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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

Tight Junction Proteins Join the Local Force for Bulk Endocytosis and Microvillus Inclusion

In order for the mammalian gastrointestinal tract to optimally perform its diverse functions including nutrient absorption and defense against microbial pathogens, intestinal epithelial cells must form a physical and biochemical barrier protecting host tissues from the external environment. These epithelial cells are held together by a series of lateral junctions (adherens, tight, and desmosomal) forming a polarized tissue with unique apical and basolateral membranes to direct the trafficking of molecules through the epithelium and prevent transfer of harmful substances to the host. Several disorders are the result of transiently or continuously impaired epithelial cell polarity and barrier integrity, including diarrhea, irritable bowel syndrome, and cancer.[1] Microvillus inclusion disease is a severe pediatric enteropathy that causes secretory diarrhea and malabsorption in infants. The pathology of microvillus inclusion disease is characterized by perturbed cell polarity, intracellular protein mislocalization, a lack of apical microvilli on enterocytes, and formation of cytoplasmic microvillus inclusions.[2] Mutations in Myosin 5b (*MYO5B*), a motor protein crucial for apical transport in intestinal epithelial cells, have been found in most patients with microvillus inclusion disease.[3] Although MYO5B deficiency has been shown to result in mislocalized apical and basolateral proteins,[4] the cellular mechanisms underlying the formation of those hallmark inclusions in patients with microvillus inclusion disease remain inconclusive.

Previous work by Engevik et al[5] demonstrated that Myo5b null mice develop cytoplasmic microvillus inclusions, which were promoted through activity-dependent bulk endocytosis, a mechanism used for synaptic vesicle uptake during intense neuronal stimulation. In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Engevik et al[6] demonstrate through continued analysis of MYO5B-deficient animals and *in vitro* models, that MYO5B deficiency leads to mislocalization of core apical polarity complexes and tight junction proteins, because both were found to localize with developing microvillus inclusions. Prior literature suggesting a redistribution of polarity proteins in patients with microvillus inclusion disease,[7] and the association of core polarity complexes with Rab11a, a MYO5B-binding small GTPase regulating apical transport,[8] led the authors to investigate how loss of MYO5B may affect the localization of polarity proteins. Using immunofluorescence and electron microscopy, the authors demonstrate that 2 major apical polarity complexes are found on cytoplasmic internalized inclusions within MYO5B-deficient mouse intestinal epithelial cells. Additionally, they found that tight junction proteins, including Claudin-2, ZO-1, and ZO-2, which are typically located at the apical junctional complex between intestinal epithelial cells, were recruited to ectopic sites where inclusion bodies develop. Interestingly, they found fully internalized inclusion bodies that were not associated with tight junction proteins, suggesting a potential novel role of these proteins for inclusion formation and scission from the apical membrane. Using a pig model of microvillus inclusion disease, in which the animals express a MYO5B mutation found in human microvillus inclusion disease patients, the author observed similar events. Finally, to explore their hypothesis that tight junction proteins coordinate scission of the inclusions for internalization, they investigated the localization of cortactin, an actin-binding protein that plays a role in endocytosis. In Myo5b null mice, the tight junction protein ZO-1 formed “caps” directly associated with the developing inclusion bodies that were also colocalized with discrete cortactin puncta. These data suggest that tight junction proteins are recruited during apical bulk endocytosis.

Engevik et al[6] continue to build on a body of work examining the importance of the actin cytoskeleton mechanoenzyme MYO5B in maintaining and establishing apical surface structure and function. Most importantly, this study confirmed that MYO5B-deficiency results in mis-trafficking of tight junction proteins and polarity-determining complexes to the forming and internalized inclusion bodies. Based on their current findings and previous literature demonstrating that Rab11 is necessary for the apical polarity protein Crumbs to properly localize,[9] the authors speculate that MYO5B is required for proper localization of Rab11a, which itself regulates the localization of apical polarity proteins.[10,11] Future studies examining such interactions will most certainly further the understanding of the mechanisms that drive and define apical structure and function.

Additionally, the authors’ data allude to potential redundant functions of apical polarity proteins. Inhibiting the core polarity protein aPKC in MYO5B-deficient intestinal organoids did not reduce the formation of microvillus inclusions, suggesting other polarity proteins may contribute to form these inclusion bodies. In the future, it would be interesting to examine the function of other apical polarity-determining components during inclusion formation, but this would likely require combinatorial null mutation strategies because of the proposed redundancies. In conjunction with existing links between the tight junction protein ZO-1 and endocytosis regulators cortactin and dynamin,[12,13] the results point to a model in which tight junction proteins are recruited to sites of inclusion where they participate in forming a structural patch that may define the site of apical membrane invagination. The authors’ findings highlight the depth of further investigation required to fully understand

the coordination and interactions among polarity and tight junction protein complexes during apical bulk endocytosis.

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Conflicts of interest
The authors disclose no conflicts.

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