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Packing in the Proteins

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In this issue of *Developmental Cell*, Park et al. (2019) demonstrate that specialized enterocytes of the developing vertebrate intestine are equipped with a broad-spectrum protein absorption machinery to meet animals' nutritional needs through intracellular protein digestion while simultaneously allowing important immune and developmental proteins to traverse the lumen unscathed.

Kids often get a bad rap for failing to behave according to adult expectations. But on closer inspection, the squirming toddler and the late-snoozing teen are just conforming to age-appropriate developmental programs. Similarly, the vertebrate intestine has long been known to be especially leaky to ingested proteins, but new work by Bagnat and colleagues (Park et al., 2019) demonstrates that what had been chalked up to immaturity is in fact a developmental program.

Developing animals are required to simultaneously function as they build themselves. This poses a challenge in the early-life digestive tract, where proteins are needed as nutrients but also serve important functional roles in development. In adult mammals, the digestion of proteins begins within the stomach and is further carried out within the lumen of the proximal intestine. However, gut-luminal digestion of proteins is stalled before weaning, allowing luminal persistence of antigens and maternal antibodies needed to train the developing immune system (Kulkarni and Newberry, 2019). This juxtaposition of attenuated luminal protein digestion with the necessity to digest dietary proteins for growth sparks the question: How does dietary protein absorption occur in the developing vertebrate gut? In this issue of *Developmental Cell*, Park et al. (2019) describe how a specialized subset of intestinal cells within the midgut of zebrafish and suckling mice absorb proteins from the intestinal lumen for intracellular digestion. With the molecular characterization of these cells' protein absorption machinery, the authors are able to show just how critical this protein-uptake pathway is for the animals' growth and survival.

The authors made use of optically transparent zebrafish larvae to first visualize the spatial localization and kinetics of fluorescent protein uptake and digestion. Their imaging led them to a subset of highly vacuolated enterocytes found within the ileum region of young vertebrate intestines, which they termed lysosomal-rich enterocytes (LREs). Although previous histological studies had implicated these cells in dietary protein absorption (Graney, 1968; Rombout et al., 1985), Park et al. (2019) provided the first live-imaging characterization of their protein uptake activity. By tracing the fate of proteins with different stabilities and pH-sensitive spectra, they built a compelling case that LREs continuously internalize and digest luminal proteins within the zebrafish intestine. Intriguingly, the authors also reported that a subset of intact fluorescent proteins ended up in distant organs including the kidney, liver, and gallbladder, suggesting a possible transcellular transport mechanism mediated by the LREs or possibly other cells of the digestive tract.

The ability to functionally label the LREs in living animals allowed Bagnat and colleagues to isolate them from the other cells of the intestine and compare their transcriptional profiles. The sequencing data revealed that LREs are enriched for genes encoding an endocytic protein machinery complex comprised of the transmembrane broad-spectrum scavenger receptor Cubulin (Cubn), the transmembrane protein Amnionless (Amn), and the intracellular endocytic adaptor protein Dab2. Subsequent experiments confirmed these genes' specific localization to the ileum region of the larval zebrafish intestine and showed a similar ileal expression of DAB2 protein in the suckling mouse. LREs had been reported to disappear from the mammalian intestine at weaning (Harper et al., 2011; Muncan et al., 2011) but to persist into adulthood in the intestines of stomachless zebrafish (Lickwar et al., 2017). These developmental patterns make sense in light of the LREs' role in protein absorption, which the authors were able to corroborate based on expression of the endocytic complex genes in published transcriptome datasets from mice and zebrafish of different ages.

The authors then performed elegant genetic experiments to test the necessity of these genes in luminal protein absorption and animal fitness. They generated germline mutations in zebrafish in all three complex members and showed each to be required for normal levels of protein uptake in LREs. Making use of patchy expression of a dominant-negative *dab2* construct, they showed that every cell expressing the defective adaptor had impaired protein absorption, demonstrating that the complex functions cell autonomously. They then used the mutants to explore the long-term fitness requirements for this protein uptake process. Reared on a protein-poor diet, the larval zebrafish lacking the endocytic machinery displayed stunted growth and poor survival. The authors went on to show a similar requirement for DAB2 in dietary protein absorption within LREs in suckling mice and found that these mutant mice, like the mutant zebrafish, exhibited stunted growth. Notably, the phenotypes of both animals with impaired protein uptake are evocative of human patients with kwashiorkor, a disease of severe malnutrition. Collectively, Park et al. (2019) have uncovered the molecular mechanism underlying the LREs' endocytic activity that serves a critical, conserved function in meeting the

nutritional and developmental requirements of vertebrates.

This work highlights how permeable developing vertebrates are to luminal proteins, with intact fluorescent proteins being readily internalized into ileal enterocytes and even disseminating into distant organs of the body. A major source of luminal proteins is the complex collection of bacteria, viruses, fungi, and archaea, collectively termed the microbiota, that assembles within the vertebrate intestine (Fischbach and Segre, 2016). How resident microbes influence host biology is an active area of microbiota research. Certain processes require the continual presence and activity of the microbiota, such as microbially assisted breakdown of fiber, undigestible to the host. Other processes may require microbial inputs during critical developmental windows, such as maturation of the immune system or establishment of tissue metabolic rates. It is tantalizing to speculate that the LREs serve as a conduit for microbiota-derived cues and that the transient nature of these cells in mammals restricts the time period during which certain cues can act. Further investigation of LRE function is likely to shed light on when and how diet and resident microbes interact to impact host development, how these programs are disrupted in the context of malnutrition, and what strategies (for example, "microbiota-directed complementary food" [Gehrig et al., 2019]) might be useful for treating intestinal pathologies of protein malnourishment.

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Epithelial Morphogenesis: Size Matters

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In this issue of *Developmental Cell*, Gibson and colleagues (Ramanathan et al., 2019) investigate the relationship between size and shape in epithelial cells. They find that cell size impacts cell shape, with unexpected consequences for epithelial growth and morphogenesis, particularly the clonal dynamics of epithelial cells.

In their new report, Gibson and colleagues (Ramanathan et al., 2019) provide insights into the relationship between cell size, cell division, and the mechanics of epithelial morphogenesis. Epithelia are the most common tissue type in animals and can develop into an astonishing variety of different sizes and shapes. How the behavior of individual cells is organized to achieve precise control of tissue growth and form is a fascinating question in biology. Many early breakthroughs have been made by *Drosophila* geneticists studying development of the fly embryo or the adult eyes, wings, and legs. For example, in the developing *Drosophila* wing, genetic marking of individual epithelial cells revealed them to grow into single cohesive clones with elongated shapes oriented along the elongating proximal-distal axis of the wing (Baena-López et al., 2005). During the early proliferating larval stages of wing development, elongation of clones was shown to require the Fat-Dachsous atypical cadherin system to planar polarize tissue tension via the Dachs myosin (Baena-López et al., 2005; Mao et al., 2011). After formation of the pupa, cells arrest proliferation, but both clone and tissue shape further elongate due to a similar pattern of tension induced by planar polarized Myosin-II (Diaz-de-la-Loza et al., 2018). Finally, long-range stretching of the tissue determines final wing shape (Etournay et al., 2015; Ray et al., 2015). These global patterns of stress and strain then influence the geometry of cell shapes (Mao et al., 2011), the orientation of cell