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Microbial control of intestinal homeostasis via enteroendocrine cell innate immune signaling

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Abstract

A community of commensal microbes known as the intestinal microbiota resides within the gastrointestinal tract of animals and plays a role in maintenance of host metabolic homeostasis and resistance to pathogen invasion. Enteroendocrine cells, which are relatively rare in the intestinal epithelium, have evolved to sense and respond to these commensal microbes. Specifically, they express G protein-coupled receptors and functional innate immune signaling pathways that recognize products of microbial metabolism and microbe-associated molecular patterns, respectively. Here we review recent evidence from *Drosophila melanogaster* that microbial cues recruit antimicrobial, mechanical, and metabolic branches of the enteroendocrine innate immune system and argue that this response may play a role not only in maintaining host metabolic homeostasis but also in intestinal resistance to invasion by bacterial, viral, and parasitic pathogens.

Keywords

intestinal microbiota; metabolism; innate immunity; enteroendocrine cell; enteroendocrine peptide; colonization resistance; *Drosophila melanogaster*

An open gastrointestinal tract is both a burden and a blessing

The gastrointestinal tracts of mammals and *Drosophila* include a mouth and an anus, proximal and distal openings to our densely populated environment, which we share with trillions upon trillions of microbes. The gastrointestinal tract is in constant communication with these microbes, which may join the intestinal microbiota to benefit the host or disrupt the intestinal community through their ability to dominate the intestinal environment, disrupt intestinal function, and/or damage the intestinal epithelium. To maintain organismal homeostasis, hosts must control and tailor the commensal population, resist pathogen

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colonization, and counteract pathogen invasions. Recently, the importance of enteroendocrine cells (EECs) in this process has been elucidated in a *Drosophila* model. In particular, a subtype of EEC within a particular region of the intestine has been shown to use innate immune signaling to respond to the intestinal microbiota [1]. This response targets antibacterial, metabolic, and mechanical functions of the host intestine. Here we review the structure and function of the *Drosophila* intestine, its commensal microbiota, and its innate immune system and suggest a model in which EECs, through their unique innate immune responses to the commensal microbiota, control the susceptibility of the host intestine to colonization by pathogens.

The dipteran and mammalian intestines compared

The *Drosophila* gastrointestinal tract consists of a foregut, midgut, hindgut, and rectum (Fig 1) [2]. The foregut functions mainly in food intake and storage, while the hindgut and rectum are specialized for water absorption. Here we focus on the midgut, which is the absorptive portion of the *Drosophila* intestine. The midgut has been divided into as many as 14 distinct functional sections based on morphology and transcriptional analysis [3, 4]. Broadly, though, the *Drosophila* midgut consists of anterior, middle, and posterior regions. In the anterior midgut, one finds the highest burden of commensal microbes as well as the highest expression of antimicrobial peptides [4–7]. Therefore, in uninfected hosts, the anterior midgut is the home of the commensal microbiota, and the innate immune response of the anterior midgut is principally elicited by this population.

Collaboration of the microbiota in host digestion of complex macromolecules is a common theme in nature. For instance, wood-eating insects do so with the aid of intestinal microbes [8, 9]. In the mammalian colon, the abundant commensal microbes participate in host digestion of complex carbohydrates, while antibacterial products keep the microbiota at a distance [10–12]. Based on its transcriptome, the *Drosophila* anterior midgut is also predicted to participate in the degradation of complex macromolecules including polysaccharides, proteins, and lipids, and the resident microbiota may participate in this process [4, 13].

The *Drosophila* middle midgut epithelium is populated by copper cells that pump protons into the intestinal lumen, lowering the pH to less than 4 [4, 14]. This compartment is analogous to the mammalian stomach. Like the stomach, this region represents a bottle neck that decreases the passage of bacteria to more distal portions of the intestine [5, 6]. The posterior midgut of the fly, therefore, harbors few bacteria. This compartment expresses proteins more consistent with a function in absorption of simple nutrients [4].

While the mammalian and dipteran gastrointestinal tracts include functionally similar compartments for digestion, acidification, and water reabsorption, their succession in the intestine is shuffled (Fig 1). After ingestion by a mammal, nutrients and bacteria first encounter the stomach, a compartment of pH 2 analogous to the middle midgut of the fly. Passage through this compartment eliminates most entering bacteria. Bile secreted into the anterior small intestine, along with rapid flow through the small intestine, further decreases bacterial residence with the result that the burden of bacteria in the small intestine is low and

increases only near the opening to the large intestine, the ileocecal valve [15]. The small intestinal epithelium secretes many digestive enzymes and functions in uptake of simple nutrients. The large intestine is essential for digestion of complex carbohydrates, and, like the anterior midgut of the fly, it harbors a dense community of microbes that participate in this function. The distinct succession of acidic, microbe-rich, and digestive compartments in the mammalian and dipteran intestines may reflect the relative dependence of each host on bacterial catabolism of complex carbohydrates for nutrition. In other words, because adult *Drosophila* depend heavily on complex carbohydrates for their nutrition, these energy sources are broken down by microbes in the most proximal portion of the midgut and can then be efficiently absorbed. Mammals derive most of their energy from dietary nutrients that can be digested and absorbed by the relatively microbe-free small intestine. Therefore, processing of indigestible complex carbohydrates by the microbiota is relegated to most distal portion of the intestine, the colon, because it contributes relatively little to a mammal's overall caloric intake [16].

The intestinal microbial community of laboratory-reared and wild-caught *Drosophila*

The intestinal microbiota of laboratory-reared *Drosophila melanogaster* consists principally of *Acetobacter* and *Lactobacillus sp.* [17, 18]. Because these bacteria are excreted into the culture medium and then re-ingested by the fly, their abundance in the intestinal microbial community is as much a reflection of their adaptation to the culture medium as to the *Drosophila* intestine. A large part of this microbial population appears to be only transiently associated with the intestine, as the population decreases rapidly when flies are passaged to sterile medium frequently [17, 19].

In the natural environment, the intestinal microbiota of *Drosophila melanogaster* is much more complex including α -, β -, and γ -Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes [18, 20, 21]. Some components of this microbiota persistently colonize the gut of laboratory-reared flies in spite of frequent passaging [19]. These include *Acetobacter thailandicus*, *Acetobacter cibinongensis* and *Leuconostoc pseudomesenteroides*. Interestingly, several colonizers of the human gastrointestinal tract such as *Enterococcus faecalis*, *Enterococcus faecium*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Pantoea agglomerans* as well as human pathogens such as *Shigella* have been identified in the intestines of wild-caught flies. While this could suggest parallels in the intestinal environments of humans and insects, future studies focused on proteins encoded and expressed within the genomes of these intestinal bacteria as well as studies of their *in situ* physiology and metabolism are necessary to establish this (see Outstanding Questions).

Although the abundance varies, bacteria in the Acetobacteraceae and Lactobacillaceae orders are present in the gut microbiota of both wild-caught and laboratory-reared flies. Interestingly, the presence of both *Acetobacter* and *Lactobacillus sp.* in the gut microbial community have been shown to accelerate *Drosophila* growth and development by distinct mechanisms that ultimately impact insulin signaling and, therefore, host nutrient utilization [1, 22–25]. A similar correlation between the gut microbiota and human development exists

[26–28]. As a result of the trove of genetic tools available, elucidation of the mechanisms underlying microbiota-associated developmental delay has come rapidly in *Drosophila*, whereas mechanistic studies in mammals have been more challenging.

Cell types in the *Drosophila* intestine with a focus on enteroendocrine cells

Three mature cell types comprise the *Drosophila* intestinal epithelium. The most abundant is the enterocyte, which synthesizes and secretes digestive enzymes into the intestinal lumen and then imports digested lipids, proteins, and carbohydrates. Enterocytes are also responsible for production of antimicrobial peptides (AMPs) and for synthesis of the intestinal covering known as the peritrophic matrix [3, 29]. More sparse cells include stem cells, which regenerate the epithelium, and EECs, which coordinate digestion. Gene expression in all of these cell types varies along the course intestinal epithelium. This suggests specialized functions for each of these cell types in each region of the intestine [3].

In both mammals and flies, EECs synthesize regulatory peptides known as enteroendocrine peptides (EEPs) and package them into small vesicles that are stored near the basolateral cell membrane. While the function of only a subset of *Drosophila* EEPs has been defined, the spatial distribution of EEP expression in the intestine has been characterized both by immunofluorescence and transcriptomic techniques [4, 13, 30–32]. Tachykinin (Tk) is found throughout the midgut. CCHamide-2 (CCHa-2) is most highly expressed in the anterior midgut. The middle midgut expresses Allatostatin C (AstC), neuropeptide F (NPF), and orchokinin (OK) most highly. AstC and OK are produced by the same EECs [31]. Finally, AstA, CCHa-1, and diuretic hormone 31 (DH31) are most abundant in the posterior midgut. Therefore, EECs are distinguished both by the EEPs they produce and by their location along the length of the intestine, and these two features likely define the role of these EECs in regulating intestinal function and systemic metabolism.

The apical surface of the EEC is exposed to the intestinal lumen. In mammals, luminal conditions are sampled by G-protein coupled receptors (GPCRs), such as GPR43 and GPR41, that respond to many small molecules including short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which are products of bacterial fermentation [33]. Activation of these receptors results in release of EEPs from the basolateral surface of the EEC. While few such GPCRs have been characterized in *Drosophila*, they are expressed in these cells and likely function similarly.

Tk and DH31 are a focus of this review. DH31 activates intestinal contractions, while Tk represses accumulation of lipid droplets in enterocytes [34, 35]. Where both are easily visualized, Tk and DH31 have been localized to the same EECs in both larvae and adults and, therefore, expression and release of these two EEPs may be activated by similar stimuli [30, 36].

The innate immune responses of the *Drosophila* intestine

The best studied defenses of the *Drosophila* midgut are reactive oxygen species produced by the enzyme dual oxidase and AMPs [37, 38]. Transcription of intestinal AMPs is regulated by the TNF-like IMD signaling pathway [39]. The IMD pathway was initially elucidated in

the fat body [40]. However, components of the IMD pathway are expressed in every intestinal cell type [3]. As IMD pathway signaling in the different cell types and regions of the intestine has been explored, there has been no indication that the components of this signaling pathway differ from that in the fat body [1,38]. However, the effectors activated by the IMD pathway appear to be tailored to match the role of each cell type in the intestinal innate immune response [1].

The IMD pathway is activated by two receptors, PGRP-LC, a membrane-associated peptidoglycan receptor, and PGRP-LE, a cytoplasmic receptor of peptidoglycan fragments [41–43]. Peptidoglycan sensing ultimately results in the phosphorylation and cleavage of the transcription factor Relish [44]. The N-terminus of Relish then translocates to the nucleus where it binds to DNA to regulate the transcription of many genes including AMPs.

Tk and DH31-expressing enteroendocrine cells of the anterior midgut coordinate the innate immune system in response to commensal microbiota

Mammalian EECs express Toll-like receptors and activate an NF-KB-mediated response when exposed to microbe-associated molecular patterns (MAMPS). This leads to secretion of cytokines and EEPs [45, 46]. As such, it has been proposed that EECs may orchestrate intestinal immunity [47]. This hypothesized role of EEC in intestinal innate immunity is strongly supported by evidence gathered using the *Drosophila* model [1]. In uninfected flies, AMPs are most highly transcribed in the proximal region of the anterior midgut, where the highest burden of microbiota is also found (Fig 1) [4–7]. The response to these microbes is mediated by IMD pathway signaling in Tk-expressing EECs of the anterior midgut [3]. This response includes increased transcription of the genes encoding AMPs, Tk, and DH31 [1]. As one might predict, attenuation of IMD pathway signaling in Tk-expressing EECs, either through elimination of the commensal microbiota or through mutation of IMD pathway components, results in decreased AMP, DH31, and Tk expression in the anterior midgut [1]. AMPs have antibacterial activity, DH31 increases intestinal contractions, and Tk has recently been shown to repress lipid synthesis in enterocytes [34, 35]. Thus, Tk and DH31-expressing EECs in the dipteran anterior midgut coordinate antibacterial, mechanical, and metabolic branches of the host intestinal innate immune response to the commensal microbiota.

Could the enteroendocrine innate immune response to commensal microbes afford the host resistance to colonization by symbionts and pathogens?

Microbial activation of the mechanical, metabolic, and antimicrobial branches of the innate immune response may contribute to the host's ability to resist pathogen invasion. AMPs are well-established to have antibacterial activity, and the intestinal contractions activated by DH31 have recently been demonstrated to limit the microbial population in the anterior midgut [48–50]. The action of Tk, which decreases lipid stores in enterocytes, may

contribute to resistance against replication of intracellular microbes [34]. Bacterial endosymbionts have been shown to utilize host lipids. *Spiroplasma* and *Wolbachia* species, both of which are endosymbionts of insects, require host lipids for growth [51–53].

The dependence of endosymbionts on host lipids suggests that depletion of lipid stores in enterocytes would limit replication of these microbes. Therefore, IMD pathway-regulated repression of lipid synthesis in anterior midgut cells may control the burden of intracellular symbionts. Similarly, we hypothesize that repression of lipid synthesis by Tk in enterocytes limits colonization by intracellular pathogens.

In insects, arboviruses that are human pathogens such as West Nile, Dengue, Chikungunya, and Zika virus replicate in the intestines of biting insects and are transmitted during blood meals. These viruses exploit host lipid droplets to enhance viral replication [54–56]. Lipid metabolism also plays a role in insect-specific intracellular infections such as flock house and *Drosophila C* virus [57, 58]. The insect parasite *Steinernema carpocapsae* also modulates host lipid stores, and this may affect parasite viability or numbers. The dependence of all these intracellular pathogens on host lipids suggests that innate immune-regulated depletion of lipid stores in enterocytes through Tk expression and release would limit replication of these microbes. There is ample indirect evidence for this in the literature as insects that are infected with *Wolbachia* are resistant to arboviruses, and this is the result of competition for intracellular lipids [59–61]. Given the importance of host lipid droplets in proliferation of intracellular microbes, we posit that IMD pathway-controlled repression of lipid synthesis in anterior midgut cells is a branch of the intestinal innate immune response that targets intracellular pathogens.

Many intracellular viruses, bacteria, and parasites that infect humans also interact with host lipid droplets, using them as lipid reservoirs and platforms for replication [62, 63]. For instance, human gastrointestinal viruses such as hepatitis C, rotavirus, and poliovirus all exploit lipid droplets as replication platforms [58, 64–66]. The human bacterial pathogens *Chlamydia trachomatis* and *Mycobacterium tuberculosis* both utilize intracellular lipid stores for their replication [62, 63, 67, 68]. Parasites such as *Leishmania*, *Trypanosoma cruzi*, and *Toxoplasma gondii* all rely on host lipids for growth [69–73]. Therefore, pathways that repress intracellular lipid synthesis could represent a target for the development of broad-range antimicrobial compounds.

The innate immune responses of the *Drosophila* anterior and posterior midgut are distinct

In EECs of the anterior midgut, the IMD pathway is activated by the microbial metabolite acetate, and the response to acetate requires the extracellular peptidoglycan receptor PGRP-LC [1, 74]. This suggests that the role of this pathway is to detect the abundance of metabolically active bacteria in the intestinal lumen, such as the commensal microbiota. In the uninfected posterior midgut, IMD pathway signaling is suppressed by Caudal, a transcription factor that is most highly expressed in this compartment [75, 76]. The pathogens *Vibrio cholerae*, *Erwinia carotovora carotovora* 15 (*Ecc15*), and *Serratia marcescens* all transit the acidic *Drosophila* middle midgut and access posterior portions of

the gut, where they grow to high numbers and activate IMD pathway signaling [74, 77, 78]. Signaling is spearheaded by the cytoplasmic peptidoglycan receptor PGRP-LE, suggesting that activation of the IMD pathway in this compartment is principally a response to invasive pathogens [74]. Taken together, these observations point to a model in which the innate immune response of the anterior midgut is active under homeostatic conditions to control the commensal microbiota, intracellular symbionts, and pathogen colonization, while that of the posterior midgut is designed to combat overwhelming and invasive infection of this more sterile environment (Fig 2, Key Figure).

Similar to what is observed when the microbiota of the anterior midgut is eliminated, *V. cholerae* proliferation in the *Drosophila* posterior midgut is accompanied by a dearth of acetate and enlargement of lipid droplets in this region [1, 79]. However, the similarities between the germ-free condition and *V. cholerae* infection go no further. It is critical to note that in the posterior midgut acetate is not essential for Tk transcription, and these lipid droplets do not form due to triacyl glyceride accumulation [1, 79]. Rather, catabolism of phospholipids causes lipid droplets to coalesce, thus minimizing their surface area and dependence on phospholipids as an interface between the hydrophobic lipid droplet interior and the hydrophilic cell cytoplasm [80].

Concluding Remarks

Activation of the *Drosophila* anterior midgut innate immune response requires both the SCFA acetate and extracellular peptidoglycan. In the absence of these signals, the fly accumulates large lipid droplets in anterior midgut enterocytes and becomes hyperlipidemic and hyperglycemic [1]. Thus, the intestinal innate immune response of the anterior midgut maintains host metabolic homeostasis in response to microbial metabolites and peptidoglycan. Here we posit that this innate immune response may also limit the burden of commensal microbes, endosymbionts, and pathogens.

Because EECs express effectors of metabolic and innate immune control that respond to bacterial metabolites and MAMPS, they are ideally positioned to maintain the delicate balance between beneficial microbial colonization of the intestine that preserves metabolic homeostasis and detrimental colonization leading to dysbiosis, poor nutrition, and infection. In mammals, the intestinal microbiota has been implicated in malnutrition, diabetes, and obesity [26, 33, 81, 82]. Elucidation of the link between intestinal microbes, EECs, and host metabolism in *Drosophila* may ultimately pave the way for novel prebiotic and probiotic therapeutics targeting these chronic metabolic diseases (see outstanding questions).

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Highlights

- The intestinal microbiota of flies and mammals resides in the most proximal and most distal portions of gastrointestinal tract, respectively
- Enteroendocrine cells express innate immune signaling pathways that respond to microbial metabolites and patterns by upregulating transcription of antimicrobial and the enteroendocrine peptides DH31 and Tachykinin.
- The IMD pathway of enteroendocrine cells controls intestinal levels of antimicrobial peptides as well as DH31-regulated intestinal contractions and Tachykinin-regulated lipid synthesis.
- Antimicrobial peptide, DH31, and Tachykinin control the antimicrobial, mechanical and metabolic branches of the intestinal innate immune system.
- Small molecules that target the enteroendocrine innate immune system may represent therapies for chronic metabolic diseases such as obesity and diabetes.

Outstanding Questions

What is the significance of the differing sequence of compartments in the *Drosophila* and mammalian intestines?

How does the metabolism and physiology of related commensal microbial species differ in the intestines of laboratory-cultured *Drosophila*, wild-caught *Drosophila*, and mammals?

How does innate immune sensing and response differ in the anterior and posterior midguts and what is the role of IMD pathway signaling in these two intestinal compartments?

What is the mechanism by which acetate activates the *Drosophila* IMD pathway and do any other microbial metabolites have a similar effect?

Do microbial metabolites activate innate immune signaling in mammalian enteroendocrine cells?

Do the enteroendocrine innate immune systems of flies and mammals play a role in pathogen colonization resistance?

Can the insect enteroendocrine innate immune signaling pathway be manipulated to reduce transmission of vector-borne illnesses?

Can the mammalian enteroendocrine innate immune signaling pathway be manipulated to improve nutrient utilization, counteract malnutrition, and treat chronic metabolic diseases such as diabetes and obesity?

Figure 1: A comparison of the dipteran and mammalian gastrointestinal tracts.

(A) The commensal microbiota of *Drosophila* gastrointestinal tract resides principally in the anterior midgut. Digestion of complex carbohydrates is predicted to occur in this compartment. Transit of microbes to the posterior midgut is restricted by the low pH of the middle midgut. Therefore, the posterior midgut is relatively microbe-free except during overwhelming infection. (B) The mammalian intestine has similar compartments. However, their order is shuffled. Ingested microbes first encounter the acidic stomach. This, along with bile secretion and rapid flow through the small intestine, ensures that the burden of microbes in the small intestine is low. The microbial burden increases as the large intestine is reached, and the density of microbes in the large intestine is extremely high. It is in the latter compartment that digestion of many complex carbohydrates occurs.

Figure 2: Key Figure: A model for the impact of the commensal microbiota on the anterior midgut immune response and the impact of pathogens on the posterior midgut immune response.

(A) Viable microbes that comprise the commensal microbiota of the anterior midgut produce acetate and shed peptidoglycan. These two products are necessary for activation of the IMD pathway within DH31/Tk-expressing enteroendocrine cells. In the anterior midgut, the membrane-associated receptor PGRP-LC is the principal sensor of peptidoglycan. IMD pathway signaling in these cells results in expression of AMPs, Tk, and DH31. The ultimate result is control of the microbiota through antimicrobial action, intestinal contractions, and inhibition of lipid synthesis within enterocytes. (B) In antibiotic-treated, germ-free, or IMD pathway mutant flies, the IMD pathway is not activated, and AMPs, Tk, and DH31 are not made. The absence of Tk expression leads to increased numbers of lipid droplets within enterocytes presumably due to de-repression of lipid synthesis. In addition, decreased levels of DH31 result in decreased intestinal contractions. (C) In the uninfected fly, there is little signaling through the IMD pathway of the posterior midgut, and expression of IMD pathway-regulated genes is repressed by the transcription factor Caudal. Because Tk expression in the posterior midgut is not under control of the IMD pathway, Tk is expressed and homeostatic levels of lipids are synthesized in enterocytes. (D) Pathogens such as *V. cholerae* gain access to the posterior midgut. In this case, peptidoglycan activates the IMD

pathway, and AMP synthesis is increased. Because *V. cholerae* activates degradation of phospholipids, lipid droplets coalesce, leading to larger lipid droplets in spite of an overall decrease in TAG stores. Abbreviations: ABX, antibiotic; TCT, tracheal cytotoxin; TKR, tachykinin receptor; VM, visceral muscle; EC, enterocyte; EE, enteroendocrine; TAG, triacylglycerol.

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