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Regulation of membrane trafficking in polarized epithelial cells

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Abstract

Polarized epithelial cells continuously sort transmembrane proteins to either apical or basolateral plasma membrane domains. Research in recent years has made tremendous progress in understanding the molecular mechanisms of the major pathways to either basolateral or apical domain. This understanding will help us elucidating how these pathways are interconnected in ensuring maintenance of cell polarity and integrity of epithelial monolayers.

Introduction

During polarization epithelial cells such as MDCK cells develop biochemically and functionally distinct apical and basolateral membrane domains, which are separated by tight junctions [1]. Therefore, to maintain cell polarity epithelial cells have to ensure proper delivery of apical and basolateral cargo to their respective target location. The sorting of transmembrane proteins into distinct transport carriers occurs either in the trans-Golgi network (TGN) during “direct” biosynthetic delivery or in common recycling endosomes (RE) during “indirect” biosynthetic delivery or recycling of cargo internalized from either plasma membrane domain (Figure 1) [1,2]. During endocytic recycling, internalized proteins enter first apical or basolateral early endosomes underlying their respective membrane domain (AEE or BEE) [3]. Subsequently, cargo may enter common recycling endosomes from which they are sorted back to the basolateral membrane or move into Rab11-positive apical recycling endosomes (ARE) for apical delivery. ARE might either be distinct from or a subdomain of common recycling endosomes [4,5].

Polarized protein delivery is aided by sorting signals contained within the transmembrane proteins themselves, which are recognized by sorting machineries. Furthermore, this process is regulated by small GTPases of the Ras superfamily. After the formation of transport vesicles, they have to be moved to the right target location where tethering proteins adhere vesicles to target membranes prior to SNARE-mediated membrane fusion. As researchers add on to the list of established proteins involved in polarized membrane trafficking, we begin to unravel the underlying molecular mechanisms, appreciate commonness and acknowledge differences. Due to space limitations, this review will focus mainly on sorting to the apical or basolateral domains along the biosynthetic pathway.

Apical pathways

Apical sorting information typically consists of either ectodomain signals (*N*-linked or *O*-linked glycans), comprises of special features of the transmembrane domain, is mediated by a

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glycosylphosphatidylinositol (GPI) anchors (GPI-anchored proteins) together with an ability of the GPI-anchored proteins to oligomerize during passage through the Golgi complex [6], or may be decoded by cytoplasmic domains [1]. A common prerequisite for apical sorting seems to be a clustering of the apical proteins into either glycolipid raft domains or non-raft carriers, perhaps with the help of lectins that recognize *N*- or *O*-linked glycans for direct delivery from the TGN to the apical membrane (pathways 1 and 2 in Figure1).

Raft-dependent apical transport

Glycolipid rafts are defined as a clustering of sphingolipids (glycosphingolipids and sphingomyelin) and cholesterol into membrane microdomains that are resistant to extraction with Triton X-100 at 4°C [7]. Proteins associated with glycolipid rafts are caveolin 1 and MAL/VIP17, which may play a role in organizing or stabilizing the raft domains [7]. Cargos that segregate into glycolipid rafts include placental alkaline phosphatase (PLAP, a GPI-anchored protein that forms oligomers), dipeptidyl peptidase IV (DPP-IV), influenza hemagglutinin (HA) and sucrase-isomaltase [6-9]. Clustering of glycoproteins into lipid rafts may be facilitated by sorting lectins. Indeed, galectin-4 was shown to bind glycosphingolipids on the luminal site of the vesicles and RNAi mediated knock down of galectin-4 led to sorting defects of DPP-IV [8].

Recently, it was suggested that the phosphatidyl(4)phosphate (PI[4]P) binding protein FAPP2 is an adaptor protein associated with apical delivery [10]. FAPP2 has a PH domain that binds PI(4)P and activated Arf1 in the TGN [11]. Knock down of FAPP2 in polarized MDCK cells resulted in a kinetic delay of apical delivery of a variety of different cargos including HA and GPI-anchored proteins [10]. Interestingly, FAPP2 also contains a glycolipid-transfer-homology domain, and it seems that FAPP2 is necessary for transport of glucosylceramide from the cis-Golgi (where it is generated) to the TGN where it serves as a precursor for the synthesis of glycosphingolipids [12]. Therefore, it has been speculated that FAPP2 might coordinate glycosphingolipid synthesis with apical transport [12].

After fission from the TGN, HA containing vesicles were found associated with the negative-end directed microtubule motor Kif3C [13], which may transport these vesicles to the apical membrane perhaps together with cytoplasmic dynein [14]. In addition, Kif3C-positive vesicles also contained annexin XIIIb [13]. Typically, annexins bind to membranes upon Ca^{2+} stimulation and might therefore be involved in regulated secretion [15]. However, annexin XIIIb is N-myristylated and binds to membranes even in the absence of Ca^{2+} [16]. Annexin XIIIb is needed for apical delivery of HA [16]. Furthermore, annexin II in complex with S100A10 was shown to play a role in apical secretion of sucrase-isomaltase [9]. Interestingly, annexins have been shown to self-aggregate and to localize to TGN/endosomes and to the apical membrane [15]. Thus, perhaps annexins serve as tethering factors between apical carriers and the apical plasma membrane [15]. Fusion at the apical membrane may then be mediated by the v-SNARE TI-VAMP and the apically localized t-SNARE syntaxin 3 [17-20].

Non-raft associated apical transport

While raft-dependent apical transport is well established, less is known about apical targeting of proteins that do not associate with raft domains such as lactase-phorizin hydrolase (LPH), gp114, and neurotrophin receptor (p75) [21]. However, clustering of non-raft proteins may still play a role in their apical delivery. Indeed, recently galectin-3 was shown to be associated with the luminal site of non-raft, LPH-containing vesicles [21]. Galectin-3 can form oligomers and is involved in clustering glycoproteins for vesicle formation [22], and knock down of galectin-3 inhibited apical delivery of LPH, gp114 and p75 [21]. Interestingly, FAPP2 knock down disrupted apically enriched staining for galectin-3 indicating that FAPP2 may also be involved in sorting of non-raft vesicles [23]. Furthermore, as in the case of the raft-dependent pathway,

the non-raft pathway depends on the t-SNARE syntaxin 3 [18]. In addition, p75 has been shown to associate with the plus-end directed microtubule motor Kif5B [24]. However, it remains to be shown whether Kif5B is also needed for apical delivery of other cargos.

Basolateral pathways

In contrast to apical targeting information, basolateral sorting information is frequently decoded in the cytoplasmic tail of transmembrane proteins in the form of small peptide motifs either tyrosine- (YxxØ) or di-leucine-based, which are recognized by cytosolic adaptor proteins and are typically cis-dominant over apical sorting information [2]. In general, these cytosolic adaptors are heterotetrameric and interact with clathrin. There are four major classes (AP-1 through AP-4), each consisting of two large subunits (α , γ , ϵ , δ and β 1 - β 4), a small subunit (σ 1 - σ 4), and a medium subunit (μ 1 - μ 4). Typically, it is the medium subunit that interacts with the tyrosine-based sorting signals, whereas the di-leucine-based signals may be recognized by the γ/σ 1 subunits of AP-1 or δ/σ 3 of AP-3 [2,25]. In addition, polarized columnar epithelial cells express the epithelial cell-specific adaptor complex AP-1B [26], which differs from its close cousin AP-1A only in the incorporation of the medium subunits μ 1B or μ 1A, respectively. AP-1B plays important functions in basolateral sorting from recycling endosomes [27-29].

Sorting through recycling endosomes

In recent years, the field started to appreciate that the recycling endosomes can serve as a bona fide – and important – sorting station during biosynthetic delivery to the basolateral plasma membrane (pathway 3 in Figure 1). Cargos that are suspected to move from the TGN into recycling endosomes are vesicular stomatitis virus glycoprotein (VSVG), a truncated version of low-density lipoprotein (LDL) receptor (LDLR-CT27) and the apical variant of VSVG, A-VSVG [30-33]. Moreover, VSVG and LDLR-CT27 are examples of cargos dependent on AP-1B during biosynthetic delivery to the basolateral domain [30,32,34], and correct sorting or mistargeting of VSVG is commonly used as a tool to investigate whether or not a cytosolic protein might be involved in regulating AP-1B-dependent trafficking (AP-1B pathway). Little is known about how trafficking between the TGN and recycling endosomes is regulated. One candidate might be protein kinase D (PKD). PKD is involved in fission of carriers emanating from the TGN and overexpression of kinase dead PKD leads to apical missorting of VSVG [35,36]. In contrast, we have a much better understanding of AP-1B-dependent trafficking from the recycling endosomes to the basolateral membrane.

AP-1B has two major functions. First, AP-1B interacts with basolateral cargos [26,32]. Second, AP-1B facilitates membrane recruitment of subunits of the mammalian exocyst for incorporation into AP-1B vesicles [28]. The exocyst is a vesicle-tethering complex comprised of eight subunits, which may be involved in fusion of basolateral carriers with the target membrane [37,38]. Atomic resolution of C-terminal domains of Exo70, Exo84, as well as Sec6 and Sec15 revealed that at least these four subunits contain long, rod-like domains, which are thought to be important for tethering [39]. The exocyst might be regulated by RalA, which directly interacts with exocyst subunits Sec5 and Exo84, however not at the same time [40]. Thus RalA may be involved in regulating exocyst assembly [41]. In agreement with these findings, RalA was shown to function in basolateral sorting of VSVG [42]. Other GTPases that are localized to recycling endosomes and interact with the exocyst are Rab11 and Arf6, however, it is not known whether these interactions are important for basolateral sorting [43, 44]. Perhaps, these interactions play a role in basolateral to apical transcytosis [45].

Fusion of basolateral carriers often takes place in the upper third of the lateral membrane and the v-SNARE cellubrevin and the t-SNARE syntaxin 4 have been implicated in the AP-1B pathway [18,32]. The fusion event may further be regulated by the mammalian homolog of

Drosophila tumor suppressor lethal giant larvae (lgl) [46]. Furthermore, the small GTPases Cdc42, Rab10 and Rab8 have been implicated in VSVG sorting along the biosynthetic pathway, most likely in sorting from recycling endosomes or the TGN [47-49]. Interestingly, Cdc42 also has a well-established function at the tight junctions where activated Cdc42 binds to the Par3/Par6/aPKC complex [50]. However, the role of Cdc42 in basolateral sorting seems to be distinct from its role at the tight junctions, because acute overexpression of mutant Cdc42 by microinjection of Cdc42 cDNA into cell nuclei led to apical missorting of VSVG without disrupting tight junctions [51].

Movement of AP-1B vesicles may be regulated by myosin VI, which interacts with Rab8 via optineurin [52]. A recent paper showed that interfering with myosin VI function resulted in apical mistargeting of VSVG and LDLR, and the authors suggested, that perhaps myosin VI was involved in guiding AP-1B vesicles through the actin meshwork surrounding recycling endosomes [53]. In addition, AP-1B might interact with the plus-end directed microtubule motor Kif13A, which was shown to interact with the $\beta 1$ subunit of AP-1 [54]. Thus, both the actin and microtubule cytoskeletal networks may be important for the delivery of AP-1B vesicles to their fusion site.

Finally, the PI(4)phosphate-5 kinase $I\gamma$ 661 (PIPKI γ) was recently shown to directly interact with $\mu 1B$ through a tyrosine-based motif [55]. PIPKI γ also directly interacted with E-cadherin, and interactions with both partners were necessary for basolateral delivery of E-cadherin [55]. However, it is not known, whether PIPKI γ plays a general role in the AP-1B pathway or has a specific function in E-cadherin trafficking.

Direct pathways

Less is known about the “direct” sorting at the TGN, perhaps because basolateral proteins travel through the TGN only once while they are typically recycled many times (pathway 4 in Figure 1). Cargos sorted along the “direct” pathway during biosynthetic delivery are Fc receptor (which has a di-leucine-based sorting motif), TfnR and an LDLR mutant [LDLR(Y18A)], in which the AP-1B-dependent sorting signal is mutated. Interestingly, sorting signals in both LDLR(Y18A) and TfnR interact with $\mu 4/AP-4$ [32,56], and expression of antisense RNA directed against $\mu 4$ led to mild sorting defects of LDLR in MDCK cells [56]. Since only steady-state distributions were analyzed in this study, AP-4's involvement in basolateral sorting may have been underestimated. Although “direct” sorting via AP-4 refers to a pathway that does not involve recycling endosomes, it is uncertain whether such a pathway may involve basolateral early endosomes (pathway 5 in Figure 1). It is also not known, whether or not other clathrin adaptors such as AP-1A or AP-3 play a role in basolateral sorting.

Conclusions

Although the major pathways to the apical and basolateral membranes are reasonably well understood, open questions in polarized trafficking remain. For example, we still know little about membrane trafficking to the primary cilium, which outgrows from the apical membrane during polarization. Interestingly, proteins localized at tight junctions (Par3/Par6/aPKC) or factors involved in sorting to the basolateral membrane (Rab8) have recently also been noted for their role in cilium biogenesis [57-60] or even regulation of apical protein localization (Rab8) a phenotype perhaps related to impaired cilia [61]. Furthermore, the exocyst complex has also been localized to the base of the cilium in addition to the lateral membrane [62]. Moreover, also proteins involved in apical sorting (FAPP2) seem to be important for healthy cilia [23]. It will be interesting to learn, how sorting to apical and basolateral domains interplays with sorting to the cilia. With our growing molecular understanding of polarized membrane trafficking, these questions can now be addressed.

Acknowledgements

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Figure 1. Membrane trafficking pathways to apical and basolateral membrane domains

This Figure depicts schematically the different pathways to either apical or basolateral plasma membrane domain: (1) raft-dependent trafficking to the apical membrane, (2) raft-independent trafficking to the apical membrane, (3) AP-1B-dependent, “indirect” sorting to the basolateral membrane via recycling endosomes, (4) “direct” sorting to the basolateral plasma membrane without traveling through any endosomes, and (5) “direct” sorting to the basolateral membrane via early endosomes. For details of proteins regulating the sorting steps refer to main text. Note that the route of “direct” sorting to the basolateral membrane is not entirely clear.

Abb.: AEE = apical early endosomes, ARE = apical recycling endosomes, BEE = basolateral early endosomes, RE = common recycling endosomes, ND = not discussed in the text.