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Gut development on a full stomach

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Abstract

Mechanical forces are important for normal gastrointestinal development and function. Now, He et al. have discovered a population of *Drosophila* midgut epithelial enteroendocrine cell (EEC) precursors that express the mechanosensitive ion channel Piezo, which generates a calcium influx that drives EEC differentiation and proliferation in response to physiological mechanical stimuli.

The gastrointestinal epithelium is a diverse community of multiple cell populations. One important population is the enteroendocrine cells (EECs). EECs are sparsely distributed throughout the gastrointestinal tract, accounting for only 1% of epithelial cells. However, en masse they comprise the largest human endocrine organ. EECs are specialized sensors of luminal signals: they convert the many stimuli from flow of nutrients and metabolites into coordinated secretion of several important hormones and neurotransmitters that regulate gastrointestinal and systemic physiology. EEC densities and functions vary along the length of the gastrointestinal tract and are altered in human diseases. Consequently, it is important to determine how EEC populations and functions are regulated in health and disease.

Our understanding of gastrointestinal epithelial development has progressed rapidly due to advances in transgenic methods, including lineage tracing of cell populations, genetic knockouts, and fundamental discoveries in invertebrate development, such as in the fruitfly *Drosophila melanogaster*. *Drosophila* midgut, which is analogous to stomach and small intestine in vertebrates, has a dedicated intestinal stem cell (ISC) population marked by *Escargot* (*Esg*) and more specifically by *Delta* (*Dl*). ISCs proliferate by regulating cytosolic calcium (Ca^{2+}) levels¹ and differentiate into precursors of absorptive enterocytes, marked by *Su(H)Gbe*, or secretory cells, marked by *Prospero* (*Pros*). Some secretory lineage precursors differentiate into EECs by a multistep process that involves a balance of multiple transcription factors. Notch signalling has a critical role in this process. High Notch signalling levels lead to the enterocyte lineage commitment, whereas low Notch commits ISCs to the secretory lineage (such as EECs). In vertebrates, mature EECs make up a heterogeneous population of hormone-secreting specialized sensory cells. Although less is known about fly EECs, they can be divided into two classes (I and II) of neuropeptide-producing cells.

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Competing interests

The author declares no competing interests.

Mechanical forces are fundamental for shaping the structure of the developing gastrointestinal epithelium. Strong in vitro evidence suggests that mature epithelium (gut and skin) remains tuned to mechanical forces. Intraepithelial forces regulate both epithelial cell proliferation² and anoikis, which is attachment-dependent apoptosis and extrusion³. Both were shown to sense intraepithelial forces by the stretch-sensitive ion channel Piezo⁴ — a transmembrane protein with a mechanically gated conductive pore that passes cations, including Ca²⁺. Indeed, mechanosensitivity and Ca²⁺ permeability make the Piezo channel an excellent candidate to have central roles in regulating cellular fates to mechanical stimuli⁵.

In the new study⁶ (FIG. 1), the authors discovered a Piezo⁺ subset of *Esg*-positive cells in *Drosophila* midgut and used multiple lineage-tracing approaches to demonstrate that this population was positive for *DI* (an ISC marker) and *Pros* (an EEC marker) but negative for *Su(H)Gbe* (an enterocyte marker). It therefore seemed that Piezo⁺ cells were a subpopulation of EEC precursors and early EECs. *Piezo*-knockout (*Piezo*^{KO}) led to a deficiency in EEC expansion normally seen with age. Indeed, *Piezo*^{KO} clones generated dramatically fewer EECs, which could be reversed by *Piezo* overexpression (*Piezo*^{OE}). As expected, bleomycin injury and Notch inhibition resulted in robust generation of EECs, whereas stimulating apoptosis transiently diminished EEC numbers. However, these interventions did not change the proportions of Piezo⁺ and Piezo⁻ EECs, suggesting that Piezo⁺ EEC progenitors are important EEC precursors, but the stimulus for Piezo⁺ EEC expansion was unclear.

As Piezo channels conduct Ca²⁺, and intracellular Ca²⁺ regulates ISC development¹, He and colleagues⁶ tested whether Piezo expression and cytosolic Ca²⁺ levels in ISCs interacted to alter EEC development. *Piezo*^{KO} or knockdown of molecules that control cytosolic Ca²⁺ levels (by Ca²⁺ influx or release from intracellular stores) decreased EEC density. By contrast, *Piezo*^{OE}, cytosolic Ca²⁺ increases by genetic modulation or Notch signalling knockdown not only increased EEC density but overcame the *Piezo*^{KO} phenotype. Conversely, increasing Notch signalling and decreasing intracellular Ca²⁺ levels could suppress the *Piezo*^{OE} phenotype. Thus, the proposed mechanism for Piezo⁺ EEC differentiation is force-dependent Piezo activation, Ca²⁺ influx and Notch inhibition. Interestingly, reducing extracellular- signal-regulated kinase (ERK) signalling decreased Piezo⁺ EEC proliferation only, suggesting that Piezo⁺ EEC differentiation and proliferation are regulated differently.

To test the physiological relevance of these findings, He et al. ‘overfilled’ fly gut with nonabsorbable methylcellulose fibre in vivo and exerted extraluminal forces by extrinsically squeezing the gut in vitro. These mechanical manipulations increased ISC, ECC and Piezo⁺ EEC numbers. In both cases, these effects were diminished by genetic inhibition of *Piezo*. These findings suggest that Piezo⁺ EEC precursors might regulate EEC density in normal physiology.

The new study by He et al.⁶ presents exciting discoveries that lead to fascinating research questions. It will be important to visualize the intraepithelial forces that guide development and regulate gastrointestinal motility, and to understand how those forces are transduced to

the resident mechanosensitive cells. Mouse and human gastrointestinal epithelium structure is exemplified by the crypt–villus axis, and this asymmetry means that intraepithelial forces are not homogeneous. Although *Drosophila* lacks the crypt–villus axis, interestingly there is physical asymmetry within *Drosophila* epithelium that drives cell division at tricellular junctions⁷ and contributes to directional regulation of Notch-directed EEC development⁸. Progress in this realm will help to determine whether the loss of normal EEC expansion in *Piezo*^{KO} is due to steady state forces in developing intestinal epithelium, such as in *Piezo* regulation of neuronal precursors⁵, or due to increased gastrointestinal motility in a growing fly. Similarly, is the ISC expansion induced by ‘overflow’ due to *Piezo*⁺ EECs, does it rely on EEC–smooth muscle signalling⁹, or are there other groups of mechanosensitive stem cells present?

We will also need to better understand the roles of *Piezo*⁺ EECs in physiology. Whereas *Drosophila* has one *Piezo* gene, vertebrates have *Piezo1* and *Piezo2* (REF.4). *Piezo1* controls epithelial force- dependent division² and extrusion³. In human and mouse small intestine epithelium, *Piezo2* was recently shown to be expressed in a population of EECs¹⁰, but their mechanosensitivity and function are not known, and it is unclear if these cells are akin to *Drosophila*’s *Piezo*⁺ EECs. As EECs regulate physiology, it will be important to determine the functional roles of *Piezo*⁺ EECs, including whether they remain mechanosensitive, what hormones and neuropeptides they contain and release and what physiological functions they regulate?

The results by He et al.⁶ in *Drosophila* midgut await translation to vertebrate models and, ultimately, humans. Yet, they raise an intriguing possibility that mechanical forces regulate EEC development and function, whereas disruption in mechanosensation might lead to changes in EEC densities seen in some human gastrointestinal pathologies, such as chronic constipation, IBS, obesity and IBD.

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Fig. 1 |. Mechanical forces trigger enteroendocrine cell differentiation in *Drosophila* midgut. Piezo⁺ enteroendocrine cell (EEC) precursors give rise to Piezo⁺ EECs when stimulated by mechanical forces.