CHAPTER 6

Recycling endosomes in apical plasma membrane domain formation and epithelial cell polarity

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Trends in Cell Biology 2010 Oct; 20(10), 618-26. Review.

ABSTRACT

Recycling endosomes have taken central stage in the intracellular sorting and polarized trafficking of apical and basolateral plasma membrane components. Molecular players in the underlying mechanisms are now emerging, including small GTPases, class V myosins and adaptor proteins. In particular, defects in the expression or function of these recycling endosome-associated and endosome-regulating proteins have been implicated in cell surface polarity defects and diseases, including microvillus inclusion disease, arthrogryposis-renal dysfunction- cholestasis syndrome, and virus susceptibility. Key findings are that recycling endosomes recruit and deliver core polarity proteins to lateral cell surfaces and initiate the biogenesis of apical plasma membrane domains and epithelial cell polarity. Here, we review recent data that implicate recycling endosomes in the establishment and maintenance of epithelial cell polarity.

INTRODUCTION

Organization of the recycling endosomal system in epithelial cells

A fundamental step in eumetazoan evolution was the interaction of epithelial cells to form isolated intercellular lumens, which give rise to tubes that ensure transport of vital fluids and nutrients throughout organ systems. The establishment of epithelial cell polarity, including distinct apical plasma membrane (apPM) and basolateral PM (blPM) domains, is important for lumen development, vectorial transport and accommodation of specific functions that are required for cells facing different environments.

Intracellular sorting and trafficking of the apPM and blPM components is important to establish and maintain these domains (Mostov et al. 2003; Mellman & Nelson, 2008; Weisz & Rodriguez-Boulan, 2009). The role of the Golgi and the trans-Golgi network (TGN) in sorting and trafficking newly synthesized PM components in the exocytotic pathways is well recognized (Rodriguez-Boulan et al., 2005). In the face of continuous endocytosis and recycling from both surface domains, generally not involving the Golgi, endosomes (Fig. 1a) must maintain the composition of PM domains (Breitfeld et al., 1989). Proteins and lipids, endocytosed from the blPM or apPM, first encounter basolateral (BEE) or apical early endosomes (AEE), respectively.

The proteins and lipids not sorted for targeting into the late endosomal/lysosomal pathway or fast recycling to the PM domain of origin, are transported to mildly acidic (Wang et al., 2000; Gagescu et al., 2000; Ohgaki et al., 2010) common recycling endosomes (CRE) (also called subapical compartment (SAC) in hepatocytes) (Wang et al., 2000; Odorizzi et al., 1996; Futter et al., 1998; Gibson et al., 1998). From CRE, proteins and lipids are delivered to either apPM or blPM via multiple pathways, with differential dependence on the small GTPases Rab11a, Rab25 and/or their effector myosin Vb (Fig. 1a). Studies of basolateral to apical transcytosis of the polymeric immunoglobulin receptor (pIgR) and its ligand dimeric immunoglobulin A (dIgA)

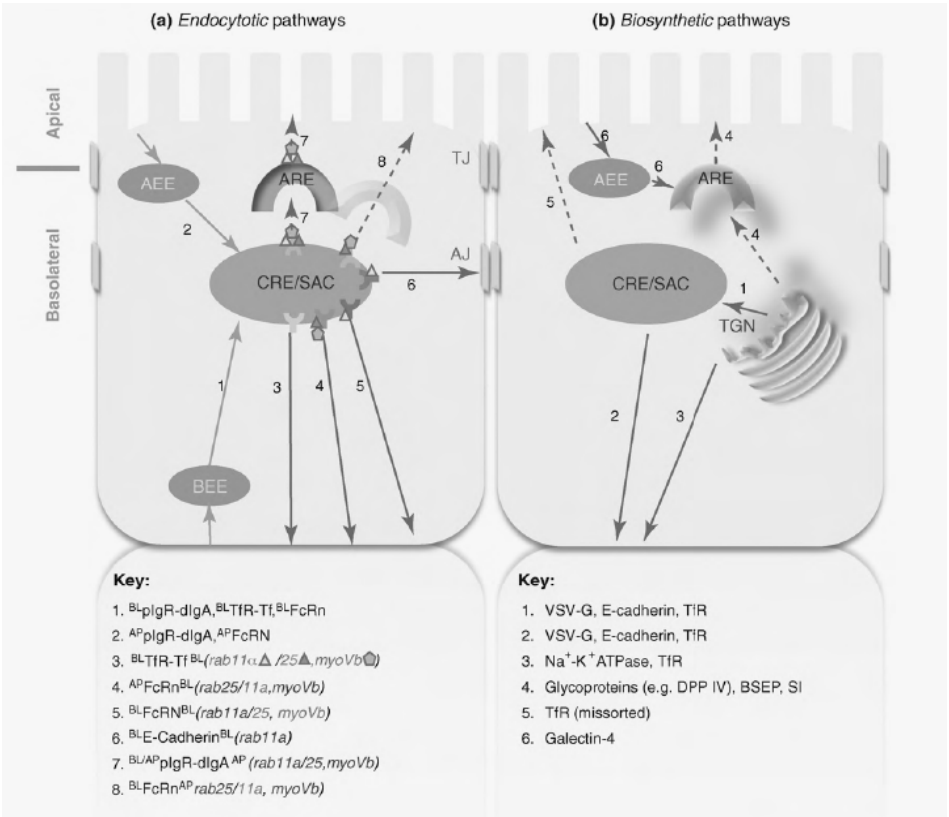

Figure 1. Endosomal pathways and compartments involved in the trafficking of (a) endocytosed and (b) newly synthesized proteins in polarized epithelial cells. (a) Proteins endocytosed from the blPM (route 1) or apPM (route 2) can travel via BEE or AEE, respectively, to CRE (also named SAC). From CRE, proteins can be targeted to the blPM or to the apPM via distinct pathways (routes 3–8), shown by their differential sensitivity to Rab11a, Rab25 and/or myosin Vb interference. Proteins that are targeted from CRE to the apical surface travel via ARE (route 7), although this is not clear for all proteins (route 8). The numbered routes have been characterized using the marker proteins listed at the bottom of the figure. The annotation APproteinBL refers to a protein that is endocytosed from the apPM and delivered to the blPM. Similarly, the annotation BLproteinBL refers to a protein that is endocytosed from the blPM and delivered to the blPM. The Rab proteins listed with the marker proteins indicate whether the Rab protein is experimentally demonstrated to be functionally involved (marked in red) or not (marked in blue) in the corresponding trafficking route. (b) Newly synthesized basolateral proteins can travel from the TGN to the CRE (route 1) and subsequently to the blPM (route 2), or bypass endosomes en route to the blPM (route 3). CRE-associated basolateral proteins, when missorted, are delivered from CRE to the apPM (route 5). Newly synthesized apical proteins can travel

from the TGN to ARE and subsequently to the apPM (route 4). Some apical proteins require apically endocytosed galectin-4 (route 6) to be delivered from ARE to the apPM. The numbered routes have been characterized using the marker proteins listed at the bottom of the figure. Unbroken and broken lines refer to routes to the blPM and the apPM, respectively. TJ, tight junction; AJ, adherens junction.

indicate that polarized delivery of apPM components from CRE involves subapically positioned apical recycling endosomes (ARE) (Gibson et al., 1998; Apodaca et al., 1994; Barroso & Sztul, 1994; Brown et al., 2000). ARE have nearly neutral luminal pH (Wang et al., 2000), distinct cup-shaped morphology (Gibson et al., 1998), are operationally defined by the presence of apPM-destined cargo and the absence of basolaterally recycling transferrin and contain Rab11a, Rab25 and myosin Vb (Wang et al., 2000; Apodaca et al., 1994; Barroso & Sztul, 1994; Brown et al., 2000).

Whether ARE are separate endosomal entities or subdomains of CRE is under debate (Brown et al., 2000; Tzaban et al., 2009; Sheff et al., 1999). One argument for ARE as a separate entity is the clear segregation of apically transcytosing dIgA-pIgR with Rab11a, myosin Vb and most Rab25 away from basolaterally recycling transferrin (Casanova et al., 1999). However, spatial segregation does not necessarily imply their trafficking to a physically separate ARE, as this could reflect pronounced lateral segregation within a continuous CRE membrane. Recent studies demonstrate that Rab11a mediates basolateral recycling of the Fc receptor (FcRN) (Tzaban et al., 2009), although not of transferrin (Wang et al., 2000; Tzaban et al., 2009).

Furthermore, Rab25 and myosin Vb control apical transcytosis of blPM-derived FcRN and basolateral transcytosis of apPM-derived FcRN (Tzaban et al., 2009). Finally, Rab11a associates with RE containing endocytosed transferrin, where it regulates sorting and delivery of E-cadherin to the blPM (Lock & Stow, 2005; Desclozeaux et al., 2008). It remains puzzling why Rab11a does not colocalize with transferrin-containing CRE in Madin-Darby canine kidney (MDCK) cells used to study pIgR-IgA transcytosis, but does colocalize in MDCK cells used to study E-cadherin trafficking. Possibly, the ectopic expression of pIgR in MDCK cells (which

do not express endogenous pIgR) induces redistribution of Rab11a and its effectors from CRE to ARE during constitutive transcytosis, which is strongly unidirectional (Jerdeva et al., 2010). This would not be unprecedented, as the ectopic expression of pIgR in polarized, hepatic WIF-B cells leads to redistribution of myelin and lymphocyte protein 2 (MAL2), which normally shuttles between BEE and CRE/SAC, to the Golgi and transcytotic intermediates occupied by the receptor (In & Tuma, 2010). While this hypothesis remains to be investigated, Rab11a, Rab25 and myosin Vb appear more promiscuous with regard to PM domain selectivity than originally thought (Casanova et al., 1999), and cannot be considered selective markers for ARE. Nonetheless, the distinct pH measured in ARE (where transcytosing dIgApIgR and Rab11a reside following segregation from basolaterally recycling transferrin (Wang et al., 2000)) suggests that ARE are physically distinct compartments. We favor a working model of the epithelial RE system (Fig. 1a) in which endocytosed apical and basolateral cargoes (and some newly synthesized basolateral cargoes) converge in CRE and are segregated and recycled to either PM domain. A physically distinct ARE compartment that contains cargoes derived from CRE (or TGN in the case of newly synthesized apical cargoes) is dedicated to delivery to the apPM. Traffic regulatory proteins, such as Rab11a, Rab25 and myosin Vb, associate with both CRE and ARE. During apical recycling or transcytosis, ARE might arise from CRE as part of an endosomal maturation process, possibly manipulated by the cargo itself. Transcytosing dIgA-pIgR, which interacts directly with Rab3b in a ligand-dependent manner to regulate its transcytotic efficiency (van IJzendoorn et al., 2002; Smythe, 2002), induces formation of membrane tubules from transferrin-positive endosomes that reach into the apical cytoplasm and from which basolateral recycling proteins are concurrently retrieved (Futter et al., 1998; Gibson et al., 1998).

Regardless of how ARE relate to CRE, the merging of endocytosed apical and basolateral cargo in CRE implicates this organelle as a polarized sorting center that appears instrumental in the establishment, maintenance and plasticity of epithelial PM domains (van IJzendoorn & Hoekstra, 1999; Hoekstra et al., 2004). Here, we review

recent work that: (i) reveals the molecular machinery controlling sorting and polarized targeting from RE; (ii) indicates a role for RE in positioning evolutionarily conserved proteins that constitute the epithelial polarity program and in the de novo biogenesis of apPM domains and epithelial polarity; and (iii) identifies the first human epithelial disease-linked mutations in RE-associated proteins.

Sorting endocytosed apPM and blPM components by lateral segregation in CRE

Electron microscopy observations of endocytosed transferrin receptors concentrated in clathrin–g-adaptin-coated buds in CRE of polarized MDCK cells (Futter et al., 1998; Gibson et al., 1998), provided the first indications of protein sorting in CRE. In addition, apically endocytosed sphingolipid analogues C6NBDsphingomyelin and C6NBD-galactosylceramide sort from C6NBD-glucosylceramide in CRE/SAC (accessible for basolaterally endocytosed IgA) and preferentially traffic to the blPM and apPM, respectively, in living polarized HepG2 cells (reviewed in van IJzendoorn & Hoekstra, 1999; Hoekstra et al., 2004). As different sphingolipid species are found in different lateral regions within the same cellular membrane (Westerlund & Slotte, 2009), it has been proposed that lateral membrane segregation in CRE/SAC gives rise to distinct microdomains for apPM- or blPM-directed transport vesicles (van IJzendoorn & Hoekstra, 1999; Hoekstra et al., 2004; Westerlund & Slotte, 2009). Using high-resolution confocal imaging and 3D reconstructions, the lateral segregation of endocytosed basolateral and apical PM proteins in a continuous CRE membrane has been visualized directly in polarized MDCK cells and neurons (Thompson et al., 2007). The lateral segregation of endocytosed apPM and blPM proteins represents cargo sorting after arrival at CRE rather than regions of entry into CRE, and this segregation does not result from differences in sorting kinetics of apPM and blPM cargoes (Thompson et al., 2007). In activated macrophages RE laterally segregate newly synthesized inflammatory cytokine interleukin-6 and tumor necrosis factor-α from endocytosed transferrin into separate regions within the same membrane (Manderson et al., 2007).

Sorting of apPM and blPM cargoes in CRE membranes requires phosphatidylinositol (PI)-3 kinase (Thompson et al., 2007), suggesting a role for phospoinositide lipids. Finally, lateral segregation of apPM and blPM components was not observed in RE of nonpolarized MDCK cells or fibroblasts transfected with apPM and blPM marker proteins (Thompson et al., 2007). It should be noted that these results predominantly come from (transfected) cell lines and should be confirmed in primary polarized epithelial cells. However, the available data suggest RE are capable of lateral segregation of membrane components, and that this ability appears restricted to polarized or activated cells.

RE are not involved in segregation of newly synthesized apical proteins from newly synthesized basolateral proteins

RE act as intermediate stations for some newly synthesized apPM and blPM proteins (Orzech et al., 2000). This raises important questions about the relationship between the TGN and RE in sorting newly synthesized apical and basolateral PM proteins (Maier & Hoekstra, 2003). In polarized MDCK cells, the basolaterally targeted vesicular stomatitis virus-G protein (VSV-G) (Ang et al., 2004; Farr et al., 2009), but not the basolaterally targeted Na^+,K^+-ATPase (Farr et al., 2009), is delivered from the TGN to CRE containing endocytosed transferrin. Chemical inactivation of the CRE (partly) inhibits delivery of newly synthesized VSV-G to the basolateral surface. Whether newly synthesized basolateral proteins travel to the blPM via CRE can be dictated by the degree of cell polarity, as shown for the fate of newly synthesized transferrin receptors in MDCK cells (Gravotta et al., 2007). Before delivery at the apical surface, the resident apical plasma membrane proteins sucrase-isomaltase (SI), lactase-phlorizin hydrolase (LPH), endolyn and influenza hemagglutinin (HA) traverse REs that are likely to differ from the CRE (Cramm-Behrens et al., 2008; Cresawn et al., 2007). Chemical inactivation of the CRE fails to inhibit apical delivery of HA or endolyn (not examined for SI or LPH) (Cresawn et al., 2007). By contrast, over-expression of a tail domain of the RE-associated protein myosin Vb, which

perturbs Rab8- and Rab11a-mediated trafficking, inhibits apical delivery of newly synthesized endolyn, but not VSVG (Cresawn et al., 2007). In agreement, knockdown of Rab8 and, to a lesser degree, Rab4 and Rab11a, inhibits apical surface delivery of SI and LPH (Cramm-Behrens et al., 2008). These data suggest that some basolateral and apical cargoes do not converge in a single endosomal compartment, but are delivered via distinct REs to epithelial surface domains (i.e. basolateral cargo via endocytosed transferrin-positive CRE and apical cargo via transferrin-negative, Rab8-/Rab11a-positive ARE) (Fig. 1b). Although Rab11a-positive ARE harboring newly synthesized apical cargo are accessible for apically endocytosed material, it is not known whether these are accessible for CRE-derived transcytosing proteins. We hypothesize that these ARE are dedicated to mediating delivery from TGN to apPM. The latter is not unprecedented, as Rab11a associates with the TGN (Chen et al., 1998; de Graaf et al., 2004; Satoh et al., 2005). The observation that newly synthesized apical and basolateral cargo are delivered via distinct RE to their respective PM domains implies that RE are not compartments where newly synthesized and TGN-derived apical and basolateral proteins are laterally segregated into distinct membrane domains.

In contrast to CRE-derived trafficking, disabling trafficking from ARE typically does not result in mistargeting of apical cargo to the blPM, which implies that the TGN is the predominant sorting station for newly synthesized apical cargo. Directing a subset of newly synthesized proteins through ARE might kinetically store and regulate signal-mediated timing and expression level at the apPM (discussed in Weisz & Rodriguez-Boulan, 2009; Cresawn et al., 2007). In addition, passage of newly synthesized proteins through ARE en route to the apPM might add an additional regulatory station, as seen for newly synthesized glycoproteins in intestinal epithelial HT29 cells, which require apically endocytosed galectin- 4 to complete their trafficking from ARE (Fig. 1b) (Stechly et al., 2009).

The benefit of successively sorting some newly synthesized basolateral cargoes in TGN and CRE remains unclear. Sorting of basolateral proteins in the TGN and CRE

both require clathrin, but are likely to involve different clathrin adaptor proteins (Deborde et al., 2008).

Proteins that regulate sorting and polarized trafficking from CRE

Clathrin-mediated basolateral sorting from CRE is controlled by the epithelial-specific clathrin adaptor protein complex 1B (AP1B) (Gan et al., 2002), which recognizes a subset of basolateral tyrosine motifs through its m1B subunit (Folsch et al., 1999). AP1B associates stably with CRE through a membrane recruitment patch in its carboxyl terminus (Fields et al., 2010), and in polarized Fisher rat thyroid cells, this association is dependent on brefeldin A-sensitive ARF-like GTPase activity (Cancino et al., 2007). Inhibition of the expression or function of AP1B in polarized epithelial cells causes mistargeting to the apPM or CRE trafficking arrest of newly synthesized and endocytosed basolateral, but not apical, cargoes (Gravotta et al., 2007; Cancino et al., 2007). The lateral segregation of endocytosed apPM and blPM cargoes in the CRE membrane occurs also in epithelial LLC-PK1 cells lacking AP1B (Thompson et al., 2007). Cleavage of the v-SNARE cellubrevin with tetanus neurotoxin causes a redistribution of AP1B from CRE and mistargeting of AP1B-dependent cargoes such as the transferrin receptor to the apPM (Fields et al., 2007). In contrast, tetanus neurotoxin does not cause mistargeting of AP1B-dependent VSV-G to the apPM, indicating that VSV-G might use alternative pathways to the blPM in the absence of functional AP1B (Fields et al., 2007). Given that clathrin is generally required for sorting basolateral proteins, other clathrin adaptor proteins (e.g. AP1A) might be involved in basolateral sorting (Deborde et al., 2008).

While AP1B mediates sorting of basolateral proteins from other proteins by recognizing tyrosine-based signals in their cytoplasmic tails, basolateral recycling of E-cadherin depends on AP1B and interaction with type Ig phosphatidylinositol 4-phosphate 5-kinase (PIPKIg) (Ling et al., 2007).

PIPKIg acts as a scaffold that interacts directly with the msubunits of AP1B and E-cadherin in transferrin-containing CRE, thereby linking E-cadherin to the AP1B

complex. Inhibition of the interaction between PIPKIg and either E-cadherin or AP1 causes mistargeting of internalized E-cadherin to the apPM (Ling et al., 2007). Basolateral targeting of newly synthesized E-cadherin from transferrin-positive CRE is Rab11a-dependent, because Rab11a mutants cause mistargeting of E-cadherin to the apPM in MDCK cells (Lock & Stow, 2005; Desclozeaux et al., 2008). A link between Rab11a and AP1B has not been demonstrated.

Recent evidence involves the Na^+/H^+-exchanger NHE-6.1 in maintaining a limited pH range in CRE, which appears important for securing polarized distribution of membrane lipids at the apPM and maintaining apical bile canaliculi and cell polarity in HepG2 cells (Ohgaki et al., 2010). Steep pH gradients exist within the RE system (Wang et al., 2000; Gagescu et al., 2000) and appear to function in apPM directed transport (Henkel, 1999). The data suggest a role for NHE-6-mediated proton flux across CRE membranes in the regulation of polarized sorting and/or trafficking at CRE, possibly by regulating recruitment of cytoplasmic components of membrane sorting machinery, including ADP-ribosylation factor 1 (Arf-1) (Gu & Gruenberg, 2000).

Local lipid metabolism controls polarized sorting at CRE

The lipid composition of CRE appears to be important in AP1B-mediated sorting of basolateral cargo. Recent work indicates that CRE are rich in phosphoinositol 3,4,5-triphosphate (PI(3,4,5)P3), which appears to require AP1B expression and might involve AP1B-facilitated recruitment of PI-kinases and TGN-derived PI(4)P as a template lipid (Fields et al., 2010). Interfering with PI(3,4,5)P3 formation, either with the pharmacological PI3 kinase inhibitor LY92004 or over-expression of the PI(3)-phosphatase PTEN, leads to displacement of AP1B from CRE and mistargeting of basolateral cargo to the apPM (Fields et al., 2010). Pharmacological inhibition of PI3 kinase with wortmannin inhibits lateral segregation of apical and basolateral cargoes in CRE, while AP1B is dispensable for this (Thompson et al., 2007). PI(4,5)P2 and PI(3,4,5)P3 function as apPM and blPM determinants and organizers, respectively

(Gassama-Diagne et al., 2006; Martin-Belmonte et al., 2007; Martin-Belmonte & Mostov, 2007). A working model emerges in which conversion of PI(4)P and/or PI(4,5)P2 to PI(3,4,5)P3 in CRE membranes promotes lateral segregation of basolateral cargo into a distinct CRE subdomain that promotes AP1B recruitment and basolateral targeting. It is tempting to propose that a (PTEN-mediated or PI3 kinase inhibition- mediated) shift towards PI(4,5)P2 in RE might promote apPM-directed trafficking. In polarized epithelial cells, CRE are enriched in the apical raft lipids sphingomyelin, cholesterol, and phosphatidylserine (Gagescu et al., 2000), and segregation of sphingolipid analogues in CRE membranes has been reported (van IJzendoorn & Hoekstra, 1999; Hoekstra et al., 2004; Maier & Hoekstra, 2003).Moreover, the reported association of PIP5KIg, a known effector of apPM and RE-associated Arf6, with endosomal membranes (Fields et al., 2010; Ling et al., 2007; Aikawa & Martin, 2003), in conjunction with the observation that local production of PI(4,5)P2 by PIP5KI (a, a' and b) promotes apPM-directed trafficking of sphingolipid/cholesterol-rich vesicles (Rozelle et al., 2000), is enticing.

Involvement of RE in apPM biogenesis and epithelial polarity development

A wealth of data substantiate the involvement of RE in maintaining surface polarity in polarized epithelial cells. New evidence implicates RE in the de novo establishment of epithelial cell polarity. It has been postulated that apPM biogenesis is initiated upon surface delivery of Rab11a/ myosin Vb-containing RE membranes (Wakabayashi et al., 2005). In agreement, imaging-based analysis of early MDCK cell polarity development suggests that insertion of membrane from nearly neutral, Rab8 and/or Rab11a-positive ARE into the contact surface between two cultured epithelial cells establishes a microvilli-containing preapical surface patch. This patch contains the apPM marker gp135/podocalyxin (Ferrari et al., 2008), which, in conjunction with Na^+/H^+-exchanger regulatory factor NHERF, has been proposed to participate in formation of the apPM domain (Meder et al., 2005; Nielsen et al., 2007). In nonepithelial cells, Rab11-positive RE recruit members of two evolutionarily

conserved apical polarity complexes, Crumbs/PALS1/PATJ and Par6/Par3/aPKC/Cdc42, supporting a link between the endocytotic system and the core cell polarity program (Balklava et al., 2007; reviewed in Shivas et al., 2010). In epithelial MDCK cells, the apical determinant and transmembrane scaffold protein Crumbs (as well as PALS1 and PATJ) was shown to be concentrated in Rab11-positive RE before onset of mitosis (Schlüter et al., 2009). These RE, which contained gp135/podocalyxin, are delivered via microtubules to the site of cytokinesis and, together with the recruitment of aPKCz, constitute the first steps in formation of the

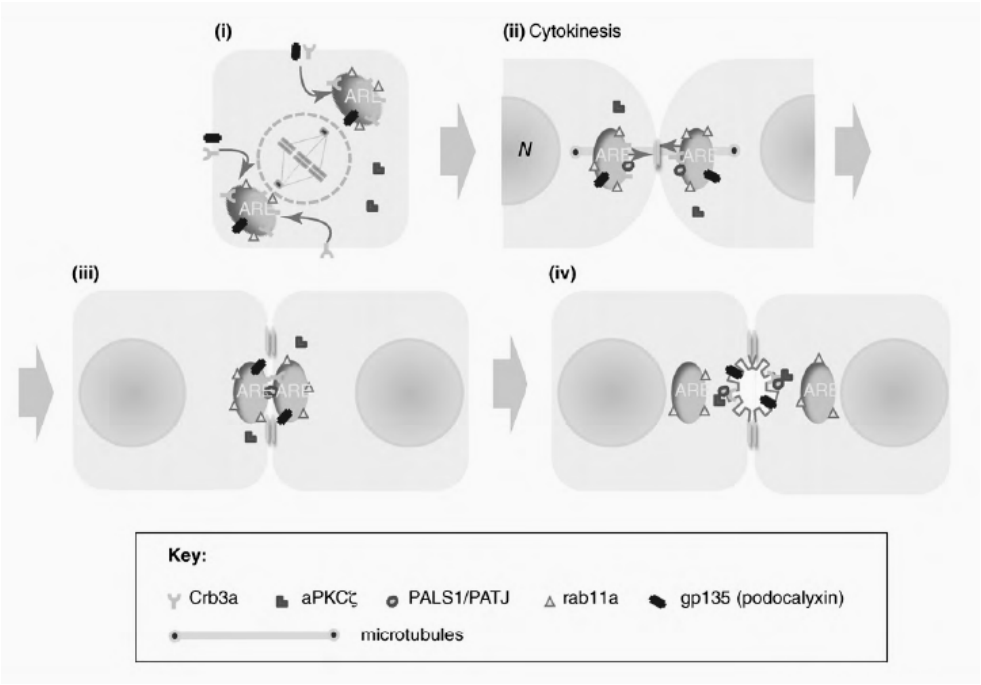

Figure 2. The role of RE in formation of the apical plasma membrane domain. During mitosis, gp135/podocalyxin, the core polarity protein and the apPM determinant Crb3a are internalized into Rab11-positive RE that also contain PATJ and PAL1 (stage I). During cytokinesis, these RE are targeted in a microtubule-dependent manner to the newly formed lateral PM domain, where they deliver Crb3a and gp135/podocalyxin (stages II and III). Crb3a recruits aPKCz and, together with gp135/podocalyxin, initiates the formation of the apPM and apical lumen (stage IV). N, nucleus.

apPM domain (Fig. 2) (Schlüter et al., 2009).

The involvement of Rab11 in maintaining apical Crumbs is seen in the Drosophila embryonic ectoderm (Roeth et al., 2009). RE, via Rab11 or Arf6, recruits members of the exocyst, which is a multiprotein complex controlling polarized PM delivery (Oztan et al., 2007). In Drosophila Exo84 mutants, Crumbs accumulates in expanded Rab11-positive RE (Blankenship et al., 2007), underscoring a requirement for RE and polarized vesicle delivery for apPM Crumbs localization (Blankenship et al., 2007; Campbell et al., 2009). The cue(s) triggering delivery of Crumbs-containing RE to the cell surface might involve signaling by proteins involved in physical cell–cell contact or circulating factors such as cytokines. Liver maturation-stimulating cytokines of the IL6 family stimulate apPM biogenesis in hepatocytes by redirecting traffic from CRE/SAC to the apical domain (van IJzendoorn et al., 2004).

RE also receive PM-derived members of apical polarity complexes. Increased expression of AMOT/angiomotin, which binds to and controls the activity of Cdc42/Rho-GAP/RICH1 and consequently that of Cdc42, causes relocalization of Crumbs and binding partners, including the membrane-associated polarity proteins PALS1 and PAR3, from the PM to Rab11-/Arf6-positive RE in epithelial cells (Wells et al., 2006). AMOT recognizes RE through a lipid-binding domain that binds selectively to monophosphorylated phosphatidylinositols and cholesterol of Rab11- and Arf6-positive RE (Heller et al., 2010). By shifting the subcellular location of polarity complex proteins from the PM to RE, AMOT and Cdc42 might influence maintenance of cell polarity, which could underlie the ability to promote migratory and invasive phenotypes. Expression of AMOT/angiomotin is typically low in differentiated epithelial cells but up-regulated during epithelial migration programs (Heller et al., 2010). These data demonstrate a role for Rab11-positive RE in AMOT/angiomotin-mediated retrieval, and in Rab11-mediated and exocyst-mediated delivery of apPM defining proteins. RE might be instrumental in the breakdown and de novo establishment of apPM domains and cell polarity.

Disease-linked mutations in epithelial RE-associated proteins

Information on the role of RE in epithelial polarity relies heavily on studies using epithelial cell lines. While the contribution of model organisms is increasing, evidence of RE involvement in epithelial polarity in humans has been lacking. Human epithelial pathologies attributed to structural and/or compositional defects in apical cell surface polarity have been reported, and development of genetic tools has enabled identification of mutant genes underlying some of these diseases. These include genes encoding proteins known to regulate polarized trafficking from RE, and understanding their function aids in understanding disease pathogenesis. Other mutant genes encode proteins not known to regulate polarized trafficking from RE. These human diseases underscore the value of cell line-based studies and contribute novel areas of further study to advance our knowledge of RE involvement in epithelial polarity.

Adenovirus susceptibility

Adenoviruses that invade via digestive or respiratory routes use a Coxackie-adenovirus receptor (CAR) protein that is typically expressed at the blPM of epithelial cells. The viruses, which are normally present at the luminal or apical epithelia, must disrupt the tight junctions to gain access to CAR. The basolateral localization of CAR is secured by the CRE-associated clathrin adaptor protein AP1B. Retinal pigment epithelial cells, which form a non-renewable single-cell layer that maintains the blood–retinal barrier, are highly susceptible to adenoviral infection because these cells do not express AP1B and mistarget CAR to the apPM (Diaz et al., 2009). An enhanced susceptibility to adenovirus infection in epithelial cell lines of intestine (Caco-2), lung (Calu-3) and kidney (MDCK) is observed when AP1B is down-regulated (Diaz et al., 2009). Reintroduction of AP1B into AP1B-silenced MDCK cells corrects targeting of CAR to the basolateral surface and decreases adenovirus infection efficiency. CRE and associated AP1B therefore guarantee adenoviral resistance of most epithelial tissues, and explain the susceptibility of retinal pigment epithelia.

Arthrogryposis-renal dysfunction-cholestasis syndrome (ARC)

ARC (OMIM #208085 and #613404) is a rare and autosomal recessive multisystem disorder characterized by neurogenic arthrogryposis multiplex congenita, renal tubular dysfunction resembling renal Fanconi syndrome and neonatal cholestasis. The majority (75%) of ARC families carries mutations in the VPS33B gene (OMIM #208085) (Gissen et al., 2004).

VPS33B encodes the Vps33 protein, which is involved in protein sorting and membrane fusion. In the 25% of ARC patients without VPS33B mutations (OMIM #613404), mutations are seen in the previously uncharacterized gene VIPAR (Cullinane et al., 2010). VIPAR (Vps33-interacting protein involved in polarity and apical protein restriction) complexes withVps33 and preferentially colocalizes and interacts with RE-associated GTP-bound Rab11a. In zebrafish embryo hepatocytes, mutant VIPAR or Vps33 fail to form a functional complex with Rab11a, causing mistargeting of the apPM resident bile salt export protein (BSEP), but not MRP2, to the blPM domain. This agrees with the notion that BSEP, but not MRP2, constitutively cycles between the apical bile canalicular membrane and Rab11a-positive RE (Wakabayashi et al., 2005). Plausibly, the mistargeting of BSEP, in which mutations cause progressive familial intrahepatic cholestasis type 2, contributes to cholestasis in ARC patients. Mistargeting of basolateral proteins to the apPM has not been observed in this syndrome. Knockdown of VIPARand Vps33 in renal inner medullar collecting duct mIMCD-3 cells causes mistargeting of the apical p75 neurotrophin receptor, but not of basolateral Na^+/K^+-ATPase. These knockdowns reduce the expression of tight junction and adherens junction proteins claudin-1 and E-cadherin, respectively, enhance monolayer permeability, increase cell proliferation and inhibit formation of luminal spheres in 3D cell culture (Cullinane et al., 2010). These data implicate Rab11a and the VIPAR–Vps33 protein complex in regulating polarized delivery of an apical protein from the RE (Fig. 3) and contributing to the proper composition and function of apical surface domains in human hepatocytes and kidney epithelial cells.

Microvillus inclusion disease (MVID)

MVID (OMIM #25185) is a rare autosomal recessive malabsorption disorder in neonates that is accompanied by intractable diarrhea within the first days (early onset) or weeks (late onset) of life. MVID is characterized by blunted villi at the tissue level, a variable loss of apical surface exposed microvilli, the intracellular appearance of microvilli-lined compartments, and intracellular accumulation of apical brush border proteins in villus enterocytes (Phillips et al., 1985; Phillips & Schmitz, 1992).

Mislocalization of basolateral proteins or blPM defects has not been observed (Ameen & Salas, 2000), and intestinal epithelial cells appear normally arranged in monolayers with visible cell–cell junctions. Most MVID patients display homozygous or (compound) heterozygous deletions, nonsense, missense or splice site mutations in the *MYO5B* gene (Szperl et al., 2011; Müller et al., 2008; Erickson et al., 2008; Ruemelle et al., 2010). The *MYO5B* gene encodes for the actin-based molecular motor protein myosin Vb, an effector protein of RE-associated Rab11a/b (Lappiere et al., 2001), Rab25 (Lappiere et al., 2001), Rab10 (Roland et al., 2009) and Rab8 (Roland et al., 2007), which regulates polarized epithelial (see Tzaban et al., 2009) protein trafficking from RE (Tzaban et al., 2009; Cresawn et al., 2007; Lappiere et al., 2001; Babbey et al., 2006; Swiatecka-Urban et al., 2007; Nedvetsky et al., 2007; Silvis et al., 2009; Mattilla et al., 2009). MVID-linked MYO5B mutations result in reduced myosin Vb mRNA levels and are predicted to result in expression of defective myosin Vb proteins (e.g. perturbed motor function or loss of Rab-binding domains) (Szperl et al., 2011). MVID-linked MYO5B mutations affect expression and/or distribution of the myosin Vb protein in enterocytes of MVID patients (Szperl et al., 2011). The spatial distribution of Rab11a-marked RE is perturbed in enterocytes of MVID patients, suggesting altered myosin Vb function (Szperl et al., 2011). Down-regulation of myosin Vb expression in polarized intestinal epithelial Caco-2 cells with siRNA mimics some of the apPM defects seen in MVID (Ruemelle et al., 2010).

Expression of a motorless tail mutant of myosin Vb in polarized hepatic WIF-B cells prevents apical bile canalicular lumen formation (Wakabayashi et al., 2005). The

mouse Rab8 conditional knockout mimics MVID clinical symptoms and apPM defects, without effect on the blPM (Sato et al., 2007). These data implicate RE-associated myosin Vb and its RE receptors Rab8 and Rab11 in apPM development and/or maintenance. Although myosin Vb is implicated in apical recycling of the cystic fibrosis transmembrane conductance regulator in lung epithelial cells (Swiatecka-Urban et al., 2007; Silvis et al., 2009) and of aquaporin water channels in kidney epithelial cells (Nedvetsky et al., 2007), MVID patients present clinical symptoms in neither lung nor kidney.

Conceivably, RE organization and function is organ-specific. Myosin V redundancy might explain why MVID patients have clinical symptoms restricted to the intestine, despite ubiquitous myosin Vb expression. Expression of *MYO5B* transcripts in murine intestine is much higher than that of MYO5C (_5500 versus _100, respectively; http://symatlas.gnf.org). Also, expression of MYO5B transcripts in lung (_1400) and kidney (_3000) are lower than that in gut (_5500). One could argue that the high demand for *MYO5B* in the intestine cannot be sufficiently compensated for by *MYO5C* in cases of *MYO5B* dysfunction. In less *MYO5B*-demanding epithelial tissues (e.g. lung), the compensatory effect of *MYO5C* might be sufficient.

The discovery of nonsense and loss-of-function mutations in RE-associated proteins in ARC and MVID patients strongly supports the importance of RE in establishment and/or maintenance of epithelial polarity, organ function and human health.

Concluding remarks

Recent years have revealed an essential role of RE in segregation and polarized targeting of newly synthesized and endocytosed PM components and epithelial cell surface polarity. Data demonstrating the involvement of ARE in de novo establishment of apical surface domains and cell–cell junctions via delivery of apical polarity complex proteins further underscore the role of RE in epithelial polarity.

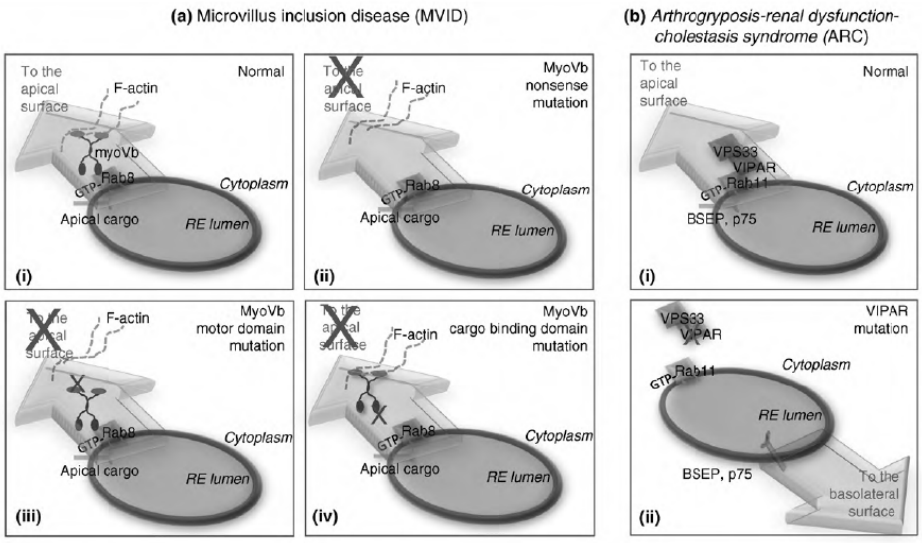

Figure 3. Human disease-linked mutations in RE-associated proteins interfere with the polarized delivery of apical PM proteins. (a) In microvillus inclusion disease (MVID), *MYO5B* gene mutations (including nonsense mutations (panel II), mutations in the F-actin-binding motor domain (panel III) or mutations in the cargo-binding tail domain (panel IV)) are correlated with the intracellular retention of apical proteins and their absence at the apPM. Deletion of Rab8 in mice reproduces the clinical and morphological phenotype of MVID, but Rab8 mutations have not been identified in MVID patients. (b) In arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome, mutations in VIPAR inhibit its interaction with RE-associated Rab11a and result in the missorting of the apPM resident bile salt export protein BSEP and p75 receptor to the basolateral surface in hepatocytes and kidney epithelial cells, respectively.

Importantly, the identification of multiple gene mutations encoding RE-regulating proteins in patients with apparent epithelial polarity defects supports a critical role of RE in proper mammalian organ function and human health. With an increasing number of regulatory proteins (e.g. Rab8, -10, -11a/b, -17 and -25) recruited in non overlapping patterns to RE, a major challenge is to unravel the organization and spatial–temporal dynamics of the RE system. This will shed light on the relationship between CRE and ARE, and the question of whether RE membranes give rise to transport vesicles or if RE elements fuse directly with target PM domains. Future studies will highlight the contribution of cargo in shaping and compartmentalizing the

RE system. High-resolution four-dimensional imaging is needed to examine the coordinated dynamics of multiple, endocytosed and/or newly synthesized, apical and basolateral cargoes, and regulatory proteins, at RE. The number of proteins known to control segregation of apical and basolateral proteins and polarized targeting from RE remain limited. New approaches are needed to obtain a detailed mechanistic picture of polarity development and maintenance. Lipid-based determination and organization of apPM and blPM domains might act at RE to segregate apical and basolateral cargoes and to preassemble apPM and blPM membranes. The dynamic interaction of regulatory proteins with RE membrane lipids appears to be a promising area of research that will benefit from careful characterization of RE lipid composition (including CRE and ARE) and detailed insight into regulation of phosphoinositide lipid metabolism in relation to raft lipids.