Effects of lifestyle and environmental factors on intestinal epithelial cell morphogenesis

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Chapter 2

Mechanisms of cell polarity-controlled epithelial homeastais and immunity in the intestine

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Abstract
Intestinal epithelial cell polarity is instrumental to maintain epithelial homeostasis and balance communications between the gut lumen and bodily tissue, thereby controlling the defense against gastrointestinal pathogens and maintenance of immune tolerance to commensal bacteria. In this review article, we highlight recent advances with regard to the molecular mechanisms of cell polarity-controlled epithelial homeostasis and immunity in the human intestine.

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Absorptive intestinal epithelial cells are the predominant cell type of are the lumen-facing layer of the intestinal wall as they cover the intestinal villi. As such, intestinal epithelial cells are responsible for the metabolism and uptake of diet-derived nutrients and for their transfer to the tissue side where they can enter the blood circulation. Intestinal epithelial cells are also the first line of defense against potential pathogens which may easily enter the intestinal lumen via the mouth. The lumen of the intestinal tract, however, is also colonized by trillions of commensal bacteria that play important roles in normal physiology. Intestinal epithelial cells are thus confronted with the challenging task to maintain good relationships with these bacteria yet prevent these bacteria from entering the body tissue. At the tissue side, the intestinal epithelial cells must maintain good relationships with the immune system which actively monitors and probes the microbiota via intraepithelial immune cells. The intestinal epithelial cells thus ensure carefully balanced communications between the gut lumen and bodily tissue, thereby controlling the defense against gastrointestinal pathogens while maintaining immune tolerance to commensal bacteria.

A brief introduction to epithelial cell polarity in the intestine

Intestinal epithelial cells are arranged as a monolayer of columnar-shaped, polarized epithelial cells. Cells predominantly communicate with their environment via proteins (receptors, transporters, channels) at their surface. The surface (or plasma membrane) of the intestinal epithelial cell is divided into one apical and one basolateral domain, which face the lumen of the gut and the intestinal tissue, respectively. The basolateral cell surface domain can be further divided in a basal and a lateral domain that face the basement membrane and neighboring cells, respectively. The lateral surface houses intercellular adhesions, including E-cadherin-based adherens junctions and Claudin-based tight junctions, which ensure monolayer strength and impermeability, respectively [1]. The basal surface is home to integrin-based cell-matrix adhesions via which the cells are attached to the basement membrane. Planar polarity mechanisms ensure that all intestinal epithelial cells orientate their apical and basal surface domains in the same direction.

The apical and basal cell surface domains are readily distinguished with regard to both structural organization and macromolecular composition. Only the apical plasma membrane displays densely packed actin filament-based finger-like projections of ~80 nm in diameter, called microvilli [2]. Because the microvilli have the appearance of a brush at the electron microscopic level, the apical plasma membrane of intestinal epithelial cells is also referred to as the brush border. In addition to their structural differences, the apical and basal plasma membrane domains are differently equipped with enzymes and transporter proteins to control the metabolism, absorption and/or secretion of nutrients between the gut lumen, cell interior and body tissue. The polarized distribution of cell surface receptors and transporter proteins allows for the vectorial transport

of circulating molecules, such as nutrients and immunoglobulins, across the intestinal epithelial monolayer. Not surprisingly, the mislocalization of apical brush border proteins leads to malnutrition, diarrheal disorders and, if untreated, to death. The mislocalization of basement membrane receptors such as integrins at the opposing basal surface domain has been correlated with loss of epithelial architecture and cancer development [3]. Further, the polarized, basal expression of cytokine receptors mediate the cytokine-mediated communication with immune cells in the lamina propria, while the polarized, apical expression of several pattern recognition receptors controls the induction of innate immunity responses. Defects in the mechanisms that control apical microvilli development or the polarized distribution of cytokine receptors or pattern recognition receptors result in the perturbation of intestinal epithelial-microbial interactions and gut immune homeostasis in mice, and often contribute to the pathogenesis of inflammatory bowel diseases in humans [4–7], as will be further discussed below. Intracellular sorting and trafficking secures the polarized distribution of integral membrane proteins at the apical and basal plasma cell surface domains [8,9]. The Golgi apparatus is well-known for its role in the polarized sorting and trafficking of proteins. In addition, the endosomal system and therewith associated proteins (e.g., the small GTPase Rab8, Rab11, Rab25 and their effectors, and the epithelium-specific polarized sorting factor adaptor protein (AP)-1B) have in the last decade emerged as important regulators of polarized sorting and trafficking of proteins [6,7,10–12]. Local interactions between proteins and the cytoskeleton secure and stabilize the polarized distribution of cell surface proteins. Intracellular sorting and trafficking also controls the constitutive or regulated polarized secretion of signaling molecules such as growth factors, cytokines and antimicrobial products.

Recycling endosomes have been implicated in the polarized secretion of cytokines in immune cells [13], but the mechanisms of polarized cytokine secretion in intestinal epithelial cells is not well understood.
Tight junctions, situated at the border between the apical and basal cell surface domains, establish tight cell-cell adhesion and restrict the lateral diffusion of apical and basal cell surface proteins, thereby securing cell surface polarity. Tight junctions, by virtue of the pore-forming Claudins, also control the paracellular transport of electrolytes and water [1,14], and restricts the localization of secreted molecules in either gut lumen or lamina propria [15,16]. In inflammatory bowel disorders, tight junctions and consequently the intestinal barrier is often impaired, allowing the translocation of pathogens or pathogen-derived molecules [14]. Tight junctions also recruit transcription factors and prevent their translocation to the nucleus, and in this way contribute to the regulation of cell proliferation [17]. The aberrant expression or distribution of several tight junction-associated proteins has been implicated in colon cancer development and progression [18,19], underscoring the importance of tight junctions in intestinal epithelial homeostasis.
The polarized intracellular trafficking machinery and tight junctions are central players in the epithelial polarity program [20]. Evolutionary conserved polarity protein complexes are the core components of this epithelial polarity program. Three polarity complexes are typically distinguished: a complex consisting of Crb3a, Pals1 and PATJ, a complex consisting of Par3, Par6, atypical (a)PKC and the small GTPase Cdc42, and a complex consisting of Lgl, Dlg and Scribble [20]. The individual loss or aberrant expression or function of most of these core cell polarity determining proteins has been correlated with impaired intestinal epithelial homeostasis [21–24] and inflammatory bowel disorders [25–30] .
Taken together, intestinal epithelial cell surface

polarity and tight junctions are instrumental to balance communications between the gut lumen and bodily tissue and control the defense against gastrointestinal pathogens and maintenance of immune tolerance to commensal bacteria [15,16,31]. Below, we highlight recent advances with regard to the molecular mechanisms of cell polarity-controlled epithelial homeostasis and immunity in the intestine.

Apical brush border dynamics contribute to epithelial homeostasis and innate immunity in the gut

Apical microvilli greatly enlarge the absorptive cell surface area while minimally influencing cellular volume. In agreement, apical microvillus atrophy has been correlated with sub-optimal absorption of diet-derived nutrients. Interestingly, recent studies have demonstrated novel roles for apical plasma membrane microvilli and their protein components in intestinal epithelial cells beyond absorptive surface expansion.

Villin is an actin modifying protein and in intestinal epithelial cells exclusively located in apical plasma membrane microvilli and the underlying subapical terminal web. [32]. Targeted disruption of the villin gene in mice did not impair the organization of apical microvilli, suggesting that Villin plays a minor or redundant role in the microvilli development [33]. Villin was demonstrated to be required to sever filamentous actin to depolarize microvilli and, in this way, allow intestinal epithelial cell migration and remodeling upon mucosal injury [34]. Villin was also shown to be subject to post-translational changes, *i.e.*, proteolysis, following gut infection and during the recovery phase of gut infection, but not in immune deficient mice, suggesting a role for immune cells **[35]**. Villin expression in intestinal epithelial cells is reduced in inflammatory bowel disorders characterized by recurring inflammation and associated lesions [36]. These results highlight

the importance of the apical polarity of intestinal epithelial cells and, specifically, the apical plasma membrane microvilli and their dynamics, in the process of gut wound healing and, hence, intestinal epithelial homeostasis and immunopathology.

While the regulated breakdown of apical microvilli appears to be necessary for gut wound healing, apical microvilli are also important for maintaining intestinal epithelial homeostasis. This is well exemplified by the deletion of the only member of the Ezrin-Radixin-Moesin (ERM) family of membrane-actin crosslinking proteins present in the intestinal epithelial cells, Ezrin. Germline as well as conditional deletion of Ezrin led to apical microvillus atrophy throughout the intestinal epithelium, and resulted in villus morphogenesis defects (*i.e.*, villus fusions) and neonatal death in mice [37,38]. Similar effects were observed in Crumbs3-deficient mice [21,22], presumably because of the functional interaction between Crumbs3 and Ezrin [21]. Moreover, villus fusions were also reported in humans with microvillus inclusion disease and carrying *MYO5B* mutations [39]. Loss of the Myosin Vb protein in these individuals was shown to inhibit the activation of Ezrin at the apical surface and lead to microvillus atrophy in their intestinal epithelial cells [39]. The loss of Ezrin-mediated microvillus atrophy in the adult mouse intestine caused defects in cell geometry, cell extrusion, cell-cell adhesion remodeling, and mitotic spindle orientation [38], which are essential for intestinal epithelial homeostasis and villus morphogenesis. By contrast, an increased expression of Ezrin has been correlated with elongated apical microvilli in the small intestine of Vitamin D receptor knock-out mice [40]. Ezrin is proteolytically cleaved in mice with intestinal inflammation in a $CD4^+$ T-cell-dependent manner [35], but the physiological or pathophysiological relevance of this phenomenon is not clear.

The intestinal brush border, in agreement with

its role in the regulation of epithelial tissue morphogenesis and architecture (see above), protects against carcinogenesis. Thus, the loss or inactivation of Myosin Ia, another abundant component of microvilli and necessary for microvilli development [41], leads to the loss of intestinal epithelial cell polarity, carcinogenic behavior of intestinal epithelial cells and tumor development in mice [42]. These results point to a functional link between apical plasma membrane architecture and epithelial cell homeostasis.

Apical microvilli are highly dynamic structures, governed by interactions between the apical plasma membrane and the underlying actin cytoskeleton. The motor activity of Myosin Ia and Myosin VI controls the microvillus tip- and base-directed movement, respectively, of the plasma membrane along the actin filaments that make up the microvillus core [43]. As such, these myosins regulates the intra-microvillus (that is, microvillus tip versus microvillus base) distribution of brush border enzymes [44–46]. Interestingly, the tips of the apical microvilli give rise to vesicles that are shed into the gut lumen [47]. These microvillus-derived vesicles are enriched in Intestinal Alkaline Phosphatase. This enzyme allows the vesicles to dephosphorylate and, thereby, detoxify bacterial lipopolysaccharide and prevent Toll-like receptor 4 (TLR4) responses on host cells. Furthermore, the exposure of intestinal epithelial cells to *Escherichia coli* induced the expression of intestinal alkaline phosphatase and the production of microvilli-derived vesicles which, in turn, inhibited *Escherichia coli* proliferation and the attachment of these bacteria to the host cells *in vitro*, albeit in an Intestinal Alkaline Phosphatase-independent manner [6]. The activity of Myosin Ia appears to be crucial for the correct assembly of these microvilli-derived vesicles, as microvilli-derived vesicles in mice and cell lines lacking Myosin Ia were not enriched in Intestinal Alkaline Phosphatase

but, rather, displayed a protein composition that was similar to the overall enterocyte brush border [6]. Together, apical brush border microvilli release membrane vesicles into the intestinal lumen which are laden with host defense machinery, and indicate a new role for apical plasma membrane microvilli in intestinal innate immunity [7]. Whether loss of Villin, Ezrin or Myosin Vb and resultant microvillus atrophy affects the release of microvilli-derived vesicles and therewith associated functions, and whether microvilli-derived membrane vesicles contribute to intestinal epithelial wound healing, homeostasis and/or tumor suppression, is not known.
In conclusion, these studies have demonstrated novel roles for apical plasma membrane microvilli and their protein components in wound healing, epithelial homeostasis and tumor suppression, and innate immunity in the gut.

Polarized endosomal sorting and trafficking mechanisms control epithelial homeostasis, tumor suppression, and innate immunity in the gut.

The endosomal system has emerged as a crucial regulator of polarized sorting and trafficking of cell surface proteins in epithelial cells [10]. In addition, the spatial distribution of endosomal system may provide polarized signalling platforms [39,48,49] that control the activation of Ezrin at the apical surface of intestinal epithelial cells and, thereby, the development of the apical brush border membrane [39]. While the endosomal system consists of a heterogeneous network of vesicular an tubular structures [50,51], two endosomal sub-compartments have been identified that appear particularly important for the correct sorting and trafficking of apical and basal proteins: the apical recycling endosome and the common recycling endosome. The apical recycling endosome is characterized by the presence of small GTPases Rab8, Rab11a, Rab25 and their common effector

protein Myosin Vb. The apical recycling endosome is predominantly accessible for resident apical proteins and functions in the dispatch of newly synthesized and apically recycling and/or transcytosing proteins to the apical brush border membrane. The common recycling endosome is characterized by its accessibility to proteins internalized from either apical or basal surface domain [52] and contains the polarized sorting factor adaptor protein AP-1B, which is believed to control the sorting of proteins to the basal cell surface. Noteworthy, most of the knowledge about the organization of the endosomal system in polarized cells is derived from Madin-Darby canine kidney epithelial cells [50,51], and whether this organization of the endosomal system is similar in intestinal epithelial cells has not been carefully addressed. Nevertheless, components of the apical and/or common recycling endosome play a role in the regulation of epithelial homeostasis, tumor suppression, and innate immunity in the gut, as outlined below.

The apical recycling endosome

Intestine-specific *Rab11a* knockout mice show intracellular accumulation and mislocalization of resident apical plasma membrane proteins to the basal surface domain, microvillus atrophy and microvillus inclusion bodies [53,54]. Some apical proteins, such as Dipeptidyl Peptidase IV and Intestinal Alkaline Phosphatase were downregulated in the intestinal epithelial cells of *Rab11a* knockout mice, while the polarized distribution of basal proteins appeared unaffected. *Rab11a* knockout mice die in the postnatal period as the consequence of starvation. Intestinal epithelial cells of intestine-specific *Rab8* knockout mice, *Myo5b*-knockout mice, and individuals with microvillus inclusion disease that carry *MYO5B* mutations show very similar intracellular accumulation of resident apical plasma membrane proteins, microvillus atrophy and

microvillus inclusion bodies, yet died of diarrhea [12,55,56]. The apical transport abnormalities in intestinal epithelial cells in *Rab8*-deficient mice may be the result of effects of *Rab8a* depletion on the secretion of Wnt ligands, which play an important role in intestinal epithelial morphogenesis [57]. The aberrant expression or function of either Rab11a, Rab8 or Myosin Vb in mice or humans affected each other's expression or subcellular distribution in the intestinal epithelial cells [12,24,39,58(p11),59,60]. Together, these results support the *in vitro* evidence that Rab11a, Rab8 and Myosin Vb operate in the same pathway that control the trafficking of apical brush border proteins and development of apical microvilli in (intestinal) epithelial cells [11,39,49].

The *RAB11A* gene is located adjacent to a Crohn's disease risk locus [4]. Intestine-specific *Rab11a* knockout mice spontaneously develop colitis, and exhibit excessive intestinal epithelial proliferation. Intestinal epithelial cells of *Rab11a* knockout mice show upregulation of interleukins-6 and -1B and monocyte chemoattractant protein-1, and show a redistribution of Toll-like receptor 9 (TLR9) from its normal brush border location to late endosome and/or lysosomes. Stimulation of TLR9 at the apical surface domain of intestinal epithelial cells was shown to counteract NF-κB activation that curtailed inflammatory responses induced by basal stimulation by other TLRs or TNF-alpha [61,62]. *TLR9* knockout mice are highly susceptible to experimental colitis [61]. In line with these observations, intestinal epithelial cells of *Rab11a* knockout mice exhibit upregulated signalling activity through NF-κB and mitogen-activated protein kinase, which is involved in inflammatory and stress responses [63]. Moreover, germ-free *Rab11a* knockout mice failed to tolerate intraluminal stimulation by microbial agonists and induced interleukin-6 when compared to wild type mice undergoing the same treatment [4]. Thus, the apical recycling endosome, by virtue of Rab11a,

controls subcellular TLR9 compartmentalization and, consequently, is crucial for the regulation of immune tolerance and inflammation.

At least in cultured epithelial Madin-Darby canine kidney cells, Rab11a and Rab8 at apical recycling endosomes together regulate the activation of the small Rho family GTPase Cdc42 [49]. In Cdc42-deficient mice, the intestinal epithelium showed gross hyperplasia, crypt enlargement, microvillus inclusions and brush border formation at the lateral surface domains, and abnormal epithelial permeability, suggesting a coordinating role for Cdc42 in the polarity, migration and differentiation of intestinal epithelial cells [23]. In other intestine-specific Cdc42-deficient mice, generated independently, impaired Rab8 activation, intestinal stem cell division, survival, and differentiation of intestinal epithelial cells was observed [24]. Also in human colorectal carcinoma cells, loss of Cdc42 caused mitotic spindle orientation defects and deranged intestinal epithelial morphogenesis [64]. Furthermore, single intestinal epithelial LS174:W4 cells show multiple brush border domains and dispersed apical recycling endosome localization [65]. Notably, in contrast to the loss of Rab11a [4], the loss Cdc42, Rab8 or Myosin Vb in mice did not elicit intestinal inflammation [23]. This suggests that the latter three proteins play a minimal or redundant role in the regulation of intestinal immunity, and it will be of interest to investigate the distribution of TLR9 in the intestinal epithelial cells of Cdc42- Rab8- and Myosin Vb-deficient mice.

In addition to Rab11a and Rab8, Rab25 is an epithelial-specific component of the apical recycling endosome [66]. Depletion of Rab25 in human intestinal Caco-2 cells resulted in disorganized apical microvilli, loss of apical villin and the intracellular retention of the brush border protein Sucrase-Isomaltase, without affecting the expression of Rab11a or Rab8 [3]. Unlike Rab11a and Rab8, Rab25 has been linked to tumor aggressiveness and

metastasis in several tissues [67]. Loss of Rab25 was demonstrated to promote the development of intestinal neoplasia and was found to be associated with human colorectal adenocarcinoma's [68]. As part of the underlying mechanism, loss of Rab25 from intestinal colon carcinoma Caco-2 cells was shown to lead to upregulated Claudin-1 expression (previously associated with perturbed intestinal epithelial homeostasis and colon cancer development [69]), increased trans-epithelial resistance, and increased invasive behavior [68]. Loss of Rab25 likely contributed to invasiveness of (colon) cancer cells by regulating basal integrin expression and/or trafficking [3,70] via Rab Coupling Protein [71] and consequent reorganization of the cortical actin cytoskeleton [72,73]. Loss of Rab25 did not appear to affect E-cadherin-based adherens junctions [3]. Together, these findings suggest that Rab25 is an important regulator of intestinal epithelial homeostasis and a tumor suppressor in colon carcinogenesis [74]. The occurrence of spontaneous colitis, defects in in the polarized distribution of TLRs or alterations in other aspects of intestinal immunity have not been reported in Rab25-deficient mice. Notably, Rab25 and Myosin Vb were demonstrated to control the transcytosis of the neonatal major histocompatibility complex class I–related IgG receptor FcRN, when ectopically expressed in Madin-Darby canine kidney cells, between the apical brush border and the basal surface [75]. In an *in vivo* setting in the intestine, the loss of Rab25 could then be predicted to impair IgG-mediated humoral immunity of the fetus or newborn, but this has not been demonstrated.

The common recycling endosome

The best-characterized regulator of polarized protein sorting at the common endosomes is AP-1B. AP-1B is downregulated in colonic epithelium of individuals with Crohn's disease [5], an inflammatory bowel disease. Further, a reduced ratio of tumor to non-

tumor tissue expression of AP-1B was correlated with nuclear translocation of b-catenin in human colorectal tumor tissue, indicative for a hyper-proliferative state [76].
Deletion of the AP-1B gene in mice resulted in the mislocalization of several basolateral proteins. For example, the low-density lipoprotein receptor accumulated in cytoplasmic vesicular structures. AP-1B deficiency led to mistargeting of a subset of basolateral cytokine receptors to the apical plasma membrane [5]. Also E-cadherin was mislocalized to cytoplasmic structures, the E-cadherin-beta-catenin interaction was inhibited, and enhanced nuclear translocation of beta-catenin was observed [77]. The mislocalization of E-cadherin was also observed in an intestinal epithelial cell line that lacked the mu1B subunit, and the ectopic expression of the latter restored the normal distribution of E-cadherin [76]. The nuclear translocation of beta-catenin was shown to stimulate the pre-proliferative beta-catenin/Tcf4 pathway, and *Ap1m2*$^{-/-}$ mice showed massive elongation of the small intestine and intestinal crypt hyperplasia with villous dysplasia as a result of excessive proliferation of epithelial cells.
Interestingly, although AP-1B is considered to be a regulator of basolateral protein sorting [78], also resident apical proteins were found missorted in the intestinal epithelial cells of *Ap1m2*$^{-/-}$ mice. For example, the resident apical proteins Villin and Sucrase-Isomaltase were mistargeted to the lateral plasma membrane. The apical surface of the intestinal epithelial cells developed sparse and disorganized microvilli, while ectopic microvillus-like structures developed at the lateral membrane. In accordance, these mice developed digestive and/or absorptive defects [77]. In *Ap1m2*$^{-/-}$ mice intestinal IgA responses were induced, but the basal to apical transcytosis of IgA from the lamina propria to the lumen of the intestine was impaired [5], possibly reflecting impaired basal sorting of the polymeric

IgA receptor. Also in the nematode C. Elegans AP-1 was shown to control an apical trafficking pathway and regulate apical polarity and intestinal epithelial morphogenesis [79,80]. The role of AP-1B in the delivery of apical proteins in intestinal epithelial cells may be explained by the observation that some apical membrane proteins, including Sucrase-Isomaltase, are transported in distinct vesicular carriers [81] and are first sorted to the basal surface, from where these are subsequently internalized and transcytosed to the apical brush border domain of intestinal epithelial cells [82–84]. In conclusion, AP-1B is required for the delivery of (at least a subset of) proteins to the apical surface in intestinal epithelial cells and, as a result, controls the function of the intestinal apical brush border surface.
Ap1m2$^{-/-}$ mice spontaneously developed colitis [5].
It was proposed that the missorting of cytokine receptors from the basal to the apical surface, the reduced expression of antimicrobial proteins, and the impaired apical release of IgA in these mice resulted in intestinal dysbiosis and increased bacterial translocation from the lumen of the gut into the lamina propria [5].
These results demonstrate the importance of the common recycling endosome-associated polarized sorting factor adaptor protein AP-1B as a crucial player in the establishment of intestinal epithelial polarity, epithelial homeostasis, and immunity in the gut.

Evolutionary conserved cell polarity determining proteins regulate epithelial homeostasis, tumor suppression, and innate immunity in the gut

Three evolutionary conserved protein complexes, initially discovered in the nematode Caenorhabditis elegans [85], play a pivotal role in the development of cell polarity, including mammalian epithelial cell polarity [20]. These proteins complexes include 1) the

Crumbs3/protein associated with tight junctions (PATJ)/protein associated with Lin Seven (Pals)1 complex, 2) the Partitioning defective (Par)3/Par6/ atypical protein kinase C (aPKC) complex, and 3) the Scribble/Lethal giant larvae (Lgl)/Discs large (Dlg) complex. Many of these proteins are tumor suppressors and therefore link epithelial cell polarity to the maintenance of epithelial homeostasis. These polarity protein complexes have been extensively studied in a variety of polarized cell systems, and much less in the intestine. Nonetheless, as outlined below, the aberrant expression, localization and/ or function of the human orthologues of these proteins have been implicated in defective epithelial homeostasis and immunity in the gut.

The Crumbs3/PATJ/Pals1 complex

Crumbs3 is localized to the apical and subapical area of epithelial cells from the mouse and human intestine [86]. Studies in intestinal epithelial Caco-2 cells demonstrated that PATJ stabilized the Crumbs3 complex and regulated the spatial concentration of several components at the border between the apical and lateral domains [87]. While PATJ or Pals1-deficient mice have not been reported, Crumbs3-deficient mice show defects in intestinal epithelial morphogenesis via its role in apical brush border organization [21,22]. Of interest, tight junctions and the barrier function of the intestinal epithelium appeared unaffected in Crumbs3-deficient mice [21].

The Par3/Par6/aPKC complex

The atypical (a)PKC is aberrantly expressed in intestinal tissue from individuals with active and inactive inflammatory bowel disease [27]. A negative correlation has been reported between the expression of active aPKC and local inflammation. Tumor necrosis factor alpha and dextran-sulfate sodium-induced inflammation in mice were shown

to disrupt the Par3/Par6/aPKC polarity complex and its activity in intestinal epithelial cells via a posttranslational mechanism which involved the degradation of aPKC as a result of inhibited chaperoning activity of BAG-1M and Hsc/Hsp70 [26,88]. Par3 and aPKC were proposed to act as inhibitors of the canonical NF-κB activation pathway in this way involved in pro-inflammatory responses [89]. Loss of aPKC or Par3 by RNA interference in cultured intestinal epithelial cells phenocopied inflammatory signaling, as evidenced by enhanced NF-κB activity and resultant Myosin light chain kinase (MLCK) expression, an enhanced TNF-alpha response, and enhanced paracellular leakage [26,89]. Genetic variants of Par3 have been associated with coeliac disease and ulcerative colitis in a Dutch cohort [90]. Reduced expression of Par3 in cultured intestinal epithelial cells was associated with altered expression and assembly of several tight junction proteins [25], the latter of which, in turn, have been associated with enhanced paracellular leakage in intestinal inflammatory diseases [91,92] including coeliac disease [25], ulcerative colitis [93] and Crohn's disease [91]. Atypical PKC, and Par6B show aberrant localization in the intestinal epithelial cells of individuals with microvillus inclusion disease carrying *MYO5B* mutations [39,48,94], which is in agreement with a reported role for apical recycling endosomes in the regulation of the subcellular distribution of these polarity proteins [10,39]. The mislocalization of aPKC-iota from the subapical domain of intestinal epithelial cells was associated with impaired activation of ezrin at the apical surface and consequent impaired brush border development [39]. Individuals with microvillus inclusion diseases do not typically show signs of intestinal inflammation [95], suggesting that the mislocalization of the Par3/Par6/aPKC complex, as such, does not necessarily compromise the intestinal barrier function and immunity in the gut. Further, hypoxic stress signaling resulted in the

angiomotin-mediated retention of Par3 and Crumbs3 in intracellular vesicles and prevented these proteins from reaching the apical cell surface, and the resulting loss of cell polarity potentiated the response to invasive cues, both *in vitro* in intestinal epithelial Caco-2 cells and *in vivo* in mice [96]. Par6 was reported to play a role in the directional, *i.e.*, polarized migration of intestinal epithelial cells as part of the intestinal wound healing response [97].
In conclusion, both the expression and localization of members of the Par3/Par6/aPKC polarity complex are important, albeit in different ways, for intestinal epithelial functions. The maintenance of proper expression levels of members of the Par3/Par6/aPKC polarity complex appears important for the barrier function of the intestinal epithelium and thereby for innate immunity, and reduced expression and/or activity of aPKC and Par3 likely contribute to epithelial barrier dysregulation in inflammatory bowel diseases [26]. By contrast, the maintenance of the proper subcellular localization of members of the Par3/Par6/aPKC complex appears important for apical brush border membrane development and preventing carcinogenesis but, based on the available data, not for the regulation of immunity in the intestine.

The Scribble/Lgl/Dlg complex

The expression of Scribble was reported to be downregulated in inflamed colonic mucosa of an individual with active Crohn's disease [28], an inflammatory bowel disease. RNA interference studies in human intestinal cell lines demonstrated that Scribble, independently of its interaction with LgL and Dlg, regulated epithelial barrier function via its effects on the *de novo* assembly and reassembly of tight junctions [28]. The depletion of Scribble did not affect E-cadherin-based adherens junctions [28]. *In vitro* exposure of intestinal epithelial cells to

interferon-gamma (IFNγ), a key pro-inflammatory cytokine in inflammatory bowel disease [98], resulted in the mislocalization of Scribble away from the tight junctions, suggesting that IFNγ-induced depletion of Scribble from tight junctions may contribute to the breakdown of the epithelial barrier during intestinal inflammation or impair the recovery of the intestinal epithelial barrier during epithelial restitution [28]. Inflammatory bowel disease is an important etiologic risk factor for the development of colorectal cancer [99]. Notably, changes in the expression patterns of Scribble and Dgl are correlated with loss of colon tissue architecture during malignant progression [100]. Further, Scribble was found to accumulate in colorectal neoplasia in association with an altered distribution of beta-catenin [101]. In addition to Scribble, the human orthologue of the Drosophila *Melanogaster* tumor suppressor gene Lgl, was found to be reduced in colorectal tumor samples in a stage-dependent manner [102], and the human orthologue of Drosophila *Melanogaster* Discs Large (Dlg) has been associated with inflammatory bowel diseases [30,103], albeit debated [104], and colon cancer [105]. Altogether these findings suggest a role for the cell polarity-regulating Scribble/Lgl/Dlg complex in the pathogenesis of inflammatory bowel disease [28] and in colon carcinogenesis [101].

Concluding remarks

The polarity of intestinal epithelial cells is essential to properly balance communications with the microbiota and immune cells at opposite sides of the epithelial barrier and, in this way, ensure effective immune responses that allows a full restoration of intestinal tissue function when inflammation is resolved. Defects in the mechanisms that control the polarity of intestinal epithelial cells are associated with impaired epithelial homeostasis, impaired innate immunity and inflammatory bowel diseases, and colon carcinogenesis.

Particularly the apical brush border membrane and the recycling endosomal system appear as prominent regulators of epithelial homeostasis, tumor suppression and immunity in the gut. Interestingly, components of both the apical recycling endosome system (Rab11a, Rab8, Rab25, Myosin Vb) and the common recycling endosome system (AP-1B) appear to be involved in epithelial homeostasis and immunity in the intestine, and further research is warranted to elucidate how these proteins and the different endosomal compartments cooperate.

Rab8 and AP-1B were initially reported as regulators of basolateral protein sorting and trafficking in cultured Madin-Darby canine kidney cells [78,106–108]. Intestine-specific deletion these proteins, however, have implicated these proteins (also) in the regulation of apical protein sorting and /or trafficking [5,12,109]. These results suggest that the function of these proteins may differ between *in vitro* and *in vivo* contexts, or may be different in intestinal epithelial cells. Regardless, the study of these proteins in intestinal epithelial cells have shed new light onto their function in cell polarity.

Notably, although Rab11a, Rab8, Rab25 all have been implicated in the regulation of intracellular trafficking via the apical recycling endosomes and all can interact with Myosin Vb, the individual depletion of these proteins from the intestine in mice gives rise to only partly overlapping and often distinctive effects on intracellular protein expression and distribution, epithelial homeostasis and immunity in the intestine. This may indicate that the apical recycling endosome and/or its molecular components display extensive functional heterogeneity with regard to the regulation of nutrient absorption, epithelial homeostasis and tumor suppression, and immunity. A caveat however is the non-uniformity of read-out between the different knock-out mice as reported in the different studies. For example, TLR mislocalization has been studied in the mouse

intestine depleted of Rab11a, but not in the mouse intestine depleted of Rab8, Rab25 or Myosin Vb (or at least not reported). A more comprehensive and uniform read-out of effects is therefore needed in order to obtain better insight into the roles of the apical recycling endosomal system and its regulators in the different processes.

Along the same lines, further studies are required to determine the organization and function of the endosomal system, expression and function of cell polarity-determining proteins and immunity-regulating cell surface proteins 1) along the vertical (crypt-to-villus) axis (e.g., [110], and [111]), 2) along the horizontal (from duodenum to colon) axis of the intestine [112], and 3) as a function of age [113,114], and to determine how variations in these contribute to the regulation of intestinal epithelial homeostasis and immunity.

An intriguing finding was that apical surface microvilli appear to give rise to extracellular vesicles that can interfere with bacteria in the lumen of the gut. It will be of interest to determine the consequences of microvillus atrophy - as a result of different causes - on the regulation of immunity in the intestine. Further, it will be of interest to determine the potential contribution of apical microvilli-derived vesicles to normal intestinal epithelial homeostasis and tumor suppression.

Perturbed immunity in the intestine and the initiation and/or progression of colon carcinogenesis have been proposed to be associated. The observation that inhibition or depletion of some polarity-determining proteins give rise to both inflammation and colon cancer are in support of this. However, in some cases defects in either immunity or tumor suppression are observed, underscoring that these events may not be necessarily linked, and offering tools to further investigate their interrelationship.

In conclusion, intestinal epithelial cell polarity is at the heart of epithelial homeostasis and immunity

in the intestine. Defects in the mechanisms that underlie intestinal epithelial cell polarity are functionally associated with inflammatory bowel diseases and colon carcinogenesis, of which the pathogenesis is not fully understood and for which cures do not exist. The further elucidation of the mechanisms that underlie intestinal epithelial cell polarity will contribute to the further elucidation of the pathogenesis of these diseases and is expected to provide potential molecular targets that may be exploited for therapeutic interventions.

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