**Abstract** Gastrointestinal infection can provoke substantial disturbance at both a local as well as at a systemic level and may evolve into a chronic disease state. Our growing knowledge of gut-pathogen interactions has been based to a large extent on the use of genetically tractable model hosts such as the fruit fly *Drosophila melanogaster*. In this review we will summarise the growing literature and critically address the advantages and disadvantages of using this model to extrapolate results from studying pathogen virulence and intestinal responses to humans.

**Highlights**

- **Comparisons of *Drosophila* and human gut**
- **Natural and non-natural pathogens and their interactions with the *Drosophila* gut**
- **Advantages and limitations in using *Drosophila* intestinal immunity as a model for human pathogens**

# Exploring interactions between pathogens and the *Drosophila* gut.

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**Abstract** Gastrointestinal infection can provoke substantial disturbance at both a local as well as at a systemic level and may evolve into a chronic disease state. Our growing knowledge of gut-pathogen interactions has been based to a large extent on the use of genetically tractable model hosts such as the fruit fly *Drosophila melanogaster*. In this review we will summarise the growing literature and critically address the advantages and disadvantages of using this model to extrapolate results from studying pathogen virulence and intestinal responses to humans.

## Introduction

The gastrointestinal tract is of paramount importance in all organisms, as what we ingest affects many processes in our bodies. Thus, it is essential to maintain intestinal homeostasis. In humans, deregulation of intestinal homeostasis from gastrointestinal infections (GI) results in acute discomfort but may also lead to chronic inflammation, intestinal bowel disease (IBD) and in rare cases to colorectal cancer. In this complex context, we need to model *in vivo* host-pathogen interactions that while representative of the human response simplifies the subject. Mice models are the obvious go-to organisms as they reflect human pathology but come with ethical concerns and are costly.

The fruit fly, *Drosophila melanogaster,* is a well-established and advanced alternative model for studies of host-pathogen interactions (reviewed in Bier and Guichard 2012). It has been used to develop statistically robust, high-throughput infection assays that are easy to perform. There are extensive similarities in innate immune signalling between flies and mammals; indeed the innate immune role of *Toll* was first described in *D. melanogaster* in response to *Aspergillus fumigatus* infection (Leimaitre *et al*, 1996). *Drosophila* feed off rotting fruit infested with millions of bacteria and thus, must effectively be able to discriminate between friend and foe to avoid gastrointestinal infections. In this context, *Drosophila* has been recently developed as a model for human GI infection and pathology (reviewed in Apidianakis and Rahme 2011; Panayidou *et al*, 2014).

## *Drosophila* vs. human gut

**Structure** There are striking similarities between the physiological and anatomical structure of the human and *Drosophila* gut (see Figure 1). The *Drosophila* gut is divided into three domains (foregut, midgut and hindgut). The foregut is of ectodermal origin and includes the crop. This is where food accumulates and digestion begins, much alike the human stomach. The cardia, found at the foregut/midgut border, is similar to the human sphincter, which controls the entry of food into the midgut. The midgut is of endothelial origin and similarly to the small intestine, is where the majority of nutrient absorption takes place. The hindgut originates from the ectoderm and is where water is mostly reabsorbed, a process resembling the large intestine in humans. The malpighian tubules is the functional equivalent of the human kidney found at the mid-hindgut (Hakim *et al.* 2010; Buchon *et al.* 2013). The basement membrane, an extracellular collagenous matrix, is located on the basal side of the epithelial cells, beneath which are circular trachea-oxygenated muscles required for peristatic movement. Similarly in humans, oxygenated musculature is also located in the outer layers of the intestine (Apidianakis and Rahme 2011).

**Immunity** Both insects and humans also have localised intestinal immune responses to maintain intestinal homeostasis. This involves sensing and responding to both beneficial and harmful pathogens and controlling the growth of gut microbiota as well as tolerating its presence. To this end localised intestinal immunity plays a crucial role in maintaining a constant intestinal environment.

The human intestine is lined with a single layer of intestinal epithelial cells known as enterocytes. Specialised enterocytes play an important role in optimising digestion while also protecting the host from infection. Goblet cells produce mucin glycoproteins that assemble into mucus that resides in a layer above the brush border (Johansson *et al.* 2008; Hooper *et al.* 2012). In the colon, there are two distinct layers of mucus. The outer layer contains bacteria, but the inner layer is impermeable to bacterial penetration (Johansson *et al,* 2008). The small intestine however, only has a single layer of mucus and as a result relies on the production of antimicrobial proteins (AMPs) for protection from harmful bacteria (Johansson *et* al, 2011). Antimicrobial activity in the mucus is partly constitutive and partly inducible (Putsep *et al,* 2000; Bradl *et al,* 2007; Hooper *et al,* 2010). It contains high concentrations of AMPs (including defensins and cathelicidins) as well as C-type lectins (the latter produced mostly by specialised epithelial cells called Paneth cells) (Maloy & Powrie 2011; Hooper *et al.* 2012).

Likewise in *Drosophila*, a chitin layer known as the peritrophic matrix is analogous to the mucus layer in humans. It is composed of chitin polymers and glycoproteins like peritrophins that protect the intestinal epithelial cells by forming a barrier from bacteria (Lehane 1997; Kuraishi *et al.* 2013). The NK-κB-driven Immune Deficiency (IMD) pathway is the main immune responder to infection in the *Drosophila* gut. Responding to peptidoglycan from Gram-negative bacteria and Gram-positive bacilli, activation of this pathway results in the production of various AMPs. Secreted peptidoglycan recognition receptor LB (PGRP-LB) has amidase activity and consequently is able to cleave peptidoglycan from the above bacteria preventing prolonged activation of IMD (Zaidman-Rémy *et al.* 2006). In this manner, IMD is further regulated by a number of intra- and

extracellular negative regulators at every step from cell surface to NK-κB-mediated transcription (reviewed in Buchon *et al.* 2009; Kounatidis & Ligoxygakis 2012). For instance, Pirk is a protein that antagonises IMD receptors PGRP-LC and PGRP-LE signalling inside gut cells to dampen IMD activity triggered by infection or the flora (Kleino *et al,* 2008; Lhocine *et al,* 2008; Aggarwal *et al*, 2008) while a number of deubiquitinase enzymes suppress activating ubiquitinilation at the cytoplasmic stage of signal transduction (Tsichritzis *et al*, 2007; Thevenon *et al* 2009; Guntermann *et al*, 2009; Fernando *et al*, 2014). In addition, Caudal and Nubbin directly suppresses AMP gene transcriptional activation in midgut nuclei (Ryu *et al,* 2008; Dantoft *et al*, 2013). In essence, all these control mechanisms prevent harmful effects of a constitutively active immune response.

At the epithelial surface in mammalian intestinal cells, secreted immunoglobulin A (IgA) limits bacteria. Intestine-specific IgA is produced by B-cells facilitated by dendritic cells (DCs), which are located along the intestine, such as in Peyers's patches. DC's sample bacteria and activate B-cells to produce IgA (Macpherson *et al,* 2000; Hooper *et al,* 2012). Bacteria that do manage to cross the intestinal epithelial cell barrier are phagocytosed by macrophages present in the lamina propria. However, a strong pro-inflammatory response is not mediated by intestinal macrophages as expected. This is possibly an evolutionary adaptation of the cells to the presence of high numbers of bacteria in the gut, a state known as "inflammation anergy" (Smythies *et al,* 2005; Hooper et al. 2012).

It is still an open question if there are any immune cells associated with the *Drosophila* intestine. However, there is evidence that hemocytes are present

116 in the *Drosophila* proventriculus (PV). These cells produce many of the markers
117 hemocyte populations express, have the characteristic hemocyte morphology
118 and are able phagocytose bacteria and dying cells. It was also determined that
119 phosphoinositide 3-kinase (PI3K) regulates the size of the gut hemocytes, their
120 localisation in the larvae and their phagocytic activity (Zaidman-Rémy *et al*.
121 2012). Presence of intestinal macrophages would be beneficial to the host if
122 pathogens cross the intestinal epithelial cell barrier. Moreover, fruit flies that are
123 deficient for the IMD pathway are still able to survive following ingestion of
124 bacteria suggesting the presence of other immune mechanisms in the gut. One
125 such mechanism includes the production of reactive oxygen species (ROS). ROS
126 plays an important role in the defence against ingested bacteria in both human
127 and insects. Following oral infection in *Drosophila*, the NADPH oxidase, Duox
128 produces ROS at the membrane surface. ROS eliminates bacteria by damaging
129 DNA, RNA and proteins. It also causes the oxidative degradation of lipids in cell
130 membranes (Ha *et al,* 2005). In mammals, ROS production is also found at many
131 mucosal sites including at the apical membrane of enterocytes along the entire
132 digestive tract (Bae *et al,* 2010; El Hassani *et al,* 2005; Kuraishi *et al.* 2013). A
133 summary of the above can be seen in Figure 2 for *Drosophila* and Figure 3 for
134 human gut cells.
135
136 **Microbiota** Both the human and the *Drosophila* gut are associated with a
137 number of microorganisms. The human gut is known to host >500 species
138 whereas *Drosophila* harbours approximately 30 different species (Broderick &
139 Lemaitre 2012). In both, the flora makes essential contributions to the health
140 and physiology of the host. In humans, the intestinal microbiota is involved in

epithetlia; cell maturation, angiogenesis and digestion (Hooper *et al,* 2001; Stappenbeck *et al,* 2002). In reference to the latter, commensal bacteria assist in the enzymatically break down of polysaccharides. For instance, the *Bacteriodes thetaiotaomicron* genome has a number of genes coding for carbohydrate degrading enzymes (Xu & Gordon, 2003). Further to this, commensal bacteria adapted to colonise the intestine outcompete other potential invading pathogens for nutrients. Additionally, some commensals are able to activate an immune response against pathogens. For example epithelial Toll-like receptors (TLRs) are activated by symbiotic bacteria in the gut in response to certain pathogens such as *Salmonella enterica* (Vaishnava *et al.* 2008; Hooper *et al.* 2012). In *Drosophila,* it has been shown that in addition to peptidoglycan recognition, discrimination between non-pathogenic bacteria and pathogenic bacteria is mediated by the detection of uracil produced by the latter, leading to the production of ROS (reviewed in Lee & Hase 2014). Moreover, absence of gut microbiota from the fly results in increased susceptibility to infection by the fungus *Candida albicans* (Glittenberg *et al.* 2011).

## Response to infection

### Natural pathogens

***Pseudomomas entomophila*** *P. entomophila* is a Gram-negative pathogen known to induce both local and systemic immune responses in *Drosophila*. Lielh and colleagues studied the oral infections in *Drosophila* with this pathogen and found that *P. entomophila* secretes a zinc metalloprotease, AprA, important for virulence (Liehl *et al,* 2006). Metalloproteases are a common mode of attack of pathogens by degrading AMPs and structural components (see review by Miyoshi & Shinoda, 2000). Expression of AprA is dependent on both PrtR and the

GacS/GacA two-component system. PrtR and GacS/GacA mutants lack AprA and PrtR only retains 30% of wild-type supernatant activity. Oral infection with this pathogen results in cessation in both larvae and adults. This is also displayed when *aprA* mutants are fed to larvae and adults. Wild-type *P. entomophila* is able to persist longer than *aprA* mutants. The IMD pathway plays an important role in eliminating *P. entomophila*. Activation of the IMD pathway 12h prior to *P. entomophila* infection protects adults whereas 6h prior to infection only contributed moderately. This suggests that *P. entomophila* is sensitive to the IMD pathway for a short period of time. *aprA* mutants were able to survive to wild-type levels in *Relish* mutants further suggesting the protective role of AprA protein against systemic immunity. Further to this, the AMP *diptericin* has previously not shown to play a major role against Gram-negative bacteria. However, this study demonstrates an essential role of *diptericin* in the defence against *P. entomophila*. It is possible that the activity of this AMP is enhanced in the gut by other factors such as lysozymes influencing the gut microenvironment (Devine, 2003). However, although systemic immunity is important against *P. entomophila*, it is the local immune response in the gut that is responsible for its clearance. *Rel* mutant flies with *Rel* only expressed in the gut survive better than *Rel* mutants, when infected orally with *P. entomophila.* Thus, *Drosophila* is able to induce a local immune response in the gut that is sufficient to clear the pathogen as with other pathogens (see below).

A recent study investigated the variation in immune, stress and regenerative processes, which they defined as gut immunocompetence, in response to oral infection with *P. entomophila*. To determine whether differences in survival was specific to *P. entomophila*, eight lines of DGRP strain were

191 infected orally with a clinical isolate of *P. aeruginosa* (*PA14*). These eight lines
192 include four resistant strains and four susceptible strains to *P. entomophila*.
193 Resistant strains to *P. entomophila* were also resistant to *PA14* and likewise with
194 the susceptible strains indicating that irrespective of the pathogen there is a
195 common molecular mechanism that mediates recovery after oral infection.
196 Further to this, clearance of the *P. entomorphila* after infection was markedly
197 faster in resistant lines than susceptible lines (Bou Seliman *et al*, 2015). Infection
198 with *P. entomophila* results in global inhibition of protein synthesis, which as a
199 result affects gut immunity and epithelial renewal. This is dependent on the
200 virulence factors AprA (mentioned above) and Monalysin, a pore-forming toxin
201 involved in damage to intestinal cells, both of which are under control of the
202 GacS-GacA two-component system (Chakrabarti *et al*, 2012). It was found that
203 resistant lines of DGRP still had functioning protein synthesis machinery unlike
204 susceptible strains and a greater number of mitotic stem cell following infection
205 with *P. entomophila*. This indicates that the genetic background is of great
206 importance in determining the response, as *P. entomophila* infection does not
207 always lead to lethality. Moreover, differences in susceptibility to *P. entomophila*
208 infection are not due to genetic relatedness. When all possible hybrid
209 combinations were generated (by crossing the eight lines with each other), it
210 revealed that susceptibility to infection was additive and depended on the
211 strains being crossed. Genome wide association study revealed many immune
212 modulators including *Gyc76C*, a membrane receptor capable of activating the
213 IMD pathway independently of its receptor PGRP-LC (Bou Sleiman *et al*, 2015)
214 (Overend *et al*, 2012).
215

***Erwinia carotovora*** *E. carotovora* is a Gram-negative bacterium that causes rotting on fruit and vegetables. *Drosophila* is a natural vector for *E. carotovora atroseptica* and *E. carotovora carotovora* (*Ecc*), which causes potato black leg disease (Agrios, 2004; Perombelon and Kelman, 1980). Both feeding and injection with the potent strain *Ecc-15*, activates a global immune response able to attenuate the effects of the pathogen (Basset *et al,* 2000). Highest levels of *diptericin* were seen in the early pupal stage three hours after larval infection, whereas none was detected in adults. *Ecc-2046* is another isolate of *Ecc*, which induces a weak immune response. After natural infection with both *Ecc-15* and *Ecc-2046*, bacterial levels decrease with time with *Ecc-15* persisting longer. The IMD pathway plays an important role in reducing bacterial levels as *Ecc-15* persists in higher numbers in mutants defective in IMD components compared with wild type flies. *Ecc-15* localisation is predominantly found in the digestive tract, particularly the foregut and anterior midgut 4-7h after natural infection. However, bacteria were also detected in the respiratory tract but not in the hemolymph (Basset *et al,* 2000). Persistence of *Ecc-15* is dependent on a single gene, the *Erwinia virulence factor* (*Evf*), which codes for a S-palmitoylated protein that binds lipid vesicles (Quevillon-Cheruel *et al,* 2009). Evf promotes colonisation of the gut and subsequent triggering of systemic immunity (Acosta Muniz *et al,* 2007). The mode of action of Evf is currently unknown although it has been shown that it is not a toxin nor does it provide protection from AMPs (Acosta Muniz *et al,* 2007).

From the side of the host, hemocytes play a vital role in the defence against *E. carotovora. Dom* and *L(3)hem* are cell proliferation mutants that significantly decrease hemocyte numbers. Oral infection of these two mutants

241 results in much lower *diptericin* levels compared to controls. However, this is not
242 seen after direct injection suggesting that in the absence of physical injury,
243 hemocytes are incapable of activating a systemic response. A possible theory is
244 that the presence of the bacteria in the hemocoel is not sufficient to induce a
245 systemic response. Rather, a second signal, either via the hemocytes or physical
246 injury, is required to induce this response. It is possible that hemocytes come in
247 contact with bacteria and mediate activation of fat body immunity through a
248 secreted factor. This communication between blood cells and fat body has been
249 indicated in the activation of the Toll pathway (Shia *et al*, 2009).
250
251 **Non-natural pathogens**
252 ***Serratia marcescens*** The route of infection (oral feeding or direct injection into
253 the body cavity) is important in determining the type of immune response that is
254 elicited in the host. This is illustrated when infecting *Drosophila* with the human
255 pathogen *Serratia marcescens*. *S. marcescens* is a Gram-negative bacterium that is
256 associated with hospital-acquired infections (HAI). A study by Nehme and
257 colleagues showed that *S. marcescens* was able to resist the systemic immune
258 response when injected into *Drosophila* (Nehme *et al,* 2007). Interestingly, when
259 Toll and IMD mutants were injected, their mortality rate was the same as wild
260 type suggesting that the systemic host defence was not making any difference in
261 the fight against such infections. The ability of the wild-type strain of *S.*
262 *marcescens* to tolerate antimicrobial peptides (AMPs) was due to the presence of
263 LPS-O-antigen, as bacterial mutants lacking this were sensitive to the systemic
264 host defence. However, when ingested, this bacterium was sensitive to the IMD-
265 mediated local immune response in the gut and wild-type flies died at a rate

dependent on the bacterial load in the food. Nevertheless, *S. marcescens* was not contained in the gut, but rather escaped into the hemolymph where it was still unable to activate systemic immunity. This was because phagocytosis was effective in eliminating escaped bacteria. If phagocytosis was blocked however, a systemic immune response ensued. Therefore, the local immune response in the gut together with phagocytosis is sufficient to control and eliminate *S. marcescens* infection (Nehme *et al,* 2007).

***Pseudomonas aeruginosa*** Some pathogens are able to cause chronic infection by producing highly organised structures known as biofilms. Biofilms are multicellular microbial communities encased in an extracellular matrix composed of extracellular DNA, multiple exopolysaccharides (EPS), proteins and lipids. As they can exist on a variety of surface types and are difficult to eradicate, they are a proven problem for human health and are associated with HAI where they are found especially on medical equipment (Bordi & de Bentzmann, 2011). *Pseudomonas aeruginosa* (*P. aeurginosa*) is able to produce biofilms and as a result to persist in the host in both HAI as well as Cystic Fibrosis patients. In *Drosophila,* following oral infection of 1-3 day adults, *P. aeurginosa* colonies were found predominantly in the crop (Mulcahy *et al*, 2011). Here, they were organised into hexagonal biofilm bacterial colonies floating within the crop, which had lost musculature and lacked an organised structure. These biofilms had the classic features found in humans with an extracellular matrix made of DNA and EPS. Moreover, the ability to form biofilms determined the fate of the pathogen within the host as well as the fate of host survival. Biofilm-forming *P. aeruginosa* was able to persist for 48h upon infection. A defective biofilm forming strain of *P. aeruginosa* (producing less EPS; *pelB::lux)* was detected in

the hemolymph much more quickly than wild-type (PAO1) and was less persistent (Mulcahy *et al*, 2011). However, *pelB::lux* was more virulent compared to PAO1 in both oral infection and injection into the hemocoel. In contrast, the hyperbiofilm forming PAZH13 was less virulent than *pelB::lux* and PAO1. Interestingly, infection with *pelB::lux* resulted in a decreased AMP gene induction compared with the other two stains. Therefore, inability to produce biofilms resulted in increased dissemination and thus, reduced host survival. Finally, the virulence of *pelB::lux* was attenuated when co-infected with PAO1 or PAZ13 (Mulcahy *et al*, 2011).

***Candida albicans*** (*C. albicans*) is a commensal fungus that can be isolated from the mucosal layers of healthy individuals (Odds *et al*, 2007). However, as an opportunistic pathogen, it can cause not only mucosal but also severe, life threatening invasive infections. Risk factors for the development of invasive *C. albicans* infections in human patients include mucosal colonization, antibiotic therapy, gastrointestinal surgery, cancer chemotherapy, and neutropenia (Andes *et al,* 2003; Odds *et al*, 2007). To cause life threatening, invasive disease, *C. albicans* has to cross the mucosal barrier, invade the tissue and evade host defenses. One of the fungal attributes important for tissue invasion is the ability to form hyphae. *C. albicans* is a dimorphic pathogen that is able to freely alternate between filamentous hyphae and yeast states depending on environmental cues (Noble *et al,* 2010; Enoch *et al,* 2006). Glittenberg and colleagues developed a model to study gastrointestinal (GI) infection in *Drosophila* larvae (Glittenberg *et al,* 2011). Oral infection of wild-type larvae with *C. albicans* resulted in few larvae surviving to adulthood. This number was further reduced when fed to larvae deficient for NF-κB-driven pathways.

316 Moreover, oral infection of *C. albicans* resulted in significant cell death of both
317 enterocytes and stem cells. This was coupled to a progressive reduction of the
318 epithelial lining (Glittenberg *et al,* 2011). Commensal bacteria in the gut were
319 shown to play a positive role in the defence against *C. albicans*. Fewer larvae
320 raised in germ-free conditions survived to adulthood compared to
321 conventionally reared larvae. Nonetheless, in both germ-free and conventionally
322 reared wild type larvae, a systemic fat body activation of *drosomycin* (*drs*) was
323 induced regardless of *C. albicans* being restricted to the gut (Glittenberg *et al,*
324 2011). It is speculated that in later developmental stages, the gut empties during
325 pupation and this could be harmful to the host, thus activating a systemic
326 response. In the larval stage however, systemic induction of *drs* was dependent
327 on hemocytes as their absence resulted in a significant reduction of *drs*. The
328 cause of activation of systemic immunity from the pathogen side could be down
329 to proteinases, of which *C. albicans* secretes one class, secreted aspartyl
330 proteinases (SAPs), with 10 members. The absence of *SAP4* and *SAP6* reduced
331 systemic induction of *drs* to three-fold compared to 13-fold induction of *dros* in
332 wild-type larvae. In addition to proteinases, from the host side nitric oxide (NO)
333 has been shown to regulate systemic AMP expression following GI infection
334 (Foley & O'Farrell, 2003; Nappi *et al,* 2000). Knockdown of NO Synthase (NOS) in
335 the gut specifically had a significant effect on *drs* expression after infection with
336 *C. albicans*, whereas absence of NOS from the fat body or hemocytes did not
337 affect *drs* expression. Thus, SAPs and NO are two aspects of GI infection of
338 *Drosophila* with *C. albicans* that controls induction of systemic immunity. The
339 systemic response is also dependent on the Toll pathway as absence of its ligand

*spatzle* (*spz*) or the upstream protease *Persephone* (*psh*) reduced *drs* expression significantly.

| | Pathogen | Mode of attack | Mode of host defence | Reference |
|---|---|---|---|---|
| **Natural** | *Pseudomonas entomophila* | Metalloprotease AprA; Pore forming toxin, Monalysin | Local immune response | Liehl *et al,* 2006; Chakrabarti *et al*, 2012 |
| | *Erwinia carotovora* | *Erwinia* virulence factor (*Evf*) | Systemic immune response prompted by hemocytes | Quevillon-Cheruel *et al,* 2009; Acosta Muniz *et al,* 2007; Basset *et al,* 2000 |
| **Non-natural** | *Serratia marcescens* | LPS-O-antigen | Local immune response, phagocytosis | Nehme *et al,* 2007 |
| | *Pseudomonas aeruginosa* | Biofilms | Systemic immunity | Mulcahy *et al,* 2010 |
| | *Candida albicans* | Secreted aspartyl proteinases (SAP) | Local immune response prompted by NO and SAPs | Glittenberg *et al,* 2011 |

**Can *Drosophila* be a meaningful GI host model for human pathogens?**

The use of *Drosophila* as a model organism to study host-pathogen interactions has allowed us to successfully understand the mode of pathogenesis of various pathogens. A summary with all the information relevant to both natural as well as non-natural pathogens used in GI infection in *Drosophila* is presented in Table 1. However, the host responses can vary from one model organism to another. From the fruit fly perspective, there is still an on-going debate of whether

353 *Drosophila* can be meaningfully used as a host model to study non-natural
354 human pathogens in the GI tract.

355 **Limitations** The *Drosophila* gut microbiota is considerably simpler than
356 that of humans. The difference in the composition as well as the scale of the flora
357 is also another key distinction. Obligate anaerobes compose a large portion of
358 the mammalian gut microbiota that is absent from *Drosophila* flora (Cox &
359 Gilmore, 2007; Rastall, 2004; Qin *et al,* 2010). Thus, the intestinal
360 microenvironment is not directly comparable. Specifically for fungal pathogens,
361 infection and maintenance of *Drosophila* at a lower temperature can be an
362 important limitation. For example, a gene that regulates the expression of the
363 nucleolar protein CgrA has a pivotal role in *Aspergillus* thermo-tolerance and a
364 *ΔCgrA* mutant displayed attenuated virulence in mice (Bhabhra *et al*, 2004).
365 However, the *ΔCgrA* mutant was fully virulent in Toll-mutant flies infected and
366 maintained at 25°C, showing that certain aspects of fungal virulence in mammals
367 might not be appropriately modelled in flies.

368 **Advantages** Nevertheless, using *Drosophila* one can *individually* screen
369 large mutant libraries of pathogens for virulence factors. To illustrate this
370 statement let us postulate the following functional genomics screen. Noble and
371 colleagues constructed a *C. albicans* homozygous deletion library with approx.
372 3,000 deletion strains affecting 674 genes (roughly 11% of *C. albicans* genome
373 see Noble *et al,* 2004). If we screened the mutant strains for virulence
374 individually, in the 3-day mouse model (da Silva Dandas *et al*, 2010) scoring host
375 survival as a virulence measure we would need for a large difference in outcome
376 score (e.g. 9) and a standard deviation of say 3.5, approx. 4-5 mice per strain, to
377 detect differences with a resolution power of 95%. This would mean the use of

15,000 animals for a first pass experiment. The strategy of grouping pathogen strains and infecting mice with the assumption that the most virulent will prevail is clearly also not satisfactory. Additional mice would be required for confirmation and reconstitution experiments. Evidently, this is untenable both ethically as well as economically. In addition, the issue is also transferable. As functional genomics resources become available for more pathogens, there is a need to develop alternatives to small mammal models that will allow us to screen these mutant libraries in their entirety. We will thus develop holistic views of the pathogen attributes required to cause disease. In addition, the sophisticated genetic tools available in *Drosophila* make it an ideal model to explore host-pathogen interactions. As soon as a less-virulent pathogen strain is found in the screened library we can identify (through genetic screening of *Drosophila* mutants) a host genetic deficiency that will make the pathogen strain virulent again. We will thus identify the host system, which interacts with this particular virulence factor.

Therefore, when modelling intestinal host-pathogen interactions in *Drosophila* the above have to be considered. Nonetheless, the fruit fly allows us to carry on first pass experiments of large pathogen libraries and identify points of interaction with the host. It subsequently makes it much easier to take these forward in a mammalian model.

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**Figure 1**

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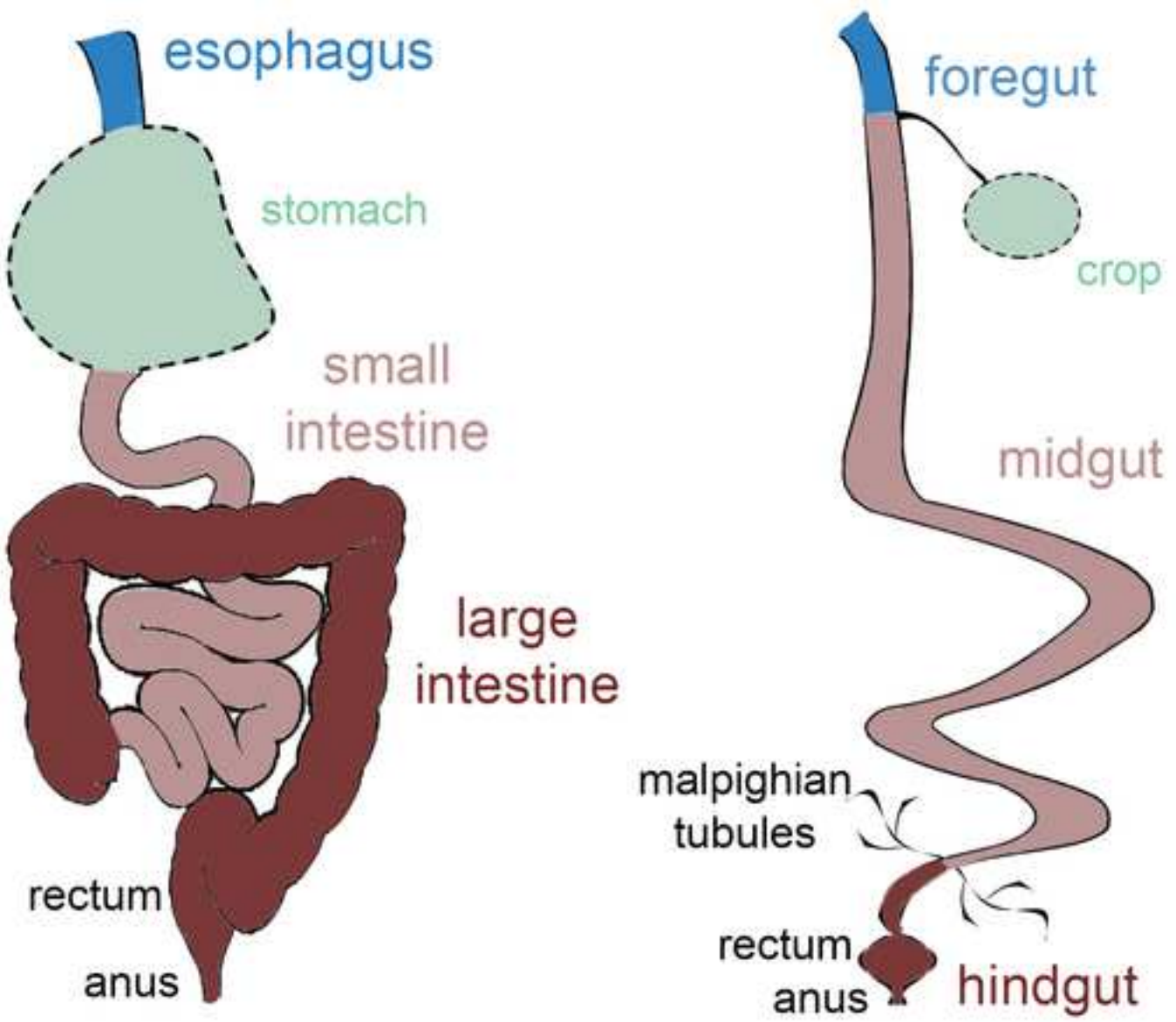

**Figure 2**

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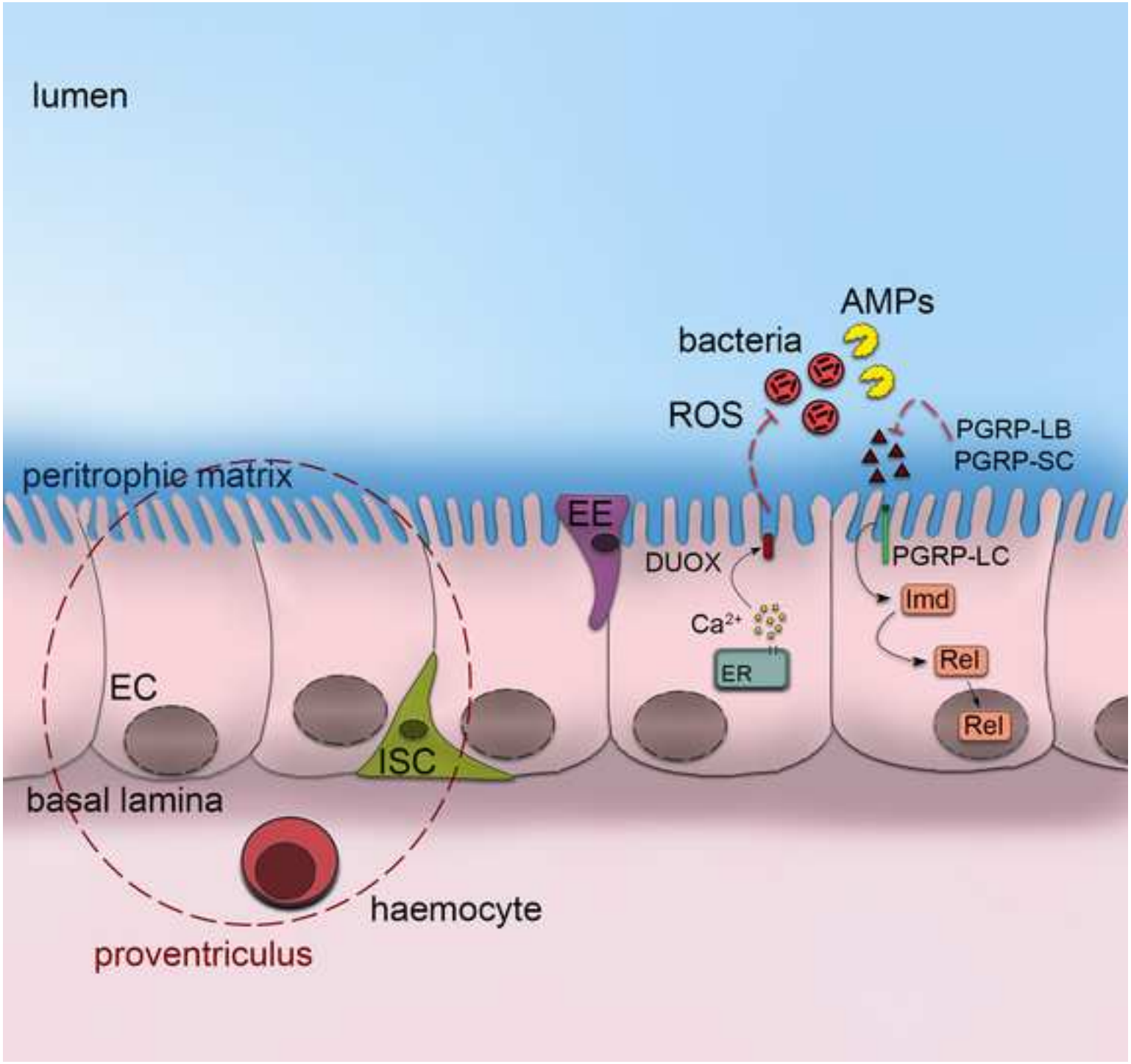

**Figure 3**

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