and/or structural roles. In contrast, deletion of the two photoreceptor membrane guanylyl cyclases (Baehr et al., 2007) or ABCA4 (Weng et al., 1999) does not block OS formation, although the abnormal and unstable OS eventually degenerates. It has been suggested that rhodopsin and peripherin-2 are independently transported to the OS (Fariss et al., 1997;Lee et al., 2006), and it is known that both peripherin-2 and Rom1 can reach the distal cilium in the absence of rhodopsin (Lee et al., 2006). However, the mechanisms that underlie protein sorting between disc and rim domains are unknown. The problem is further complicated by the fact that another OS membrane protein, the cyclic nucleotide gated channel (CNG), is targeted to

the plasma membrane (Cook et al., 1989), which requires additional sorting mechanisms between the disc and plasma membrane.

The Connecting Cilium is a Transition Zone Between Membrane Domains

The early stages of the photoreceptor cilium development are similar to other developing cilia in that a 9 + 0 axoneme emerges from a basal body associated with apical cell surface (Greiner et al., 1981; Knabe and Kuhn, 1997). In photoreceptors, the portion of the cilium between the basal body and the disc-forming region, generally referred to as the connecting cilium, is actually the structural equivalent of the transition zone of other cilia and flagella (reviewed in (Besharse and Horst, 1990). Here Y-shaped cross-linkers (see Figure 4) connect the doublet microtubules with integral membrane structures comprising the ciliary necklaces (Rohlich, 1975; Besharse et al., 1985; Horst et al., 1987; Besharse and Horst, 1990). In photoreceptors, ciliary necklaces seen in freeze fracture images and globular membrane structures seen in the plasma membrane (Figure 4) have an average longitudinal spacing of about 32 nm (Besharse et al., 1985) suggesting that they are both part of the same structure. The cytosolic space in the transition zone between inner and outer segments is reduced to ~0.25 μm in diameter, and the axoneme and cross-linking structures further crowd that space (Figure 4). Since all movement between the segments must proceed through this narrow and structurally complex space, the connecting cilium is effectively a bottleneck and a potential regulatory site. For example, it has been suggested that centrins, which localize within the transition zone and exhibit Ca^{++}-activated binding to the α/β subunits of photoreceptor transducin, regulate translocation of transducin between the segments (Pulvermüller et al., 2002; Giessl et al., 2006).

Early work on the structural organization of the Y-shaped cross-linkers showed that they consist of a trans-membrane assemblage containing glycoproteins with extracellular domains that maintain their association with the axoneme after extraction with detergent (Horst et al., 1987; Horst et al., 1990), high salt, and chaotropic agents (Muresan and Besharse, 1994). Interest in these structures has re-emerged recently with the finding that nephrocystin 1 and RPGR, both proteins associated with photoreceptor degenerative diseases, are concentrated in the connecting cilium (Hong et al., 2003; Fliegauf et al., 2006). Furthermore, Ush2a and Ush2c, two proteins associated with Usher's syndrome, a combination of hearing impairment and blindness, have long ectodomains extending between the inner segment plasma membrane and ciliary surface suggesting that they may be associated with the cross-linkers (Liu et al., 2007; Maerker et al., 2008). Both proteins have been proposed to form fibrous links between the two membranes, analogous to the ankle links between adjacent stereocilia in hair cells of the inner ear (Adato et al., 2005; McGee et al., 2006). This could explain how mutations in these genes lead to reduced stability and loss of both hair cells and photoreceptors in humans.

The structural order within the transition zone is likely to be directly related to its role as a gatekeeper between the distinct inner and outer segment membrane domains (Horst et al., 1987; Muresan and Besharse, 1994). Membrane proteins such as the CNG channel (Huttl et al., 2005), membrane guanylyl cyclase (Baehr et al., 2007), and rhodopsin are highly enriched in the OS (Papermaster et al., 1985) but are found at negligible levels in the inner segment. On the other hand, the inner segment membrane Na+/K+ ATPase is not present in the OS (Schneider et al., 1991). Although membrane continuity exists at the connecting cilium, mixing between the two domains does not appear to occur unless the ciliary barrier is disrupted (Spencer et al., 1988). This suggests that the transition zone can serve as both a gatekeeper between the inner outer segment membrane domains and as a corridor for transport of membrane proteins to the OS.

The Photoreceptor Axoneme Extends Deep into the OS

In mature rods the axoneme extends more than half the length of the OS (Brown et al., 1963; Kaplan et al., 1987; Sale et al., 1988), and in some cases may extend most of its length (Luby-Phelps et al., 2008). In cones the axoneme extends the full length of the OS and turns over during the process of disc shedding of the distal OS tip (Eckmiller, 1996). Within the proximal one third of the OS, the axoneme looses the B subfiber of the doublet to form singlet microtubules (Brown et al., 1963; Steinberg and Wood, 1975; Roof et al., 1991; Knabe and Kuhn, 1997; Insinna et al., 2008). Singlets are found through much of the length of the OS. Photoreceptor OS resemble sensory cilia in the olfactory epithelium (Reese, 1965), kidney (Webber and Lee, 1975), and *C. elegans* sensory neurons (Inglis et al., 2007) in the use of singlet extensions. This structural similarity is hypothesized to be related to common molecular mechanisms dedicated to extend and maintain distal extensions of the cilium (Evans et al., 2006; Insinna et al., 2008).

Rp1, a doublecortin domain containing protein that is mutated in a form of human retinitis pigmentosa, binds to singlet microtubules in cultured cells and is uniquely localized to the distal axoneme (Liu et al., 2004) suggesting that it may associate with and stabilize distal singlets in the OS. Since Rp1 also binds to disc rims, this protein appears to play an important role in OS assembly by mediating vertical alignment of discs along the axoneme (Liu et al., 2004).

Intraflagellar Transport in Photoreceptors

A critical role for IFT in OS assembly is supported by both cell biological and genetic data. First, multiple subunits of kinesin II (Beech et al., 1996; Muresan et al., 1997; Muresan et al., 1999; Whitehead et al., 1999; Marszalek et al., 2000) are associated with photoreceptor axonemes, and all three subunits of the kinesin II motor co-immunoprecipitate with IFT proteins in retinal extracts (Baker et al., 2003). Furthermore, both Dhc1b/Dhc2 heavy chain and the light intermediate chain (Lic3/D2Lic) of the dynein 2 retrograde motor are localized along bovine photoreceptor cilia (Mikami et al., 2002). In addition to the IFT motors, multiple IFT proteins are associated with OS axonemes in mouse, frog and zebrafish photoreceptors (Pazour et al., 2002a; Tsujikawa and Malicki, 2004; Insinna et al., 2008; Luby-Phelps et al., 2008). These findings are also supported by co-immunoprecipitation and sucrose density gradient sedimentation data (Baker et al., 2003) showing that multiple IFT complex B proteins from bovine photoreceptor OS fractionate as a large ~17S particle with properties similar to that of the IFT B complex of *Chlamydomonas*(Cole et al., 1998; Lucker et al., 2005). Finally, the functional importance of these findings has been demonstrated in mice deficient in the Kif3A subunit of kinesin II (Marszalek et al., 2000; Jimeno et al., 2006) and in $Tg737^{orpk}$ mice deficient in the IFT complex B protein, IFT88 (Pazour et al., 2002a). In both cases, photoreceptors are progressively lost after failure of OS morphogenesis and mislocalization of rhodopsin. Furthermore, similar results have been reported in zebrafish photoreceptors carrying the ovl mutation in IFT88, or after antisense morpholino knockdown of IFT52 or IFT57 (Tsujikawa and Malicki, 2004).

Although current data strongly support a role for anterograde IFT in photoreceptors, direct evidence for retrograde IFT, which would return OS components to the inner segment, has not been reported. Furthermore, the fact that the photoreceptor OS turns over about 10% of its volume daily by disc shedding at its distal tip (LaVail, 1976) implies that a retrograde pathway would not play a major role in the transport of essential disc membrane components. Nonetheless, the cytoplasmic dynein 2 motor is associated with OS axonemes (Mikami et al., 2002), and there is ample reason to think that a retrograde process would be required in photoreceptors. Retrograde IFT could play a role in support of photoreceptor axoneme

dynamics at the distal tip (Roof et al., 1991; Pedersen et al., 2005), return of anterograde IFT kinesins to the inner segment (Signor et al., 1999), transport of a soluble component of the phototransduction machinery to the inner segment (Calvert et al., 2006), or for IFT mediated signaling (Wang et al., 2006). IFT mediated signaling could be used to coordinate OS trafficking and OS length. Further studies on the retrograde IFT motor and its most closely associated proteins, the IFT complex A (Pedersen et al., 2005), are needed in photoreceptors.

Kif17, a homologue of OSM-3, is Required for OS Assembly

Kinesin II, the canonical anterograde IFT motor, (Kozminski et al., 1995; Rosenbaum and Witman, 2002; Scholey, 2008), is clearly required for OS assembly (Marszalek et al., 2000; Jimeno et al., 2006). However, we have recently found that an additional kinesin called Kif17 is also required for OS formation (Insinna et al., 2008). To put this into perspective the terminology becomes important. Kinesin II is a heterotrimeric member of the kinesin 2 family that in vertebrates is composed of Kif3A, Kif3B, and Kap3 subunits (Lawrence et al., 2004). A conditional deletion approach in photoreceptors involved deletion of the Kif3A subunit (Marszalek et al., 2000), and work at the protein level shows that all three kinesin II subunits co-IP with IFT proteins from retina (Baker et al., 2003). Kif17, also a kinesin 2 family member, is a homodimeric kinesin that is known in mammals for its involvement in dendritic trafficking in neurons (Setou et al., 2000; Guillaud et al., 2003). However, Kif17 is the homologue of the homodimeric OSM-3 kinesin in *C. elegans*, which serves as an accessory IFT motor (Snow et al., 2004; Evans et al., 2006; Pan et al., 2006). Work in *C. elegans* amphid channel cilia revealed a functional coordination between kinesin II and OSM-3 in which both kinesins are involved in elongation of the middle segment composed of doublet microtubules (MT). However, OSM-3 was found to be essential for elongation of MT singlets in the distal segment. In this model, cargo is moved along the middle segment by both IFT motors, while OSM-3 alone is responsible for transport in the distal segment.

Since photoreceptor OS (see above), like *C. elegans* sensory cilia, have singlet extensions of their axoneme (Figure 5), we have begun to test the potential role of Kif17, the Osm-3 homologue, in OS formation (Insinna et al., 2008). Kif17 co-localizes with IFT proteins along the axoneme and co-immunoprecipitates with IFT proteins. Furthermore, depletion of Kif17 using a morpholino knockdown approach in zebrafish embryos prevents OS development (Figure 6). In the most severe cases, extension of the connecting cilium was stopped at the level of the basal body. In less severe cases small, disorganized OS formed and the cells showed mislocalization of the cone visual pigment. These results demonstrate that Kif17 is associated with IFT proteins and is necessary for OS assembly. Although a role for Kif17 in singlet extension is a plausible explanation for short OS phenotype in cases of partial knockdown of Kif17, the complete failure of OS formation with more complete knockdown suggests that Kif17 is required for some additional feature of early OS formation prior to OS elongation. Further experiments are needed to define which steps involve Kif17.

An interesting feature of the Kif17 knockdown phenotype in zebrafish embryos is that the pronephros showed normal development with formation of motile cilia of normal length (Insinna et al., 2008). This indicates that Kif17 is not necessary for elongation of kidney epithelial cilia. In cultured mammalian kidney epithelial cells a dominant negative Kif17 protein blocked delivery of an exogenous olfactory cyclic nucleotide gated channel (CNG) into the distal tip of the cilium (Jenkins et al., 2006). Although this suggests a role for Kif17 in trafficking of the membrane protein, the dominant negative construct had no apparent effect on cilium elongation. Normal cilium elongation in both zebrafish and cultured cells would be expected according to the *C.elegans* model if their axonemes are dominated by doublet MT. Future analysis evaluating a role for Kif17 would be aided by a thorough structural analysis of the distal axoneme and evaluation of the lengths of singlet extensions.

Regulation of Photoreceptor IFT

Emerging data suggest the existence of multiple IFT accessory proteins of direct relevance to photoreceptor IFT. For example, the *fleer* (*flr*) mutation in zebrafish results in multiple cilium related defects including failure of photoreceptor OS formation (Pathak et al., 2007). At the EM level, these phenotypes are associated with disorganization of the B-subfiber of doublets and a deficiency in polyglutamylation of tubulin. Positional cloning has shown that *flr*, which encodes a tetratricopeptide repeat protein, is the homologue of *C. elegans* DYF-1. This is an interesting connection because in *C. elegans* DYF-1 is thought to be necessary for the association of OSM-3 kinesin and the IFT particle; in the absence of DYF-1 OSM-3 is inactive and IFT is driven by kinesin II alone (Ou et al., 2005). This raises the possibility that *flr* may be directly associated with the function of the homologue of OSM-3, Kif17, in photoreceptor OS formation. The nature of the association is unclear, however. Unlike the Kif17 knockdown (Insinna et al., 2008), the *flr* mutation results in cilium defects in multiple cilium types including the pronephros (Pathak et al., 2007). The fact that *flr* is associated with doublets that are deficient in polyglutamylated tubulin has led to the suggestion that the low activity of OSM-3 kinesin in DYF-1 mutants could be the result of its sensitivity to reduced levels of polyglutamylated tubulin. Alternatively, it is also possible that *flr* functions as both a cohesion factor and a regulator of polyglutamylation in axonemes.

Proteins encoded by genes associated with Bardet-Biedl syndrome (BBS) are thought to play a role at critical steps of IFT transport and are likely to be relevant in photoreceptors because retinal degeneration, along with obesity and polydactyly, are key components in the syndrome. Seven of the 12 BBS proteins have been shown to form a core protein complex, called the BBsome, that associates with the basal body, promotes trafficking of vesicles, and is associated with ciliary membrane expansion (Nachury et al., 2007). It is of some interest that BBS7 and BBS8 are among the BBS proteins associated with the BBsome because mutations in the homologous genes in *C. elegans* lead to defective IFT (Blacque et al., 2004). Furthermore, functional analysis suggests that they serve as cohesion factors between IFT sub-complexes A and B and the associated kinesin motors (Ou et al., 2005). The additional BBsome components, BBS4 (Abd-El-Barr et al., 2007) and BBS1 (Davis et al., 2007), also exhibit a photoreceptor OS degeneration in mice. Although BBS proteins are not stable components of IFT proteins complexes, current data strongly suggests that they play an important accessory role in trafficking to the OS. In the case of mice with mutant BBS4 (Abd-El-Barr et al., 2007) vesicles accumulate in the inner segment at the base of the cilium and rhodopsin is mis-localized. These findings suggest a direct relationship between IFT, BBS proteins, and OS trafficking in photoreceptors.

OS Membrane Proteins as IFT Cargo

Although the components of the OS axoneme are expected to be transported by IFT (Qin et al., 2004), a major question is whether OS specific proteins are IFT cargo? Current data favors the idea that at least two OS membrane proteins, rhodopsin and GC1, are transported by IFT. Rhodopsin is mislocalized in mice deficient in both Kif3A subunit of kinesin II (Marszalek et al., 2000) and in $Tg737^{orpk}$ mice deficient in IFT88 (Pazour et al., 2002a). Similar results have been obtained for membrane guanylyl cyclase 1 (GC1) in $Tg737^{orpk}$ mice (unpublished data). These data are in keeping with a growing body of evidence showing a role for IFT in the trafficking ciliary membrane proteins such as polycystins 1 and 2 (Pazour et al., 2002b; Bae et al., 2006; Follit et al., 2006), the TRPV channel (Qin et al., 2005), and the olfactory cyclic nucleotide gated channel (Jenkins et al., 2006). However, mis-localization data alone does not provide molecular insight into the details of coupling of cargo to IFT particles. Accordingly, we have used immunoprecipitation and pull down assays to identify IFT associated proteins. This approach shows that both rhodopsin and GC1 co-immunoprecipitate with IFT proteins

and that the co-chaperones, HSC70 and MRJ (DnajB6), are associated with these complexes (unpublished data). The chaperone complex may play a role in loading or stabilization of IFT cargo complexes.

IFT is Not the Only Pathway into the OS

Although the idea that IFT can deliver OS membrane proteins is attractive and consistent with emerging data from other sensory cilia, other pathways into the OS including diffusion and additional facilitated mechanisms are likely. Mutations in myosin VIIa (Ush1B) underlie a form of Usher's syndrome. Myosin VIIa and an actin filament network are associated with the photoreceptor cilium (Liu et al., 1997). Mice carrying a mutation in myosin VIIa have altered OS disc assembly kinetics and accumulate large amounts of rhodopsin in the connecting cilium. This has led to the proposal that myosin VIIa, in addition to kinesin II, is involved in OS trafficking of rhodopsin (Liu et al., 1999; Williams, 2002). How these two distinct motors and cytoskeletal systems are coordinated remains unknown. It is likely, however, that they work successively. For example, kinesin II could play a role in transport to the disc-forming region with myosin VIIa playing a role in reshaping disc membranes or sorting disc proteins at the base of the OS. Consistent with this idea, disruption of actin filaments with cytochalasin D disrupts the formation of closed discs in rod cells without disrupting the expansion of the OS membrane (Williams et al., 1988).

Simple diffusion may be sufficient for some soluble proteins such as transducin and arrestin and provides an attractive alternative to IFT for their rapid translocation between the segments in response to light (Sokolov et al., 2002; Calvert et al., 2006; Strissel et al., 2006). Green fluorescent protein which does not bind to other cellular components readily equilibrates between the segments via the connecting cilium (Peet et al., 2004). The high diffusion coefficient for soluble, globular proteins would be sufficient to account for the magnitude and rate of translocation seen in photoreceptors (Calvert et al., 2006). Furthermore, it has been pointed out that the extraordinary large number of arrestin molecules moving into the OS in light would likely saturate any motor driven mechanism (Calvert et al., 2006; Strissel et al., 2006).

Diffusion alone would lead to an equilibrium distribution and would still require a mechanism such as binding to a cellular compartment to establish a non-equilibrium condition. This idea is implicit in a recent suggestion that Ca++ regulated binding of centrins to the βγ subunit of transducin regulates their translocation (Pulvermüller et al., 2002; Giessl et al., 2006). Centrins are found in the connecting cilium where their binding to transducin βγ subunit could reduce diffusion. Quantitative analysis of GFP-arrestin distribution compared to free GFP in darkness shows that arrestin is in a non-equilibrium distribution in the inner segment (Peet et al., 2004), and its redistribution to the OS in light has been reported to be energy independent (Nair et al., 2005). Arrestin is known to bind to both tubulin and photo-activated rhodopsin, which has led to the idea that in light it redistributes by diffusion from microtubule binding sites in the inner segment to photo-activated rhodopsin binding sites in the OS (Nair et al., 2005). Although the idea that diffusion coupled with an exchange of binding sites remains attractive, the identity of the binding sites remains in question. Quantitative analysis has shown that photo-activated rhodopsin cannot account for the full magnitude of translocation because the amount of arrestin would exceed the available rhodopsin binding sites by more than an order of magnitude (Strissel et al., 2006).

Summary and Perspective

Rapid progress is being made in understanding the molecular details of IFT transport pathways and ciliogenesis in multiple cell types. Each system has a combination of general features along with cell specific features. The cell specific features of the photoreceptor sensory OS illustrated

in this overview make it an important system that can provide new insights into the function of IFT in trafficking of cell specific cargo. Further studies of photoreceptors will likely yield new molecular insights into the details of how membrane proteins are moved into cilia and how two different kinesin motors, kinesin II and Kif17, are coordinated. In addition to providing insight into membrane protein trafficking by IFT, the high degree of conservation of the IFT through evolution suggests that use of simple IFT model systems will aid in understanding how defects in trafficking can lead to photoreceptor degeneration, and more broadly, to other cilium based diseases.

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Figure 1a

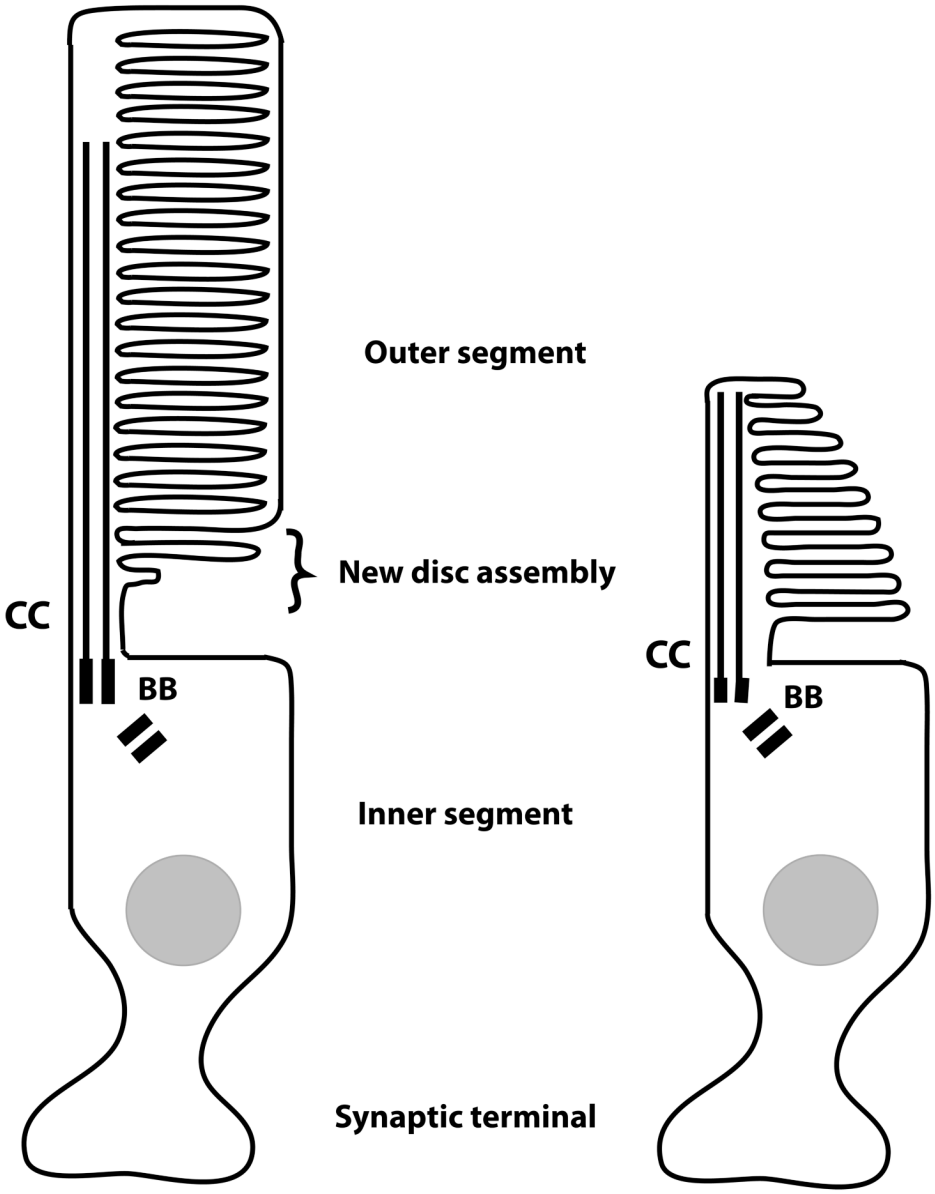

Figure 1b

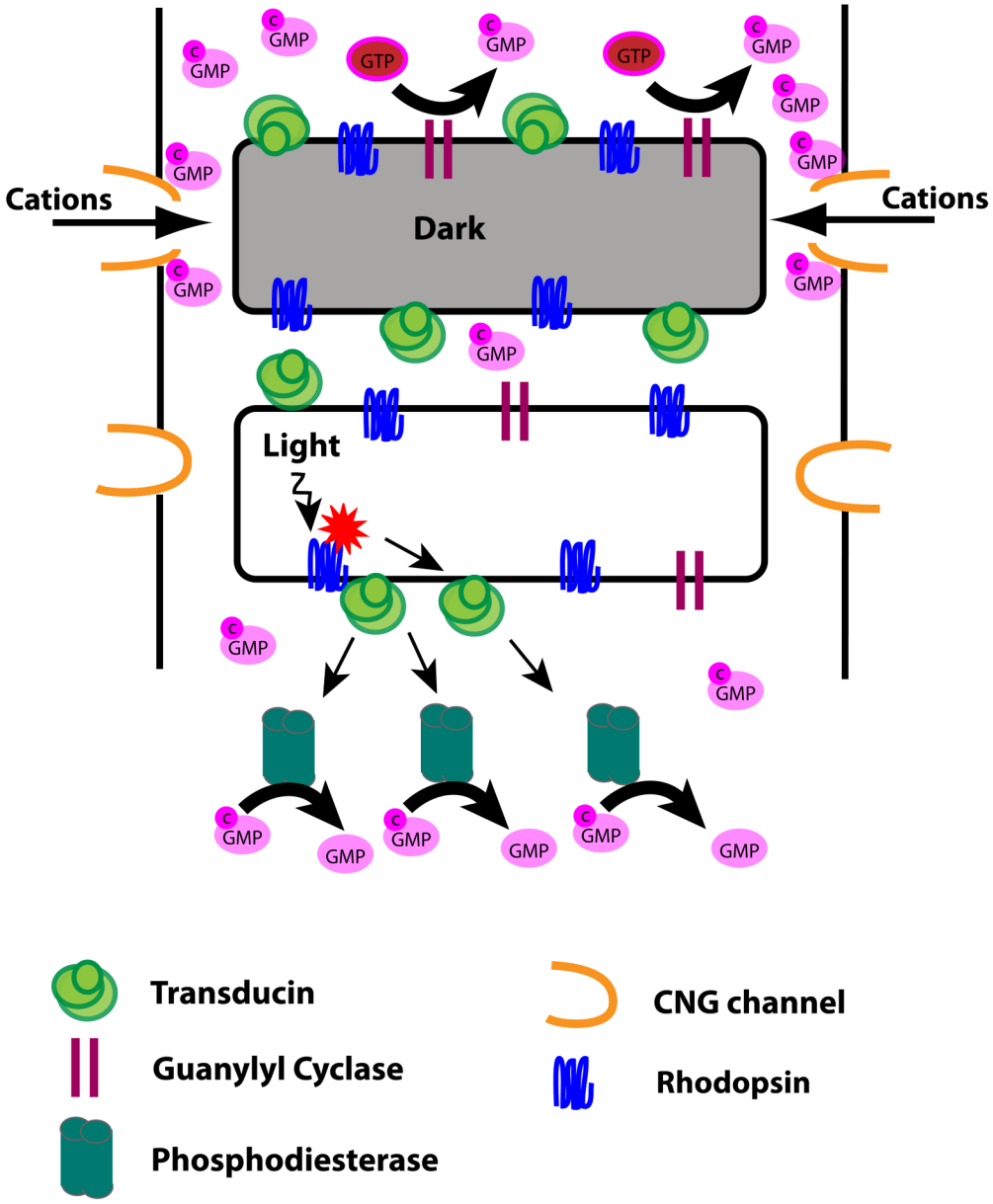

Figure 1. The photoreceptor sensory OS

A. Schematic illustrating the structure of rod and cone photoreceptors. The sensory organelle is composed of a stack of discs that initiated from the ciliary membrane at the base of the cilium. The dendritic portion extending from the basal body (BB) to the nucleus is called the inner segment. The cell body includes the nucleus and a short axon that terminates into a synapse. **B**. Simplified diagram of the phototransduction cascade taking place in the OS in the dark or upon light activation. This version does not include events involved in the deactivation of rhodopsin.

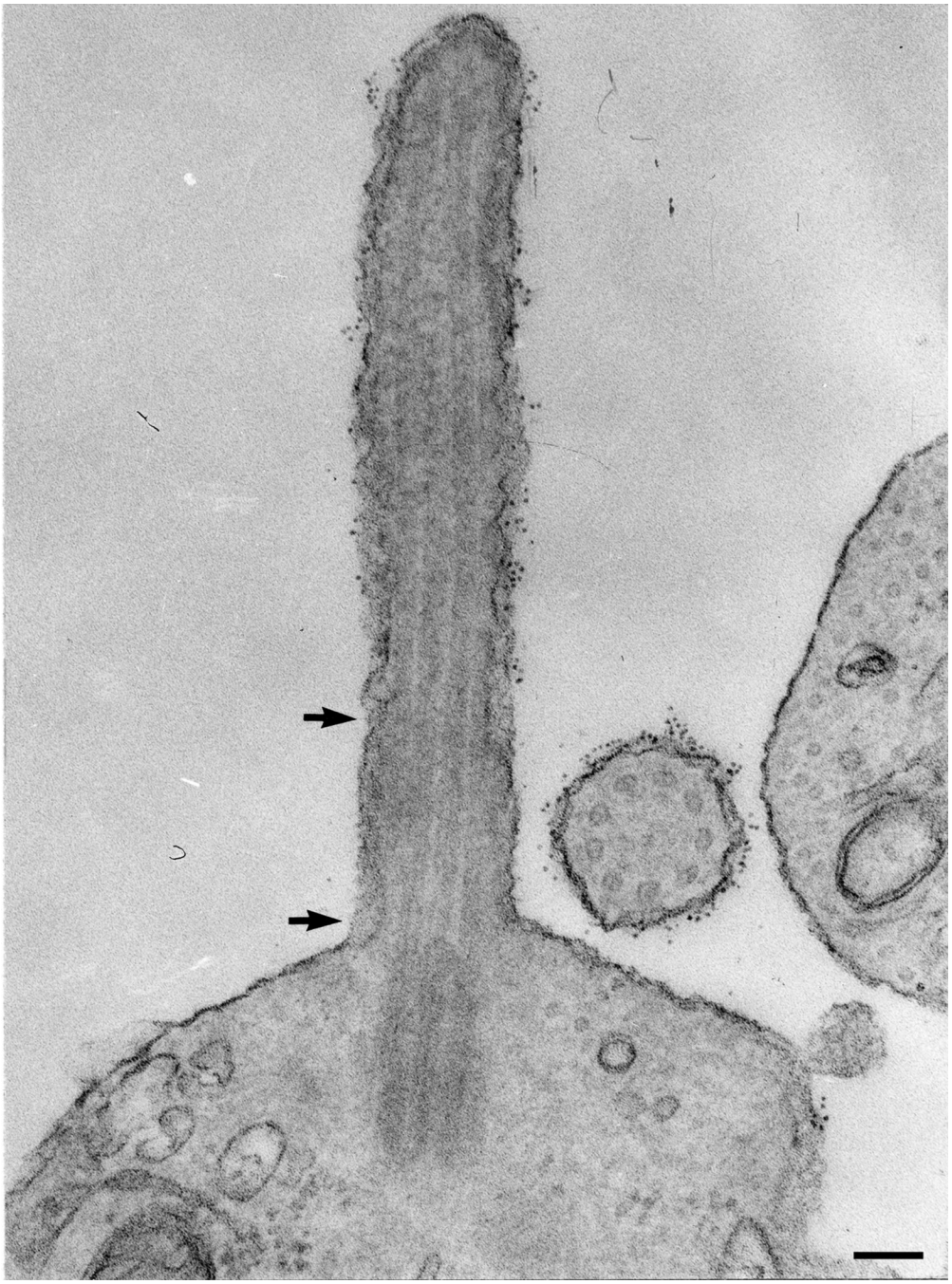

Figure 2. The photoreceptor cilium has a distinct transition zone and distal domain enriched in rhodopsin prior to the formation of discs

Rhodopsin labeling indicated by ferritin is enriched in the distal end and is much less abundant in the transition zone (between the arrows). Scale bar: 0.5 μm. Image was published previously (Besharse, 1986) and is reproduced here with permission of the publisher.

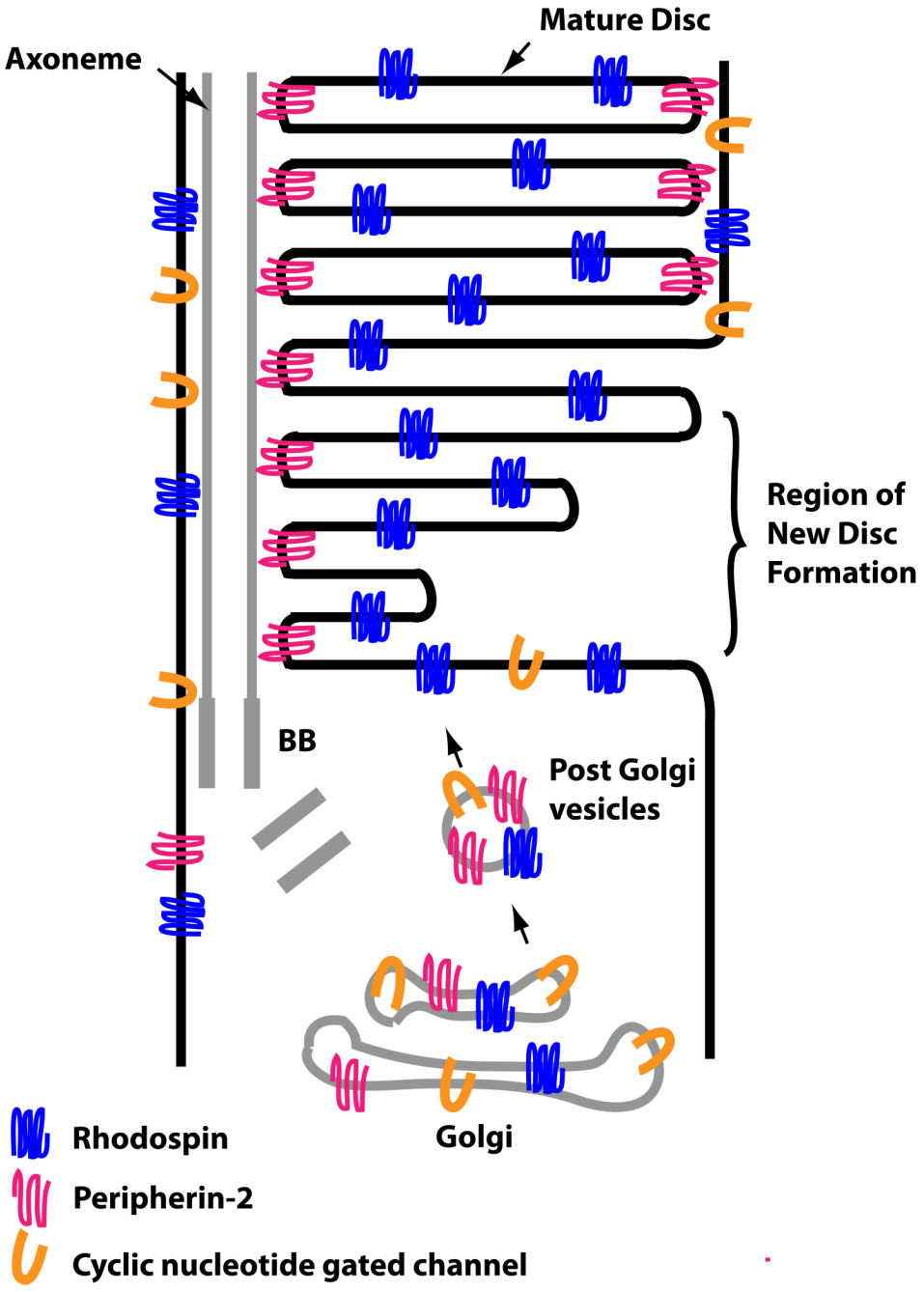

Figure 3. Simplified diagram illustrating the sorting problem for OS membrane proteins during disc formation

Membrane proteins are transported into vesicles from the Golgi complex to the base of the cilium. Once in the OS, they segregate to distinct compartments of the discs or the plasma membrane.

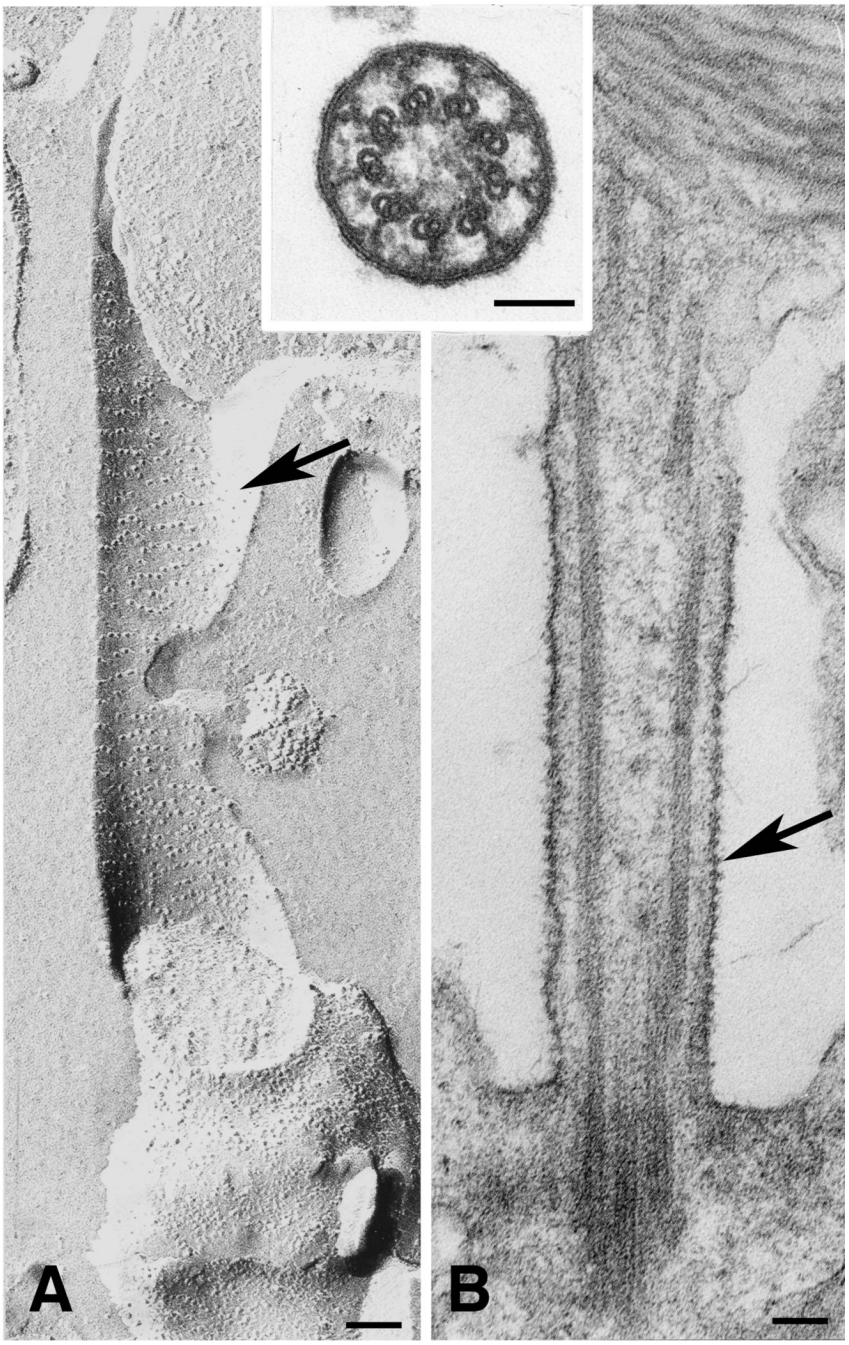

Figure 4. The structure of the connecting cilium is that of a transition zone

Images of a freeze fracture replica (A) and a conventionally stained EM section (B) oriented with the OS above and the inner segment below. Arrows indicate ciliary necklaces in A and bead-like membrane structures in B. Note the presence of fibrils in the extracellular space extending from the cilium membrane. The inset is a cross section showing doublet microtubules and the Y-shaped microtubule-membrane cross-linkers. Magnifcation bars are 0.1 μm for all three images. Image was published previously (Besharse, 1986) and is reproduced here with permission of the publisher.

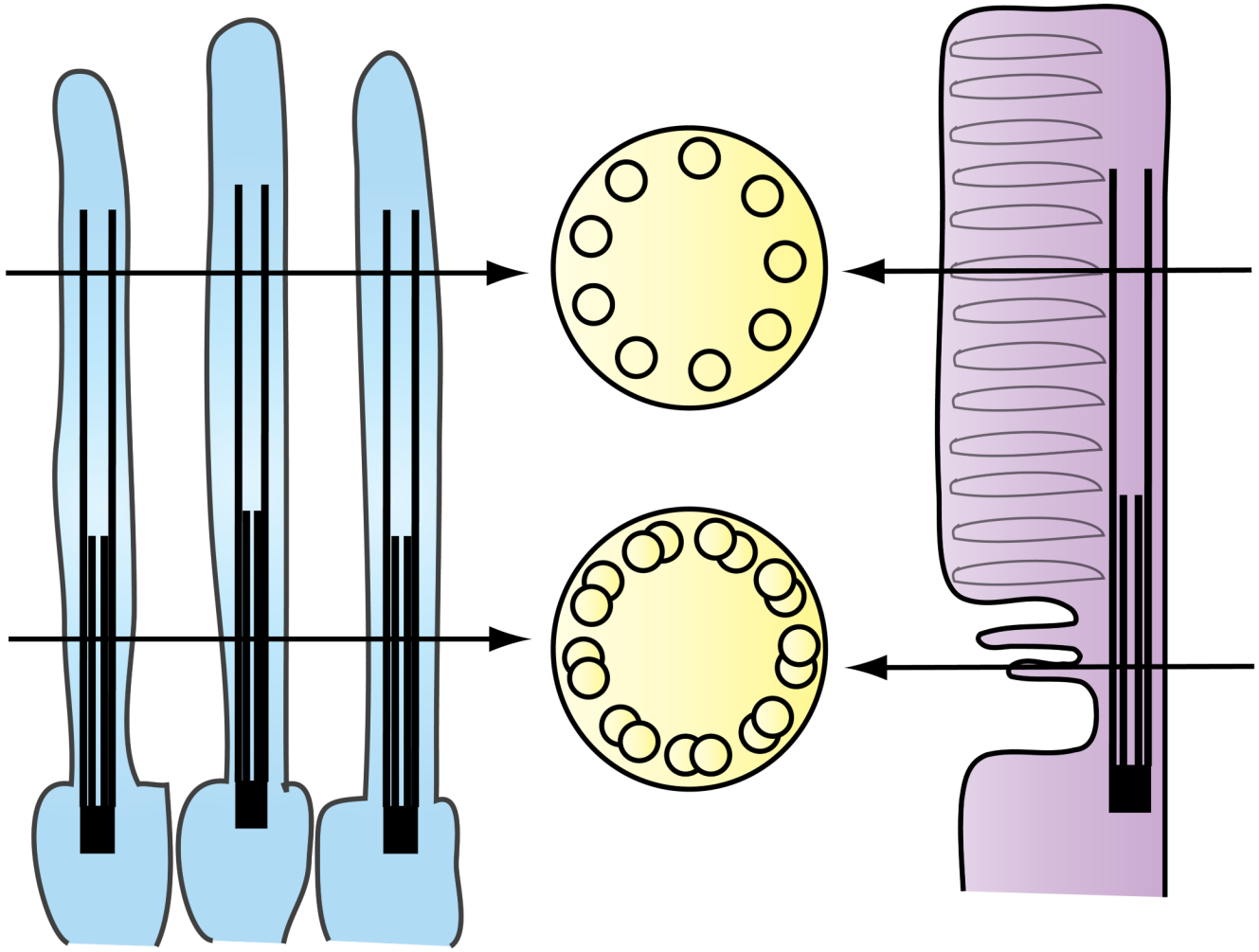

Figure 5. Diagram showing similarities between *C.elegans* amphid channel cilia (left) and the photoreceptor cilium (right)

Both sensory cilia present a bipartite structure with a proximal segment composed of nine microtubule doublets with singlets towards the distal end.

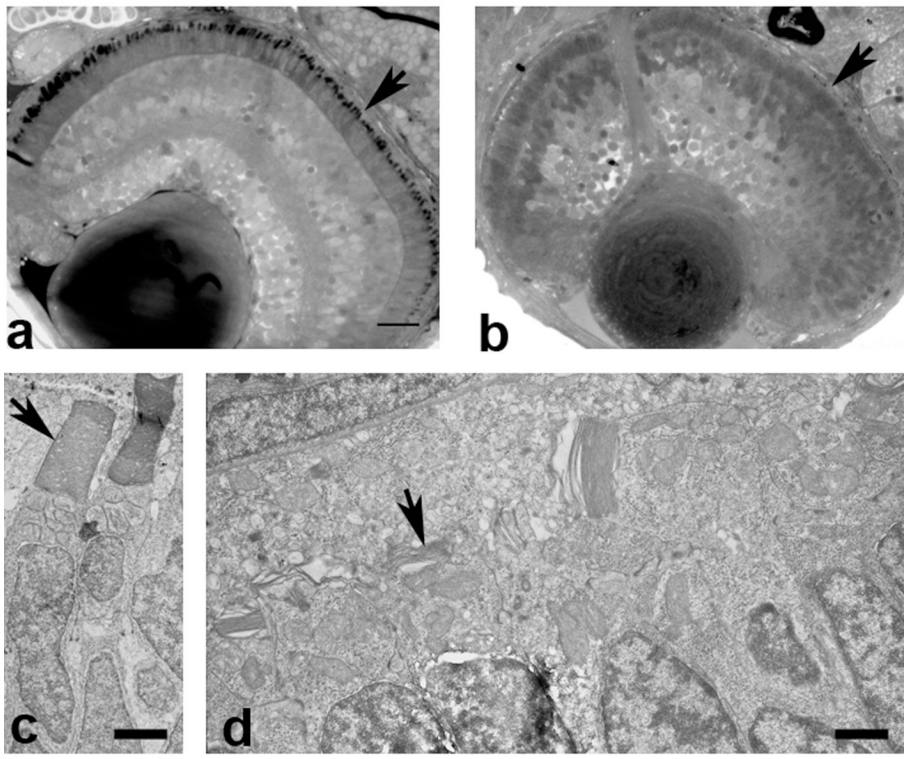

Figure 6. Failure of OS formation in zebrafish embryos depleted of Kif17 protein

A. The retina of a 3 days old wild type embryo develops normal OS (arrowhead). **B**. Embryos injected with an antisense morpholino directed against Kif17 mRNA fail to develop OS (arrowhead) and present a small degree of disorganization of the retina layers. Scale bar in A 10 μm. **C**. EM picture of wild type photoreceptors with normal OS (arrowhead). Scale bar: 3.4 μm. **D**. EM picture of a morphant with very short and abnormal OS. Scale bar: 1.4 μm. Similar images are presented in Insinna, et al. (2008).