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Kinesin-2 family motors in the unusual photoreceptor cilium

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ABSTRACT

This review focuses on recent advances in the understanding of kinesin-2 family motors in vertebrate photoreceptor development. Zebrafish photoreceptors develop by the 3rd day of embryogenesis, making it possible to study mutant phenotypes without the use of conditional alleles. Recent work using a zebrafish *kif3b* mutant allele validates the concept that the heterotrimeric kinesin II motor is generally required for ciliogenesis. In zebrafish photoreceptors, however, loss of *kif3b* function delays but does not block cilium formation. This is thought to occur because both *kif3b* or *kif3c* can dimerize with *kif3a* and function redundantly. The second ciliary kinesin thought to function in photoreceptor cells is *kif17*. Prior work has shown that either morpholino knockdown of this gene or the overexpression of its dominant negative form can reduce or delay photoreceptor cilium development without any evident impact on ciliogenesis in general. This has led to the idea that kif17 may play an important role only in some specialized cilium types, such the one in photoreceptor cells. In a recently identified *kif17* mutant, however, photoreceptor outer segments are formed by 5 dpf and an obvious delay of outer segment formation is seen only at the earliest stage analyzed (3 dpf). This work suggests that *kif17* plays a significant role mainly at an early stage of photoreceptor development. Taken together, these studies lead to an intriguing concept that as they differentiate photoreceptors alter their kinesin repertoire.

In vertebrate photoreceptors, the cilium is the photosensitive compartment of the cell. Photoreceptor cilia are exceptionally bulky and structurally complex compared to those in other cells (Fig. 1A). The most abundant protein component of the vertebrate photoreceptor cilium (traditionally referred to as "photoreceptor outer segment (OS)") is the visual pigment: rod opsin in rods and cone opsins in cones. It is estimated that ca. a billion opsin molecules are stored at any time in fully differentiated photoreceptor cilia (Pugh & Lamb, 2000). To accommodate such a huge quantity of the photopigment, the cone photoreceptor ciliary membrane is dramatically expanded and arranged into hundreds of parallel folds (Kennedy & Malicki, 2009). Similarly, rod photoreceptor cilia contain an extensive array of parallel membranes, which, in contrast to cones, form as stacks of flattened vesicles, and are referred to as discs. Thus both in rods and in cones, the cilium features a long stack of membranes oriented parallel to each other and perpendicular to the ciliary axis.

In addition to the visual pigment, structural proteins and the components of the phototransduction apparatus that function downstream of the photopigment are abundantly present in photoreceptor cilia. Similar to those in other cells, photoreceptor cilia lack the ability to synthesize proteins, and so all their protein components are transported from the cell body. This occurs throughout the lifetime of the cell, as the photoreceptor membrane is removed continuously from the distal portion of the cilium. In the mouse or rat rod photoreceptor, about 10% of membrane is eliminated daily via the phagocytic activity of retinal pigment epithelium from the distal portion of the cilium (LaVail, 1973). Data on opsin density in discs (Calvert et al., 2001; Pugh & Lamb, 2000) and this level of turnover, which amounts to about 80 discs per day (about 2.3 μm of OS length), would require that roughly 100 rhodopsin molecules are transported into the outer segment every second or 8–9 $\times$ 10^6 per day. It should be emphasized that while such estimates are indicative of a high rate of transport, those values are based on the very small diameter of rodent discs. In other vertebrates, such as *Xenopus laevis* with comparable assembly rates but larger discs, the estimate for rhodopsin transported per day can be as much as 10-fold higher (Papermaster et al., 1985).

Although bulky along most of its length, the photoreceptor cilium features a narrow constriction at its base. Cross-sections through this region reveal nine microtubule doublets arranged in a circle, and closely juxtaposed to the ciliary membrane. This segment of the photoreceptor cilium has been historically referred to as "the connecting cilium" (Fig. 1A). Electron microscopy of this region reveals that it contains Y-shaped elements linking ciliary microtubule doublet with the ciliary membrane (Besharse,

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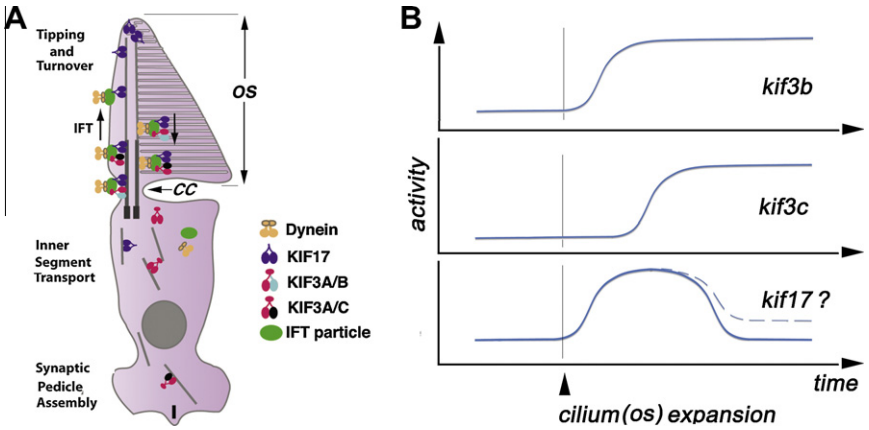

Fig. 1. Possible functions and temporal deployment of kinesin-2 motors during photoreceptor outer segment expansion. (A) Two heterotrimeric motors based on *kif3b* and *kif3c* associated with *kif3a* and one homodimeric (*kif17*) motor are illustrated. Their possible roles in trafficking within the inner segment and synaptic pedicle or in IFT trafficking within the outer segment. In some cilia, kif17 uniquely accumulates at cilium distal tips in a phenomenon called tipping. (B) Plots illustrate that the onset of *kif3b* function occurs earlier than that of *kif3c*. Current data support a role for *Kif17* only during the early phase of cilium formation. This model is proposed primarily for cone photoreceptors. The function of *kif3c* in rods is less clear, as these cells die rapidly in zebrafish *kif3b* mutants. *OS*, outer segment; *CC*, connecting cilium, IFT, intraflagellar transport.

Forestner, & Defoe, 1985; Besharse & Horst, 1990). These structures, known as Y-links, are typical of the ciliary transition zone (Reiter, Blacque, & Leroux, 2012). It is widely believed that all ciliary proteins are transported through this constriction into the more bulky distal part of the photoreceptor cilium.

The arrangement of microtubule doublets at the base of the photoreceptor cilium is typical of the majority of cilia, and suggests that transport mechanisms that operate in this region are also similar to those that function in most cilia. In contrast to other cells however, the sheer size of the photoreceptor cilium suggests that it may require atypical transport mechanisms to efficiently translocate all of its proteins, opsin in particular.

Numerous genetic studies have documented that the heterotrimeric kinesin-II, known to function in cilia of all organisms investigated so far, also plays a major role in vertebrate photoreceptors. As mutations in the kif3a and kif3b subunits of the heterotrimeric kinesin-II are lethal in the mouse, its function in vertebrate photoreceptors has to be studied using conditional alleles. The first study of this type used the IRBP promotor to drive the *cre* recombinase, and inactivate *kif3a* function in both rods and cones (Marszalek et al., 2000). It demonstrated that the loss of *kif3a* function results in the mislocalization of opsin and arrestin, but not transducin and peripherin. The changes in protein localization were followed by photoreceptor degeneration. A more in-depth analysis that followed nearly a decade later used different cre drives to eliminate *kif3a* function separately in rods and cones (Avasthi et al., 2009). Interestingly, this study revealed that *kif3a* functions somewhat differently in rods and cones. The excision of *kif3a* from cones resulted in the mistargeting of outer segment membrane proteins, including opsin, to the cell body. In contrast to that, this phenotype was substantially less pronounced in rod photoreceptors. Nonetheless, rods degenerated more rapidly, compared to cones in these mutants. Although this study clearly implicated *kif3a* in cone opsin transport, it raised questions about its role in the delivery of rod opsin into the rod outer segment. More recent studies support the concept that *kif3a* is required for both rod and cone opsin transport as well as for cell viability (Lopes et al., 2010; Trivedi et al., 2012).

The heterotrimeric kinesin-II consists of two motor subunits, encoded by the *kif3a* gene and either *kif3b* and *kif3c* loci (reviewed in Malicki (2012)). In addition, it contains an accessory non-motor subunit, encoded by *kap3*. Curiously, in contrast to mutations in *Kif3a* and *Kif3b*, knockout of *Kif3c* function in the mouse does not produce any obvious phenotype in photoreceptor cells (Jimeno et al., 2006). Further insights into the function of the heterotrimeric kinesin-II in photoreceptor cilia came from the studies of zebrafish mutant for the *kif3b* motor subunit (Zhao et al., 2012). In contrast to the mouse, and due to the absence of early embryonic lethality, the analysis of zebrafish photoreceptor defects in kinesin-II mutants does not require conditional mutant alleles. This simplifies the interpretation of mutant phenotypes as *kif3b* function is absent from the very outset of photoreceptor differentiation in zebrafish. Surprisingly, *kif3b* mutant cones differentiate cilia as well as relatively robust outer segments. The photoreceptor cilia are shorter, compared to the wild-type, but nonetheless their formation does not appear to require the *kif3b* function. This suggests that another gene functions redundantly with *kif3b* in zebrafish photoreceptors.

The *kif3b* and *kif3c* protein products are closely related, and both bind the *kif3a* protein but not each other (Muresan et al., 1998; Yang & Goldstein, 1998). These observations led to the idea that they may function redundantly as binding partners of the *kif3a* subunit. The *kif3c* protein expression data in photoreceptor cells argued, however, against such a possibility, perhaps delaying experimental tests of the redundancy hypothesis, which was proposed over a decade ago (Muresan et al., 1998; Yang & Goldstein, 1998). Nonetheless, when the redundancy was finally tested by morpholino knockdown of *kif3c* function in *kif3b* mutant zebrafish, it revealed that these two genes, indeed, function partially redundantly in photoreceptor cilia. This is not the case in the majority of other tissues, in which cilia do not form at all in the absence of *kif3b* function alone.

The study of the *kif3b* mutant zebrafish also revealed that cone outer segments form with a delay in this mutant background. This suggested that the *kif3c* kinesin function is not present at the very beginning of cilia formation in vertebrate photoreceptor cells, but, instead, becomes active at a later stage of differentiation. This possibility is supported by the results of *kif3c* overexpression, which induces earlier formation of cilia in *kif3b* mutant background (Zhao et al., 2012). Thus the photoreceptor cell is also unusual in that the repertoire of ciliary kinesins changes as the photoreceptor differentiates.

Although the heterotrimeric kinesin II motor is widely regarded as the canonical IFT kinesin in all cilia and flagella, homodimeric members of the kinesin-2 family (Osm-3, KIN5 and Kif17) have been implicated as ciliary kinesins in multiple systems, including