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Drosophila melanogaster as a model for human intestinal infection and pathology

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Recent findings concerning *Drosophila melanogaster* intestinal pathology suggest that this model is well suited for the study of intestinal stem cell physiology during aging, stress and infection. Despite the physiological divergence between vertebrates and insects, the modeling of human intestinal diseases is possible in *Drosophila* because of the high degree of conservation between *Drosophila* and mammals with respect to the signaling pathways that control intestinal development, regeneration and disease. Furthermore, the genetic amenability of *Drosophila* makes it an advantageous model species. The well-studied intestinal stem cell lineage, as well as the tools available for its manipulation in vivo, provide a promising framework that can be used to elucidate many aspects of human intestinal pathology. In this Perspective, we discuss recent advances in the study of *Drosophila* intestinal infection and pathology, and briefly review the parallels and differences between human and *Drosophila* intestinal regeneration and disease.

Introduction

Vertebrate and invertebrate animals alike are in continuous contact with a diverse array of resident microorganisms termed microbiota (Qin et al., 2010). Host-microbe interactions occur primarily along skin and mucosal surfaces, and one of the largest interfaces in humans is the intestinal mucosa (Qin et al., 2010). Mucosal immune responses to microbes must distinguish commensal bacteria and mutualists from pathogenic bacteria. In genetically predisposed individuals, some microbe components can disrupt intestinal homeostasis and contribute to the pathogenesis of intestinal disorders (Apidianakis et al., 2009; Melmed and Targan, 2010). Thus, the intestine is increasingly being recognized for its importance in human health and infections, as well as in inflammatory and neoplastic diseases (Markowitz and Bertagnolli, 2009).

To maintain intestinal homeostasis, specific commensals and mutualists must be maintained, microbial pathogens must be eliminated, and inflammatory responses and tissue regeneration must be tightly controlled (Garrett et al., 2010; Ryu et al., 2008). Acute or chronic dysregulation of these processes leads to several diseases, including gastrointestinal infections, metabolic imbalances, inflammatory bowel diseases (IBDs) and colorectal cancer (Garrett et al., 2010). According the American Cancer Society, colorectal cancer is the second leading cause of cancer-related deaths in the United States. IBDs, which include ulcerative colitis and Crohn's disease, are conditions in which the colon is inflamed over a long period of time, increasing the risk of developing colorectal cancer (Garrett et al., 2010). Data from the National Institutes of Health indicate that 1 out of 500 people in the USA suffers from IBD. Thus, the substantial healthcare burden of intestinal cancer and IBD underscores the need for developing robust research approaches in the area of intestinal homeostasis.

The advancement of biomedical research has relied heavily on the use of model organisms for the study of human diseases (Helfand and Rogina, 2003; Hergovich et al., 2006). An ideal model system should reflect human biology (specificity) while reducing the complexity of the human disease of interest, such that it can be studied and manipulated effectively (feasibility). In the case of intestinal pathology, mouse models of cancer and IBD offer good specificity in terms of mimicking human pathology. However, research involving mammals is expensive, complex and fraught with ethical concerns, so alternative models for studying intestinal infection and immunopathology are needed.

Given that *Drosophila melanogaster* feeds on rotten microbe-carrying fruit and other decaying food and can survive for more than 2 months in tropical environments, it is not surprising that it has evolved an elaborate defense system against microbes. Insects defend themselves against a diverse array of ingested and invading microbes via a combination of physical (mucus, peritrophic membrane) (Dessens et al., 2001; Sampson and Gooday, 1998; Wang and Granados, 1997) and molecular [antimicrobial peptides (AMPs), lysozymes, dual oxidase] (Apidianakis et al., 2005; De Gregorio et al., 2001; Ha et al., 2005) mechanisms. In addition, fly responses to wound infections are remarkably fast and efficient, and are elicited both locally and systemically (Apidianakis et al., 2007; Buchon et al., 2009b; Lemaitre and Hoffmann, 2007). The fact that these responses are conserved, combined with the amenability of *Drosophila* to genetic manipulation, make this organism ideal for modeling certain aspects of human intestinal pathology (Box 1). Nevertheless, *Drosophila* model systems of intestinal pathology should be chosen carefully, considering

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Box 1. Advantages of using Drosophila models of intestinal infection and pathology

- Considerable conservation between *Drosophila* and mammalian intestinal pathophysiology and regeneration, and signaling pathways that control them
- Various human intestinal pathogens and alterations in intestinal microbiota can cause intestinal pathology in flies
- Relative to mammalian genomes, the *Drosophila* genome contains fewer paralogs of intestinal regeneration-controlling genes and a simpler cellular structure
- Amenability to reverse genetics owing to availability of whole-genome RNAi libraries, molecular markers and other genetic tools
- In contrast to mammalian-cell-based drug screenings, *Drosophila* assays provide the complex cellular composition of a real intestine and opportunity to assess toxicity in a whole organism; relatively low cost

similarities and disparities between fly and mammalian pathophysiology. Not all manifestations of human intestinal disease can be studied in *Drosophila* (or even in mice); rather, this organism is best suited for studying specific and well-conserved aspects. For example, epithelial regeneration as a response to enterocyte-produced growth and innate immune factors can be assessed in flies upon intestinal injury and/or infection, instead of assessing the combinatorial effects of cellular immunity and regeneration that are usually studied in mice. This Perspective highlights recent advances in our understanding of insect intestinal infection and pathology, and briefly reviews the parallels and disparities between human and *Drosophila* intestinal infection, homeostasis and disease. The validity of the fly gut as a model in which to study human intestinal pathophysiology is discussed.

Similarities and disparities between mammalian and *Drosophila* intestinal physiology

Food passage

In mammals, swallowed food passes through the esophagus to the stomach, where food accumulates and digestion proceeds; it then moves to the small intestine for nutrient absorption and later to the large intestine for further nutrient, water and electrolyte absorption. Finally, it reaches the rectum and anus for excretion (Fig. 1) (Thompson and Malagelada, 1981). Similarly, in *Drosophila*, ingested food passes through the foregut to be stored temporarily in the crop; it then moves to the anterior midgut where nutrient absorption commences (Edgecomb et al., 1994). Passing through the middle midgut, which contains the iron and copper cells (Fe/Cu cells), a region of low pH, it transits through the posterior midgut for further absorption and through the hindgut and rectum, where water and electrolytes are exchanged, and finally reaches the anus for excretion. In addition, renal-like structures known as malpighian tubules connect to the midgut-hindgut junction. They absorb solutes, water and waste from the surrounding hemolymph, and release them in the gut in the form of solid nitrogenous compounds (Fig. 1) (Demerec, 1994).

Intestinal anatomy

Fly and human intestines also share similar tissue, anatomy and physiological function (Pitsouli et al., 2009; Rubin, 2007). Developmentally, both the mammalian gut and *Drosophila* midgut are of endothelial origin (Kedinger et al., 1987; Tepass and Hartenstein, 1994). They comprise an epithelial monolayer of columnar or cuboidal cells called enterocytes (Figs 2 and 3). To maximize its surface area, mammalian intestinal epithelium has a series of sequential depressions (the crypts of Lieberkühn) along the small and large intestine, and has protruding villi along the internal surface of the small intestine (Crosnier et al., 2006) (Fig. 2A). Extensive folding does not occur in the *Drosophila* intestine (Fig. 2C), possibly owing to its small size. Nevertheless, cytoplasmic extensions, called microvilli, of the apical side of enterocytes and intestinal stem cells (Shanbhag and Tripathi, 2009) do increase the cellular surface area facing the gut lumen in both flies and mammals. Microvilli extend parallel to each other towards the lumen, creating the brush border (Baumann, 2001; Gartner, 1970; Shanbhag and Tripathi, 2009) (Fig. 2A,C). Above the brush border, a layer of mucus protects the host from intestinal microbes; in the *Drosophila* gut, there is also a chitin layer – the peritrophic matrix – that helps to sequester microbes from coming in contact with the midgut and hindgut (Gooday, 1999; Vodovar et al., 2005).

In flies and mammals, the epithelial monolayer is aligned on its basal side on an extracellular collagenous matrix called the basement membrane (Sengupta and MacDonald, 2007) (Fig. 2A-D). Underneath the *Drosophila* basement membrane, there is a checkerboard of innervated and trachea-oxygenated longitudinal and circular muscles that drive peristaltic movements (Jiang and Edgar, 2009) (Fig. 2C,D). A similar arrangement of intestinal external musculature is found in the outer layers of mammalian intestine (Fig. 2A,B), and this musculature is also innervated and oxygenated by a plexus of vasculature (Kvietys and Granger, 1986; Rhee et al., 2009). In mammals, the space between the outer musculature and the basement membrane is filled with three additional layers (Fig. 2A,B):

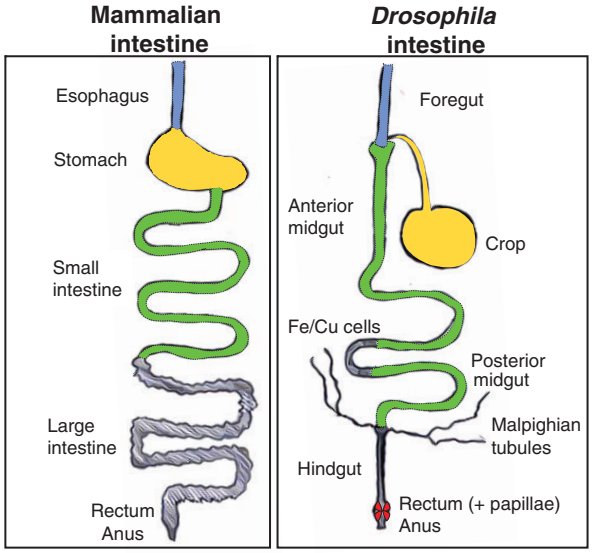

Fig. 1. Similarities between the mammalian and *Drosophila* intestines. There is similarity between the esophagus and the foregut (blue), the stomach and the crop (yellow), the small intestine and the midgut (green), and the large-intestine–rectum–anus and the hindgut-rectum-anus (gray) in mammals and *Drosophila*. Differences include the presence of the fly kidney-like malpighian tubules, which empty into the gut, the rectal papillae (red), which are used for water absorption (not present in mammals), and the fly Fe/Cu cells, which are found in a region of low pH that seems to be functionally distinct from the low-pH stomach of mammals.

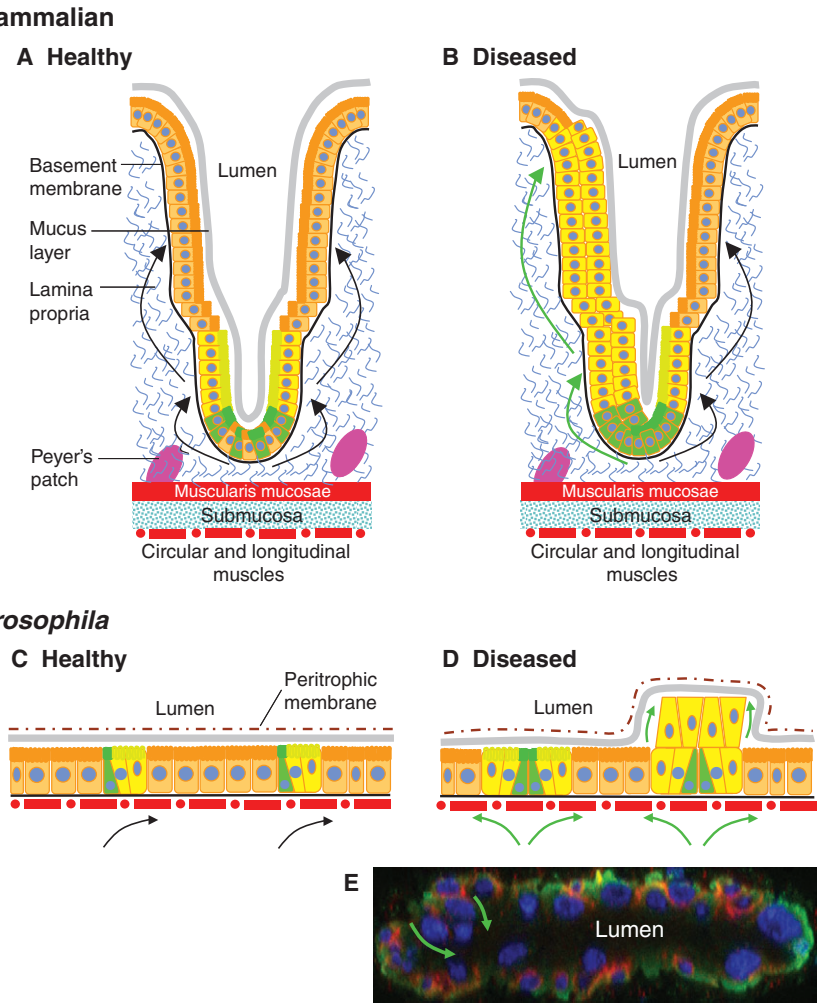

Fig. 2. Mammalian intestinal crypts and villi, and the *Drosophila* midgut, in healthy and diseased animals. (A,B) Healthy and diseased mammalian intestinal crypts are shown made up of green, yellow and orange cells, and villi are shown made up of orange cells. (A) ISCs (green) and Paneth cells (orange) are located in the bottom of the crypts in healthy tissue. TA cells produced by ISCs move upwards while gradually maturing (black arrows). (B) High levels of ISC division owing to inflammation or a genetic predisposition create an overabundance of TA cells that move upwards (green arrows), causing crypt enlargement. (C,D) Healthy and diseased *Drosophila* midgut. Paneth cells are not present in flies, but ISCs are located basally in fly midgut epithelium. As in the mammalian gut, transient and/or differentiating cells (yellow) are consistently adjacent to ISCs and mature enterocytes (orange). ISCs of the *Drosophila* midgut are found basally in the epithelium and the transient cells that they produce (enteroblasts) move upwards (black arrows) before growing to their mature size in healthy tissue. (D) Similarly to human intestinal disease, in the *Drosophila* midgut, infection, aging or genetic predisposition can lead to overproduction of differentiating cells that move basolaterally (infection, aging) or even upwards (e.g. in Wg and Ras1 overexpression) (green arrows), in which case multilayering and tissue dysplasia ensues. (E) *x-z* section of an infected and genetically predisposed *Drosophila* gut for a *Ras1* oncogene. ISCs or progenitor cells (green) spread out in the epithelium and additional layers of cells (nuclei in blue) protrude into the lumen (green arrows). Also, the lateral junction protein Armadillo (red) is subcellularly mislocalized, expanding from the lateral to the apical side of ISCs or progenitor cells.

(1) the submucosa, a dense layer of connective tissue containing nerves and lymphatic and blood vessels; (2) an additional muscle layer called the muscularis mucosae; and (3) the lamina propria, which underlies the intestinal epithelium and contains connective tissue, lymph nodes (known as Peyer's patches), immune cells (leukocytes, and dendritic and mast cells), vessels and myofibroblasts (Komuro and Hashimoto, 1990). It has been implied that there are immune cells in the *Drosophila* midgut (Lemaitre and Hoffmann, 2007), but these cells have yet to be identified.

Intestinal cell differentiation and regeneration

Both fly and mammalian guts have adult intestinal stem cells (ISCs) (Barker et al., 2008; Barker et al., 2007; Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006; Sangiorgi and Capecchi, 2008; Takashima et al., 2008), and there are also similarities in cell composition and the signaling pathways (described in the next section) that control intestinal regeneration (Figs 2 and 3). Nutrient-absorbing enterocytes and hormone-producing enteroendocrine cells are found in both flies and mammals (Crosnier et al., 2006; Ohlstein and Spradling, 2006). Other types of secretory cells that are present in the mammalian gut – namely the mucus-producing goblet cells and the AMP-producing Paneth cells – have not been found in *Drosophila* midgut, although both mucus and AMPs are produced by the *Drosophila* intestine (Buchon et al., 2009b; Vodovar et al., 2005). Mammalian enterocytes and secretory cells differentiate from ISCs, which are located at the bottom of the crypts (Fig. 2A) and specifically express the stem cell markers Lgr5 and/or Bmi1. Both of these cell types can give rise to all lineages of intestinal cells, including the transient amplifying (TA) cells that lie immediately above ISCs and move progressively upwards while maturing to eventually reach complete maturation close to the opening of the crypts. There is a continuous turnover of TA cells, which are either shed or become apoptotic upon maturation (Crosnier et al., 2006; Scoville et al., 2008).

The *Drosophila* posterior midgut has a very simple lineage, with only one type of mature absorptive cell, the enterocyte (as in mammals), and one main type of secretory cell, the enteroendocrine cell. The *Drosophila* midgut lacks TA cells under normal homeostatic conditions, but such cells might exist under pathogenic conditions. Although asymmetric ISC divisions in the midgut produce transient cells called enteroblasts, these cells do not undergo further cell division and remain close to the ISC before maturation. Despite the absence of crypts and villi, *Drosophila* midgut ISCs are situated basally and are broadly

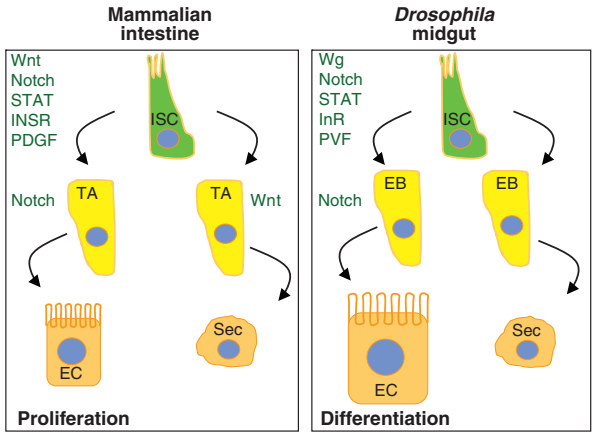

Fig. 3. ISC lineages of mammalian intestine and *Drosophila* midgut, and the common pathways that control them. Apart from the Wnt/Wg and STAT pathways that are necessary for ISC division, additional similarities between the mammalian and *Drosophila* midgut ISCs have been noted. For example, midgut ISCs divide when the InR pathway is activated (i.e. following drug exposure) and in the presence of PVF growth factors (during aging); the analogous INSR and PDGF pathways, respectively, control mouse ISC homeostasis. In addition, ISCs are controlled by the Notch pathway; however, Notch activates ISC proliferation in mammals but causes them to differentiate in *Drosophila* midgut. Nevertheless, Notch signaling is similarly required for the specification of enterocyte (EC) versus secretory (Sec) fate during the commitment of TA cells and enteroblasts (EBs). Wnt/Wg, which is needed for determination of secretory cell fate differentiation in mammals, is apparently not crucial for similarly specifying cell fate in the fly midgut.

dispersed in the intestinal epithelium. Meanwhile, mammalian ISCs are typically intermingled with Paneth cells and comprise a composite population of Lgr5-positive (Lgr5+) and Bmi1+ cells (Fig. 2A,B).

The cellular composition and regeneration observed in the *Drosophila* hindgut are strikingly similar to those of mammals. As in mammals, the ISCs of the hindgut are specified anteriorly and move posteriorly, as TA cells do, before their further differentiation in the posterior hindgut (Takashima et al., 2008). Notwithstanding a recent study describing the effects of hindgut cell death and concomitant regeneration (Fox and Spradling, 2009), the *Drosophila* hindgut has not been examined as extensively as the midgut, which has been studied comprehensively under pathogenic conditions and thus far has served as the prototype *Drosophila* tissue for the study of intestinal pathology (Amcheslavsky et al., 2009; Apidianakis et al., 2009; Buchon et al., 2009a; Buchon et al., 2009b; Cronin et al., 2009; Jiang et al., 2009).

Conserved signaling pathways involved in mammalian and *Drosophila* intestinal regeneration and disease

Wnt/Wg pathway

As mentioned above, significant homology exists between the mammalian and fly signaling pathways that control ISC proliferation. *Drosophila* wingless (Wg) is required for maintenance of ISCs and is secreted from muscle cells located adjacent to the basal epithelium (Lin et al., 2008) (Fig. 2C, Fig. 3). Its mammalian counterpart, Wnt, is similarly highly active at the bottom of the crypts and is believed to be largely responsible for maintaining ISCs and TA cells in an undifferentiated state (Crosnier et al., 2006). As they move away from the Wnt source, newly produced cells differentiate gradually into either enterocytes or secretory cells (Fig. 2A, Fig. 3).

Strikingly, overactivation of the Wg pathway increases ISC mitosis in a manner similar to that seen in mammals. Furthermore, conditional mutations in the *Apc* gene, which is a negative regulator of the Wg pathway in flies, lead to ISC mitosis and epithelial multilayering, similarly to mammalian *APC* mutations, which promote gastrointestinal cancer (Cordero et al., 2009; Lee et al., 2009) (Fig. 3B,D). Importantly, Wnt signaling cooperates with Jun NH_2-terminal kinase (JNK) and insulin receptor (INSR) pathways in regulating ISC proliferation in mammals. Indeed, the INSR pathway can activate Wnt signaling in Bmi1+ ISCs (Scoville et al., 2008), whereas JNK activation in the intestinal epithelium activates Wnt signaling and leads to tumor formation (Nateri et al., 2005).

Notch

Notch signaling also plays a very important role in *Drosophila* ISC homeostasis (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006; Ohlstein and Spradling, 2007). It is used in a context-dependent manner to induce either differentiation or proliferation during fly development (Ligoxygakis et al., 1999). In mammals, the Notch pathway is similarly involved: it induces proliferation of ISCs and drives differentiation of hair follicle stem cells (Crosnier et al., 2006). In contrast to its action in mammalian ISCs, Notch activation promotes the differentiation of fly ISCs (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006) (Fig. 3). Nevertheless, Notch is required in a similar manner in flies and mammals to specify the enterocyte versus secretory fate of intestinal cells (Crosnier et al., 2006; Ohlstein and Spradling, 2007) (Fig. 3).

PDGF/PVF, JNK and INSR/InR pathways

Within the last 2 years, several groups have identified aging, oxidative radicals and drugs as potent inducers of ISC and/or progenitor proliferation. Indeed, flies that are more than 20 days old (Biteau et al., 2008; Choi et al., 2008a; Choi et al., 2008b), or flies feeding on hydrogen peroxide or paraquat (Biteau et al., 2008; Chatterjee and Ip, 2009; Choi et al., 2008b), or on the cell-damaging agents dextran sulfate sodium and bleomycin (Amcheslavsky et al., 2009), exhibit increased ISC and/or progenitor cell divisions. However, there are differences in the effects induced by the different stressors: cell-damaging agents are thought to induce a regenerative response against the damage, whereas aging has been linked to disruptions of intestinal homeostasis caused by perturbations of the differentiation program in intestinal cell progenitors (Biteau et al., 2008). These studies implicated the platelet-derived growth factor (PDGF; PVF in *Drosophila*) and JNK pathways in promoting ISC or progenitor cell divisions (Fig. 3), causing changes in the intestinal cell population during aging or oxidative stress (Biteau et al., 2008; Choi et al., 2008b). Induction of the INSR (InR in *Drosophila*) pathway plays an important role in ISC responses following ingestion of dextran sulfate sodium or bleomycin (Amcheslavsky et al., 2009). Mammalian PDGF ligand is activated in the crypt base, signaling the nearby mesenchyme to shape the villi (Crosnier et al., 2006; Geokas et al., 1985), a role that

is apparently distinct from that of the PVF ligand in *Drosophila* because flies lack intestinal mesenchyme.

K-Ras/Ras1 pathway

Gain-of-function (GOF) mutations in the *K-Ras/Ras1* gene behave as oncogenes in both flies and mammals (Pagliarini and Xu, 2003; Uhlirova et al., 2005). Recently, our group showed the *Ras1* GOF mutations in combination with *Pseudomonas aeruginosa* infection predispose flies to intestinal dysplasia, a pre-malignant condition characterized by profound ISC or progenitor proliferation, impaired differentiation, epithelial multilayering and alterations in apicobasal polarity (Apidianakis et al., 2009). Moreover, a role for Ras1 in *Drosophila* ISC maintenance is possible because this pathway also controls the proliferation of adult midgut ISCs or progenitors (Jiang and Edgar, 2009).

JAK-STAT pathway

An array of recent reports has provided further support for the value of the *Drosophila* midgut as a model of intestinal disease: these studies established that, upon feeding of flies with the *Drosophila* pathogens *Erwinia carotovora, Serratia marcescens* or *Pseudomonas entomophila*, the cytokines Upd1, Upd2 and Upd3 are secreted by the damaged cells and activate the JAK-STAT pathway in ISCs (Buchon et al., 2009b; Cronin et al., 2009; Jiang et al., 2009). This leads to a robust increase in ISC divisions and concomitant intestinal hyperplasia. Interestingly, the homologous pathway in mammals, which involves the cytokine interleukin-6 (IL-6) and STAT3, plays a role in the induction of intestinal inflammation and cancer (Atreya and Neurath, 2008; Iliopoulos et al., 2009). In addition to providing clues on the roles of cytokines and JAK-STAT signaling in intestinal regeneration and homeostasis, these studies support the hypothesis that inflammation and concomitant cytokine production in mammals might be similar to the epithelium-induced cytokine activities that occur in *Drosophila*. This similarity might hold true not only for Upd and IL-6 cytokines, but also for the production of reactive oxygen species (ROS), which are produced by mammalian epithelia and immune cells, as well as by *Drosophila* intestinal cells, upon infection (Buchon et al., 2009b; Ha et al., 2009a; Ha et al., 2009b; Ha et al., 2005).

NFκB pathways

Both flies and humans show localized intestinal as well as systemic responses to microbes in order to control a gut infection or fight it systemically if the epithelial barrier is breached (Girardin and Philpott, 2004; Lemaitre and Hoffmann, 2007; Lemaitre et al., 1996; Nehme et al., 2007; Rosetto et al., 1995). Activation of nuclear factor-κB (NFκB) pathways is a common response to intestinal injury or infection (Buchon et al., 2009b). In flies, Imd signaling (one of the two NFκB signaling pathways) is induced during intestinal infection, eliciting various AMPs (Buchon et al., 2009b). Although AMP production is considered to be beneficial for the host (because it contributes to fighting infection), substantial evidence suggests that induction of the Imd pathway must be controlled in the gut to prevent detrimental effects of prolonged activation of the immune response. Indeed, the intestinally produced peptidoglycan-processing enzyme PGRP-LB is produced during intestinal infection to inhibit activation of the Imd pathway (Zaidman-Remy et al., 2006). In addition, activation of this pathway seems to have detrimental effects for the host in a *Drosophila* model of *Vibrio cholerae* intestinal infection (Berkey et al., 2009).

The role of intestinal microbiota in mammalian and *Drosophila* gut homeostasis

Adult fly and human intestines come into contact with a plethora of microbes, including the innocuous microbes of their commensal and mutualist flora, as well as pathogens (Deneve et al., 2009; Preidis and Versalovic, 2009; Ryu et al., 2008; Vodovar et al., 2005). Both types of hosts have evolved to recognize, defend against and control the proliferation of these microbes (Ryu et al., 2008; Slack et al., 2009). Intestinal homeostasis is disrupted when the indigenous microbiota is altered, and numerous microbiome studies have started assessing human and *Drosophila* microbiota in health and disease (Corby-Harris et al., 2007; Cox and Gilmore, 2007; Frank and Pace, 2008; Macfarlane et al., 2009; Ren et al., 2007; Ryu et al., 2008; Qin et al., 2010). For example, IBD has been linked to a loss of prevalent microbiota bacterial species in humans (Frank and Pace, 2008; Macfarlane et al., 2009). Likewise, a reduction of AMP expression in the fly midgut causes a shift in intestinal microbiota and concomitant intestinal pathology (Ryu et al., 2008). Furthermore, among human intestinal and opportunistic pathogens, *V. cholerae, S. marcescens*, *P. aeruginosa* and *Enterococcus faecalis* can disrupt *Drosophila* intestinal homeostasis (Apidianakis et al., 2009; Apidianakis and Rahme, 2009; Berkey et al., 2009; Cox and Gilmore, 2007; Nehme et al., 2007). Nevertheless, the relationships between intestinal microbiota and intestinal pathology remain unclear in both flies and mammals.

Midgut toolbox: advantages of using *Drosophila* models of intestinal pathology and infection

Intestinal cell markers and spatiotemporally controlled transgene expression

Many markers of intestinal cell identity and genetic construct combinations have been developed for studies of intestinal homeostasis. These are invaluable tools for future research (Table 1). A crucial aspect of intestinal physiology is the study of cell composition upon a given treatment or genetic mutation. Our laboratory has stained flies of the *myo1A-GAL4 UAS-GFP/esg-lacZ* genotype with anti-β-galactosidase and anti-Prospero antibody to simultaneously show the ratio of enterocytes (GFP positive), ISCs and/or progenitors (*lacZ* positive) and enteroendocrine cells (Prospero positive) during infection (Apidianakis et al., 2009). These markers are convenient because they collectively mark all posterior midgut cells, without detectable overlap between them, in the presence or absence of infection (Fig. 4). An accurate percentage of each cell type can thus be calculated (by obtaining the ratio, multiplied by 100, of cells stained positively for a given cell type over the total number of stained cells) as a function of a particular experimental condition. In addition, using the *myo1A-GAL4* or *esg-GAL4* mutation in combination with a *tub-GAL80ts* transgene, one can activate the expression of any transgene under the *UAS* promoter in enterocytes or ISC and/or progenitor cells, respectively, at any stage during the fly's life by simply transferring flies containing these transgenes from a permissive (GAL80 inhibition on; 18°C) to a restrictive (GAL80 inhibition off; 25-29°C) temperature (Jiang et al., 2009; Micchelli and Perrimon, 2006).

Table 1. Toolbox for *Drosophila* midgut cellular biology analysis

Tool	Application	Comments	Reference
Anti-phospo-histone H3	M-phase cell marker	Marks cell division	Apidianakis et al., 2009
Anti-Delta	ISC marker	Might also mark progenitors	Apidianakis et al., 2009
Anti-Prospero	Enteroendocrine marker	All enteroendocrine cells	Apidianakis et al., 2009
Anti-Allatostatin	Enteroendocrine cell marker	A subset of enteroendocrine cells	Ohlstein and Spradling, 2006
Anti-Tachykinin	Enteroendocrine cell marker	A subset of enteroendocrine cells	Ohlstein and Spradling, 2006
Anti-Pdm1	Enterocyte marker	–	Lee et al., 2009
DAPI dye	Nuclear marker	Size of nuclei indicates maturation	Apidianakis et al., 2009
EdU dye	S-phase cell marker	Marks division, endoreplication	Cronin et al., 2009
vkg-GFP	Basement membrane marker	–	Amcheslavsky et al., 2009
esg-lacZ	ISC and/or progenitor marker	β-galactosidase staining	Apidianakis et al., 2009
Gbe-Su(H)-lacZ	Enteroblast marker	β-galactosidase staining	Micchelli and Perrimon, 2006
esg-GAL4/UAS-GFP	ISC and/or progenitor driver/marker	Live imaging possible	Micchelli and Perrimon, 2006
myo1A-GAL4/UAS-GFP	Enterocyte driver/marker	Live imaging possible	Jiang et al., 2009
cad-GAL4/UAS-GFP	Posterior midgut driver/marker	Live imaging possible	Apidianakis et al., 2009
UAS-FLP;tubFRT-FRT-lacZ/tubGAL80ts esg-GAL4 UAS-GFP	Differentiation and proliferation rate indicator	Clone cells express *lacZ*; for assessment of number and maturation of cells per flip-out clone	Apidianakis et al., 2009
hs-FLP;FRT-lacZ/tub-FRT	Indicator of ISC maintenance and proliferation rate	Clone cells express *lacZ*; for assessment of number of cells per mitotic clone	Ohlstein and Spradling, 2006
hs-FLP;act-GAL4/UAS-GFP;FRT/FRT-tub-GAL80	Indicator of ISC maintenance and proliferation rate	Clone cells express GFP; for assessment of number of cells per mitotic clone	Lee et al., 2009
Delta-GAL4	ISC driver	*Delta* gene insertion expressed specifically in ISCs	Zeng et al., 2010
Gbe-Su(H)-GAL4	Enteroblast driver	*Gbe-Su(H)* transgene expressed specifically in enteroblasts	Zeng et al., 2010

Flip-out clones in progenitor cell differentiation and proliferation assessments

Flip-out clones of cells emanating from ISCs can be created in the midgut epithelium by transferring flies of the *UAS-FLP;act-FRT-FRT-lacZ/tubGAL80ts esg-GAL4 UAS-GFP* genotype from 18°C to 25-29°C to initiate clone production (Apidianakis et al., 2009; Jiang et al., 2009). GFP in these flies marks the ISC and/or progenitor cell population, whereas β-galactosidase (*lacZ*) staining positively marks all cells of each clone. In this respect, *lacZ*-positive, GFP-negative cells are cells of the clone that have proceeded to differentiation. We have used this genetic combination to assess the percentage of total clones containing cells that lack the ISC and/or progenitor cell marker GFP, in the presence or absence of infection (Apidianakis et al., 2009). Differentiation of GFP-negative cells towards the enteroendocrine and enterocyte fate can be further assessed by anti-Prospero antibody staining (enteroendocrine fate) and by observing large nuclei and anti-Pdm1 antibody staining (enterocyte fate) (Lee et al., 2009). Clone size – that is, the number of cells per clone – is a measure of ISC and/or progenitor cell proliferation and can be conveniently measured in fly intestines of the same genotype (*UAS-FLP;actFRT-FRT-lacZ/tubGAL80ts esg-GAL4 UAS-GFP*). Using this method, we observed that flies produce larger clones upon *P. aeruginosa* infection (Apidianakis et al., 2009).

Mitotic clones to assess ISC number and maintenance

There are two alternative strategies that are currently used for marking clones: the β-galactosidase method and the MARCM (mosaic analysis with a repressible cell marker) method (Harrison and Perrimon, 1993; Wu and Luo, 2006). Flies of the *hs-FLP;FRT-lacZ/tub-FRT* or the *hs-FLP;act-GAL4/UAS-GFP;FRT/FRT-tub-GAL80* genotype can be shifted for 1 hour from a standard (18-21°C) to a heat-shock (37°C) temperature to induce mitotic clones in the midgut. Comparisons of such clones can be made between flies of different genetic backgrounds, or between flies subjected

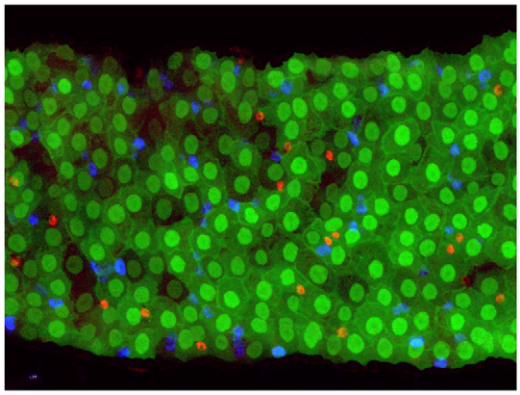

Fig. 4. Identifying cell types in the *Drosophila* posterior midgut using available tools. Visualization of ISCs or progenitor cells (using *esg-lacZ*; blue), enteroendocrine cells (using anti-Prospero; red) and enterocytes (using *myo-GAL4 UAS-GFP*; green) to distinguish the three cell types in the *Drosophila* posterior midgut.

to different treatments. Similar to the flip-out clone strategy, clone size can be used as a measure of cell proliferation. An important feature of mitotic clones, however, is that they emanate specifically from dividing cells, namely the ISCs, rather than a given GAL4-line-expressing cell population as in the case of flip-out clones. Thus, the number of mitotic clones per midgut can be used as an index of ISC number or maintenance over time (Lee et al., 2009; Lin et al., 2008).

Collective analysis and perspectives

What has fly intestinal pathology taught us about biology?

There are many unknowns regarding the cells and signals of the mammalian intestinal stem cell niche, but recent discoveries in *Drosophila* show that the muscle underlying the intestinal epithelium serves as a niche. Wg and JAK-STAT pathway signals emanating from the muscle sustain the ISC population (Lin et al., 2008; Lin et al., 2010). In addition, enteroblasts (ISC progeny) seem to provide a transient niche during development (Mathur et al., 2010). Therefore, muscle and myofibroblasts, as well as the neighboring TA cells underlying ISCs, might also provide the niche in mammals.

Evidence from fly models suggests that cytokines and ROS are not necessarily produced owing to local infiltration of immune cells but might also be produced owing to enterocyte responses to infection or toxic drugs (Buchon et al., 2009a). From this point of view, intestinal responses to infection cannot only be attributed to immune cell (e.g. hemocyte) infiltration but also to direct responses of the local cells. Genes and cellular mechanisms shown to be involved in *Drosophila* ISC homeostasis following infection (e.g. the JAK-STAT pathway and ROS production) can subsequently be studied in mammals because the roles of cytokines and ROS produced in mammals (either as a result of immune cell infiltration or local tissue activation) might approximate similar events in the intestinal epithelium of flies.

Although we know that, upon maturation, intestinal cells become apoptotic or are sloughed, results of fly studies tell us that enterocyte apoptosis can precede and actually induce regeneration (Apidianakis et al., 2009). Conversely, excessive regeneration seems to increase the size of the intestinal cell pool, although it is not clear whether this leads to increased apoptosis. Finally, the role of polarity genes in cell proliferation has been nicely elucidated in several fly studies [referred to in Humbert et al. (Humbert et al., 2008)], including studies of fly intestine (Apidianakis et al., 2009), paving the way for investigations of similar roles in mammals (Rothenberg et al., 2010).

Finally, in addition to *Drosophila* studies, work in other insects has provided important insights into intestinal pathology with potential relevance to human disease (Box 2).

Which properties of *Drosophila* validate its use for modeling mammalian intestinal pathologies?

Overall, intestinal epithelium anatomy and regeneration time (5-7 days) are similar in flies and mammals (Creamer, 1967; Ohlstein and Spradling, 2006). At the molecular level, the role of the Wnt/Wg and K-Ras/Ras1 pathways in ISC homeostasis and tumor formation, respectively, most strongly resemble the regulation of mammalian intestinal regeneration. In addition, the

Box 2. Lessons from other insects about intestinal pathophysiology

Mosquito-Plasmodium or mosquito–West-Nile-virus interaction

Studies of other insects have revealed many novel insights into microbial-intestinal interactions. Barillas-Mury and co-workers showed that, on infection of the mosquito *Anopheles stephensi*, *Plasmodium berghei* ookinetes must escape rapidly from midgut epithelial cells that are about to die in order to establish a systemic infection (Han et al., 2000). The midgut epithelium of this organism can rapidly heal damaged cells without losing its integrity using an actin-mediated budding-off mechanism (Han et al., 2000). Conversely, in *Aedes aegypti*, the midgut epithelium is repaired during infection with the same pathogen by a unique zipper-like mechanism that involves the formation of a cone-shaped actin aggregate at the base of the cell that closes sequentially, expelling the cellular contents into the midgut lumen as it brings together healthy neighboring cells (Gupta et al., 2005). In addition, Girard et al. showed that West Nile virus dissemination in orally infected *Culex pipiens* mosquitoes is mediated by cell-to-cell spread in the midgut epithelium and infection of both circular and longitudinal muscles of the same organ (Girard et al., 2004). The authors of this study proposed that muscle tissue serves as a conduit for virus dissemination and contributes to virus amplification, particularly late in infection.

Lepidoptera and viruses

Fascinating work has been done to dissect the mechanisms used by viruses to spread from the midgut of Lepidopterans (an order of insects that includes moths and butterflies) systemically through the immune cells and the trachea, a system that is analogous to mammalian lymphatics and vasculature. Infection of the larval tracheal system provides a major conduit for baculoviruses to pass from the intestine through the basal laminae and to spread throughout the host (Engelhard et al., 1994). Similarly, *Bombyx mori* (silkworm) larvae that ingest the virus show infection in the midgut epithelial cells within 24 hours. The primary spread of the viral infection occurs through the trachea and is widespread in other tissues by 48 hours, indicating the role of tracheae in spreading the infection (Rahman and Gopinathan, 2004). Slightly different results were obtained by Barrett et al., who showed that the virus infected the midgut columnar cells but also traveled through the midgut epithelial layer, entered the hemocoel and infected the hemocytes (Barrett et al., 1998). The budded virus that was released from the infected hemocytes into the hemolymph caused a secondary infection within the tracheal epithelial cells and, via this route, various other tissues (Barrett et al., 1998). Other studies of Baculoviridae showed that efficient establishment of tracheal infections counteracts infection clearance by midgut cell sloughing (Washburn et al., 1999). Strikingly, the Lepidopteran ortholog of *Drosophila* breathless [fibroblast growth factor receptor (FGFR) in mammals] is a receptor for the baculovirus FGF, which mediates host cell chemotaxis and presumably tracheogenesis of the midgut (Katsuma et al., 2006). *B. mori* FGFR is abundantly expressed in the trachea and midgut (Katsuma et al., 2006). This is particularly important owing to the analogous role of human FGF proteins in the healing of intestinal injury and the induction of angiogenesis (Katoh and Katoh, 2006).

Tsetse-trypanosoma interaction

Studies in tsetse flies indicate that stress influences the fitness of parasites in the host. For example, nutritional stress enhances maturation of a *Trypanosoma brucei* infection to the infectious metacyclic stage (Akoda et al., 2009). Other findings have shown that induction of tsetse innate immunity by the mutualist intestinal bacterium *Wigglesworthia* inhibits trypanosome transmission (Wang et al., 2009).

induction of conserved innate immune pathways (such as the Imd and STAT pathways) and the production of AMPs and ROS as basic components of the intestinal resistance to infection support the utility of this model for examining basic mechanisms of mammalian intestinal epithelial homeostasis, regeneration and disease. Last, the finding that the levels of specific indigenous bacteria must be controlled to prevent intestinal damage in both *Drosophila* and mammals (Deneve et al., 2009; Macfarlane et al., 2009; Ryu et al., 2008; Slack et al., 2009) provides further support that *Drosophila* is a relevant model for the study of microbial intestinal pathology.

What will *Drosophila* intestinal models be unable to teach us about mammalian pathophysiology?

The absence of lamina propria (Fig. 2), which includes fibroblasts and immune cells, and the lack of an adaptive immune system make flies inappropriate for studying non-epithelial intestinal inflammation and the involvement of T cells in IBD and intestinal cancer. In addition, because fly intestinal microbiota seems to be devoid of obligate anaerobes (Cox and Gilmore, 2007), which dominate the mammalian intestine (Qin et al., 2010), modeling human intestinal microbiota accurately in flies might be problematic. Nevertheless, human intestinal Proteobacteria, and other facultative aerobic or aerotolerant and microaerophilic bacteria, could be studied in flies. At the molecular level, in contrast to its action in mammalian ISCs, Notch pathway activation promotes the differentiation of fly ISCs (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006), meaning that results in flies cannot be extrapolated to explain the role of the Notch pathway in mammalian ISCs. Nevertheless, it is still possible to use ISC proliferation as a readout of Notch pathway activity in flies. Finally, as mentioned above, mammalian PDGF ligand is activated in the crypt base, signaling the nearby mesenchyme to shape the villi (Crosnier et al., 2006), a role that is apparently distinct from that of the homologous PVF ligand in flies, mainly owing to the absence of villi or other analogous structures.

What can flies teach us about intestinal infection and pathology in the future?

The conservation of molecular, cellular and tissue structures between flies and mammals means that flies can be used to investigate several aspects of biology that are relevant to human disease, including epithelial regeneration in the context of cancer and IBD, as well as the oxygenation of intestinal epithelium in normal and in pathogenic conditions (including infection, drug exposure and genetic alterations). Although hemocyte infiltration has not been documented in *Drosophila*, Upd, Wg and other cytokines and growth factors might act in a similar manner in *Drosophila* infection and in the context of chronic regeneration and tissue hyperplasia that occurs during chronic injury and/or infection of the mammalian intestine. Therefore, the well-documented link between chronic inflammation and cancer in mammals might be effectively modeled and studied in chronically injured or infected flies. Oncogenes and tumor suppressors are also highly conserved between flies and mammals (Fortini et al., 2000; Sutcliffe et al., 2003), justifying their study in *Drosophila* ISC or progenitor and enterocytes biology and relating it to mammalian cancer and infection. For example, *Drosophila* homologs of mammalian disease-associated genes [e.g. Wnt/Wg and K-Ras/Ras1, which act as a driving force in intestinal tumorigenesis (Markowitz and Bertagnolli, 2009)] can be studied in terms of ISC symmetric divisions. In addition, the role of human aerobic, aerotolerant, facultative anaerobic or microaerophilic intestinal bacteria and their metabolites can be studied in the context of fly intestinal regeneration. Finally, there are also outstanding questions regarding whether communication between the *Drosophila* intestine and other conserved organs, such as the fat body (paralleling the mammalian liver), muscle and brain, via cytokines and growth factors occurs, and the role of diet in this context.

Although inherent differences between the fly and human intestine will define the limitations of *Drosophila* model systems for resolving human pathology, they will also point to evolutionarily disparate strategies of intestinal physiology. We anticipate and hope that, in the following years, fly models of intestinal pathology, in addition to uncovering newly identified genes and basic biology mechanisms, will also highlight the most conserved aspects of human intestinal biology. As a result, fly models will contribute to translational research investigating drug effects, and microbial and host genetic component analyses, leading to biological findings that are broadly applicable to human health and disease.

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COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

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