KIF16B delivers for transcytosis

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The EMBO Journal (2013) 32, 2093–2095. doi:10.1038/emboj.2013.153; Published online 28 June 2013

Protein sorting pathways control correct delivery of membrane proteins to specific compartments of the plasma membrane and are required to maintain the physiological functions in all epithelia. Most clathrin-dependent cargoes require the adaptor protein complexes AP-1A and AP-1B for proper sorting to the basolateral plasma membrane. In this issue of *The EMBO Journal*, Perez Bay *et al* (2013) shed light on the mechanism of basal-to-apical protein transport, or transcytosis, of the transferrin receptor in natively AP-1B-deficient epithelia. In AP-1B-deficient epithelia, the transferrin receptor transcytoses through the apical recycling endosome, and requires Rab11. Furthermore, they characterize a novel and specific role for the endosomal microtubule motor Kinesin KIF16B in transferrin receptor apical transport. These findings constitute the first characterization of a specific microtubule motor involved in basal-to-apical transcytosis in epithelia.

Epithelial cells present a compartmentalized plasma membrane, where the composition of each compartment is tightly controlled by a precise protein and lipid sorting machinery (Folsch, 2008). The two most conspicuous compartments are the apical and basolateral domains, which generate and segregate from each other through the formation of apically localized junctional complexes. Protein sorting mechanisms ensure delivery of newly synthesized or recycled, protein components to their proper localization in either the apical or basolateral plasma membrane domains. Vectorial transport of proteins requires sorting determinants that are present in the cytoplasmic, transmembrane or extracellular domains. Most of the information that we have about these sorting determinants comes from the basolateral traffic, which depends on clathrin adaptor proteins (APs) AP-1A/B, AP-3 and AP-4 (Gonzalez and Rodriguez-Boulant, 2009). Specific APs bind to cytoplasmic sorting motifs in transmembrane proteins and recruit clathrin-coat components, which sequentially induce membrane curvature, clathrin oligomerization, vesicle budding and fission (Ohno, 2006; Hirst *et al*, 2011). Mammalian cells present five different AP complexes (AP1–5), each constituted by a heterotetramer of one α -, γ -, δ -, ϵ - or ζ -subunit, one β (1–5) subunit, one σ (1–5) subunit and one μ (1–5) subunit. How these clathrin-coated vesicles deliver membranes to precise compartments in the cell to regulate protein sorting is still poorly understood. The AP1

complex is a key regulator of basolateral polarity (Folsch *et al*, 1999; Gan *et al*, 2002; Gravotta *et al*, 2012). The AP1 complex μ -subunit presents two isoforms μ 1A and μ 1B, which define the formation of two different complexes, AP-1A and AP-1B, both required for basolateral polarity. AP-1A is ubiquitously expressed in different tissues and localizes mainly to the *trans*-Golgi network. In contrast, AP-1B is primarily localized to common recycling endosomes (CRE) and is specifically expressed in the majority of epithelial tissues, with the remarkable exception of retinal pigment epithelium and the proximal convoluted tubule in the nephron, which sort most of the basolateral cargo to the apical surface.

A wide array of model membrane proteins requires AP-1B to properly localize to the basolateral membrane, including the low-density lipoprotein receptor (LDLR), the VSV-G protein and the transferrin receptor (TfR). Furthermore, the expression of μ 1B in μ 1B-deficient epithelial cell line LLC-PK1 is sufficient to prevent apical sorting of TfR, indicating that AP-1B is a main player in this clathrin-mediated basolateral sorting pathway. Interestingly, the results of the present study suggest that transcytosis (a membrane trafficking pathway that transports apical or basolateral proteins to the opposite domain in the plasma membrane) is the main mechanism for apical transport of clathrin-dependent cargoes in AP-1B-deficient cells. Basal-to-apical transcytosis of the polymeric IgA receptor (pIgAR) is the best-known transcytotic pathway, and requires several steps in which the receptor complex traverses multiple compartments before reaching a Rab11-positive apical recycling compartment, from where it is sorted to the apical plasma membrane (Golachowska *et al*, 2010). Polymeric IgA receptor transcytosis requires the function of cytoskeletal proteins for its correct delivery to the apical membrane, including microtubules and actin binding motors. However, no specific microtubule motor has ever been described associated with transcytosis.

In the present study, Perez Bay *et al* (2013) analyse how the TfR is transported to the apical membrane in μ 1B-deficient epithelia using as model system the retinal pigment epithelium cell line, which lacks AP-1B, and MDCK cells. They show that basolateral administration of labelled Tf results in its endocytosis and transcytosis towards the apical membrane in AP-1B-depleted MDCK cells, following a pathway that involves Rab11-positive apical recycling

Figure 1 KIF16B controls basal-to-apical transcytosis of transferrin receptor in AP-1B-deficient epithelia. In AP-1B-expressing epithelia (such as MDCK cells), transferrin receptor (TfR) is endocytosed and sorted to common recycling endosomes, where AP-1B-clathrin-vesicles assemble and transport the protein to the basolateral plasma membrane. In AP-1B-deficient epithelia (such as RPE cells), internalized TfR is instead sorted by the plus-end directed microtubule motor KIF16B towards the ARE, and then transcytosed to the apical plasma membrane through a Rab11-regulated pathway. Polymeric IgA receptor is internalized into the same basolateral endosomes, but it uses a KIF16B-independent pathway to reach the apical membrane.

endosomes (AREs), and requires Rab11 for its correct delivery. Additionally, they find that TfR transport into AREs depends on microtubules and the kinesin KIF16B, a specific microtubule motor present in the CRE (Figure 1). KIF16B is a plus-end microtubule motor that binds to *PtdIns(3)P* and GTP-bound Rab14 and regulates the distribution of early endosomes (Hoepfner *et al*, 2005; Ueno *et al*, 2011). Surprisingly though, apical transport of pIgAR is not affected by the expression of a KIF16B-dominant negative mutant, which suggests that assembly of KIF16B/TfR carriers occurs downstream of cargo separation during transcytosis. It is also tempting to speculate that more than one transcytosis pathways are at play, and while TfR uses the KIF16B-dependent pathway, pIgAR is transported through a KIF16B independent mechanism. This article is the first study of KIF16B in epithelial cells, and the first showing involvement of a microtubule motor in transcytosis, more than 20 years after the pioneering studies that characterized the role of microtubules in this process (Hunziker *et al*, 1990).

As a whole, this paper represents a significant advance in our understanding of the protein sorting machinery in epithelial cells, and importantly, opens new questions that will be addressed in future studies. First, is the KIF16B-dependent recycling/sorting pathway required for other cargoes, especially in AP-1B-positive epithelia? Second, why TfR, but not pIgAR, requires KIF16B for correct sorting? Although KIF16B is not required for pIgAR transcytosis, its transport route still requires microtubules, thus opening the possibility for discovery of additional microtubule motors involved in transcytosis. And finally, what is the mechanism of KIF16B binding to TfR-positive recycling endosomes? It is possible that the mechanism depends on the activation of Rab14, which has been characterized as a regulator of lipid-raft transport from the Golgi apparatus to recycling endosomes (Ueno *et al*, 2011).

Conflict of interest

The authors declare that they have no conflict of interest.

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