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Review

Bioelectric regulation of intestinal stem cells

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Proper regulation of ion balance across the intestinal epithelium is essential for physiological functions, while ion imbalance causes intestinal disorders with dire health consequences. Ion channels, pumps, and exchangers are vital for regulating ion movements (i.e., bioelectric currents) that control epithelial absorption and secretion. Recent *in vivo* studies used the *Drosophila* gut to identify conserved pathways that link regulators of Ca^{2+}, Na^{+} and Cl^{-} with intestinal stem cell (ISC) proliferation. These studies laid a foundation for using the *Drosophila* gut to identify conserved proliferative responses triggered by bioelectric regulators. Here, we review these studies, discuss their significance, as well as the advantages of using *Drosophila* to unravel conserved bioelectrically induced molecular pathways in the intestinal epithelium under physiological, pathophysiological, and regenerative conditions.

Highlights

Ion channels, pumps, and exchangers regulate the transport of ions, such as Na^{+}, Cl^{-}, K^{+}, and Ca^{2+}, in and out of cells, and gap junctions allow the movement of ions across cells.

Ion imbalance is highly associated with intestinal disorders and even cancer; therefore, a genetic model capable of decoding *in vivo* how ion regulation affects intestinal stem cell proliferation could have great therapeutic value.

Recent advances in the adult *Drosophila* gut identify different molecular mechanisms by which conserved ion channels regulate intestinal stem cell proliferation.

The *Drosophila* gut is an attractive *in vivo* model to decipher how ion regulation and bioelectric currents affect conserved molecular pathways that drive proliferation during physiological and pathophysiological states.

Ion regulation in the gut

The gut is an organ dedicated to digestion and nutrient absorption. Under physiological conditions, the **gut epithelium** (see Glossary), which comprises polarized epithelial cells, secretes digestive enzymes and regulates the passage of a variety of nutrients, electrolytes, and water from the lumen into the body and *vice versa*. The ability of intestinal epithelial cells to absorb nutrients and fluids is essential to body hydration and, by extension, the animal lifespan. **Ion channels**, **pumps,** and **exchangers** are instrumental in electrolyte absorption and secretion and thereby regulate the movement of water in the gut. These epithelial ion channels, pumps, and exchangers include: (i) Na^{+} and Cl^{-} channels, which regulate water absorption and secretion; (ii) K^{+} channels, which are responsible for the negative membrane potential that is necessary for ion secretion; and (iii) ion pumps and exchangers, which help maintain the ionic gradient and osmotic equilibrium [1,2]. The combined functions of these ion channels, pumps, and exchangers move charged ions in and out of the epithelium, giving rise to the membrane potential. In addition, some receptors, such as **cholinergic receptors**, regulate Ca^{2+} signaling, which, in turn, stimulates ion transport [3,4]. We refer to these as **bioelectric regulators** due to their ability to regulate epithelial ion dynamics.

Bioelectricity (the flow of electric currents carried by charged ions, such as Na^{+}, Ca^{2+}, K^{+}, and Cl^{-} influences diverse processes, including tissue development and patterning [5–7]. In particular, voltage changes in the membrane potential affect biological functions, such as cell cycle regulation (reviewed in [8]), tissue patterning [9], and cytoskeletal organization in *Drosophila* [10]. In addition, endogenous ion currents triggered by ion channels and pumps influence tissue development [11,12], zebrafish fin growth [13], and wound healing [14,15]. Thereby, bioelectricity is considered an instrumental component of morphogenesis, growth, and even tissue remodeling and regeneration [7]. How bioelectric currents are transduced in molecular pathways capable of modulating with precision cellular functions so that organs develop to their 'correct' form and size is not clear [7]. Nevertheless, Ca^{2+}-mediated signal transduction [16] often links bioelectric changes to canonical regulatory networks [5,17]. For example, downstream Ca^{2+}-dependent pathways triggered by K^{+} channels regulate human T -cell activation and proliferation [18],

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while promoting morphogenesis in the *Drosophila* wing [19]. In addition, membrane voltage changes act through Ca^{2+} signaling to drive anterior gene expression in planaria [20].

Over the past decade, the *Drosophila* midgut, which we refer to here as the gut for simplicity, has emerged at the forefront of stem cell studies [21–29]. The *Drosophila* gut is a single layered epithelium comprising ISCs that give rise to daughter cells, the enteroblasts (EBs), which, in turn, differentiate into two distinct epithelial cell populations: enterocytes (ECs) and enteroendocrine cells (EEs) [28,29]. ECs, which form ~80% of the epithelium, are large polyploid cells specialized in absorption, whereas EEs are small secretory cells [30]. The simple architecture and cellular composition of the *Drosophila* gut, combined with the state-of the art genetic toolset available in *Drosophila* [31,32], facilitated several breakthrough studies that revealed conserved mechanisms underlying the regulation of ISC proliferation [21–29]. Specifically, ISC proliferation is triggered by the activity of several conserved pathways, which include the JAK-STAT, epidermal growth factor (EGF), bone morphogenetic protein (BMP), and Wingless pathways [21–27]. During gut regeneration, these pathways are activated by autonomous and non-autonomous release of **cytokines** from various sources, including the EBs, ECs, ISCs, and hemocytes (*Drosophila* immune cells), as well as the surrounding visceral muscle. The regenerative signaling pathways prompting ISC proliferation have been extensively reviewed elsewhere [33,34].

Although bioelectrically controlled regeneration in the gut is not yet established, ion channel dysregulation in the intestine is associated with several diseases, including inflammatory bowel diseases (IBDs) and cancer [1,35]. In this review, we highlight current knowledge relating to the control of ISC proliferation in the *Drosophila* gut by bioelectric regulators and examine how studies in flies could further our understanding of the role of bioelectric dynamics in gut homeostasis, regeneration, and human diseases.

Glossary

Bioelectric regulators: ion channels, ion pumps, ion exchangers, or receptors regulating epithelial ion dynamics.
Cholinergic receptors: receptors activated by acetylcholine; include the nicotinic and muscarinic receptors.
Cytokines: any substances secreted by innate immune cells, as well as epithelial cells; and affect other cells.
Gap junctions: specialized intercellular connections between adjacent cells allowing the flow of ions and electrical impulses.
Gut epithelium: single cell layer of intestinal epithelial cells.
Hemichannel: half of a gap junction formed by the oligomerization of six subunits: innexins in invertebrates and connexins in vertebrates.
Ion channel: proteins forming pores that allow the flow of ions across membranes.
Ion exchanger: bidirectional ion transporter.
Ion pump: active channels that use ATP hydrolysis to transfer ions from one side of the membrane to the other.
Nicotinic receptors: ligand-gated ion channels, activated by acetylcholine.
Serotonin receptors: G-protein-coupled receptors and ligand-gated ion channels activated by serotonin.

Conserved regulators of ion dynamics in the *Drosophila* gut

Recent studies in the *Drosophila* gut have generated single cell RNA-sequencing (RNA-seq) data sets[i] and bulk RNA-seq data sets for specific cell populations[ii] or regions[iii], either under physiological conditions or following bacterial infection [36–38]. A cell atlas for the whole fly based on single nuclei sequencing has also been generated [39]. These invaluable data sets revealed that conserved ion channels, pumps, and exchangers are expressed in the *Drosophila* gut, offering the opportunity to explore the physiological roles of these bioelectric regulators in the fly gut. In addition, current studies (Table 1) have identified how ISC proliferation is controlled by ion channels, pumps, as well as a Ca^{2+}-regulating receptor [40–44]. Here, we review the significance

Table 1. Bioelectric regulators in the *Drosophila* gut reviewed in this study

ISC/EBs	ECs	EEs	Ionic regulation	Function	Effects on proliferation
GluR			Cytosolic Ca^{2+} increase	Regulates proliferation based on nutritional needs [40]	Activation increases proliferation through CREB pathway [40]
TrpA1		TrpA1	Ca^{2+} influx	Promotes response to injury and noxious bacteria expulsion [42,67]	Activation increases proliferation via RAS/ERK MAPK and EGFR signaling [42]
Piezo		Piezo	Ca^{2+} influx	Promotes EE differentiation in response to mechanical pressure [41]	Activation increases proliferation via ERK signaling [41]
PMCA	PMCA	PMCA	Ca^{2+} efflux	Promotes Ca^{2+} balance [40]	PMCA decrease in progenitor cells triggers proliferation in CREB-dependent manner [40]
	ppk4/ENaC		Na^{+} influx	Regulates epithelial water movement [43]	ppk4/ENaC increase causes EC osmotic stress that triggers proliferation [43]
	CFTR		Cl^{-} transport	Regulates nutrient and water epithelial absorption [44]	Reduction increases proliferation via non-autonomous JAK-STAT and EGFR signaling [44]

of these studies and, using the available data sets, provide examples of potential regulators of K^+, Cl^-, Na^+, and Ca^{2+} dynamics in the fly gut that appear worthy of further exploration (Figure 1) based on their conservation, downstream molecular pathways (Table 1), and association with human pathologies.

Ca^{2+} regulation

mGluR

Recent work in the *Drosophila* gut identified that Ca^{2+} oscillations in ISCs increase in response to mitogenic and dietary signals [40]. This study revealed that administration of dietary glutamate (L-Glu) increases cytosolic Ca^{2+} concentrations in ISCs by activating the metabotropic glutamate G-protein-coupled receptor (mGluR) and promoting proliferation through the conserved calcineurin/CREB-regulated transcriptional co-activator (CRTC) pathway [40]. Thus, mGluR-induced Ca^{2+} dynamics are proposed to link gut proliferation to the nutrient needs of the animal [40]. The association of calcineurin with Ca^{2+} dynamics is conserved across animals, but the cellular function can vary

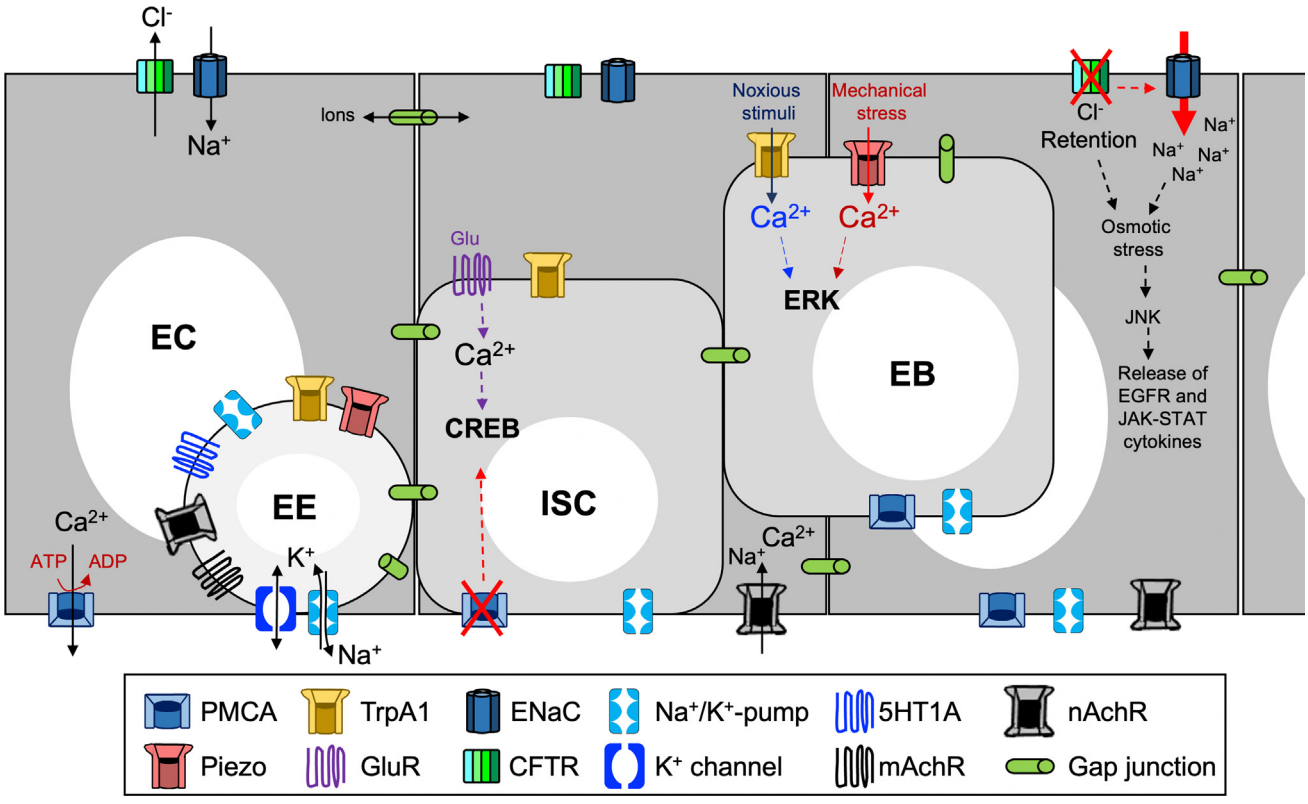

Figure 1. Intestinal stem cell (ISC) proliferation under the control of bioelectric regulators in *Drosophila*. Recent advances in the *Drosophila* gut have identified bioelectric regulators (ion channels, pumps, exchangers, and receptors) that, by triggering downstream conserved molecular pathways, regulate ISC proliferation. Dietary glutamate (Glu) activates Glu G-protein-coupled receptors (GluRs) in ISCs, elevating cytoplasmic Ca^{2+}, which promotes proliferation in a CREB-dependent manner [40]. In addition, plasma membrane Ca^{2+} ATPase (PMCA) reduction triggers proliferation in a CREB-dependent manner [40]. Mechanical pressure in a Piezo-expressing subpopulation of enteroblasts (EBs) triggers Ca^{2+} influx, which promotes ERK-dependent proliferation [41]. Upon noxious stimuli, transient receptor potential A1 (TrpA1) promotes Ca^{2+} increase and ERK-dependent proliferation [42]. Cystic fibrosis transmembrane conductance regulator (CFTR) reduction in enterocytes (ECs) leads to Cl^- retention, increased Na^+ uptake followed by JNK induction, and release of EGFR and JAK-STAT proliferative cytokines [43,44]. The schematic depicts additional conserved bioelectric regulators the expression of which in the *Drosophila* gut was detected in gut profiling studies [36–38] and are worth exploring further for their roles in ion transport and gut proliferation. These include Na^+/K^+ pump, K^+ channels in enteroendocrine cells (EEs), cholinergic receptors [muscarinic (m)AchR and nicotinic (n)AchR], serotonin receptors (5HT1A), and gap junctions. The location of the bioelectric regulators is not representative of their subcellular localization, which in most cases has not been reported. In addition, ion exchange is not indicated in all cases for simplicity. Abbreviation: ENaC, epithelial sodium channel.

for different tissues. For example, in skeletal myoblasts, Ca^{2+}-dependent calcineurin activity promotes differentiation [45].

Ca^{2+} is a particularly significant and pleiotropic downstream component that impacts almost every aspect of cellular life [16]. Ca^{2+} signaling in intestinal epithelial cells is a major regulator of ion secretion [3,4]. In addition, Ca^{2+} carries out significant non-autonomous functions; for example, elevated cytosolic Ca^{2+} triggers the bioelectric communication of adjacent cells, electrically coupling them such that they function as a unit [46,47]. Thus, it is not surprising that the gut uses Ca^{2+} dynamics in ISCs to adapt intestinal epithelial integrity to varying amounts of nutrients [40]. Future studies should examine whether mGluR interacts with other ion channels not only in ISCs, but also in the adjacent EBs, ECs, or EEs via bioelectric or chemical signaling. Such studies could unravel the underlying mechanisms that allow the gut to precisely adjust its physiological functions to match nutritional needs.

Piezo

The conserved mechanosensitive channel Piezo is a clear example of how Ca^{2+} dynamics maintain intestinal homeostasis in response to mechanical stress, which occurs regularly during digestion [41,48]. Piezo ion channels are shaped as a propeller [49–51]. When closed, the center of the propeller has three blades, which curve the membrane, generating a dome-like structure. It has been proposed that, as tension on the membrane increases due to mechanical pressure, the dome flattens, the blades straighten, and the channel opens [49–51]. Opening of the Piezo channel in the plasma membrane mediates extracellular Ca^{2+} influx, whereas its opening in subcellular organelles, such as the endoplasmic reticulum/sarcoplasmic reticulum, mitochondria, or nucleus, elevates Ca^{2+} levels by release from internal Ca^{2+} stores [52].

Two recent studies, one in mammalian cells and another in the *Drosophila* gut, highlighted how Ca^{2+} signaling through the Piezo channel triggers cell population adjustments in the intestinal epithelium so that normal gut function is maintained despite the mechanical stress that occurs during digestion [41,48]. Both studies found that Piezo activation increases ISC proliferation by stimulating Ca^{2+}-dependent phosphorylation of ERK [41,48], indicating that the mechanism controlling proliferation during mechanical stimulation in the gut is conserved. Moreover, a unique *Piezo*-expressing subpopulation of EBs was identified in the *Drosophila* gut, which, upon mechanical stimulation, proliferates and differentiates into secretory EEs [41]. Although Piezo-induced proliferation is ERK dependent in the *Drosophila* gut, Piezo-induced EE differentiation is ERK independent and possibly regulated through Notch signaling [41].

Although most studies focus on Piezo-induced Ca^{2+} currents [41,48], opening of Piezo channels generates cationic nonselective currents, such that Piezo can also result in the flux of Na^{+} or K^{+} [53]. Therefore, Piezo-induced nonselective currents in the epithelium can affect regulation of ion transport and water movement. Perhaps not surprisingly, abnormalities in gut mechanosensation are associated with defecation disorders, obesity, and colon cancer [54–56]. Further work in the *Drosophila* gut could help identify interactions of Piezo with other bioelectric regulators and unravel how the ion and osmotic balance across the intestinal epithelium is preserved despite mechanical pressure.

TrpA1

TrpA1 (also known as the wasabi receptor) belongs to the highly conserved transient receptor potential (TRP) multigene superfamily and is found in various tissues, including the gut. Similar to most TRPs, TrpA1 is a Ca^{2+}-permeable nonselective cation homotetramer channel that localizes to the plasma membrane [57]. Each TrpA1 subunit comprises six transmembrane regions

flanked by amino-terminal ankyrin repeats in the N- terminal tails [57]. TrpA1 has been extensively studied in nociceptor neurons as a detector of irritants or tissue injury and can be activated by a range of chemical, thermal, and mechanical stimuli [58]. It can also be activated by proinflammatory mediators, including oxidative stress byproducts, which directly modify cysteine residues in TrpA1 [59,60]. Upon inflammation, TrpA1 is reported to be upregulated, extensively trafficked, and translocated to the membrane [58,61]. In the mammalian intestinal epithelium, TrpA1 is found in ECs, enterochromaffin cells, and EEs [58]. Although the function of TrpA1 in mammalian gut cells is not as well understood as its function in neurons, TrpA1 has been proposed to regulate intestinal motility, aid in the digestion of specific foods, and regulate mechanosensation [62–66].

The expression of TrpA1 is conserved in the *Drosophila* gut, with potential roles in EEs and ISC/EBs [42,67]. TrpA1 is proposed to sense microbicidal-reactive chlorine and trigger bacterial expulsion as a protective response following the activation of the uracil/Duox pathway by pathobionts [67]. In addition, transcriptome analysis of the *Drosophila* gut[ii] revealed that *TrpA1* is mainly expressed in EEs in steady-state conditions but this changes after bacterial infection, with *TrpA1* also upregulated in EBs [37]. This resembles the upregulation of TrpA1 after inflammation in mammals [58,61] and suggests that, in the *Drosophila* gut, TrpA1 senses noxious substances and promotes bacteria expulsion from EEs and EBs. Moreover, TrpA1 is proposed to be one of the Ca^{2+} channels that drive ISC proliferation during *Drosophila* gut injury [42]. Specifically, TrpA1, together with the ryanodine receptor (RyR), increases intracellular Ca^{2+} in response to oxidative stress. This, in turn, activates the Ras/ERK MAPK pathway and induces secreted EGF cytokine to promote ISC proliferation [42]. Thus, Ca^{2+} influx from TrpA1 is associated with the release of mitogenic signals and proliferation [42]. Future studies should explore whether the same Ca^{2+}-induced pathway triggered by TrpA1 in response to mitogenic signals is concurrently promoting bacterial expulsion during infection or, alternatively, whether TrpA1 regulates different Ca^{2+}-induced pathways. A comprehensive understanding of the molecular and bioelectric roles of TrpA1 in the *Drosophila* gut could be relevant to gastritis, for which TrpA1 has been proposed as a potential therapeutic target [68].

PMCA and Calx

The plasma membrane Ca^{2+} ATPase (PMCA) is an ATP-driven pump that removes cytosolic Ca^{2+} from cells. This function is essential in all eukaryotic cells for the maintenance of a low resting Ca^{2+} concentration [69]. In the small intestine, PMCA1 is considered the principal driver of Ca^{2+} extrusion and is linked with Ca^{2+} absorption deficiency, reduced bone mineralization, and hyperparathyroidism [70–72]. In the *Drosophila* gut, knocking down *PMCA* in gut progenitor cells led to Ca^{2+} oscillation impairment, cytosolic Ca^{2+} increases, and ISC overproliferation in a CREB-dependent manner [40]. In addition, *PMCA* reduction in *piezo*-deficient guts was sufficient to restore the normal number of EEs [41]. Together, these data support the idea that PMCA could be the main driver of Ca^{2+} extrusion in progenitor cells.

Another Ca^{2+} efflux pathway found in almost all cells is the Na^{+}/Ca^{2+} exchanger-mediated efflux pathway. In *Drosophila,* Calx is the homolog of the mammalian Na^{+}/Ca^{2+} exchanger (NCX) [73]. *Calx* is expressed in the *Drosophila* gut and, based on profiling data sets[i,ii], *Calx* expression is as abundant as that of *PMCA* in EEs and ECs but less so in ISCs [36,37]. It might be that Ca^{2+} removal is regulated by a different mechanism among gut cells, reflecting the different physiological requirements of Ca^{2+} between progenitor and epithelial cells. This idea would be worth exploring in the *Drosophila* gut, especially under pathophysiological conditions.

Na^+ regulation

ENAC

The epithelial sodium channel (ENaC) belongs to the voltage-independent, Na^+-selective ENaC/DEG superfamily, which regulates Na^+ and water homeostasis in the epithelium [74]. In epithelial cells, ENaC is the main route for Na^+ entry and, therefore, constitutes a vital regulatory mechanism for intracellular Na^+ influx [75]. ENaC comprises heterotrimeric subunits that harbor protease-sensitive domains critical for opening and closing of the channel. When proteases cleave peptidyl tracts from the extracellular domain, ENaC opens and allows Na^+ to pass into the cell [76–79].

In *Drosophila,* the ENaC/DEG superfamily comprises 31 family members, the 'pickpocket' (*ppk*) genes [80], which are important regulators of *Drosophila* wing development [81]. *Ppk genes* are also expressed in the intestinal epithelium [43,82] and recent work revealed that *ppk4/ENaC* expression is downregulated in ECs by the small noncoding RNA *miR-263a* [43]. Depletion of *miR-263a* increased *ppk4/ENaC* mRNA levels, leading to a stress response in ECs due to dehydration and elevated Na^+ uptake [43]. This in turn triggered ISC proliferation as well as hyperplasia and promoted increased bacterial load and expression of antimicrobial peptides [43]. Thus, elevated Na^+ uptake triggers responses that resemble the elevated ENaC activation associated with the multiorgan disease cystic fibrosis (CF), rendering the *Drosophila* intestinal epithelium a potential tissue to model CF [43].

ENaC has also been proposed to regulate wound healing [15,75], with *in vitro* studies reporting that ENaC increases the levels of Ca^{2+} through interactions with the Na^+/Ca^{2+} exchanger, generating a slow Ca^{2+} wave that controls the rate of wound healing [75]. Future work in the *Drosophila* gut could help further our understanding of ENaC-stimulated Ca^{2+} dynamics by studying the interactions of Calx with ppk4/ENaC during epithelial injury and by exploring *in vivo* the role of cytokines and other inflammatory factors in modulating ppk4/ENaC function.

Na^+/K^+ pump

Transcriptome analysis of the *Drosophila* gut[i,ii] revealed the conserved expression of the Na^+/K^+-pump [36,37]. This ATPase pump utilizes ATP to move Na^+ and K^+ against their concentration gradients across the plasma membrane to maintain ion balance. In detail, Atpα, which is the essential membrane cation antiporter, forms heterodimers with the β subunits, which are noncatalytic components of ATPase, and, through ATP hydrolysis, regulates the number of Na^+ ions shuttled out of the cell while K^+ ions are shuttled in. Based on *Drosophila* gut profiling[i,ii], the Na^+/K^+-pump subunit α (Atpα) together with the three β subunits [Nervana (Nrv) 1–3] are enriched in EEs compared with ECs and ISC/EBs [36,37], indicating different Na^+ efflux and K^+ influx requirements among different gut cell types.

Na^+/K^+-pump dysregulation is associated with diseases, such as IBDs and CF. It is proposed that IBD-related diarrhea is attributed to reduced Na^+/K^+-pump activity combined with downregulation of ENaC [1,83]. Together, they potentially promote accumulation of intracellular Na^+, reducing Na^+ absorption and leading to the water and ion imbalance that causes diarrhea [1,83]. Moreover, patients with CF have elevated Na^+/K^+-pump activity across the airway epithelium, and *in vitro* experiments found this activity to be mediated by ENaC [84,85]. Future studies in the *Drosophila* gut could help reveal conserved molecular pathways that are regulated by the Na^+/K^+-pump and explore how interactions with conserved Na^+ channels, such as ENaC, contribute to disease development.

Cl^- regulation

Chloride channels and transporters have vital roles in water absorption and ion balance in the gut [86]. The movement of Cl^- throughout the gut has been proposed to control cell membrane

potential and cell volume, to maintain the cellular pH, and to regulate the balance of electrolytes [86]. Among known Cl^- families of channels and transporters, CF transmembrane conductance regulator (CFTR) is one of the most well studied due to its role in CF.

Mammalian CFTR is a Cl^- channel regulated by ATP-binding and protein Kinase A (PKA)-dependent phosphorylation [87]. CFTR comprises a single polypeptide with two transmembrane domains, two nucleotide-binding motifs, and a cytoplasmic regulatory domain that controls channel activity [87,88]. This regulatory domain needs to be phosphorylated by PKA for the CFTR channel to be able to open. Once phosphorylated, ATP binding opens CFTR, and ATP hydrolysis then closes the channel [88–90]. CFTR is highly expressed in the airway epithelium and the intestinal epithelium [91]. Mutations in CFTR lead to CF, which is characterized by infection and damage in the lung and is also associated with microbiota imbalance and inflammation in the gut [92–95].

A recent study identified the *Drosophila* equivalent of human CFTR (Dmel\CFTR) [44]. This Cl^- channel shares structural and functional properties with the human channel and is expressed in ECs [44]. ECs in the *Drosophila* gut are required for nutrient and water absorption, and knocking down Dmel\CFTR in these cells led to a disruption of Cl^- transport, abnormal cellular swelling, EC damage, and increased expression of mucin genes [44], reminiscent of CF. These CF-related phenotypes are associated with release of cytokines and with non-autonomous activation of JAK/STAT and EGFR pathways, which trigger extreme ISC proliferation and hyperplasia in the *Drosophila* gut [44].

In the mammalian epithelium, CFTR is proposed to interact with other ion channels, such as **nicotinic receptors** and the epithelial Na^+ channel ENaC to maintain ion balance [3,96,97]. In support of the idea that the ion balance in *Drosophila* and mammalian epithelial cells share similar mechanisms, Dmel\CFTR depletion increased ENaC-mediated Na^+ uptake and osmotic stress in ECs [44]. Future studies in the *Drosophila* gut focusing on the relationship between CFTR with other ion channels could help reveal conserved intracellular pathways associated with CF-related symptoms.

Based on *Drosophila* gut profiling data sets[i,ii], other chloride channels, such as chloride channel-a (ClC-a) and ClC-b, are expressed in ECs, EEs, and ISC/EBs [36,37] but their roles in the fly gut epithelium have not yet been established. Future studies in the *Drosophila* gut could explore whether additional Cl^- channels together with CFTR maintain ion balance and regulate ISC proliferation through JAK/STAT and EGFR pathways [44]. Having a comprehensive understanding of how Cl^- channels regulate proliferation is important since CFTR has been proposed to act as a tumor suppressor in intestinal cancer [98] and chloride channels in general are proposed to have roles beyond homeostatic ion balance [86].

K^+ regulation

K^+ channels are another type of ion channel found in the gut. These channels comprise a primary pore-forming α-subunit that controls K^+ transport and is often associated with a regulatory β-subunit that senses a variety of stimuli [99]. In general, K^+ channels maintain K^+ homeostasis by regulating K^+ influx and efflux and have significant roles in diverse cellular functions, including cell volume regulation, differentiation, and apoptosis [100]. In the gastrointestinal tract, K^+ channels are involved in the production of gastric acid and regulation of secretion [101–103], and changes in K^+ channel activity are associated with intestinal diseases, such as IBDs [104,105].

There are four families of K^+ channels: (i) Ca^{2+}- and Na^+-activated K^+ channels ($K_{ca/Na}$); (ii) inwardly rectifying potassium channels (K_{ir}); (iii) two-pore domain K^+ channels (K_{2P}); and (iv) voltage-gated

potassium channels (K_v) [99,104,106]. Profiling of the *Drosophila* gut [36,37][i,ii] identified the expression of a variety of K^+ channels and revealed that the expression of genes encoding K_v channels, such as Shaker (Sh) and Shaker cognate l (Shal), is enriched in secretory EEs compared with other gut cells. Therefore, it would be worth exploring whether K_v channels regulate gut-hormone release from EEs, and consequentially affect intestinal lipid metabolism [107], feeding [108], and ISC proliferation [109].

In addition, gut profiling after infection [37][ii] revealed that *Sh* and *Shal* levels increase in EBs. K^+ channels have been reported to regulate cell cycle progression and proliferation (reviewed in [110]); thus, their upregulation in the infected gut could suggest potential roles during damage-induced EB mitosis [111]. Since K^+ channels have been implicated in carcinogenesis [112,113], future studies in the *Drosophila* gut should explore the role of K^+ channels under pathophysiological conditions, such as damage-induced regeneration, and help identify conserved signaling pathways triggered by K^+ channels, which may contribute to tumor development.

Cholinergic and serotonin receptors

A group of major regulators for ion transport in the gut epithelium are the cholinergic receptors [4]. Not surprisingly, disruption of cholinergic signaling in the gut is associated with several intestinal diseases, including IBDs and cancers [4,114–116]. Cholinergic receptors fall into two categories: (i) the G-coupled muscarinic receptors, which, upon activation, are proposed to raise intracellular Ca^{2+}, causing Ca^{2+}-dependent K^+ outflow and subsequent Cl^- secretion; and (ii) the ligand gated ion channels, nicotinic receptors, which, upon acetylcholine (Ach) activation, become permeable to cations [4]. *Drosophila* gut profiling [36–38][i,ii,iii] revealed the expression of mAchR subtypes and different nAchR subunits, resembling the mammalian gut and suggesting that the role of the cholinergic pathway in regulating epithelial ion transport and water movement may be conserved. Moreover, *Drosophila* EC subpopulations, such as aEC1 and pEC3, express nAchR subunits α5 and β3 together with the Cl^- channel CFTR. Since mammalian nAchRs in airway epithelial cells are proposed to regulate Cl^- channels, such as CFTR [96], such interactions may be conserved and worth exploring for their role in promoting ion transport and for their contributions to CF-related symptoms in the *Drosophila* intestinal epithelium [44].

Single cell *Drosophila* gut profiling [36][i] revealed that a specific subpopulation of EEs co-expresses cholinergic and **serotonin receptors**, also found in the mammalian gut [117]. Specifically, the serotonin G-protein-coupled receptor 5-HT1A, together with cholinergic receptors mAchR-A, mAchR-C, nAchRα3, and nAchRα5, are expressed in a subpopulation of EEs that secrete the peptide allatostatin-A (Asta-A), which is reported to regulate K^+ transport [118]. In addition, serotonin and cholinergic receptors are being investigated as potential therapeutic targets for IBDs [119–121], although they are very rarely studied together. Future studies in the *Drosophila* gut should explore whether the intersection of serotonergic- and cholinergic-induced pathways in Asta-A expressing EEs have any role in maintaining K^+ balance in the intestinal epithelium, especially since increased K^+ secretion is common in patients with IBDs [122].

Perspectives on bioelectric signaling in the *Drosophila* gut

Ion channels and pumps are vital for initiating bioelectric signaling (endogenous ion currents), which is followed by the flow of electric currents across adjacent cells through electrical synapses known as **gap junctions**. Gap junctions open and close in response to a variety of regulatory inputs, including voltage changes and shifts in intracellular pH or Ca^{2+} concentrations [47]. When open, gap junctions allow electric currents to flow between cells, causing them to electrically couple. This bioelectric coupling has been proposed to help cells cooperate toward larger scale outcomes, such as promoting proper growth and patterning during development [6,7].

Gap junctions comprise two hexameric **hemichannels** that connect the cytoplasm of neighboring cells. The composition of gap junctions varies because each connecting hemichannel can be the same (homotypic) or different (heterotypic) per cell and comprises transmembrane proteins, which assemble in homomeric or heteromeric forms. In *Drosophila,* the transmembrane proteins that form gap junctions belong to the Innexin (Inx) family [123]. Roles for innexins have been reported in development [124–127], and cell proliferation and differentiation during spermatogenesis[128]. Profiling data in the *Drosophila* gut[i,ii] showed diverse Inx expression [36,37]. Specifically, Inx3, Inx7, and Inx2 are expressed in progenitor cells (ISC/EBs); Inx2, Inx7, and Inx1 in ECs; and only Inx2 and Inx7 are found in EEs. Although the functions of Innexins in the adult *Drosophila* gut remain understudied, in other invertebrate systems, namely *Caenorhabditis elegans*, gap junctions between intestinal cells propagate Ca^{2+} waves to promote defecation[129]. Similar waves could exist in the adult *Drosophila* gut and their roles in stimulating physiological functions, such as defecation, as well as potential interactions with channels, including TrpA1 or Piezo, should be further explored.

Bioelectric signaling during regeneration and wound healing is receiving a lot of attention because of the exciting prospect of using electric interventions as therapeutics [6,7,130,131]. However, having a comprehensive understanding *in vivo* of the impact of ion currents in the regulatory networks of a regenerative tissue can be challenging given the pleotropic nature of these currents (e.g., Ca^{2+} signaling). The extensive understanding of the molecular pathways regulating *Drosophila* gut regeneration combined with state-of-the art genetic tools has the potential to make the *Drosophila* gut an excellent bioelectric model during regeneration. For example, sophisticated tools available for *Drosophila*, such as genetically encoded calcium indicators (GECIs; e.g., GCAMP) [132–134], voltage indicators (GEVIs; e.g., ArcLight) [135], FRET-based indicators (e.g., Cl^- reporter Clomeleon [136]), and activated cation channels (e.g., CsChrimson) [137], make *Drosophila* an attractive *in vivo* model for exploring ion current propagation via gap junctions as well as for studying the initiation of endogenous ion flows by different bioelectric regulators. In addition, the multiple binary expression systems available for *Drosophila* (i.e., Gal4/UAS, LexA/LexAop, and QF/QUAS) [32,138,139] allow specific temporal and spatial *in vivo* perturbations that could facilitate the precise dissection of pathways induced by gap junctions and bioelectric regulators in different cells. Together, these tools could link with great specificity bioelectric networks to molecular pathways and expand our current view of gut regeneration and healing.

Concluding remarks

Studies in the *Drosophil*a gut, building on previous studies [40–44,67], provide an opportunity to further dissect the bioelectric roles of ion channels, pumps, and receptors and explore *in vivo* their interactions with gap junctions during physiological, pathophysiological, and regenerative conditions (see Outstanding questions). For example, recent advances indicate that *Drosophila* ingestion involves the activation of mGluR- and Piezo-inducing Ca^{2+} dynamics in progenitor cells [40,41]. Dietary Glu has been reported to activate mGluR, which increases cytosolic Ca^{2+} in ISCs, triggering CREB-induced proliferation, while food-induced mechanical pressure elevates Ca^{2+} influx in Piezo-expressing EBs, triggering proliferation through ERK signaling [40,41]. Therefore, what is perceived as steady-state gut physiological function likely involves two independent Ca^{2+} regulators in adjacent progenitor cells concurrently triggering proliferation through different Ca^{2+}-dependent molecular pathways. Further work studying the interactions of mGluR and Piezo in the *Drosophila* gut under physiological conditions will expand our understanding of how nutritional needs and mechanical pressures in the gut are decoded to regulate proliferative responses.

In addition, transcriptome analysis upon bacterial infection in the *Drosophila* gut [37,38][ii,iii] revealed that the expression of various ion channels is altered across the epithelium. *TrpA1*,

Outstanding questions

How do mGluR- and Piezo-induced Ca^{2+} dynamics work together to regulate proliferation during ingestion?

How do TrpA1-induded Ca^{2+} dynamics in the damaged epithelium promote proliferative and bacteria expulsion responses?

How do bioelectric interactions of ENaC with the Na^+/Ca^{2+} exchanger and the Na^+/K^+-pump contribute to gut regeneration?

What are the interactions of nAchRs with CFTR or other Cl^- channels during the development of CF-like symptoms?

What is the function of K^+ channels in EEs and do they affect gut regeneration?

Which bioelectric regulators help the intestinal epithelium meet the variable ion demands during gut regeneration and disease development?

Do bioelectric regulators in the gut trigger endogenous ion currents during physiological and regenerative conditions? What is the nature of the molecular pathways that are affected in response to these bioelectric currents? What are the interactions between bioelectric regulators and gap junctions during these intestinal bioelectric currents?

Sh, and *Shal* are all increased in EBs during infection, indicative of elevated Ca^{2+} and K^{+} demands when proliferation is triggered. Future studies in the *Drosophila* gut could help decipher which bioelectric regulators are vital in helping the intestinal epithelium meet the varying ion demands during pathophysiological conditions, such as regeneration, infection or CF-like development. The advanced genetic tools available in *Drosophila*, could help identify *in vivo* which bioelectric regulators and conserved molecular pathways are involved per cell type and find roles for gap junctions and bioelectric currents across the epithelium in different regenerative and disease-like states. Together, this new information could ultimately have great therapeutic value for diseases, including IBDs, CF, and cancer.

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Declaration of interests

The authors declare no competing or financial interests.

Resources

[i]www.flyrnai.org/scRNA/gut/

[ii]http://flygutseq.buchonlab.com/

[iii]https://flygut.epfl.ch/

References

1. Magalhaes, D. *et al.* (2016) Role of epithelial ion transports in inflammatory bowel disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 310, G460–G476
2. Rajendran, V.M. *et al.* (2018) Ion channels of the gastrointestinal epithelialcells. In *Physiology of the Gastrointestinal Tract* (6th edn) (Said, H.M., ed.), pp. 1363–1404, Elsevier
3. Yang, X. *et al.* (2018) Molecular mechanisms of calcium signaling in the modulation of small intestinal ion transports and bicarbonate secretion. *Oncotarget* 9, 3727–3740
4. Hirota, C.L. and McKay, D.M. (2006) Cholinergic regulation of epithelial ion transport in the mammalian intestine. *Br. J. Pharmacol.* 149, 463–479
5. Levin, M. and Martyniuk, C.J. (2018) The bioelectric code: an ancient computational medium for dynamic control of growth and form. *Biosystems* 164, 76–93
6. Harris, M.P. (2021) Bioelectric signaling as a unique regulator of development and regeneration. *Development* 148, dev180794
7. Levin, M. (2021) Bioelectric signaling: reprogrammable circuits underlying embryogenesis, regeneration, and cancer. *Cell* 184, 1971–1989
8. Blackiston, D.J. *et al.* (2009) Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle. *Cell Cycle* 8, 3527–3536
9. Emmons-Bell, M. and Hariharan, I.K. (2021) Membrane potential regulates Hedgehog signalling in the *Drosophila* wing imaginal disc. *EMBO Rep.* 22, e51861
10. Schotthofer, S.K. and Bohrmann, J. (2020) Analysing bioelectrical phenomena in the *Drosophila* ovary with genetic tools: tissue-specific expression of sensors for membrane potential and intracellular pH, and RNAi-knockdown of mechanisms involved in ion exchange. *BMC Dev. Biol.* 20, 15
11. Atsuta, Y. *et al.* (2019) L-type voltage-gated Ca(2+) channel CaV1.2 regulates chondrogenesis during limb development. *Proc. Natl. Acad. Sci. U. S. A.* 116, 21592–21601
12. Panakova, D. *et al.* (2010) Wnt11 patterns a myocardial electrical gradient through regulation of the L-type Ca(2+) channel. *Nature* 466, 874–878
13. Daane, J.M. *et al.* (2018) Bioelectric–calcineurin signaling module regulates allometric growth and size of the zebrafish fin. *Sci. Rep.* 8, 10391
14. Fraire-Zamora, J.J. and Simons, M. (2018) Vacuolar ATPase is required for ERK-dependent wound healing in the *Drosophila* embryo. *Wound Repair Regen.* 26, 102–107
15. Justet, C. *et al.* (2013) ENaC contribution to epithelial wound healing is independent of the healing mode and of any increased expression in the channel. *Cell Tissue Res.* 353, 53–64
16. Clapham, D.E. (2007) Calcium signaling. *Cell* 131, 1047–1058
17. Levin, M. (2009) Bioelectric mechanisms in regeneration: unique aspects and future perspectives. *Semin. Cell Dev. Biol.* 20, 543–556
18. Lin, C.S. *et al.* (1993) Voltage-gated potassium channels regulate calcium-dependent pathways involved in human T lymphocyte activation. *J. Exp. Med.* 177, 637–645
19. Dahal, G.R. *et al.* (2017) Inwardly rectifying potassium channels influence *Drosophila* wing morphogenesis by regulating Dpp release. *Development* 144, 2771–2783
20. Beane, W.S. *et al.* (2011) A chemical genetics approach reveals H,K-ATPase-mediated membrane voltage is required for planarian head regeneration. *Chem. Biol.* 18, 77–89
21. Cordero, J.B. *et al.* (2012) Inducible progenitor-derived Wingless regulates adult midgut regeneration in *Drosophila*. *EMBO J.* 31, 3901–3917
22. Guo, Z. *et al.* (2013) Injury-induced BMP signaling negatively regulates *Drosophila* midgut homeostasis. *J. Cell Biol.* 201, 945–961
23. Jiang, H. *et al.* (2009) Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell* 137, 1343–1355
24. Karpowicz, P. *et al.* (2010) The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. *Development* 137, 4135–4145
25. Ayyaz, A. *et al.* (2015) Haemocytes control stem cell activity in the *Drosophila* intestine. *Nat. Cell Biol.* 17, 736–748
26. Biteau, B. and Jasper, H. (2011) EGF signaling regulates the proliferation of intestinal stem cells in *Drosophila*. *Development* 138, 1045–1055
27. Buchon, N. *et al.* (2010) *Drosophila* EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. *BMC Biol.* 8, 152
28. Ohlstein, B. and Spradling, A. (2006) The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. *Nature* 439, 470–474

29. Micchelli, C.A. and Perrimon, N. (2006) Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature* 439, 475–479
30. Miguel-Aliaga, I. *et al.* (2018) Anatomy and physiology of the digestive tract of *Drosophila melanogaster*. *Genetics* 210, 357–396
31. del Valle Rodriguez, A. *et al.* (2011) Power tools for gene expression and clonal analysis in *Drosophila*. *Nat. Methods* 9, 47–55
32. Brand, A.H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401–415
33. Jiang, H. *et al.* (2016) Intestinal stem cell response to injury: lessons from *Drosophila*. *Cell. Mol. Life Sci.* 73, 3337–3349
34. Karin, M. and Clevers, H. (2016) Reparative inflammation takes charge of tissue regeneration. *Nature* 529, 307–315
35. Anderson, K.J. *et al.* (2019) Role of ion channels in gastrointestinal cancer. *World J. Gastroenterol.* 25, 5732–5772
36. Hung, R.J. *et al.* (2020) A cell atlas of the adult *Drosophila* midgut. *Proc. Natl. Acad. Sci. U. S. A.* 117, 1514–1523
37. Dutta, D. *et al.* (2015) Regional cell-specific transcriptome mapping reveals regulatory complexity in the adult *Drosophila* midgut. *Cell Rep.* 12, 346–358
38. Buchon, N. *et al.* (2013) Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila*. *Cell Rep.* 3, 1725–1738
39. Li, H. *et al.* (2022) Fly Cell Atlas: a single-nucleus transcriptomic atlas of the adult fruit fly. *Science* 375, eabk2432
40. Deng, H. *et al.* (2015) Signal integration by Ca(2+) regulates intestinal stem-cell activity. *Nature* 528, 212–217
41. He, L. *et al.* (2018) Mechanical regulation of stem-cell differentiation by the stretch-activated Piezo channel. *Nature* 555, 103–106
42. Xu, C. *et al.* (2017) Oxidative stress induces stem cell proliferation via TRPA1/RyR–mediated Ca(2+) signaling in the *Drosophila* midgut. *Elife* 6, e22441
43. Kim, K. *et al.* (2017) miR-263a regulates ENaC to maintain osmotic and intestinal stem cell homeostasis in *Drosophila*. *Dev. Cell* 40, 23–36
44. Kim, K. *et al.* (2020) *Drosophila* as a model for studying cystic fibrosis pathophysiology of the gastrointestinal system. *Proc. Natl. Acad. Sci. U. S. A.* 117, 10357–10367
45. Friday, B.B. *et al.* (2000) Calcineurin activity is required for the initiation of skeletal muscle differentiation. *J. Cell Biol.* 149, 657–666
46. Moore, K.B. *et al.* (2020) Localized calcium signaling and the control of coupling at Cx36 gap junctions. *eNeuro* 7, ENEURO.0445-19.2020
47. Peracchia, C. (2004) Chemical gating of gap junction channels; roles of calcium, pH and calmodulin. *Biochim. Biophys. Acta* 1662, 61–80
48. Gudipaty, S.A. *et al.* (2017) Mechanical stretch triggers rapid epithelial cell division through Piezo1. *Nature* 543, 118–121
49. Zhao, Q. *et al.* (2018) Structure and mechanogating mechanism of the Piezo1 channel. *Nature* 554, 487–492
50. Guo, Y.R. and MacKinnon, R. (2017) Structure-based membrane dome mechanism for Piezo mechanosensitivity. *Elife* 6, e33660
51. Saotome, K. *et al.* (2018) Structure of the mechanically activated ion channel Piezo1. *Nature* 554, 481–486
52. Liao, J. *et al.* (2021) Upregulation of Piezo1 (piezo type mechanosensitive ion channel component 1) enhances the intracellular free calcium in pulmonary arterial smooth muscle cells from idiopathic pulmonary arterial hypertension patients. *Hypertension* 77, 1974–1989
53. Coste, B. *et al.* (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* 330, 55–60
54. Acosta, A. *et al.* (2015) Quantitative gastrointestinal and psychological traits associated with obesity and response to weight-loss therapy. *Gastroenterology* 148, 537–546 e534
55. Neshatian, L. *et al.* (2015) Ranolazine inhibits voltage-gated mechanosensitive sodium channels in human colon circular smooth muscle cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 309, G506–G512
56. Fernandez-Sanchez, M.E. *et al.* (2015) Mechanical induction of the tumorigenic beta-catenin pathway by tumour growth pressure. *Nature* 523, 92–95
57. Paulsen, C.E. *et al.* (2015) Structure of the TRPA1 ion channel suggests regulatory mechanisms. *Nature* 525, 552
58. Talavera, K. *et al.* (2020) Mammalian transient receptor potential TRPA1 channels: from structure to disease. *Physiol. Rev.* 100, 725–803
59. Takahashi, N. *et al.* (2008) Molecular characterization of TRPA1 channel activation by cysteine-reactive inflammatory mediators. *Channels (Austin)* 2, 287–298
60. Trevisani, M. *et al.* (2007) 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc. Natl. Acad. Sci. U. S. A.* 104, 13519–13524
61. Liu, J. *et al.* (2020) Tumor necrosis factor-alpha regulates the TRPA1 expression in human odontoblast-like cells. *J. Pain Res.* 13, 1655–1664
62. Cho, H.J. *et al.* (2014) Identification of enteroendocrine cells that express TRPA1 channels in the mouse intestine. *Cell Tissue Res.* 356, 77–82
63. Brierley, S.M. *et al.* (2009) The ion channel TRPA1 is required for normal mechanosensation and is modulated by algesic stimuli. *Gastroenterology* 137, 2084–2095
64. Moparthi, L. and Zygmunt, P.M. (2020) Human TRPA1 is an inherently mechanosensitive bilayer–gated ion channel. *Cell Calcium* 91, 102255
65. Yang, Y. *et al.* (2019) TRPA1-expressing lamina propria mesenchymal cells regulate colonic motility. *JCI Insight* 4, e122402
66. Nozawa, K. *et al.* (2009) TRPA1 regulates gastrointestinal motility through serotonin release from enterochromaffin cells. *Proc. Natl. Acad. Sci. U. S. A.* 106, 3408–3413
67. Du, E.J. *et al.* (2016) TrpA1 regulates defecation of food-borne pathogens under the control of the Duox pathway. *PLoS Genet.* 12, e1005773
68. Tziatzios, G. *et al.* (2020) Gut microbiota dysbiosis in functional dyspepsia. *Microorganisms* 8, 691
69. Bruce, J. (2010) Plasma membrane calcium pump regulation by metabolic stress. *World J. Biol. Chem.* 1, 221–228
70. Liu, C. *et al.* (2013) Impaired intestinal calcium absorption in protein 4.1R-deficient mice due to altered expression of plasma membrane calcium ATPase 1b (PMCA1b). *J. Biol. Chem.* 288, 11407–11415
71. Hoenderop, J.G. *et al.* (2005) Calcium absorption across epithelia. *Physiol. Rev.* 85, 373–422
72. Ryan, Z.C. *et al.* (2015) Deletion of the intestinal plasma membrane calcium pump, isoform 1, Atp2b1, in mice is associated with decreased bone mineral density and impaired responsiveness to 1, 25-dihydroxyvitamin D3. *Biochem. Biophys. Res. Commun.* 467, 152–156
73. Wang, T. *et al.* (2005) Light activation, adaptation, and cell survival functions of the Na+/Ca2+ exchanger CalX. *Neuron* 45, 367–378
74. Garty, H. and Palmer, L.G. (1997) Epithelial sodium channels: function, structure, and regulation. *Physiol. Rev.* 77, 359–396
75. Chifflet, S. and Hernandez, J.A. (2016) The epithelial sodium channel and the processes of wound healing. *Biomed. Res. Int.* 2016, 5675047
76. Noreng, S. *et al.* (2018) Structure of the human epithelial sodium channel by cryo-electron microscopy. *Elife* 7, e39340
77. Vallet, V. *et al.* (1997) An epithelial serine protease activates the amiloride-sensitive sodium channel. *Nature* 389, 607–610
78. Vallet, V. *et al.* (2002) Cell-surface expression of the channel activating protease xCAP-1 is required for activation of ENaC in the *Xenopus* oocyte. *J. Am. Soc. Nephrol.* 13, 588–594
79. Vuagniaux, G. *et al.* (2002) Synergistic activation of ENaC by three membrane-bound channel-activating serine proteases (mCAP1, mCAP2, and mCAP3) and serum- and glucocorticoid-regulated kinase (Sgk1) in *Xenopus* oocytes. *J. Gen. Physiol.* 120, 191–201
80. Zelle, K.M. *et al.* (2013) The genetic architecture of degenerin/epithelial sodium channels in Drosophila. *G3 (Bethesda)* 3, 441–450
81. George, L.F. *et al.* (2019) Ion channel contributions to wing development in *Drosophila melanogaster*. *G3 (Bethesda)* 9, 999–1008

82. Liu, L. *et al.* (2003) Drosophila DEG/ENaC pickpocket genes are expressed in the tracheal system, where they may be involved in liquid clearance. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2128–2133
83. Sullivan, S. *et al.* (2009) Downregulation of sodium transporters and NHERF proteins in IBD patients and mouse colitis models: potential contributors to IBD-associated diarrhea. *Inflamm. Bowel Dis.* 15, 261–274
84. Scambler, T. *et al.* (2019) ENaC-mediated sodium influx exacerbates NLRP3–dependent inflammation in cystic fibrosis. *Elife* 8, e49248
85. Peckham, D. *et al.* (1997) Na+/K+ ATPase in lower airway epithelium from cystic fibrosis and non-cystic-fibrosis lung. *Biochem. Biophys. Res. Commun.* 232, 464–468
86. Kim, H.J. *et al.* (2022) Chloride channels and transporters: roles beyond classical cellular homeostatic pH or ion balance in cancers. *Cancers (Basel)* 14, 856
87. Zhang, Z. *et al.* (2018) Molecular structure of the ATP-bound, phosphorylated human CFTR. *Proc. Natl. Acad. Sci. U. S. A.* 115, 12757–12762
88. Liu, F. *et al.* (2017) Molecular structure of the human CFTR ion channel. *Cell* 169, 85–95
89. Csanady, L. *et al.* (2005) Functional roles of nonconserved structural segments in CFTR's NH2-terminal nucleotide binding domain. *J. Gen. Physiol.* 125, 43–55
90. Gunderson, K.L. and Kopito, R.R. (1995) Conformational states of CFTR associated with channel gating: the role ATP binding and hydrolysis. *Cell* 82, 231–239
91. Strong, T.V. *et al.* (1994) Localization of cystic fibrosis transmembrane conductance regulator mRNA in the human gastrointestinal tract by in situ hybridization. *J. Clin. Invest.* 93, 347–354
92. Tam, R.Y. *et al.* (2022) Intestinal inflammation and alterations in the gut microbiota in cystic fibrosis: a review of the current evidence, pathophysiology and future directions. *J. Clin. Med.* 11, 649
93. Crites, K.S. *et al.* (2015) CFTR Knockdown induces proinflammatory changes in intestinal epithelial cells. *J. Inflamm. (Lond.)* 12, 62
94. Meeker, S.M. *et al.* (2020) CFTR dysregulation drives active selection of the gut microbiome. *PLoS Pathog.* 16, e1008251
95. Perez, A. *et al.* (2007) CFTR inhibition mimics the cystic fibrosis inflammatory profile. *Am. J. Physiol. Lung Cell Mol. Physiol.* 292, L383–L395
96. Maouche, K. *et al.* (2013) Contribution of alpha7 nicotinic receptor to airway epithelium dysfunction under nicotine exposure. *Proc. Natl. Acad. Sci. U. S. A.* 110, 4099–4104
97. Berdiev, B.K. *et al.* (2009) Assessment of the CFTR and ENaC association. *Mol. BioSyst.* 5, 123–127
98. Than, B.L.N. *et al.* (2017) CFTR is a tumor suppressor gene in murine and human intestinal cancer. *Oncogene* 36, 3504
99. Kuang, Q. *et al.* (2015) Structure of potassium channels. *Cell. Mol. Life Sci.* 72, 3677–3693
100. Heitzmann, D. and Warth, R. (2008) Physiology and pathophysiology of potassium channels in gastrointestinal epithelia. *Physiol. Rev.* 88, 1119–1182
101. Grahammer, F. *et al.* (2001) The cardiac K+ channel KCNQ1 is essential for gastric acid secretion. *Gastroenterology* 120, 1363–1371
102. Dedek, K. and Waldegger, S. (2001) Colocalization of KCNQ1/KCNE channel subunits in the mouse gastrointestinal tract. *Pflugers Arch.* 442, 896–902
103. Sausbier, M. *et al.* (2006) Distal colonic K(+) secretion occurs via BK channels. *J. Am. Soc. Nephrol.* 17, 1275–1282
104. Han, J. *et al.* (2016) Potassium channelopathies and gastrointestinal ulceration. *Gut Liver* 10, 881–889
105. Anbazhagan, A.N. *et al.* (2018) Pathophysiology of IBD associated diarrhea. *Tissue Barriers* 6, e1463897
106. Cosme, D. *et al.* (2021) Potassium channels in intestinal epithelial cells and their pharmacological modulation: a systematic review. *Am. J. Physiol. Cell Physiol.* 320, C520–C546
107. Song, W. *et al.* (2014) Control of lipid metabolism by tachykinin in *Drosophila*. *Cell Rep.* 9, 40–47
108. Yoshinari, Y. *et al.* (2021) The sugar-responsive enteroendocrine neuropeptide F regulates lipid metabolism through glucagon-like and insulin-like hormones in *Drosophila melanogaster*. *Nat. Commun.* 12, 4818
109. Amcheslavsky, A. *et al.* (2014) Enteroendocrine cells support intestinal stem-cell-mediated homeostasis in *Drosophila*. *Cell Rep.* 9, 32–39
110. Urrego, D. *et al.* (2014) Potassium channels in cell cycle and cell proliferation. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 369, 20130094
111. Tian, A. *et al.* (2022) Damage-induced regeneration of the intestinal stem cell pool through enteroblast mitosis in the *Drosophila* midgut. *EMBO J.* 41, e110834
112. Girault, A. *et al.* (2020) Roles for Ca(2+) and K(+) channels in cancer cells exposed to the hypoxic tumour microenvironment. *Biochim. Biophys. Acta Mol. Cell Res.* 1867, 118644
113. Pardo, L.A. and Stuhmer, W. (2014) The roles of K(+) channels in cancer. *Nat. Rev. Cancer* 14, 39–48
114. Muller, I. *et al.* (2021) Cholinergic signaling attenuates pro-inflammatory interleukin-8 response in colonic epithelial cells. *Front. Immunol.* 12, 781147
115. Konishi, M. *et al.* (2019) Role of muscarinic acetylcholine signaling in gastrointestinal cancers. *Biomedicines* 7, 58
116. Rueda Ruzafa, L. *et al.* (2021) Nicotinic acetylcholine receptor involvement in inflammatory bowel disease and interactions with gut microbiota. *Int. J. Environ. Res. Public Health* 18, 1189
117. Guzel, T. and Mirowska-Guzel, D. (2022) The role of serotonin neurotransmission in gastrointestinal tract and pharmacotherapy. *Molecules* 27, 1680
118. Vanderveken, M. and O'Donnell, M.J. (2014) Effects of diuretic hormone 31, drosokinin, and allatostatin A on transepithelial K(+) transport and contraction frequency in the midgut and hindgut of larval *Drosophila melanogaster*. *Arch. Insect Biochem. Physiol.* 85, 76–93
119. Motavallian, A. *et al.* (2019) Anti-inflammatory effects of alosetron mediated through 5-HT3 receptors on experimental colitis. *Res. Pharm. Sci.* 14, 228–236
120. AlSharari, S.D. *et al.* (2020) The alpha9alpha10 nicotinic acetylcholine receptors antagonist alpha-conotoxin RgIA reverses colitis signs in murine dextran sodium sulfate model. *Eur. J. Pharmacol.* 883, 173320
121. Maruta, K. *et al.* (2018) Nicotine treatment ameliorates DSS-induced colitis by suppressing MAdCAM-1 expression and leukocyte recruitment. *J. Leukoc. Biol.* 104, 1013–1022
122. Barkas, F. *et al.* (2013) Electrolyte and acid-base disorders in inflammatory bowel disease. *Ann. Gastroenterol.* 26, 23–28
123. Sanchez, A. *et al.* (2019) Gap junction channels of innexins and connexins: relations and computational perspectives. *Int. J. Mol. Sci.* 20, 2476
124. Lehmann, C. *et al.* (2006) Heteromerization of innexin gap junction proteins regulates epithelial tissue organization in *Drosophila*. *Mol. Biol. Cell* 17, 1676–1685
125. Sahu, A. *et al.* (2017) A gap junction protein, Inx2, modulates calcium flux to specify border cell fate during *Drosophila* oogenesis. *PLoS Genet.* 13, e1006542
126. Holcroft, C.E. *et al.* (2013) Innexins Ogre and Inx2 are required in glial cells for normal postembryonic development of the *Drosophila* central nervous system. *J. Cell Sci.* 126, 3823–3834
127. Bauer, R. *et al.* (2002) The *Drosophila* gap junction channel gene innexin 2 controls foregut development in response to Wingless signalling. *J. Cell Sci.* 115, 1859–1867
128. Smendziuk, C.M. *et al.* (2015) Bi-directional gap junction-mediated soma-germline communication is essential for spermatogenesis. *Development* 142, 2598–2609
129. Peters, M.A. *et al.* (2007) A calcium wave mediated by gap junctions coordinates a rhythmic behavior in *C. elegans*. *Curr. Biol.* 17, 1601–1608
130. McLaughlin, K.A. and Levin, M. (2018) Bioelectric signaling in regeneration: mechanisms of ionic controls of growth and form. *Dev. Biol.* 433, 177–189
131. Tyler, S.E.B. (2017) Nature's electric potential: a systematic review of the role of bioelectricity in wound healing and regenerative processes in animals, humans, and plants. *Front. Physiol.* 8, 627
132. Dana, H. *et al.* (2019) High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. *Nat. Methods* 16, 649–657

133. Simpson, J.H. and Looger, L.L. (2018) Functional imaging and optogenetics in *Drosophila*. *Genetics* 208, 1291–1309
134. Chen, Y. *et al.* (2022) Ultra-sensitive responsive near-infrared fluorescent nitroreductase probe with strong specificity for imaging tumor and detecting the invasiveness of tumor cells. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 268, 120634
135. Cao, G. *et al.* (2013) Genetically targeted optical electrophysiology in intact neural circuits. *Cell* 154, 904–913
136. Grabe, V. *et al.* (2020) Odor-induced multi-level inhibitory maps in *Drosophila*. *eNeuro* 7, ENEURO.0213-19.2019
137. Klapoetke, N.C. *et al.* (2014) Addendum: independent optical excitation of distinct neural populations. *Nat. Methods* 11, 972
138. Yagi, R. *et al.* (2010) Refined LexA transactivators and their use in combination with the Drosophila Gal4 system. *Proc. Natl. Acad. Sci. U. S. A.* 107, 16166–16171
139. Potter, C.J. *et al.* (2010) The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. *Cell* 141, 536–548