Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*

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Abstract | Intestinal homeostasis is achieved, in part, by the integration of a complex set of mechanisms that eliminate pathogens and tolerate the indigenous microbiota. *Drosophila melanogaster* feeds on microorganism-enriched matter and therefore has developed efficient mechanisms to control ingested microorganisms. Regulatory mechanisms ensure an appropriate level of immune reactivity in the gut to accommodate the presence of beneficial and dietary microorganisms, while allowing effective immune responses to clear pathogens. Maintenance of *D. melanogaster* gut homeostasis also involves regeneration of the intestine to repair damage associated with infection. Entomopathogenic bacteria have developed common strategies to subvert these defence mechanisms and kill their host.

Drosophila melanogaster has proved to be a powerful genetic model to decipher the innate immune responses to bacterial, fungal and viral infections[1]. More recently, *D. melanogaster* has emerged as an equally powerful model to study host–microorganism interactions in the gut[2–4]. This is due, in part, to the natural ecology of the fruitfly: it feeds on microorganisms that develop in and live on decaying matter, mostly fruits. Therefore, *D. melanogaster* ingests the diverse and abundant microorganisms that can occupy this environment, and transmits them to new habitats. Some of these microorganisms, especially yeast species, have well-established roles as food[5–7]. The bacterial species that are most commonly associated with *D. melanogaster* are thought to constitute the normal gut microbiota, and a few less commonly associated microorganisms have been shown to be pathogenic in laboratory settings. Although the lines separating these categories are not entirely clear, especially in differentiating between the gut microbiota and dietary microorganisms, it has been suggested that the interactions with these microorganisms have shaped *D. melanogaster* evolution[8–11]. As a result, *D. melanogaster* exhibits a range of both gut-specific and systemic immune responses that help to combat infection. In addition, the species possesses mechanisms that maintain intestinal tissue homeostasis by both dampening gut immune responses to non-pathogenic microorganisms and promoting regeneration of the tissue following intestinal damage.

Our understanding of the gut epithelial response to indigenous and infectious bacteria has been greatly expanded by an increased knowledge of *D. melanogaster* gut structure and function[12] (BOX 1), as well as by the recent identification of both commensal bacterial species associated with the *D. melanogaster* gut, and pathogens that can infect and damage the gut when ingested, such as *Erwinia carotovora* subsp. *carotovora* 15 (REF. 13), *Pseudomonas entomophila*[14] and *Serratia marcescens*[15]. To date, no specific mechanism to recognize commensal bacteria has been identified in *D. melanogaster*. Despite the fact that indigenous and pathogenic microorganisms activate similar mechanisms, the level of the immune response that is activated, as well as the amount of damage that is inflicted on the gut epithelium, is significantly lower in the case of the gut microbiota[16]. This indicates that *D. melanogaster* has evolved regulatory mechanisms that prevent a deleterious induction of the immune response under basal conditions, but allow a rapid elimination of microorganisms on pathogenic infection.

In this Review, we describe the range of interactions between host and bacteria in the *D. melanogaster* gut, as revealed by recent studies. We discuss how the gut microbiota affects host physiology, as well as the mechanisms used by *D. melanogaster* to limit microbial infection while ensuring intestinal homeostasis, and what occurs when these responses are dysregulated.

The microbiota promotes host homeostasis

D. melanogaster feeds on microorganisms that grow in overripe and decaying fruit, and thus is constantly exposed to dietary and environmental microorganisms. Recently, several studies have used 16S rRNA analysis to determine the diversity of bacteria commonly associated with *D. melanogaster*[17–22]. A major goal of these studies has been to

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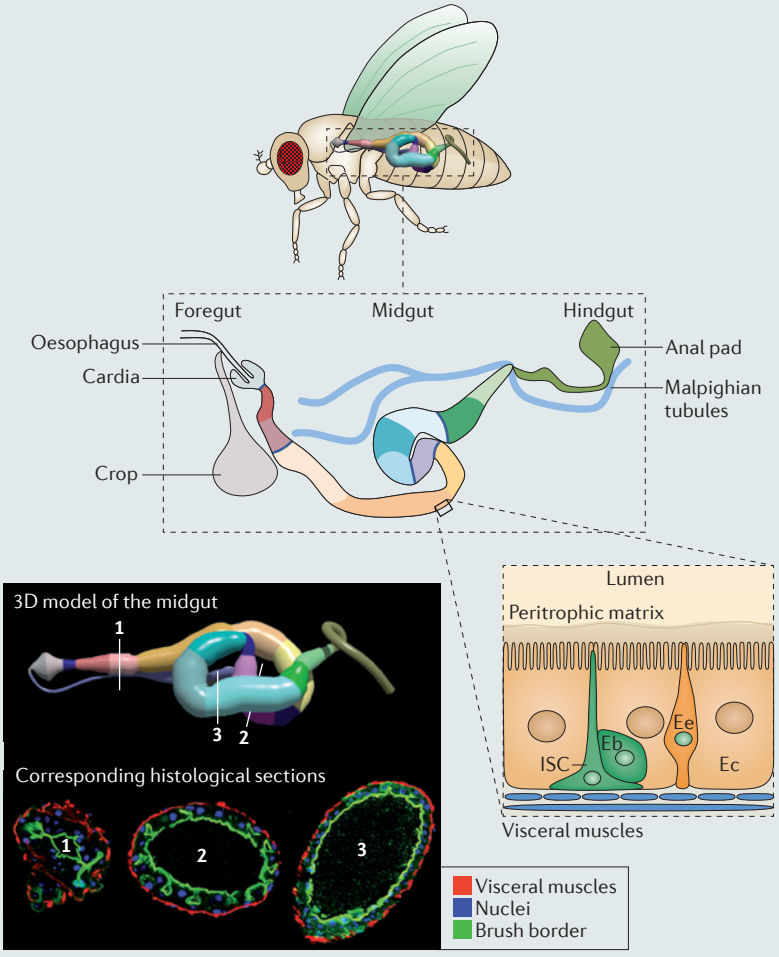
Box 1 | **The gut of *Drosophila melanogaster***

A structured organ

The *Drosophila melanogaster* gut has a structure and organization that resemble those of the mammalian gut. It is a tubular epithelium composed of a monolayer of cells surrounded by visceral muscles and tracheae[112]. This tube is divided into three distinct compartments defined by their developmental origin: the foregut, midgut and hindgut (see the figure). Both the foregut and hindgut originate from the ectoderm, whereas the midgut originates from the endoderm. A layer of cuticle lines the foregut and hindgut. In the midgut, a semipermeable chitinous layer, the peritrophic matrix, protects the epithelium from physical damage and regulates the passage of particles between the lumen and enterocytes. The foregut is subdivided into the pharynx, the oesophagus and a diverticulum called the crop, which stores food. The midgut extends from the cardia (a valve located at the midgut–foregut junction) to the junction with the hindgut, where the Malpighian tubules (the principal excretory and osmoregulatory organ) connect to the gut. The midgut is the main site of digestion, and nutrients are absorbed in the midgut and hindgut. The midgut is compartmentalized into regions with distinct histological and physiological properties[12]. Despite the important role of the gut in digestion and physiology, the mechanisms that control these processes are largely unknown.

A dynamic organ

The gut of *D. melanogaster*, despite its apparent simplicity, is a complex and dynamic organ. One specific feature of the adult gut is that it is renewed constantly throughout the lifespan of the fly. The adult midgut is composed of two types of cells: large absorptive enterocytes (Ecs) and small secretory enteroendocrine cells (Ees). Like the mammalian digestive tract, the fly gut epithelium is maintained by pluripotent intestinal stem cells (ISCs) that divide and self-renew, giving rise to two cells: a new ISC and a progenitor cell, called the enteroblast (Eb), which is devoid of mitotic capability[113,114]. Enteroblasts are maintained transiently in the epithelium or differentiate into either enterocytes or enteroendocrine cells[115].



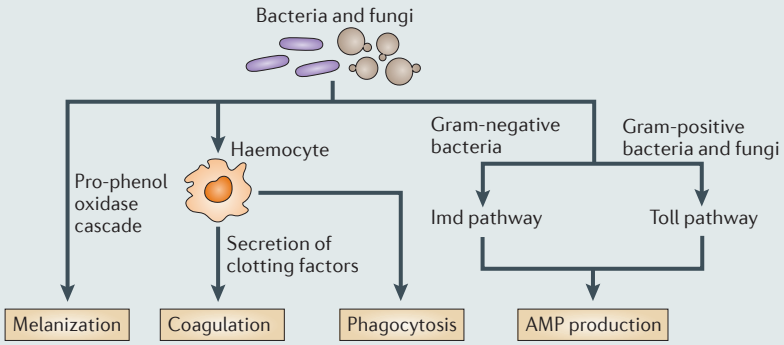
define the core microbiome of this species. Collectively, these studies, which examined both wild-caught and laboratory fly stocks, indicate that the *D. melanogaster* gut is an environment of low bacterial diversity (1–30 species) and that the most commonly associated bacterial species are members of the *Lactobacillus* and *Acetobacter* genera. However, these bacteria also grow in and on the food substrates of the fly (fruit in nature and an artificial diet in the laboratory), and it is not fully understood how transient these bacterium–fruitfly associations are or whether a microbiota is actively growing and maintained in the gut (see REF. 23 for further discussion). What is evident is that *D. melanogaster* associates with a narrow range of bacteria that is transmitted to new environments through regurgitation or defecation and can be acquired by larvae through ingestion of contaminated embryo surfaces or fruit tissue. Furthermore, these bacteria are not essential for host development or survival, as axenic fly cultures (produced by decontaminating the embryo surface with bleach) can be easily maintained on an adequate diet in the laboratory for generations[24].

The ability to derive axenic fly lines and thus establish gnotobiotic cultures has allowed researchers to investigate the effects of the gut microbiota and dietary microorganisms on *D. melanogaster* (with the caveat that observed differences could be due to direct effects on the gut, on the fly medium, or on both). As a result, a growing number of studies are showing that the *D. melanogaster* microbiota can influence several attributes of host physiology both locally, by changing intestinal homeostasis, and systemically, by modulating host physiology as a whole. The gut of axenic flies displays a lower mitotic index than that of flies with a gut microbiota[16,25], suggesting that this microbiota basally stimulates intestinal turnover and helps to establish basal tissue homeostasis.

It has also been observed that changes in diet (high sugar versus high starch) that shift the composition of the gut microbiota affect mating preference, possibly by altering the levels of cuticular hydrocarbon sex pheromones[26]. In addition, *D. melanogaster* larvae that lack a gut microbiota are more susceptible than wild-type flies to infection with *Candida albicans*, highlighting a role for the microbiota in host defence[27]. This effect of the microbiota is also seen in immunodeficient flies, suggesting that the protective effect of the gut microbiota is through its impact on other aspects of host physiology (rather than the immune system) or through direct competition with pathogens[27]. For example, the gut microbiota might help to protect the host by inducing stress and tolerance mechanisms or altering the gut physiology to create a more inhospitable gut environment (through changes in pH and levels of digestive enzymes). Alternatively, the gut microbiota might limit the persistence of pathogens in the gut or the food substrate by physically occupying available niches, outcompeting for available resources, or producing antimicrobials. Indeed, studies have shown that the presence of larvae in the substrate reduces the diversity of dietary yeast species[28] and limits the growth of pathogenic fungi[29]. It remains unclear how this is achieved and whether the gut microbiota contributes to these interactions.

Box 2 | **The systemic immune response**

The direct introduction of bacteria or fungi into the body cavity of *Drosophila melanogaster* elicits multiple defence mechanisms, collectively termed the systemic immune response[1]. Early after injury, two reactions, melanization and coagulation, are induced to limit the spread of potential pathogens. The melanization reaction generates microbicidal reactive intermediates through the pro-phenol oxidase cascade, and the coagulation reaction forms a clot around the site of injury that can also trap the pathogen and block further invasion into the haemocoel. Circulating blood cells, called haemocytes, participate in clot formation through the release of secreted clotting factors, but their major contribution to immunity is to clear microorganisms through phagocytosis or to encapsulate larger foreign bodies. The last line of defence is the production of antimicrobial peptides (AMPs), mostly by the fat body (the analogue of the mammalian liver); these AMPs are released into the haemolymph. Two pathways are activated by the recognition of microorganisms; the immune deficiency (Imd) pathway is activated following the detection of products from Gram-negative bacteria (diaminopimelic acid-type peptidoglycan (DAP-PGN) by the membrane-bound peptidoglycan recognition protein LC (PGRP-LC), and the Toll pathway is activated by the detection of fungi and Gram-positive, lysine-type peptidoglycan (Lys-PGN)-containing bacteria by circulating receptors, which then initiate a proteolytic cascade leading to the activation of the cytokine Spatzle (Spz) and its recognition by the receptor Toll. For a complete review of the systemic immune response of the fly, see REF. 1.



To date, the most consistent effect of the gut microbiota on host physiology has been linked to larval growth. It has been known for some time that larvae raised in axenic conditions exhibit a delay in development compared with gnotobiotic or conventionally reared larvae[24] (for an extensive review, see REF. 23). More recently, three independent studies have further characterized this effect of the *D. melanogaster* gut microbiota on larval growth. They found that, although this effect is more pronounced in conditions of nutrient scarcity[25,30], axenic larvae reared on a fully adequate diet also show delayed development[31]. This growth effect depends on insulin signalling[25,30].

Interestingly, two studies demonstrated that different members of the gut microbiota can regulate larval growth. In one study, *Lactobacillus plantarum* was shown to modulate the target of rapamycin (Tor) pathway, a major sensor of the nutritional status of the cell, and to increase the release of insulin-like peptides, which increase the larval growth rate[30]. In the second study, *Acetobacter pomorum* was identified as the member of the gut microbiota that has the greatest impact on host responses, ranging from growth and developmental time of larvae to body size and metabolism, through the impact of the bacterium on insulin signalling[25]. It was further demonstrated that the pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) activity of *A. pomorum* is capable of activating the insulin pathway, which leads to increased larval growth and body size. Notably, the reduced intestinal stem cell proliferation observed in axenic flies (see above) was also found to be mediated by decreased insulin signalling[25], which modulates stem cell proliferation either cell autonomously[32,33] or through the modulation of enterocyte growth[34]. The phenotypes of axenic flies can be rescued either by genetically enhancing insulin signalling or by supplementing the diet with acetic acid, a metabolic product of PQQ-ADH activity. However, this supplementation is effective only in the presence of the PQQ-ADH-mutant bacterium, suggesting that additional metabolic inputs from this bacterium are required[25].

The antimicrobial response in the gut

Among the microorganisms that *D. melanogaster* encounters and ingests, some have the potential to become pathogenic. To ensure its survival, *D. melanogaster* has developed a range of defence responses, both throughout the body and specifically in the gut. In contrast to the systemic response (BOX 2), which ensures the sterility of the body cavity and haemolymph, intestinal immune responses must tolerate the presence of the gut microbiota and dietary microorganisms while responding to and eliminating potential pathogens. This presents a conundrum, as the immune system has to either be able to distinguish between pathogens and beneficial microorganisms, or at least be capable of adapting its response to the type of microorganisms present in the gut. Recent studies have shown that the gut immune response includes physical barriers that limit exposure to all microorganisms in the gut, as well as several tightly regulated inducible antimicrobial defences (FIG. 1).

Physical barriers: the peritrophic matrix, mucus and epithelial integrity. The peritrophic matrix is a mixed grid-like structure composed of chitin polymers and proteins such as peritrophins[35,36]. Studies with different insect species have suggested that the peritrophic matrix establishes a first line of defence by preventing contact between bacteria and the intestinal epithelium, and thus potentially blocking the injection of bacterial effectors by type III secretion systems, a common strategy used by enteropathogenic bacteria of mammals[37]. In addition, the peritrophic matrix acts as a sieve that restricts the passage of not only bacteria but also bacterial toxins and food particles[36] (FIG. 1). Recently, a defensive role of the peritrophic matrix in *D. melanogaster* was shown through genetic manipulation[38]. Specifically, mutation of *Crystallin*, which encodes a chitin-binding protein expressed in the gut, was found to be associated with a decrease in the thickness of the peritrophic matrix and a higher susceptibility to infection with *P. entomophila* or to its pore-forming toxin, Monalysin (encoded by *pseen3174*). Ingestion of bacteria induced a higher level of expression of antibacterial peptides in *Crystallin* mutants, indicating that the peritrophic matrix might also restrict immune activation in the gut.

Axenic
Pertaining to animals: raised under sterile conditions.

Mitotic index
The proportion of proliferating cells in a tissue.

Ectoderm
The outermost germ cell layer in the metazoan embryo.

Endoderm
The innermost germ cell layer in the metazoan embryo.

Haemolymph
The circulatory fluid of arthropods.

Type III secretion systems
Specialized syringe-like bacterial structures that inject effectors into host cells.

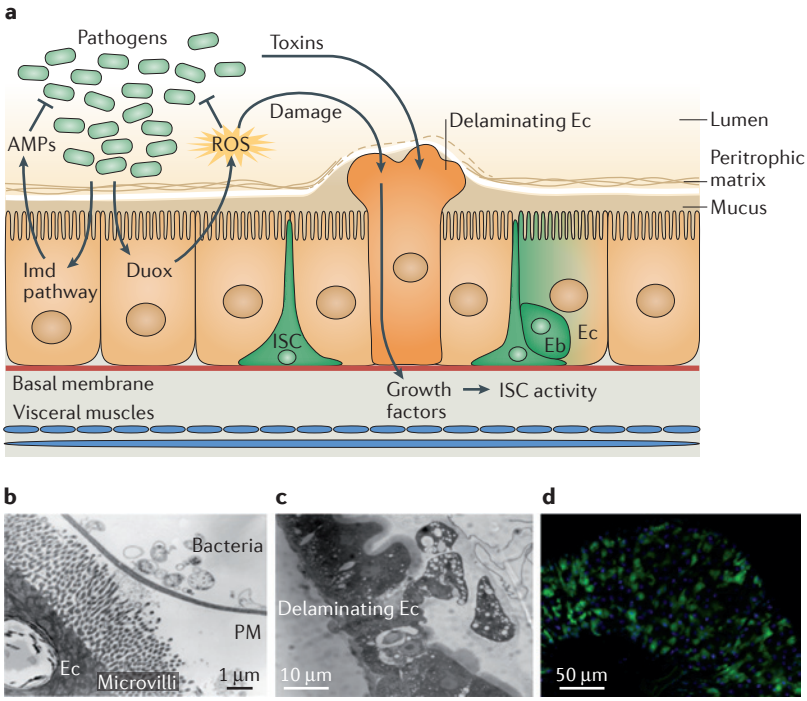


Figure 1 | **Immune and repair mechanisms contribute to the resolution of infection.** **a** | Physical barriers, such as the peritrophic matrix (a chitinous layer that separates epithelial cells from luminal contents), as well as the chemical conditions of the gut (low pH and the presence of digestive enzymes) provide the first barrier to infection. Microorganisms that are able to cross the peritrophic matrix then activate defence mechanisms, including the production of antimicrobial peptides (AMPs; through the immune deficiency (Imd) pathway) and reactive oxygen species (ROS; by Duox), which limit the growth of pathogens. In some cases, the host response, especially the production of ROS, has secondary consequences on the host, eliciting delamination of enterocytes from the epithelium. This damage is compensated for by the induction of epithelial renewal: the secretion of growth factors from enterocytes promotes the differentiation of quiescent enteroblasts (Ebs), to immediately repair damage, and the division of intestinal stem cells (ISCs), to fully replace damaged cells and return to homeostatic conditions. **b** | An electron micrograph of the fly gut, showing the peritrophic matrix (PM). **c** | A histology section showing delamination of an enterocyte. **d** | A live-cell micrograph showing proliferation of ISCs in a fly line containing an inducible *GFP* gene under the control of the upstream activation sequence (UAS) enhancer, and *Gal4* (which drives expression from the UAS enhancer) coupled with *escargot* (*esg*), a gene that is expressed specifically in progenitors (ISCs and enteroblasts). Part **b** image is reproduced, with permission, from REF. 116 © (2000) Elsevier. Part **c** image is reproduced, with permission, from REF. 54 © (2010) BioMed Central Ltd. Part **d** image is reproduced, with permission, from REF. 16 © (2009) Cold Spring Harbor Laboratory Press.

In vertebrates, the intestinal epithelium is separated from the external environment by mucus layers composed of polysaccharides and proteins (mucins) that restrict bacteria to the lumen. The peritrophic matrix has a similar role in insects, but *D. melanogaster* also has a mucus layer that lines enterocytes along the midgut[12]. Although more than 30 *D. melanogaster* genes have been annotated as encoding mucin-like proteins[39], the functional relevance of these genes or, more generally, of mucus in host defence in the *D. melanogaster* gut is not known. A transcriptomic analysis of guts from flies infected with *E. carotovora* subsp. *carotovora* 15 shows that many genes linked to peritrophic matrix metabolism and mucus production are regulated by infection, suggesting that these two barriers are remodelled during infection[40]. In addition, mutations affecting the strength of enterocyte septate junctions (for example, in the gene *big bang*) or the structure of the brush border (for example, in *Myo61F*, the gene encoding Myosin IB) cause higher susceptibility to bacterial infection, indicating that epithelial integrity contributes to resistance to bacterial pathogens[41,42].

Imd signalling and AMP production. One of the best characterized responses of *D. melanogaster* to infection is the production of antimicrobial peptides (AMPs) to eliminate bacteria[1]. Two major signalling pathways, immune deficiency (Imd) and Toll, control the expression of AMPs in the body cavity[1], but in the case of the midgut, AMP production is induced in response to Imd signalling[40,43,44] (FIG. 1). Flies deficient for Imd activation are more susceptible to infection with pathogenic bacteria such as *S. marcescens* and *P. entomophila*, suggesting that the Imd pathway has a fundamental role in intestinal defence[15,45].

In the gut, the Imd pathway is activated by two pattern recognition receptors of the peptidoglycan recognition protein (PGRP) family: the membrane-bound cell surface receptor, PGRP-LC (which mainly acts in the foregut, the anterior midgut and the hindgut), and the cytoplasmic intracellular sensor, PGRP-LE (which functions in the midgut)[46,47] (FIG. 2). These receptors sense diaminopimelic acid (DAP)-type peptidoglycan, which is found in the cell wall of all Gram-negative bacteria and certain Gram-positive bacteria (bacilli)[48–50]. Both peptidoglycan monomers (also called tracheal cytotoxin (TCT)) and peptidoglycan polymers can activate the Imd pathway[46,47,51] through binding to PGRP-LC and PGRP-LE in different gut compartments. The cell walls of *Acetobacter* and *Lactobacillus* spp., the dominant members of the *D. melanogaster* microbiota, contain DAP-type peptidoglycan, indicating that these species can be recognized by the gut immune response. In agreement with this, the expression of AMPs is significantly reduced in the gut of axenic flies, indicating that the microbiota induces the Imd pathway in the gut[16,17,52].

Several mechanisms ensure that the Imd pathway is not overactivated in response to pathogens or by the presence of the microbiota (FIG. 2). A first layer of control relies on the fact that bacterial sensing is achieved through the recognition of peptidoglycan. In Gram-negative bacteria, this bacterial cell wall component is restricted to the periplasmic space, which is hidden by the outer layer of lipopolysaccharide and is therefore less accessible for detection by the host immune system[53]. In the case of the gut microbiota, the density of bacteria in the gut is significantly lower than that observed during experimental infections with pathogens[16–18,54], so less immune-stimulating peptidoglycan would be present in the gut under normal conditions. As peptidoglycan fragments are mostly released when the cell wall is remodelled during bacterial division, it is also possible that the microbiota proliferates more slowly in the gut than pathogens and that high rates of division would reflect an acute infection. The fact that the Imd pathway is more

Brush border
The microvillus-covered surface of the epithelium.

Pattern recognition receptors
Host receptors (such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs)) that can sense pathogen-associated molecular patterns and initiate signalling cascades which lead to an innate immune response. These receptors can be membrane bound (such as TLRs) or soluble and cytoplasmic (such as NLRs).

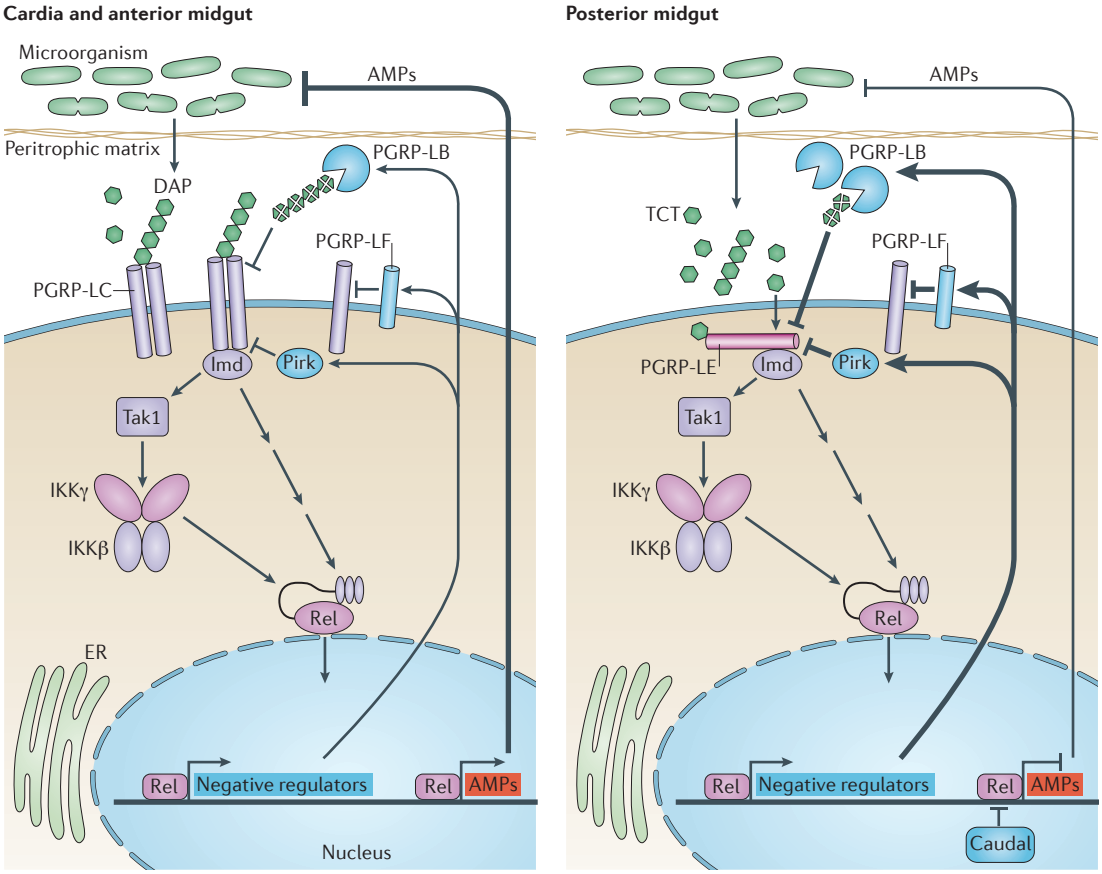


Figure 2 | **Regulation of the immune deficiency pathway in the *Drosophila melanogaster* midgut.** The immune deficiency (Imd) pathway is a key component of the response to infection in the fly gut. This pathway is basally activated by the gut microbiota and ingested microorganisms, and strongly induced by microbial infection. In addition, it has a demonstrated regional specificity depending on the tissue responding to infection (cardia and anterior midgut (left) versus posterior midgut (right)). The pathway is activated by the recognition of diaminopimelic acid-type peptidoglycan (DAP) by the cell surface-expressed peptidoglycan recognition protein LC (PGRP-LC; which has a predominant role in the anterior midgut, notably the ectodermal cardia) and the recognition of DAP monomers (also called tracheal cytotoxin (TCT)) by the intracellular receptor, PGRP-LE (which has a predominant role in the endodermal posterior midgut). Activation of the receptors triggers a signalling cascade that ultimately results in the production of antimicrobial peptides (AMPs). Multiple negative regulators of the Imd pathway have been identified that decrease the amount of immune-reactive compounds (as in the case of PGRP-LB), decrease the activity of the receptor (as in the case of PGRP-LF and Pirk (poor Imd response upon knock-in)) or modulate the transcriptional response of the transcription factor Relish (Rel) (as in the case of Caudal). Bold lines indicate the dominant pathways in each region of the gut. IKKβ, inhibitor of NF-κB kinase subunit-β (also known as Ird5); IKKγ, IKK subunit-γ (also known as Key); Tak1, transforming growth factor-β-activated kinase 1.

highly induced by peptidoglycan monomers (TCT) allows an additional level of discrimination, as TCT is found in Gram-negative bacteria, but not in DAP-type Gram-positive bacteria, such as *Lactobacillus* spp., which tend to be more consistently associated with flies and are present at higher numbers in the gut than the Gram-negative, TCT-containing *Acetobacter* spp.[21,22]. Furthermore, the predominant role of the intracellular peptidoglycan receptor, PGRP-LE, in activating the Imd pathway in the midgut — which is the region most accessible to bacterial compounds from the gut lumen — could ensure a lower level of immune reactivity in normal conditions[46,47].

A second layer of control is provided by the expression of negative regulators that downregulate the Imd pathway at virtually all levels of the cascade. These regulators are often themselves activated by the Imd pathway, establishing a negative feedback loop that adjusts the amplitude of the immune response to both the gut microbiota and pathogens. One class of negative regulators is the amidase PGRPs (PGRP-LB, PGRP-SB1, PGRP-SB2, PGRP-SC1a, PGRP-SC1b and PGRP-SC2), which cleave peptidoglycan and reduce the levels of immunostimulatory compounds in the gut lumen[51,55–57]. Among these, PGRP-LB has a predominant role in the gut to downregulate the Imd pathway[51,58]. In addition, PGRP-LB and, to a lesser extent, the PGRP-SC proteins scavenge immunostimulatory molecules in the gut lumen, thereby limiting their passage into the body cavity and preventing aberrant activation of the systemic immune response to bacteria that have not crossed the gut barrier[51,58]. Another class of negative regulators

Peptidoglycan recognition protein
(PGRPs). Pattern recognition receptors that bind peptidoglycan from the cell wall of bacteria. Recognition PGRPs bind peptidoglycan and activate the immune response. Catalytic PGRPs degrade peptidoglycan and thereby act either as negative regulators of the immune response or as immune effectors.

targets intracellular components of the Imd pathway. The gene *pirk* (*poor Imd response upon knock-in*), which is induced in response to Imd signalling, encodes a protein that relocalizes PGRP-LC from the cell membrane to an intracellular compartment, thus disrupting its interaction with Imd and limiting Imd signalling[52,59,60]. Similarly, PGRP-LF, a transmembrane receptor with two external PGRP domains but no intracellular signalling domain, binds PGRP-LC and decreases Imd signalling, probably by sequestering a fraction of the PGRP-LC isoforms[61,62]. In addition, multiple proteins (including the Cullin 1 (also known as Lin19)–SkpA complex, USP36 (also known as Scny), Cylindromatosis, POSH, Defence repressor 1 (Dnr1) and Caspar) have been shown to decrease Imd signalling by modulating different steps of the signalling pathway, for example by promoting ubiquitylation and subsequent degradation of Imd pathway components[63,64]. These multiple layers of negative regulation allow the host to match the level of immune induction to the level of immune stimulus, thus avoiding chronic activation of the Imd pathway by the gut microbiota or a damaging immune response to pathogens. Although there is no evidence for a specific dialogue between the gut microbiota and the host, the gut immune system in the fly affords an adapted and proportionate response to control both microbiota and pathogens and to avoid deleterious immune induction.

Another layer of control is provided by the compartmentalization of the antimicrobial response along the gut. Although the Imd pathway is activated all along the gut in response to bacteria, AMPs are differentially produced along the digestive tract[12,40,43]. This patterned AMP expression is mediated by regionalized transcription factors that can restrict AMP expression to specific segments of the gut. For instance, the homeobox protein Caudal is expressed in the posterior midgut and blocks the expression of AMP-encoding genes, but does not affect PGRP-LB expression in this region[17]. Thus, Imd pathway effectors, such as AMPs, and regulators, such as PGRP-LB, can have distinct patterns of regulation in different gut regions. This indicates that AMP expression along the gut is regulated both by inducible signals from the Imd pathway and by regional cues.

Production of ROS in response to microorganisms. Surprisingly, flies that are deficient for the Imd pathway, which are unable to activate an AMP response, still survive oral infection with most bacteria. This suggests that complementary immune mechanisms are active in the gut[44]. Indeed, ingestion of *E. carotovora* subsp. *carotovora* 15 induces the production of reactive oxygen species (ROS) in the gut[65] (FIGS 1,3). RNAi studies indicate that ROS in the *D. melanogaster* gut are produced by the NADPH oxidase Duox[65,66]. This enzyme has been proposed to eliminate bacteria through the direct bactericidal effect of ROS, which cause damage to DNA, RNA and proteins, and promote the oxidative degradation of lipids in cell membranes. However, the possibility that Duox has additional roles cannot be excluded. Along these lines, studies carried out in the mosquito *Anopheles gambiae* have suggested that the ROS-generating activity of Duox modulates peritrophic matrix sclerotization, which reduces gut permeability and induction of the immune response[67]. This role could be conserved in *D. melanogaster*, as Duox has recently been shown to contribute to stabilization of the cuticular structure of the wing[68]. Finally, ROS generated by Duox have been shown to regulate the activation of wound healing responses, suggesting that in addition to a bactericidal role, ROS act as signalling molecules to induce repair responses or other homeostatic pathways[69,70].

Both the expression and ROS-producing activity of Duox are upregulated by the detection of microorganisms[71,72]. Duox-mediated ROS production is directly increased following the release of intracellular calcium from the ER[71]. Biochemical studies have shown that, on the detection of bacteria in the lumen, the adaptor guanine-nucleotide-binding protein q subunit-α ($G\alpha_q$) and phospholipase Cβ (PLCβ; also known as NorpA) induce the synthesis of inositol-3-phosphate, which mobilizes intracellular calcium, ultimately leading to Duox activation[71]. On infection, the transcription of *Duox* is also increased through the activation of a Mekk1–p38a (also known as Mpk2) mitogen-activated protein kinase (MAPK) pathway, which activates the *Duox*-targeting transcription factor Atf2 (REF. 72). Of note, flies bearing mutations in p38a and p38b are susceptible to oral microbial infection, illustrating the importance of stress signalling pathways in the gut[73]. In the case of Duox, Mekk1–p38a activation depends on both the recognition of peptidoglycan by the Imd pathway and the recognition of uracil, which is proposed to be released by pathogenic bacteria in the gut and probably activates a G protein-coupled receptor (GPCR) upstream of $G\alpha_q$–PLCβ[72,74]. These results suggest that *D. melanogaster* can detect infection through the recognition of at least two different types of microbial products in the gut: peptidoglycan and uracil.

Duox is also basally activated by the gut microbiota and dietary yeast species. In the presence of the gut microbiota, the activity of the p38 MAPKs is buffered by MAPK phosphatase 3 (Mkp3), which shuts down Duox activity[72]. Mkp3 is expressed in a PLCβ- and Calcineurin B-dependent manner, indicating that the same pathway that is responsible for Duox induction during infection shuts down Duox activity in basal conditions. Thus, like the Imd response, Duox activity is induced according to the level of stimulus and is tightly regulated to reduce excessive activation by the gut microbiota. Deletion of Mkp3 leads to a shorter fly lifespan and increased apoptosis in the gut. This phenotype is fully suppressed by the knockdown of Duox, suggesting that flies lacking Mkp3 die owing to excessive ROS levels[72]. Interestingly, uracil is released by pathogenic bacteria such as *E. carotovora* subsp. *carotovora* 15 and by the microbiota-derived pathobiont *Gluconobacter morbifer* (see below) at higher quantities than by other indigenous microorganisms[74]. This suggests that the amount of uracil released by bacteria allows the fly to differentiate between benign and pathogenic microorganisms in the gut.

Sclerotization
The process of cuticle hardening in insects.

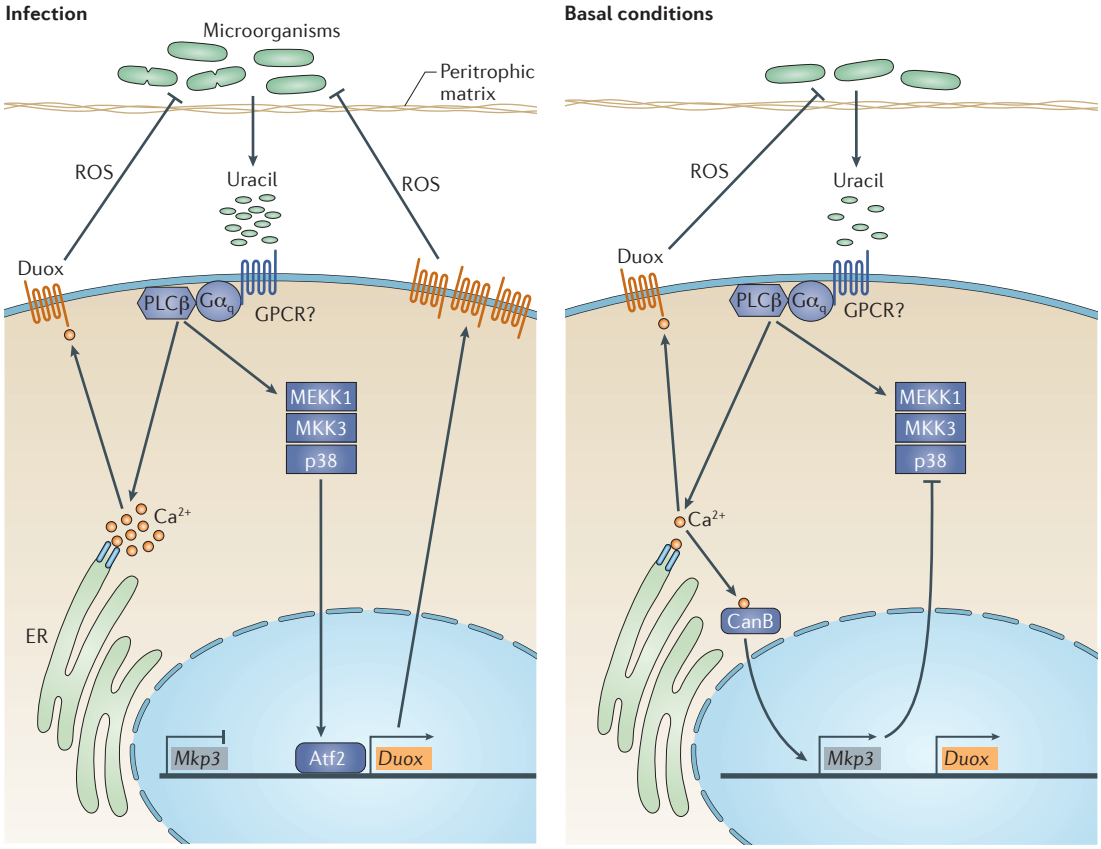


Figure 3 | **Regulation of reactive oxygen species production in the *Drosophila melanogaster* midgut.** The production of reactive oxygen species (ROS) is another core component of the immune response in the gut of the fly. As with the immune deficiency (Imd) pathway, the ROS response is basally activated by the gut microbiota and ingested microorganisms, and strongly induced by microbial infection. Microbially derived uracil triggers the adaptor guanine-nucleotide-binding protein q subunit-α ($G\alpha_q$) and phospholipase Cβ (PLCβ) to induce the synthesis of inositol-3-phosphate, which in turn mediates the release of intracellular calcium and the transcription of the oxidase-encoding gene *Duox*. *Duox* transcription can also be induced by the p38 mitogen-activated protein kinase (MAPK) pathway and the transcription factor Atf2. The Duox pathway is also tightly regulated under basal conditions, when the p38-mediated transcription of *Duox* is downregulated by MAPK phosphatase 3 (Mkp3); *Mkp3* transcription is induced under basal conditions by PLCβ and Calcineurin B (CanB). GPCR, G protein-coupled receptor; IKKβ, inhibitor of NF-κB kinase subunit-β (also known as Ird5); IKKγ, IKK subunit-γ (also known as Key); Mekk1, MAPK and ERK kinase kinase 1; MKK3, MAPK kinase 3 (also known as Lic); Tak1, transforming growth factor-β-activated kinase 1.

Other defence mechanisms. Although Duox and the Imd pathway provide two complementary lines of defence[44], they are not the only mechanisms that control microorganisms in the fly gut. In addition to AMPs regulated by the Imd pathway, a group of AMPs known as Drosomycin-like peptides (Drsl2, Drsl3 and DrlI4) is induced in the anterior part of the gut, under the control of the JAK–STAT pathway[40,75], and is thought to have antifungal but not antibacterial activity[76,77] (FIG. 4). The expression of Drosomycin-like peptides depends on the JAK–STAT ligands Upd2 and Upd3, which are released by the epithelium following damage[40,75] (see below), suggesting that the production of antifungal peptides in the gut is activated on intestinal damage rather than in response to microbial products, as occurs with antibacterial peptides.

Recently, genes involved in the Toll pathway and melanization were shown to be expressed in the foregut and hindgut, which are the ectodermal segments of the digestive tract[15] (BOX 1), but whether or how these genes contribute to host defence is not known. Likewise, despite the fact that a population of phagocytes has been found to reside in the larval cardia, there is no evidence of the involvement of blood cells in the gut response to ingested microorganisms[78].

Gut epithelium renewal on infection

Recent studies have shown that an efficient and rapid recovery from a bacterial infection is possible only when the immune response is coordinated with epithelial renewal to repair the damage caused by infection[40,79–81]. This is in line with the notion that the ability to survive an infection relies not only on resistance mechanisms that eliminate the pathogen but also on tolerance mechanisms (such as gut repair) that increase the capacity of the host to endure the infection[82].

Cardia
A valve-like structure that separates the fore- and midgut in insects.

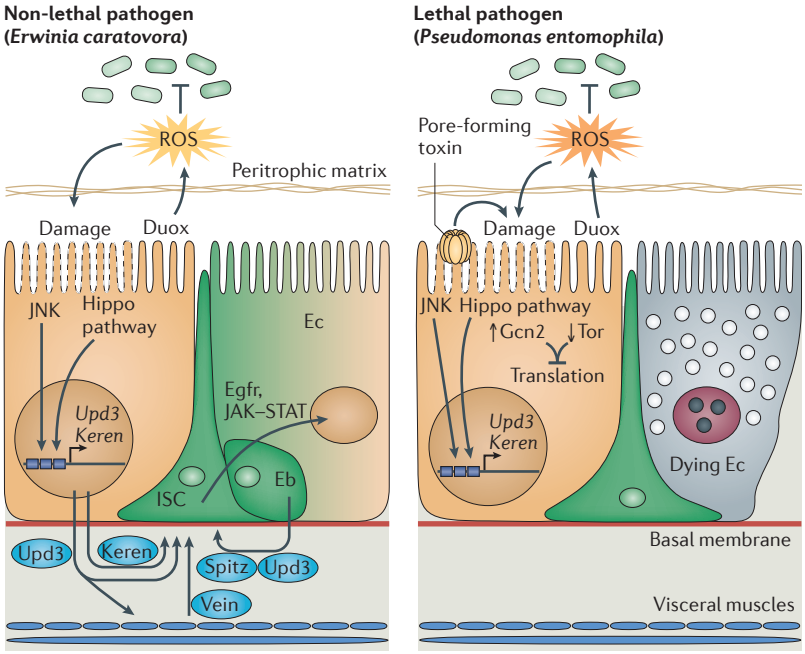


Figure 4 | **Epithelial renewal establishes a homeostatic loop required to tolerate infection.** A major factor in the likelihood of a host surviving pathogen infection is the ability of that host to repair damage. In response to ingestion of the non-lethal pathogen *Erwinia carotovora* subsp. *carotovora* 15, stressed enterocytes (Ecs) delaminate from the epithelium but are replaced by newly synthesized cells. New enterocytes originate from the quick differentiation of progenitor cells called enteroblasts (Ebs) and the induced proliferation of intestinal stem cells (ISCs). One of the initial events in this process is the expression and release of the JAK–STAT ligand Upd3 and the epidermal growth factor Keren by stressed enterocytes, which is thought to be induced through signalling from the JNK and Hippo pathways. Induction of the JAK–STAT pathway in progenitors increases their differentiation and promotes the synthesis of Upd3 and the epidermal growth factor Spitz. In addition, JAK–STAT activation in the surrounding visceral muscles promotes the synthesis of the epidermal growth factor ligand Vein. Together, these factors induce the Epidermal growth factor receptor (Egfr) pathway in ISCs to increase the ISC proliferation rate. By contrast, the high epithelial stress that is inflicted by ingested *Pseudomonas entomophila* decreases global translation in enterocytes by inducing the kinase Gcn2 and decreasing target of rapamycin (Tor) signalling. This decrease in translation results in a lack of epithelial repair and the subsequent death of the fly. This overwhelming stress response is dependent on the high levels of reactive oxygen species (ROS) that are generated in the gut in response to the pathogen, as well as the bacterial production of pore-forming toxins.

Several studies have shown that ingestion of bacterial pathogens damages the epithelium, resulting in the death of intestinal cells[16,40,54,80,81,83]. This damage is caused both by bacterial toxins that directly target the epithelium and by the immune response itself (for example, the ROS burst caused by Duox activation)[16]. Gut homeostasis is maintained because the loss of enterocytes is compensated for by the activation of intestinal stem cells, which proliferate and differentiate into new enterocytes to rebuild the gut[40,54,80] (FIG. 4). Genetic studies have shown that this response depends on induction of the Wingless, JAK–STAT and Epidermal growth factor receptor (Egfr) pathways in progenitor cells, which stimulates their proliferation and increases their differentiation into enterocytes[16,54,80,84–86]. Indeed, when the progenitor cells are mutated for the JAK–STAT or Egfr pathways, the mutant flies die because they are unable to repair the damage associated with infection[16,75,80]. JAK–STAT and Egfr signalling is activated by the release of JAK–STAT ligands (Upd2 and Upd3) or growth factors (epidermal growth factors and Wingless), respectively, by stressed enterocytes and activated progenitors, establishing a compensatory homeostatic loop[16,40,54,75,80,84,85]. Interestingly, visceral muscles that surround the epithelium contribute to this response by producing the epidermal growth factor Vein in response to JAK–STAT activation[54,84,85,87].

A key aim of research in this field is to decipher the signals that lead to the production of early-secreted factors, as such factors are the first line of regulation for epithelial renewal. Initial studies have shown that the induction of Upd3 depends on the activation of both stress kinases, such as JNK (also known as Bsk), and pathways that sense epithelial integrity, such as the Hippo pathway[80,88–91]. However, knockdown of JNK does not fully block Upd3 induction, suggesting that other regulators exist[80].

In basal conditions, the gut microbiota also promotes epithelial renewal, but to a lesser extent than infectious bacteria, suggesting that the microbiota causes less stress and damage to the gut than infectious bacteria. Interestingly, the level of intestinal stem cell activity seems to be a good indicator of gut health, and pathologies of the gut are often associated with either a blockage to or chronic activation of stem cell activity.

Ruptures in gut homeostasis

Rupture of gut homeostasis by the microbiota. Although *D. melanogaster* immune responses are tightly controlled in the absence of infection, these responses can sometimes become dysregulated and lead to situations in which the gut microbiota contributes to high immune activation and loss of gut homeostasis. Specifically, loss of gut homeostasis is observed in flies carrying mutations that alter gut compartmentalization. For instance, disruption of the transcription factor Caudal in the posterior midgut is associated with increased production of AMPs, which alters the composition of the gut microbiota and promotes the dominance of an otherwise minor member, *G. morbifer*[17]. The dysbiosis induced by *caudal* mutation causes damage to the gut and results in a loss of intestinal homeostasis, increased cell death and reduced lifespan. However, this situation does not appear to be specific to Caudal, as chronic expression of AMPs and high epithelial cell turnover are observed in several mutants in which gut organization is altered[12]. Together, these results indicate that gut structure and its organization into regions with distinct immune and metabolic properties are essential to the interactions of *D. melanogaster* with microorganisms.

Along these lines, there is evidence that the gut microbiota has a role in the initiation and progression of intestinal age-related disease. Old flies show gut dysplasia, accumulate undifferentiated intestinal cell progenitors and have a high rate of intestinal stem cell proliferation[92,93], all of which are associated with a loss in intestinal barrier integrity[94]. This aberrant epithelial renewal results from chronic activation of the stress-responsive

Dysbiosis
The condition that results from an imbalance in the microbiota.

kinase JNK and increased levels of ROS in the gut[93]. Interestingly, old flies also have higher bacterial loads in their gut, and axenic flies display attenuated intestinal dysplasia, suggesting that bacteria contribute to the disorganization of the gut in aged flies, possibly by modulating epithelial turnover[16]. Furthermore, intestinal dysplasia is associated with gut barrier dysfunction and higher expression of immune genes by the fat body in old flies[94], suggesting that the interaction between the microbiota and the intestine influences the lifespan of *D. melanogaster*.

Finally, dysregulation of the Imd pathway can also disrupt gut homeostasis. Flies lacking multiple negative regulators of the Imd pathway (for example, PGRP-LB and Pims) have a shorter lifespan owing to the continuous stimulation of the Imd pathway by the gut microbiota[58]. Collectively, these studies highlight the fact that diverse factors which lead to dysbiosis induce pathologies with similar hallmarks: higher stem cell activity, derepression of immune genes and altered lifespan. Future work should decipher the causal links between these factors and how they contribute to organismal health.

Disruption of gut homeostasis by pathogens. As mentioned above, to survive infection by a pathogen, the host must activate immune defences, as well as repair mechanisms that maintain tissue integrity. However, in some circumstances these mechanisms fail, leading to a rupture of intestinal homeostasis and, eventually, death of the host.

One mechanism by which pathogens can prevent the re-establishment of homeostasis is through excessive activation of stress-induced pathways (FIG. 4). This mechanism was recently shown to underlie the pathogenesis induced by *P. entomophila*, a newly described bacterium[95] that is lethal to multiple insect species[14]. In adult *D. melanogaster*, infection with *P. entomophila* leads to overactivation of the kinase Gcn2 and inhibition of the Tor pathway by AMP-activated protein kinase (AMPK; also known as SNF1A). These stress-responsive pathways decrease protein translation rates, thereby blocking the synthesis of immune effectors and of growth factors that regulate epithelial repair[96]. As a consequence, epithelial renewal is disrupted, and the intestinal damage caused by infection cannot be repaired, leading to fly death[96]. Gcn2 and AMPK are activated by a variety of stresses, such as starvation and oxidative stress, and translation inhibition is generally proposed to be a beneficial stress response that allows a cell to pause protein translation and instead use its resources to repair damage[97,98]. Accordingly, this response is protective following infection with non-lethal pathogens such as *E. carotovora* subsp. *carotovora* 15 and helps the host to survive infection[96]. Thus, bacterial pathogenesis can occur through the excessive activation of stress-responsive pathways that normally protect the host. Therefore, one feature that might differentiate lethal and non-lethal entomopathogens is the severity of damage that the pathogen can inflict on the host, and whether that damage reaches a threshold which compromises host responses. In the case of *P. entomophila*, damage is caused by secreted virulence factors such as the pore-forming toxin Monalysin[99] and by an excessive production of ROS by the host[96]. Interestingly, infection with *P. entomophila* is also associated with a strong activation of the Imd pathway in the fat body[45]. It remains to be determined whether this systemic response is a direct consequence of gut damage, which would allow passage of bacterial compounds into the haemolymph, or a secondary consequence of the translation blockage, which would result in decreased synthesis of negative regulators (such as Pirk and PGRP-LB) that normally restrict the immune response to the gut[51,96].

Other bacteria are lethal because they cross the gut barrier or form biofilms in the gut. For instance, the mechanisms of infection of two other pathogens, *S. marcescens* subsp. *marcescens* str. Db11 and *Pseudomonas aeruginosa*, are clearly distinct from that of *P. entomophila*, as oral infection with these bacteria involves crossing of the gut epithelium and systemic infection. When ingested, *S. marcescens* persists in the gut and induces the local production of AMPs, after which it crosses the intestinal barrier to reach the haemocoel[15]. However, it is unclear where *S. marcescens* proliferates; moreover, the associated bacteraemia, which kills the fly in 4–8 days, does not elicit a systemic immune response[15]. It has been proposed that *S. marcescens* is not detected by the fly immune system because there is a low release of peptidoglycan into the haemolymph[15]. Both the local production of AMPs in the gut and the phagocytosis of *S. marcescens* in the haemolymph contribute to the host response to this bacterium[15,100,101]. A pangenomic RNAi screen carried out in *D. melanogaster* suggests that, as in the case of *P. entomophila* and *E. carotovora*, the host response to *S. marcescens* encompasses both immune and repair mechanisms[79]. *P. aeruginosa* also induces enterocyte death on ingestion and can cross the gut barrier of *D. melanogaster*[83,102].

Finally, studies using the human pathogen *Vibrio cholerae* have shown that the ability of a pathogen to form a biofilm can be lethal to *D. melanogaster*. Contrary to its effect on mammals, ingestion of cholera toxin alone does not kill *D. melanogaster*[103]. However, when the bacterium itself is ingested, the gut environment activates the production of *V. cholera* polysaccharides that allow bacterial colonization of the hindgut, which is necessary for lethality[104]. Surprisingly, Imd-deficient flies are more resistant to infection with *V. cholerae*[103], suggesting that the immune response contributes to *V. cholerae* pathogenesis.

Concluding remarks

Much recent research has focussed on the gut-associated bacteria of *D. melanogaster* and on oral models of infection for the fruitfly, and the findings from this research have highlighted the fact that gut epithelial responses to microorganisms are complex and diverse, whether those organisms be benign, beneficial or pathogenic. To date, the only factor that distinguishes the gut microbiota from pathogens in *D. melanogaster* is the quantity of uracil released in the gut. Instead, regulatory mechanisms

Fat body
An insect organ with immune and metabolic functions similar to those of the mammalian liver and adipose tissue.

Haemocoel
The haemolymph-containing body cavity of insects.

such as those downregulating the Imd pathway ensure an appropriate level of immune reactivity in the gut to accommodate the presence of the microbiota and dietary microorganisms (which have numerous effects on host physiology) while allowing effective elimination of pathogens.

Although the role of AMPs and ROS in the gut immune response is well established, additional mechanisms are likely to be involved. For instance, most studies have dissected the response to Gram-negative bacteria, and it remains unknown whether the gut mounts specific responses to Gram-positive bacteria, which contain lysine-type peptidoglycan, or to fungi, protozoa and viruses. In addition, a range of gut physiological functions might also limit the effects of ingested microorganisms. For example, the low pH of specific gut regions, as well as digestive enzymes such as lysozyme and proteases, might have antimicrobial effects. Even gut peristalsis might assist by moving ingested pathogens into regions that are less hospitable (acidic regions or areas of higher AMPs concentration) or eliminating them by excretion. As observed in other insects, some ingested pathogens can cause a malaise that is associated with the cessation of feeding[105]. The contribution of this response to disease pathology and the underlying molecular mechanisms that govern this response are not completely understood. However, the malaise response suggests that there is a sort of communication between the gut and the nervous system that remains to be characterized. The recent demonstration that flies have a dedicated nervous circuit to detect the volatile compound geosmin, which is produced by mould fungi and actinobacteria, indicates the ability of flies to identify potential pathogens[106]. Thus, pathogen detection and avoidance might have an important role in the survival of *D. melanogaster* to pathogens, as has been shown in *C. elegans*[107].

Another important lesson from studies on the *D. melanogaster* gut response to bacterial infection is the importance of the stress response and repair mechanisms that maintain tissue integrity[40,73,80,96]. How stress and repair programmes are integrated with the immune response remains largely unknown and promises to be a rich area of study. As illustrated above, studies using pathogenic bacteria and the oral route of infection have begun to identify common virulence mechanisms that can disrupt gut homeostasis. Some of these mechanisms are likely to be used by other entomopathogenic bacteria, and their investigation in *D. melanogaster* could help decipher how bacteria evolve as entomopathogens. For instance, the implication of Monalysin in *P. entomophila* virulence, together with the well-characterized action of crystal toxins from the most widely used organic pesticide, *Bacillus thuringiensis*[108], highlights the key role of pore-forming toxins in bacterium-mediated pathogenesis of insects. These toxins could represent an adaptation to the insect gut structure, as their action does not require direct contact between the bacterium and host cells and because they can reach intestinal cells despite being limited by the peritrophic matrix.

Many aspects of the *D. melanogaster* gut response are likely to be relevant to other host organisms, including mammals. For instance, mucosal defences in mice also rely on the production of antimicrobial peptides and ROS[109]. A recent report indicates that *Salmonella enterica* subsp. *enterica* serovar Typhimurium infection in mice increases the level of stem cell proliferation, suggesting that, similarly to in the *D. melanogaster* gut, both immune and repair mechanisms contribute to host defence in the mammalian gut[110,111]. These studies suggest that *D. melanogaster* and its powerful genetics can highlight some important facets of intestinal homeostasis that are relevant to vertebrates and could provide insights into the molecular determinants that link chronic infection, stem cell activity and cancers of epithelial origin. Thus, studies on the *D. melanogaster* gut response to microorganisms should not only illuminate many facets of gut–microorganism interactions that are conserved in other insects, including insect pests and vectors affecting human health, but also increase our understanding of the general mechanisms used by animals to maintain intestinal homeostasis in a microbial world.

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The authors declare no competing financial interests.