

Chapter 28

Mutations in the Dynein1 Complex are Permissible for Basal Body Migration in Photoreceptors but Alter Rab6 Localization

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Abstract The photoreceptor outer segment is a specialized primary cilium, and anchoring of the basal body at the apical membrane is required for outer segment formation. We hypothesized that basal body localization and outer segment formation would require the microtubule motor dynein 1 and analyzed the zebrafish *cannonball* and *mike oko* mutants, which carry mutations in the heavy chain subunit of cytoplasmic dynein 1 (*dync1h1*) and the p150^{Glued} subunit of Dynactin (*dctn1a*). The distribution of Rab6, a player in the post-Golgi trafficking of rhodopsin, was also examined. Basal body docking was unaffected in both mutants, but Rab6 expression was reduced. The results suggest that dynein 1 is dispensable for basal body docking but that outer segment defects may be due to defects in post-Golgi trafficking.

Keywords Zebrafish · Retinal development · Dynein · Basal body · Rab

28.1 Introduction

The formation of cilia, including photoreceptor outer segments, requires the migration of a mature centriole to the apical cell surface, where it docks and forms the basal body. Basal body docking requires an intact apical actin network and elements of the planar cell polarity pathway (Boisvieux-Ulrich et al. 1990; Park et al. 2006).

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Further, disruption of the microtubule network by nocodazole did not prevent basal body migration, but did block cilia growth, suggesting microtubule-based motors may function in vesicle-mediated trafficking (Boisvieux-Ulrich et al. 1989). Nevertheless, the identity of the molecular motors and the precise cellular mechanism(s) governing basal body migration remain unclear. As cilia defects cause disorders termed “ciliopathies,” of which retinal degeneration is often a symptom (Kim et al. 2004), it is critical to understand the mechanisms directing basal body localization.

Cytoplasmic dyneins are multisubunit, minus end-directed microtubule motors (Kardon and Vale 2009). Cytoplasmic dynein 1 (Dynein1) controls all minus-end directed microtubule transport within the cytoplasm, while cytoplasmic dynein 2 (Dynein2) transports cargo along the ciliary axoneme. We and others have shown that photoreceptor outer segment formation requires both Dynein1 and Dynein2, but the precise mechanisms remain poorly defined (Tai et al. 1999; Krock et al. 2009; Insinna et al. 2010). We hypothesized that Dynein1 contributes to outer segment development by promoting apical migration of the centriole and tested this hypothesis in zebrafish lacking components of the Dynein1 complex. The zebrafish *cannonball* (*cnb*) mutant contains a null mutation in the heavy chain of Dynein1 (*dync1h1*) (Insinna et al. 2010) while the zebrafish mutant *mikre oko* (*mok*) disrupts the p150^{Glued} subunit of the dynactin complex. Both mutants show outer segment and nuclear positioning defects (Tsujiikawa et al. 2007). We investigated basal body localization in the zebrafish Dynein1 mutant *cnb* and the dynactin mutant *mikre oko*. We also explored the alternate hypothesis that outer segment disruption is due to impaired post-Golgi trafficking by examining the distribution of Rab6 in photoreceptors.

28.2 Materials and Methods

28.2.1 Animal Husbandry

Adult zebrafish were maintained at 28.5°C in recirculating water systems (Pentair, Apopka, FL). The *cannonball* mutant *dync1h1^{mw20}* and the *Tg(-5actb2:cetn4-GFP)* line (Randlett et al. 2011) were gifts from Dr. Brian Link (Medical College of Wisconsin), while the *mikre oko* mutant, *dctn1a^{m632}* was obtained from the Zebrafish International Resource Center (Eugene, OR). All experiments were approved by the IACUC at the Cleveland Clinic and conformed to the ARVO policy on animal care.

28.2.2 Basal Body Localization in Dynein Mutants

Larvae were fixed in 4% paraformaldehyde in PBS, followed by infiltration with 30% sucrose and embedding in Tissue Freezing Medium. Cryosections (10 µm) were stained with Alexa-568 phalloidin (Life Technologies, 1:100) and DAPI. Im-

aging was performed on a Zeiss AxioImager Z.2 fluorescence microscope with ApoTome.2 attachment and AxioCam MRm camera. Images were exported to ImageJ, and basal bodies in single Z slices were categorized as being present in the ONL or apical to it. The ONL was defined as the region of DAPI staining between phalloidin reactivity at the outer limiting membrane and outer plexiform layer. Samples for electron microscopy were prepared as described (Sukumaran and Perkins 2009), although wash and dehydration steps were carried out in a BioWave Pro (Pelco).

28.2.3 Genetic Mosaics and Immunohistochemistry

In vitro transcribed RNA encoding mCherry with a nuclear localization signal (nls-mCherry) was prepared using the Message Machine kit (Ambion) and 50 pg of RNA was injected into *cnb;Tg(-5actb2:ctn4-GFP)* and *mok;Tg(-5actb2:ctn4-GFP)* embryos at the 1-cell stage. Embryos were grown to the 1000 cell stage and cells were transplanted to age-matched wild-type embryos. Donor embryos were genotyped by high-resolution melt curve analysis on a BioRad CFX96 real-time PCR machine. Retinal cryosections of mosaic fish were stained with a polyclonal mCherry antibody (BioVision, 1:500) and imaged as described above. For Rab6 immunostaining, sections were stained with rabbit polyclonal Rab6 antibodies (Santa Cruz, 1:1000), followed by Alexa conjugated secondary antibodies and imaged as described above.

28.3 Results

We examined basal body localization in *cnb* and *mok* larvae harboring the *Tg(-5actb2:ctn4-GFP)* transgene, which expresses a centrin-GFP fusion protein from the actin promoter and labels centrioles and basal bodies. At 2.5 days post fertilization (dpf) phalloidin staining was disorganized and photoreceptor nuclei failed to form an orderly layer in the mutants. By 4 dpf both mutants exhibited a significant degree of retinal degeneration, with rounded nuclei and disorganized lamination (Fig. 28.1a–c). Despite this phenotype, both mutants contained areas of well-preserved apical actin network, in which basal bodies were localized near the OLM. Outside of these areas, basal bodies could be occasionally observed among photoreceptor nuclei in the ONL. Mislocalized basal bodies usually colocalized with ectopic actin staining (Fig. 28.1c, arrow). Semi-quantitative analysis of mutant retinas showed significant mislocalization of basal bodies in 3 dpf *cnb* fish (Fig. 28.1d). Transmission electron microscopy (TEM) revealed properly formed basal bodies and cilia near the apical membrane, but we were unable to locate any mislocalized basal bodies by TEM (Fig. 28.1e–g).

Closer examination of the mislocalized basal bodies revealed that they were apical and adjacent to nuclei that were similarly displaced (Fig. 28.2a–c), sug-

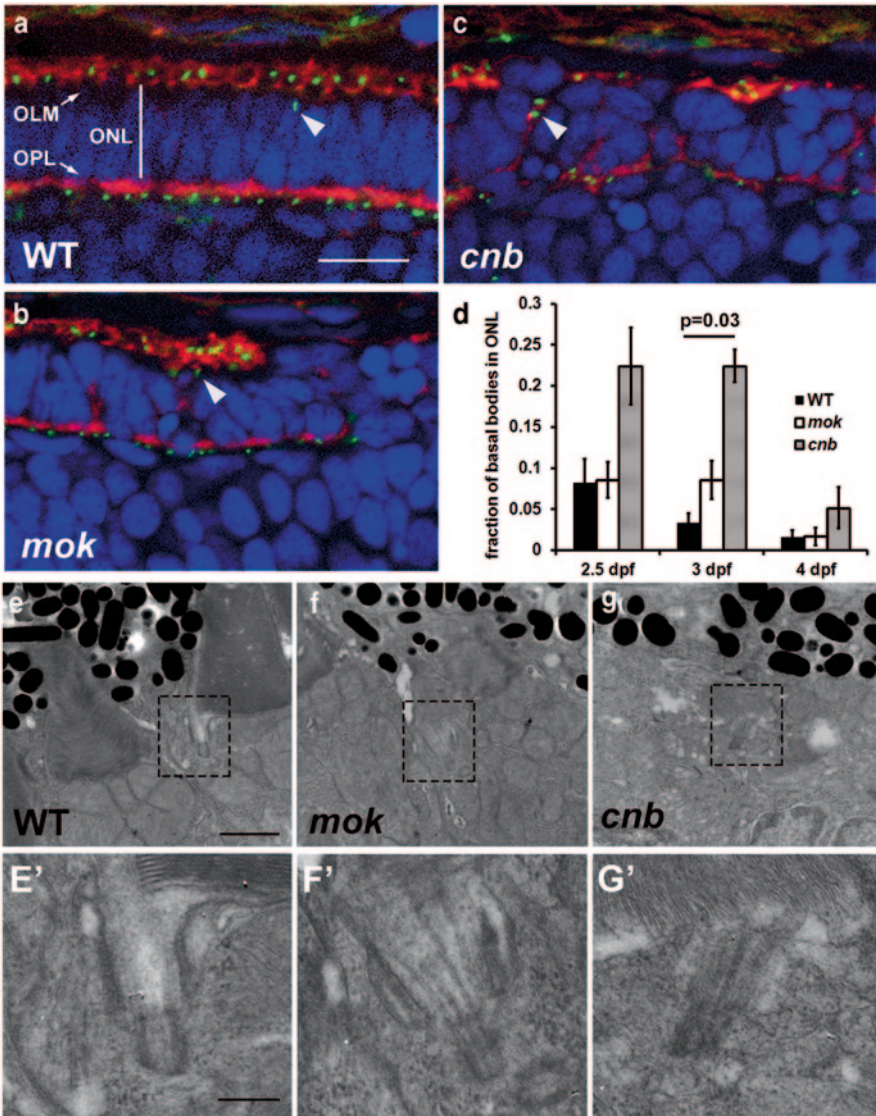


Fig. 28.1 **a–c** Representative images of basal body positioning in 3 dpf larvae. *Green*=centrin-GFP, *red*=phalloidin, *blue*=DAPI. *OLM* outer limiting membrane, *ONL* outer nuclear layer, *OPL* outer plexiform layer. Bar=10 μm. **d** Quantification of basal bodies in the ONL. **e–g** Electron microscopy images of 3 dpf larvae. Bar=1 μm. Boxed areas are magnified in **e'–g'** to show basal bodies (bar=250 nm)

gesting that basal bodies were properly positioned in the displaced cells. To test this hypothesis, mosaic animals were generated by blastula transplantation to assign individual basal bodies to their nuclei. *cnb*; *Tg(-5actb2:cetn4-GFP)* and *mok*; *Tg(-5actb2:cetn4-GFP)* embryos were injected with RNA encoding NLS-mCherry

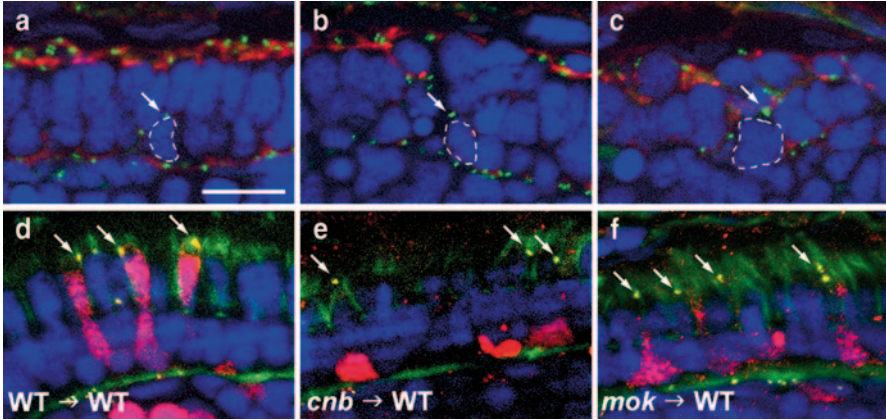


Fig. 28.2 **a–c** Basally displaced nuclei (*blue, outlined*) are adjacent to mislocalized basal bodies (*green, arrows*). Phalloidin staining (*red*). **d–f** Genetically mosaic 5 dpf fish expressing centrin-GFP (*yellow*) and nuclear mCherry (*red*) in donor cells. Basally displaced photoreceptor nuclei (*asterisks*) are associated with properly localized basal bodies (*arrows*). Sections are stained with phalloidin (*green*) and DAPI (*blue*). Bar=10 μ m

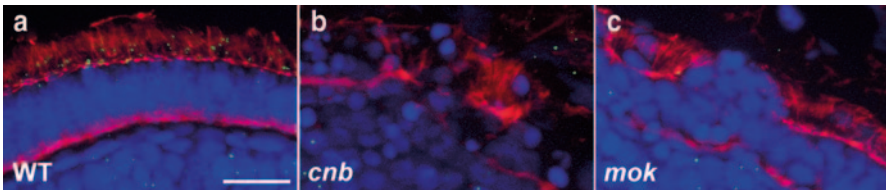


Fig. 28.3. Rab6 immunostaining (*green*) is reduced in *cnb* and *mok* larvae at 4 dpf. *Red*=phalloidin, *blue*=DAPI. Bar=10 μ m

to label nuclei. Transplanted donor cells from these embryos had mCherry-labeled nuclei and GFP-labeled basal bodies in an unlabeled wild-type host. While the nuclei of mutant donor cells were frequently positioned at the basal extent of the ONL, consistent with previous observations (Insinna et al. 2010), the basal bodies of these cells were not only apical relative to the cell body but also properly positioned near the OLM (Fig. 28.2d–f). This indicated that the apical domain remained intact despite the majority of the cell’s volume being displaced, and suggested that *dync1h1* and *dctn1a* are dispensable for basal body migration in retinal photoreceptors.

An alternative hypothesis for the disruption of outer segment formation in *cnb* and *mok* larvae is that loss of Dynein1 activity blocks ciliary transport of post-Golgi vesicles. Rab6 is present in the trans-Golgi and in rhodopsin transport carriers, and interacts with the dynein complex and the dynein light chain DYNLRB1. Staining with Rab6 antibodies revealed fewer Rab6-positive foci in *cnb* and *mok* retinas at 4 dpf, suggesting that post-Golgi trafficking is disrupted in mutant cells (Fig. 28.3).

28.4 Discussion

Our finding that basal body positioning at the apical membrane during ciliogenesis is independent of Dynein1 function is somewhat surprising, especially given the role of dynein in other centriole functions such as spindle positioning (Kiyomitsu and Cheeseman 2013). However, evidence from multiciliated epithelial cells suggests that basal body positioning depends on an intact actin network, suggesting a myosin motor (Boisvieux-Ulrich et al. 1990). In the developing retinal epithelium, basal bodies remain apically polarized except during M phase, after which they quickly return to the apical membrane. This phenomenon is conserved even after *centrin2* knockdown, which destabilizes tubulin (Norden et al. 2009). These observations, when combined with the results from genetic mosaic animals presented here, argue against a role for microtubule-based motors in basal body migration.

We evaluated a role for dynein-based motility on post-Golgi trafficking in zebrafish photoreceptors. The dynein light chain Tctex-1 binds rhodopsin (Tai et al. 1999), and minus-end directed motors are thought to transport rhodopsin from the Golgi (Troutt and Burnside 1988). Moreover, Rab6 and Rab11 label rhodopsin-containing vesicles and interact with components of the dynein-dynactin complex (Short et al. 2002; Wanschers et al. 2008; Mazelova et al. 2009). Our finding that Rab6 immunoreactivity is decreased in *cnb* and *mok* photoreceptors indicates that interactions between the dynein complex and post-Golgi trafficking machinery are critical for outer segment development, and may explain why mutant outer segments fail to elongate.

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