

An old dog learns new tricks: novel functions of the exocyst complex in polarized epithelia in animals

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Abstract

The role of the exocyst complex has been studied mainly in the context of basolateral sorting of cargos in polarized cells. Recent developments indicate an extended yet specific function of the exocyst in the outgrowth of the primary cilium from the apical membrane, thereby highlighting a role for the exocyst in ensuring membrane trafficking to important signaling stations in the cell, the tight junctions, and the cilia.

Introduction and context

Cell polarity is maintained in part by targeted vesicular trafficking to the apical and basolateral membrane domains as well as the primary cilium of polarized cells. The cilium is a rod-like organelle featured on the apical plasma membrane domain on most epithelial cells in vertebrates, although the composition of the cilium membrane is not well understood. Increasing evidence indicates that polarized cell homeostasis is strongly affected by the 'health' of primary cilia, perhaps because cilia are needed for numerous cell signaling pathways such as polycystin, Wnt, and Hedgehog signaling [1,2]. Acute deciliation can result in the loss of polarity in cells, implying an important role for the cilium in maintaining cell polarity [3]. Furthermore, the loss of ciliary function is associated with cystic livers and kidneys as observed in Bardet-Biedl syndrome (BBS) and autosomal dominant polycystic kidney disease (ADPKD) [1].

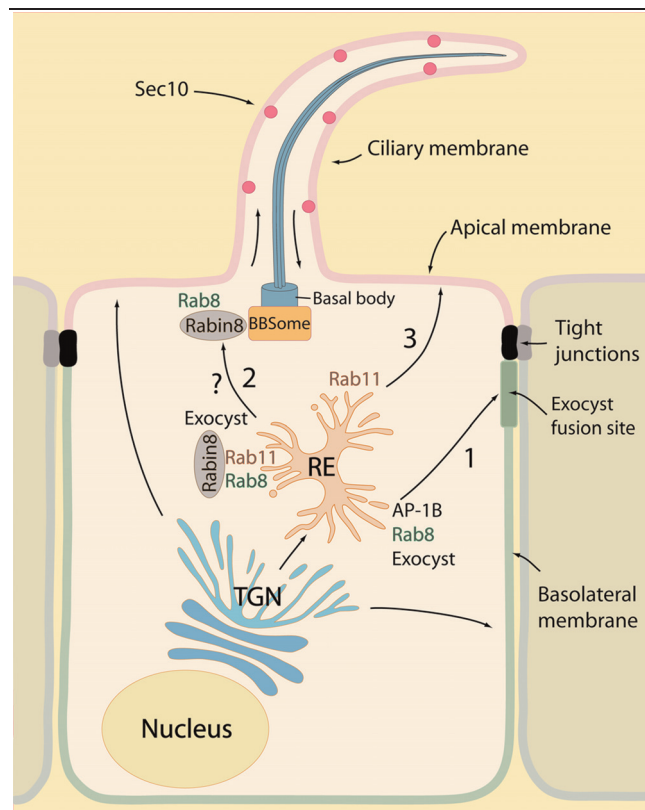
The exocyst is a highly conserved complex with important roles in mediating targeted vesicular trafficking in polarized cells. The exocyst is an eight-subunit complex (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84) that was first identified as a requirement for exocytosis of vesicles at the bud tips of yeast [4]. In addition, the small GTPase Sec4 is essential for post-

Golgi trafficking in yeast [5], and the exocyst complex functions as an effector for Sec4 [6]. Biochemical evidence further indicates that yeast Sec3 interacts with the activated form of Cdc42 [7]. A current model of the exocyst delineates its function as a tethering complex in the trafficking of vesicles from a post-Golgi compartment, the recycling endosome, to the basolateral plasma membrane in columnar epithelial cells [8,9]. To accomplish this task, the exocyst associates with vesicles that contain the epithelial cell-specific clathrin adaptor complex AP-1B [10]. This process is regulated by the small GTPases Rab8, Rab10, Cdc42, and RalA [11,12]. Biochemical data indicate that both Sec5 and Exo84 interact with RalA in its GTP-bound form [13-16], and structural and biochemical work further reveals that Sec5 and Exo84 competitively bind to active RalA [17,18], suggesting that RalA plays an important role in regulating exocyst assembly. These data established a role for the exocyst in basolateral sorting of cargo in polarized epithelial cells (Figure 1, arrow 1).

Major recent advances

Over the last couple of years, there has been mounting evidence that the exocyst also plays a role in vesicular trafficking to the cilium. Rogers *et al.* [19] were the first to show localization of the exocyst subunits Sec6/8 at the base of the primary cilium in addition to its known

Figure 1. Hypothetical model of the different roles of exocyst complexes in polarized epithelial cells



The sorting of biosynthetic cargos to different plasma membrane domains takes place either at the trans-Golgi network (TGN) or in recycling endosomes in polarized epithelial cells. Vesicular trafficking to the cilium may originate in recycling endosomes where Rab8 or Rab11 or both may regulate the organization of distinct subdomains. The exocyst has been shown to play a role in vesicular trafficking from the recycling endosomes (RE) to the basolateral membrane (arrow 1) as well as in trafficking to the cilium in association with Rab8, Rabin8, and Rab11 (arrow 2). Vesicles may be directed to the base of the cilium by the interaction of Rabin8 and BBS1. Rab11 also has a known role in sorting to the apical membrane (arrow 3).

localization just below the tight junctions. They also demonstrated that Sec6/8 are overexpressed in ADPKD primary cell cultures and immortalized cell lines, suggesting a link between the ciliopathy ADPKD and the exocyst [19]. A recent study then demonstrated a direct role for the exocyst in primary ciliogenesis and cystogenesis in Madin-Darby canine kidney (MDCK) cells [20]. Knockdown of Sec10 in MDCK cells resulted in a decrease in cilium length which could be rescued with exogenous Sec10 expression [20]. In addition, knockdown of Sec10 resulted in decreased expression levels of Sec8 and Exo70, indicating that Sec10 may play a central role in exocyst organization at the cilium [20].

Moreover, knockdown of Sec10 decreased the levels of the intraflagellar transport protein 88 (IFT88), suggesting that trafficking of IFT88 to cilia may be exocyst-dependent [20]. In the future, it will be interesting to learn whether similar findings will be observed with genetic mutations of Sec10 or other exocyst components.

Notably, the exocyst is not the only component with a dual function in targeting to the basolateral membrane and cilia. Rab8a, which was described as a GTPase involved mainly in regulating cargo sorting to the basolateral membrane [21], was recently shown to localize to the primary cilium membrane in human retinal pigmented epithelial cells and to function in primary cilium formation [22]. Similarly, in cilia-derived sensory organelles, the rod outer segments, Rab8 plays a role in docking and fusion of rhodopsin transport carriers (RTCs) [23], and Rab8 co-localizes with Sec8 in the vicinity of RTC fusion sites in *Rana berlandieri* frog cells [24]. Interestingly, the Rab8 guanine nucleotide exchange factor Rabin8 interacts directly with the BBS1 subunit of a multimeric complex, the BBSome, that localizes at the base of the cilium and regulates ciliary membrane biogenesis [25]. It is Rabin8 that provides a link between Rab8 and the exocyst complex. Biochemical data indicate that Rabin8 may interact with Sec15 and GTP-loaded Rab11 (Wei Guo, personal communication). Furthermore, Rab11 has been shown to bind to the C-terminus of Sec15 [26-28], indicating a cooperative action between the exocyst and Rab8 and Rab11 GTPases in regulating vesicular trafficking to the primary cilium (Figure 1, arrow 2).

The role of the exocyst in sorting to cilia or cilia-derived structures seems to be conserved from invertebrates to vertebrates, although the majority of epithelial cells in invertebrates do not contain cilia. However, *Drosophila* photoreceptor cells have a light-sensing microtubule structure on their apical stalk membrane. Sorting of rhodopsin1 to rhabdomeres and rhabdomere formation are also dependent on Sec6, Sec15, and Rab11 function [27,29], highlighting the importance of the exocyst in cilia formation.

Future directions

Models describing vesicular trafficking into the cilia are sparse in the literature. However, this should soon change with the identification of players involved in this pathway. In the effort to establish a model, certain questions become apparent. For example, it is intriguing to note that the exocyst and Rab8 are used for both cilium outgrowth and sorting to the basolateral membrane, whereas Rab11 plays a role in cilium biogenesis in

addition to its known function in regulating apical delivery of the polymeric IgA receptor as it transcytoses from the basolateral to the apical membrane (Figure 1, arrow 3) [26]. How are these different pathways delineated? Given the known localization of Rab8, Rab11, and the exocyst in different areas of recycling endosomes [10,21,26,30], it seems conceivable to assume that the pathway to the cilium involving these components may be originating in recycling endosomes. Perhaps the recycling endosomes are organized in three subdomains, each regulating a specific trafficking step and thereby preventing missorting of apical or basolateral cargos into the cilium. Indeed, the organization of recycling endosomes into apical and basolateral domains is so dramatic that they are often referred to as 'apical recycling endosomes' and 'central recycling endosomes' (for a detailed discussion of this topic, see [31]). Perhaps Rab8 and Rab11 are involved in forming such subdomains. Future studies will be necessary to analyze whether this is indeed the case. Another unknown is where vesicles that are targeted to the cilia fuse with the ciliary membrane. Perhaps they are directed first to the base of the cilium via the interaction between Rabin8 and BBS1. From the base of the cilium, vesicles may be directed into the cilium on microtubule tracks because Sec10 stains the entire length of the cilia membrane [20], implying that fusion of vesicles takes place along the ciliary membrane; however, it is not clear whether such fusion might occur as there is currently no evidence for the presence of transport vesicles in cilia. Alternatively, fusion of vesicles may occur at the base of the cilium, thereby leading to the outgrowth of ciliary membrane. As researchers continue to make strides toward understanding the role of the exocyst in polarized epithelial cells, mechanisms of how one complex may specifically regulate multiple membrane trafficking steps will be unraveled.

Abbreviations

ADPKD, autosomal dominant polycystic kidney disease; BBS, Bardet-Biedl syndrome; IFT88, intraflagellar transport protein 88; MDCK, Madin-Darby canine kidney; RTC, rhodopsin transport carrier.

Competing interests

The authors declare that they have no competing interests.

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