Title: Thinking small: zinc-sensing by the gut epithelium

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Abbreviations: ligand gated ion channels, LGIC; *hold on, don't rush'*, Hodor; major histocompatibility complex, MHC; target of rapamycin, TOR.

Main text

Intestinal sensing is critical to maintain homeostasis. The gut epithelium is predominantly inhabited by enterocytes that, in addition to their barrier and absorptive function, may play a surveillance role during nutrient uptake. Even insects such as *Drosophila melanogaster* possess a sophisticated nutrient-sensing system. This suggests that enterocytes translate nutrient cues into signals that regulate the local intestinal epithelium and likely impact the entire organism (growth and development, immunity, *etc.*).

Miguel-Aliaga's group has recently investigated nutrient sensing by the absorptive enterocytes in *Drosophila melanogaster*.[1] They demonstrated that enterocyte-specific deletion of the protein transporter CG11340 (or pHCl-2) caused developmental delay in larvae. For this reason, they named it Hodor (*'hold on, don't rush'*). Hodor is a pH-sensitive, zinc-gated chloride channel that belongs to the Cys-loop subfamily of ligand-gated ion channels (LGIC). Hodor loss resulted in: brain insulin-like peptide 2 accumulation; increased autophagy; decreased food intake and lower activation of target of rapamycin (TOR); and the acidification of autophagic compartments in the copper cell region of the gut (acidic region of the middle midgut in *Drosophila* constituted by copper cells and Hodor-expressing interstitial cells). These observations indicate that TOR activation in Hodor-expressing interstitial cells regulates feeding and larval growth by promoting food intake and systemic insulin signaling.[1]

Initially, LGICs were thought to be neurotransmitter-gated receptors. However, this concept has been updated with the discovery of prokaryotic homologs, a zinc-activated channel[2,3] and the detection of LGIC in non-neuronal tissues.[4,5] The recent identification of Hodor as a new LGIC expressed in the gut of some invertebrates, and its activation by zinc, strengthens the notion that LGICs were incorporated by the nervous system for fast synaptic signaling.

Absorptive enterocytes are equipped with major histocompatibility complex (MHC) class II for presentation of extracellular antigens.[6] Considering that under basal conditions enterocytes express low levels of co-stimulatory molecules (*e.g.*, CD80, CD86, CD40), [7]oral tolerance to foods may be partly induced by epithelial-cell antigen presentation in the healthy gut. During nutrient transport, some luminal antigens are endocytosed and processed in the lysosomal compartment, binding to MHC-II in late endosomes. Miguel-Aliaga's group shows that Hodor was specifically enriched in late endosomes or lysosomes with apical localization.[1] For adequate antigen presentation, these antigens must be partly hydrolyzed, which requires lysosomal pH acidification for optimal protease activity. The authors found that Hodor deficiency results in defective acidification of these compartments in *Drosophila*.[1] Therefore, if a similar zinc-sensing mechanism was expressed in humans, zinc deficiency could alter lysosomal antigen degradation in enterocytes, potentially affecting antigen presentation.

Hodor-related alterations of lysosomal pH may have opposed effects in terms of antigen presentation. On the one hand, Hodor dysfunction could limit lysosomal antigen degradation, potentially reducing antigen presentation. On the other hand, it could prevent excessive antigen degradation into immune-silent small peptides, potentially increasing antigen presentation. The outcome is presumably affected by the cell type and other regulatory mechanisms. As the enterocyte is a highly degradative cell type specialized in nutrient supply, zinc shortage may protect luminal antigens from full lysosomal degradation enabling antigen presentation at the gut epithelium.

The role of zinc in mucosal integrity is well known, particularly as it pertains to the barrier function of intestinal epithelial cells. For example, zinc deficiency has been reported in patients with chronic diarrhea or inflammatory bowel disease. In this context, it is possible that the lack of zinc (*i.e.*, reduced Hodor signaling) exacerbates pathology via increased enterocyte-driven antigen presentation.[8,9] Especially considering that intestinal inflammation or exposure to certain food antigens (*e.g.,* gliadin in celiac disease) has been shown to upregulate enterocyte expression of MHC-II and co-stimulatory molecules.

The intestinal epithelium is being increasingly recognized as a multifaceted player of the gastrointestinal tract. It plays a central role in nutrient absorption while providing a first line of luminal surveillance that guides mucosal immune responses. The interdisciplinary investigation of enterocyte nutrient-sensing pathways by which enterocytes communicate to other cells will advance the understanding of gut homeostasis and immune function.

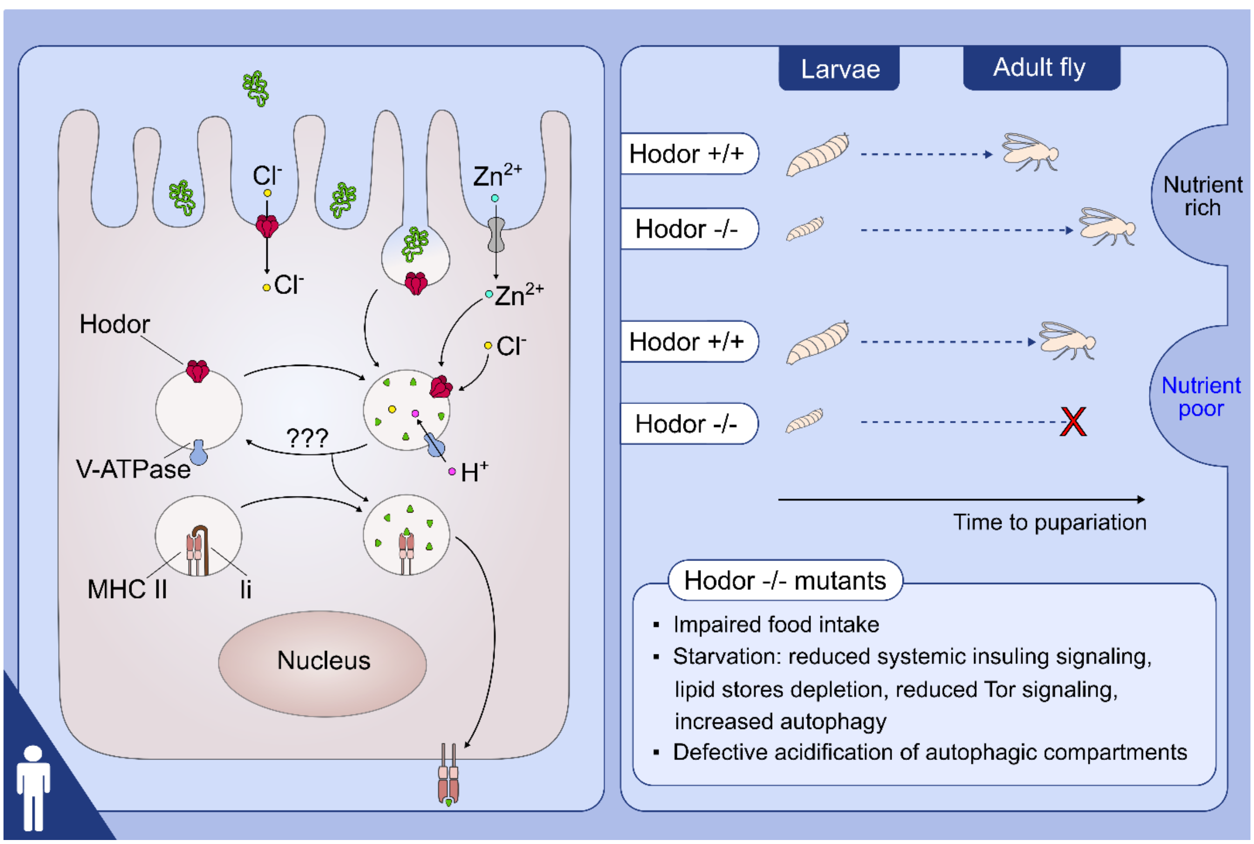

Figure 1. Hodor was specifically enriched in the late endosomes or lysosomes with apical localization and in the brush border of interstitial cells of *Drosophila melanogaster*. Zinc-sensing

by Hodor promotes chloride transport in/out cytoplasm and lysosomes. If expressed in humans, Hodor-mediated chloride transport across lysosomal membranes could sustain the activity of the proton-pumping vacuolar-type ATPase (V-ATPase) that maintains lysosomal acidity. During luminal transport of antigens by absorptive enterocytes, some endocytosed antigens can be processed in the acidified lysosomal compartment. Later these peptides bind to MHC-II in late endosomes upon invariant chain (li) removal.

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