Plasmolipin—a new player in endocytosis and epithelial development

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Polarized vesicle sorting is essential not only for epithelial cell function but also for cell polarization and tissue morphogenesis. Endocytosis is a key determinant of the surface abundance of plasma membrane proteins and is highly regulated. In an important recent paper, Rodríguez-Fraticelli *et al* (2015) identify a new player in apical endocytosis—a previously uncharacterized protein called Plasmolipin. They report not only its mechanism of action through binding to an epsin, but also highlight an essential role in regulating Notch signaling, which controls epithelial differentiation.

See also: **AE Rodríguez-Fraticelli *et al***
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Despite decades of work by multiple laboratories on the mechanisms of endocytosis, the full complement of proteins involved in this process likely remains incomplete. This is particularly the case for polarized epithelia, which possess multiple endocytic pathways. In addition, most studies on vesicle traffic in higher organisms have been performed on cell lines grown in 2D cultures, which probably lack some of the components needed by functional tissues *in vivo*.

To address this issue, Rodríguez-Fraticelli and colleagues recently screened for genes expressed in the zebrafish midgut that were induced during lumen formation and expansion (Rodríguez-Fraticelli *et al*, 2015). They identified a gene called plasmolipin (*pllp*), which encodes a tetraspanin protein of unknown function. Plasmolipin (PLLP) contains a MARVEL domain that is associated with proteins involved in vesicle traffic (Sanchez-Pulido *et al*, 2002), and is highly

expressed in the brain where it is associated with myelin, but is also found in the apical region of epithelial cells. The authors exploited the power of zebrafish genetics to create transgenic animals either defective in *pllp* or that express a PLLP-GFP fusion protein. They found expression of *pllp* in the posterior midgut and specifically in the apical region of the intestinal epithelial cells. PLLP-GFP localized to vesicles, apical microvilli, and basal endosomes. Zebrafish mutant for *pllp*, created using TALEN gene editing, showed defects in intestinal absorption, and the intestinal epithelial cells contained enlarged endosomes. Moreover, PLLP partially co-localized with Rab11—a marker of the apical recycling endosome (ARE) compartment—and in the absence of PLLP, Rab11 was mislocalized. Taken together, these data strongly suggested an essential function for PLLP in apical endocytosis.

The authors confirmed this hypothesis using MDCK cells and found that the over-expression of PLLP is sufficient to enhance the formation of the ARE. Their next goal was to determine the molecular mechanism underlying this function. The authors used the powerful BioID method (Roux *et al*, 2012) to biotinylate and isolate proteins that might interact with PLLP. Of 42 candidates, 20 were associated with vesicle sorting. Two major interactors were an epsin, EpsR, which is required for retrograde transport from late endosomes, and Syntaxin 7 (Stx7), which is a known cargo for EpsR and drives fusion with endosomal membrane (Miller *et al*, 2007). Silencing of either EpsR or Stx7 phenocopied loss of PLLP in MDCK cells, strongly suggesting that the interaction with these proteins is of functional importance.

What are the consequences of disrupting apical endocytosis through PLLP? In addition

to absorption of nutrients by the intestine, and ion transport in the kidney, the morphogenesis of epithelial tissues itself is dependent on the traffic of apical vesicles. The abundance of the polarity protein Crumbs (Crb) at the apical cortex, for example, controls the size of the apical domain, and defects in either delivery or endocytosis would likely alter its abundance with deleterious consequences to epithelial function. Indeed, the *pllp*-mutant zebrafish exhibits abnormal apical accumulation of Crb3 in the intestinal epithelium, and a similar phenotype was found in MDCK cells, together with a defect in the enrichment of Crb3 at the tight junctions. The authors used an elegant methodology called RUSH, developed by Franck Perez and colleagues, to address the dynamics of Crb3 delivery, in which a biotin-tagged protein is trapped in the endoplasmic reticulum through association with an ER-resident streptavidin (Boncompain *et al*, 2012). Addition of biotin to the cells releases the tagged protein, so its subsequent transport through the vesicular system of the cell can be tracked. In this case, the Crb3 could be observed to arrive first at the apical surface, and later became enriched at the tight junctions. This enrichment was lost in the absence of PLLP. Inversely, the over-expression of PLLP resulted in decreased apical Crb3.

As a second example of the importance of PLLP-dependent apical transport, the authors examined Notch signaling, which is required for the differentiation of intestinal epithelia (Fre *et al*, 2011) and which in *Drosophila* is known to involve Stx7 (Vaccari *et al*, 2008). Importantly, the mutant *pllp* zebrafish showed reduced Notch signaling and defects in intestinal epithelial differentiation, a phenotype that could be recapitulated by a zebrafish mutant in the

Figure 1. PLLP is required for Notch signaling and zebrafish midgut differentiation.

PLLP regulates Notch signaling through endocytosis and results in correct patterning of the posterior midgut. In the absence of PLLP, Notch signaling is impaired and induces defects in intestinal epithelial cell differentiation, model based on Rodríguez-Fraticelli *et al* (2015).

Notch pathway or by chemical inhibition of Notch signaling.

Ligand engagement of Notch induces the proteolytic cleavage of Notch to release an intracellular domain (NICD), and NICD levels were significantly reduced by silencing of PLLP or EpsR, which suppressed Notch1 endocytosis. Notch ligands are also membrane associated. When MDCK cells expressing Notch1 were co-cultured above cells that express the ligands Delta-1 or Jagged-1, the silencing of PLLP expression specifically blocked activation of Notch signaling by Jagged-1 (Fig 1). This is perhaps surprising since it is the basal surface rather than the apical surface of the MDCK cells that would presumably make contact with the ligand-expressing cells. The authors also co-cultured MDCK cells expressing Jagged-1 with the Notch1 MDCK cells and found again that PLLP or EpsR knockdown suppressed signaling—even though it would be the lateral membranes that would contact one another in this situation rather than the apical membranes. These data suggest that PLLP function is not confined to the apical domain, but is also needed for basolateral endocytosis.

Together, this interesting study identifies a novel function for the uncharacterized PLLP protein in the promotion of epithelial endocytosis, and reveals the importance of this function in fine-tuning Notch signaling, which is essential for the proper development and differentiation of the intestinal epithelium, particularly of the posterior gut absorptive cells. It will be of great interest to explore PLLP function in other tissues where it is expressed at high levels, such as the brain and kidney, and to determine if it is coupled to Notch signaling in these other situations. It will also be important to determine if PLLP is regulated solely by expression level or is subject to post-translational modifications, whether its function is restricted to specific cargoes, and to explore further the mechanism through which it promotes endocytosis.

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