INTRODUCTION

As a barrier epithelium, the intestinal epithelium has to coordinate physiological functions like digestion and nutrient resorption with the control of commensal bacteria and the prevention of pathogenic infections. It can therefore mount powerful innate immune and inflammatory responses, while, at the same time, maintaining tissue homeostasis through regenerative processes. How these different functions are coordinated remains unclear, and further insight is required to understand the age-related loss of homeostasis in this system, as well as the etiology of inflammatory and proliferative diseases of the gut. Recent work in *Drosophila melanogaster* has provided important new insight into the regulation of regenerative activity, innate immune homeostasis, commensal control, as well as age-related dysfunction in the intestine. Interestingly, many of the identified processes and mechanisms mirror similar homeostatic processes in the vertebrate intestine. This review summarized the current understanding of how innate immune responses, changes in commensal bacteria, and other challenges influence regenerative activity in the aging intestinal epithelium of flies and draws parallels to similar processes in mammals.

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tissue dysfunction by impacting regenerative and homeostatic processes.

The interactions between microbiota, stress and immune signaling in epithelial cells, as well as regenerative processes in the epithelium, represent a complex and wide-ranging field of study, and simple animal models are needed to provide fundamental insight into these interactions. The availability of powerful genetic tools for D. melanogaster, as well as its short lifespan and the relative simplicity of its intestine, but also the presence of complex epithelial interactions with commensals and of regenerative processes that resemble similar processes in mammals, have recently elevated the fly to a model organism of choice in this context. A large number of studies have already provided important mechanistic insight into epithelial/commensal interactions, as well as age-related changes in these interactions and their consequences for epithelial homeostasis (Buchon et al., 2009a, 2013a; Apidianakis and Rahme, 2011; Hochmuth et al., 2011; Karpac et al., 2011; Rera et al., 2011; Lee and Brey, 2013).

In the wild, D. melanogaster feeds on rotten fruits and vegetables. Such feeding behavior exposes them to repeated interactions with a variety of microbes. Distinct mechanisms have therefore evolved in fruit flies that enable them to maintain intestinal tissue homeostasis and survive in a microbe-rich environment (Ferrandon, 2013). In older flies, however, a widespread growth of intestinal microbial populations is associated with hyperplasia and misdifferentiation of intestinal stem cells (ISCs) and their progeny, leading to loss of tissue homeostasis (Buchon et al., 2009a; Biteau et al., 2010). Interestingly, over-expression of stress-protective genes in ISCs is sufficient to rescue this age-related loss of homeostasis and to increase Drosophila lifespan (Biteau et al., 2010; Rera et al., 2011, 2012). This finding supports the notion that managing the loss of intestinal homeostasis is critical for health and lifespan in metazoans, and highlights the usefulness of flies as models for inflammatory diseases of the gut. In this review we will summarize the current understanding of the interaction between innate immune responses, commensal microbiota, and proliferative homeostasis in the aging intestinal epithelium in D. melanogaster.

THE Drosophila INTESTINE

The gastrointestinal tract in Drosophila can be subdivided into the crop, foregut, midgut and hindgut (Figure 1). The crop is a food storage organ which is attached to the distal end of the foregut, via a thin tube. The midgut can further be divided into anterior, middle and posterior regions. The anterior midgut (AM) encompasses the proventriculus, and opens into the acidic middle midgut (MM; also called copper cell region). The posterior midgut, in turn, extends from the MM to a fusion point where it is connected to the hindgut and to malpighian tubules (Buchon et al., 2013b; Marianes and Spradling, 2013).

The Drosophila intestinal epithelium is a monolayer composed of three types of cells; the polyploid enterocytes (EC) form the majority of the midgut cell population, followed by hormone secreting enteroendocrine (EE) cells and the proliferating ISCs. ECs are absorptive cells but also secrete digestive enzymes in some parts of the gut, and play a central role in mounting innate immune responses to infection and in managing the commensal population. Proteases, lipases (such as LipA), carbohydrate-binding catalytic peptidoglycan recognition proteins (PGRPs) and lysozymes are among the digestive enzymes secreted by midgut cells (Sieber and Thummel, 2012; Lemaître and Miguel-Aliaga, 2013). The MM, in turn, contains acid secreting copper cells, most likely to aid digestion.

Regenerative processes in the intestinal epithelium differ along the gastrointestinal tract, and are influenced by local signals in each compartment (Buchon et al., 2013b; Guo et al., in press; Li et al., 2013; Marianes and Spradling, 2013). Interestingly, this compartmentalization seems to decline in the aging intestine, causing widespread deregulation of stem cell activity (Buchon et al., 2013b).

Regeneration of the posterior midgut epithelium is best understood so far. ISCs in this area can mount rapid and widespread regenerative responses to damage. During this renewal, ISCs divide asymmetrically to produce a population of non-differentiated progenitors called enteroblasts (EBs) (Michelli and Perrimon, 2006; Ohlstein and Spradling, 2006), EBs are not mitotically active, and differentiate into either an EC or an EE cell, depending on differential Notch signaling activity (Ohlstein and Spradling, 2007; Biteau et al., 2011a; Perdigoto et al., 2011; Cordero and Sansom, 2012; Kapuria et al., 2012). ISCs are also known to divide symmetrically to expand their own
population (O’Brien et al., 2011; Goulas et al., 2012). The ISCs are located close to the basal membrane (BM) of the epithelium and are in close proximity to the surrounding circular visceral muscle. The BM and visceral muscle, but also EBs and ECs, influence ISC proliferative activity and maintenance (Bardin et al., 2010; Biteau and Jasper, 2011; Xu et al., 2011; Cordero et al., 2012a; Goulas et al., 2012; Zhou et al., 2013).

**CONTROL OF ISC PROLIFERATION**

The proliferative activity of ISCs is very plastic. While low levels of homeostatic proliferation are generally observed in young, healthy guts, strong regenerative activity is observed in response to insults that damage the epithelium. EGF, Insulin/IGF (IIS), and p38MAPK signaling pathways are essential for ISC proliferation (Park et al., 2009; Biteau et al., 2010, 2011a; Biteau and Jasper, 2011). Constitutive activation of EGF receptor (EGFR) or insulin receptor (IIR) increases the rate of ISC proliferation, indicating that RTK signaling can modulate ISC activity in accordance with the metabolic status of the animal (Biteau and Jasper, 2011; Karpac et al., 2011; O’Brien et al., 2011; Xu et al., 2011). Long-term stem cell maintenance is further ensured by mechanisms that prevent activation of Target of Rapamycin (TOR) signaling (Amcheslavsky et al., 2011; Kapuria et al., 2012; Quan et al., 2013), and by muscle—derived Wingless (Sackton et al., 2007; Lin et al., 2008; Takashima et al., 2008; Cordero and Sansom, 2012; Cordero et al., 2012b).

While the signaling pathways listed above are required for homeostatic proliferation and maintenance of ISCs, various stress signaling pathways have been identified that govern induction of ISC proliferation when the intestinal epithelium is exposed to a stress or is injured. Stressors that trigger ISC proliferation include oxidative stress (Biteau et al., 2008; Choi et al., 2008; Buchon et al., 2009a), bacterial infection (Apidianakis et al., 2009; Buchon et al., 2009b; Cronin et al., 2009; Jiang et al., 2009; Guo et al., 2013), DNA damage (Amcheslavsky et al., 2011; Guo et al., 2013), aging (Biteau et al., 2008, 2010, 2011b; Choi et al., 2008; Buchon et al., 2009a; Karpac et al., 2009; Hochmuth et al., 2011), and factors that cause apoptosis and damage to ECs (Jiang et al., 2009; Amcheslavsky et al., 2011). Jun-N-terminal Kinase (JNK) (Biteau et al., 2008; Choi et al., 2008), JAK/Stat signaling (Buchon et al., 2009a; Cronin et al., 2009; Jiang et al., 2009) and the Hippo/Yorkie pathway (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010; Staley and Irvine, 2010) are all critical for stress-induced ISC proliferation [reviewed in Biteau et al. (2011a)]. The integration of these inductive signals with signaling pathways that play a permissive role for proliferation, as well as the exact cellular interactions during a regenerative response, are only beginning to be understood. Following bacterial infection or an injury, interleukin-6-like cytokines of the Unpaired (Upd) family, especially Upd 2 and 3 are induced in and secreted by damaged and dying ECs (Jiang et al., 2009; Osman et al., 2012; Zhou et al., 2013). Upds activate JAK/Stat signaling, either in ISCs directly (Buchon et al., 2009a; Cronin et al., 2009; Jiang et al., 2009), or in visceral muscle, where it induces the EGF-like ligand Vein, which in turn stimulates ISC proliferation (Jiang and Edgar, 2009; Buchon et al., 2010; Biteau and Jasper, 2011; Xu et al., 2011; Zhou et al., 2013).

The JNK pathway also plays a dual role in stimulating ISC proliferation: JNK is activated by reactive oxygen species (ROS) in both ECs and ISCs. Its activation in ISCs induces their proliferation by phosphorylating the AP-1 transcription factor Fos (Biteau et al., 2008; Biteau and Jasper, 2011; Hochmuth et al., 2011). Interestingly, Fos is phosphorylated both by JNK and EGFR pathways, and thus integrates growth factor and stress signals to induce ISC proliferation (Ciapponi et al., 2001; Biteau and Jasper, 2011). JNK activation in ECs, on the other hand, can stimulate Upd induction and induce ISC proliferation, but does not seem to be required for the regenerative response to a challenge (Jiang et al., 2009) nor for survival of the host upon pathogenic infection (Buchon et al., 2009a). Forced activation of JNK in ECs induces Upd expression by promoting Yorkie nuclear localization (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010; Staley and Irvine, 2010).

The need for epithelial renewal after pathogenic infections suggest that to maintain homeostasis, signaling mechanisms that control innate immune and inflammatory responses and signaling pathways that regulate ISC proliferation have to be highly coordinated. Recent years have seen tremendous progress in our understanding of the cellular and molecular mechanisms governing this coordination (Buchon et al., 2013a).

**INTESTINAL IMMUNITY**

The *Drosophila* intestine contains physical and chemical barriers to prevent microbial infections [reviewed in Ferrandon (2013)]. As a first barrier, a peritrophic matrix, consisting of chitin and glycoproteins covers the intestinal epithelium, preventing direct contact of microbes and other lumen contents with epithelial cells. The peritrophic matrix is secreted by the proventriculus with possible contributions by ECs (Hegedus et al., 2009). Loss of peritrophic matrix components renders flies susceptible to infections, highlighting the importance of the peritrophic matrix as a physical barrier against bacteria (Kuraishi et al., 2011).

A second line of defense is the secretion of antimicrobial peptides (AMPs) by ECs in the intestinal epithelium (Tzou et al., 2000; Liewhl et al., 2006; Ryu et al., 2006; Nehme et al., 2007; Buchon et al., 2009b). Invading bacteria are recognized by their peptidoglycans (PGN; structural components of the bacterial cell wall) (Zaidman-Remy et al., 2006). PGNs bind to PGRP-LC and -LE resulting in activation of the IMD/Relish (but not the Toll) pathway (Bosco-Drayton et al., 2012; Neyen et al., 2012), which in turn induces AMP transcription [immune signaling in *D. melanogaster* is comprehensively reviewed in Ferrandon et al. (2007); Lemaître and Hoffmann (2007); Ha et al. (2009a); Davis and Engstrom (2012); Buchon et al. (2013a); Lee and Brey (2013)]. Relish belongs to the family of highly conserved Nuclear Factor-kB (NF-kB) transcription factors, and is a required component of the IMD pathway, which is related to the mammalian tumor necrosis factor receptor (TNFR) pathway (Hoffmann, 2003). NF-kB and TNFR pathways are critical for epithelial immunity in mammals (Xavier and Podolsky, 2007; Meylan et al., 2011; De Jong et al., 2012). NF-kB activation in epithelial cells modulates immune responses to environmental challenges and microbial infections (Pasparakis, 2012), and cytokines and chemokines secreted by epithelial cells act on immune and
non-immune cells to modulate the cellular immune response. Chronic activation of NF-κB and of the TNFR pathway in epithelial cells results in the development of intestinal inflammation (Meylan et al., 2011; Wullaert et al., 2011; De Jong et al., 2012).

In flies, the IMD/Rel pathway is kept inactive in normal, homeostatic, conditions by a variety of negative regulators, including Caudal (Ryu et al., 2008), PGRPs of the SC, LB and LF class (Zaidman-Remy et al., 2006; Maillet et al., 2008; Paredes et al., 2011), USP36 (Thevenon et al., 2009) and PIRK (Lhocine et al., 2008) (Figure 2). These regulators are of particular importance in the maintenance of not only the commensal population, but also of proliferative homeostasis in the intestinal epithelium: loss of the homeobox transcription factor Caudal, for example, leads to a shift in commensal populations in the fly intestine, eliminating beneficial bacterial species and allowing outbreaks of pathogenic species. At the same time, stress signaling is ectopically activated, and stem cell proliferation is strongly induced, resulting in dysplasia-like phenotypes (Ryu et al., 2008; Buchon et al., 2009a; Biteau et al., 2010). These conditions are reminiscent of the dysplasia and inflammation observed in aging flies, where microbial expansion is associated with hyperactivation of the IMD, JAK/Stat and JNK signaling pathways, and with epithelial dysplasia (Buchon et al., 2009a).

The third part of the intestinal immune response against microbes is the production of ROS by ECs. ROS are produced by the transmembrane protein dual oxidase (DUOX), a member of the NADPH oxidase family, which is transcriptionally induced in ECs and activated in response to a bacterial challenge (Ha et al., 2005; Ryu et al., 2010). Under homeostatic conditions, ROS are produced at moderate levels in response to the interaction of the epithelium with resident autochthonous bacterial species. During infection with transient allochthonous bacteria, however, production of ROS is increased by two mechanisms: an unknown G-Protein Coupled Receptor (GPCR) activates Phospholipase C-β (PLCβ) and triggers inositol-1,4,5-trisphosphate (IP3)-induced Ca2+ release. Ca2+ is bound by EF-hands in DUOX, stimulating its activity (Ha et al., 2009a). A second mechanism involves activation of p38 MAPKinase, which transcriptionally induces Duox (Ha et al., 2009a,b). Young flies are believed to protect themselves from the cytotoxic effects of ROS by secreting an extracellular immune-related catalase (IRC), which neutralizes ROS (Ha et al., 2005). However, excessive ROS are generated and accumulate in the intestine of aged flies, presumably as a consequence of constant stimulation by immune resistant intestinal microbes. This excessive ROS accumulation is a likely cause of the age-related loss of epithelial homeostasis (Buchon et al., 2009a; Hochmuth et al., 2011).

**INTESTINAL MICROBIOTA**

A young and healthy *Drosophila* intestine contains a relatively simple microbiota comprising about 5–20 microbial species. Major constituents of these commensals are beneficial microbes, such as *Acetobacter pomorum* and *Lactobacillus plantarum* which promote growth and development in flies when reared on a restricted diet (Ryu et al., 2008; Chandler et al., 2011; Shin et al., 2011; Storelli et al., 2011; Wong et al., 2011). These microbes do not activate the intestinal immune system, allowing colonization of the gut. Resident pathobionts or invading potential pathogens, on the other hand, are readily recognized. One example of such a pathobiont, *Glucanobacter moribier*, constitutes only a minor proportion of the healthy intestinal community. Under favorable conditions, however, it can take over the gut, causing gut pathologies and lethality of the host (Ryu et al., 2008). Moreover, many negative regulators have also been identified that prevent chronic activation of the IMD pathway induced by indigenous microbes (Lhocine et al., 2008; Paredes et al., 2011; Bosco-Drayon et al., 2012).

Until recently, it was not known how the *Drosophila* immune system differentiates between friends and foes. Recent elegant work by the Lee lab shows, however, that pathogenic bacteria, in contrast to beneficial symbionts, are constantly secreting Uracl, which is recognized by the *Drosophila* immune system. Uracl is recognized by an unknown GPCR, which activates the PLCβ/IP3/Ca/Duox pathway discussed above to produce ROS. Many opportunistic pathogens such as *Vibrio fluvialis*, *Klebsiella pneumonia*, *Erwinia carotovora* carotovora, *Shigella sonnei*, *Pseudomonas aeruginosa* and *Serratia marcescens*, but not symbionts like *A. pomorum*, *L. plantarum* and *Commensalibacter intestini*, secrete significant quantities of Uracl (Lee et al., 2013).
Mono-association of flies with *G. morbifer* leads to chronic inflammation, induces apoptosis and shortens *Drosophila* lifespan, while these effects were not observed in germ free control flies or in flies mono-associated with a mutant *G. morbifer* lacking Uracil secretion. Uracil-producing pathobionts may also contribute significantly to the age-related increase in epithelial Duox-mediated ROS production, and thus to the age-associated dysfunction in epithelial homeostasis. It is clear that understanding causes and consequences of age-related changes in the commensal microbiome is an important task for future studies.

**INFLAMMATION REGENERATION CROSS TALK**

The Duox-induced innate immune response has thus important implications for the etiology of the dysfunction of the intestinal epithelium observed in aging flies. When flies are raised on a conventional diet, internal and external microbial populations expand with age (Ren et al., 2007; Guo et al., in press) and this expansion correlates with the age-associated accumulation of ROS in the gut (Buchon et al., 2009a). The increasing concentration of ROS stimulates ISC proliferation directly by activating JNK or inhibiting the Nrf2 homolog CncC (Biteau et al., 2008; Hochmuth et al., 2011), or indirectly by damaging ECs and stimulating Upd expression (Jiang et al., 2009). Although increased ISC activity is essential for regeneration in young epithelia in response to an insult, excessive ISC proliferation in aging animals results in the accumulation of mis-differentiated cells and the loss of tissue homeostasis, and is thus deleterious to animal health (Biteau et al., 2008, 2010; Hochmuth et al., 2011). Accordingly, overexpressing antioxidants or other stress-protective factors within ISCs not only rescues this age-related dysplasia, but also extends lifespan in *Drosophila* (Biteau et al., 2010; Hochmuth et al., 2011; Rera et al., 2011). These observations further support the notion that age-associated changes in the intestinal microbiota play a critical role in the development of age-related pathologies of the intestine, a concept that further studies in the fly should be able to test.

Why does the commensal microbiota expand in aging animals? It is unclear whether malfunctioning of the host immune response causes commensal populations to overgrow, or if expansion of immune-resistant intestinal commensals is the initiating event that causes the described aged-related intestinal pathology. Deregulation of innate immune signaling in aging epithelia can be observed, and may be brought about by age-related activation of stress signaling, in particular of the JNK signaling pathway (Buchon et al., 2009a; Karpac et al., 2009, 2013). The interaction between the IMD pathway and JNK is multifactorial and complex: JNK-mediated activation of the transcription factor Foxo can induce Rel expression in larvae (Karpac et al., 2011). In larval fatbodies, activation of TAK1 by infection not only promotes Relish nuclear localization, but also activates Hemipterous (JNK), which phosphorylates and activates Basket (JNKK), which phosphorylates and activates Basket (JNK) (Silverman et al., 2003; Park et al., 2004; Kallio et al., 2005). Another IMD pathway component, DREDD, may also directly activate JNK upon immune stimulation (Guntermann and Foley, 2011). Furthermore, JNK and Foxo have been shown to induce AMP transcription, in part independently of Relish (Delaney et al., 2006; Becker et al., 2010). Recent work in larvae and adults highlights the need to study these interactions in a spatially- and temporally-resolved manner in order to characterize the complex interactions between innate immune responses and stress and inflammatory signals *in vivo* (Karpac et al., 2011, 2013). Interestingly, a recent study from our lab identified age-related activation of Foxo in ECs as a driving force in the disruption of innate immune homeostasis, resulting in immune senescence. Foxo inhibits the expression of PGRP-SC2, resulting in chronic, excessive activation of Relish, and impairing the ability of the intestine to clear bacteria (Guo et al., in press).

While germ-free conditions can rescue age-related dysplasia (Buchon et al., 2009a), and pharmacological inhibition of the NFκB signaling pathway can reportedly extend *Drosophila* lifespan (Moskalev and Shaposhnikov, 2011), the evidence for a role of intestinal microbiota in influencing fly longevity remains controversial (Brummel et al., 2004; Ren et al., 2007). It is likely that rearing flies under sterile conditions results in the removal of not only deleterious species, but also of beneficial commensals, and experiments assessing fly lifespan under germ-free conditions may thus result in variable outcomes. However, moderate down-regulation of ISC proliferation has been shown to not only rescue the age-related intestinal disruption but also to extend lifespan (Biteau et al., 2010; Hochmuth et al., 2011). Conditions that can keep the commensal bacterial population in check, promoting innate immune homeostasis and proliferative homeostasis in the intestinal epithelium, are thus expected to be beneficial for the animal’s health. Accordingly, we find that managing the commensal population by preventing the age-related loss of PGRP-SC2 expression is sufficient to limit age-related dysplasia and extend lifespan (Guo et al., in press).

**CONCLUSION**

To maintain intestinal homeostasis, a highly selective immune response has to ensure that pathogenic microorganisms are eliminated, while commensals can thrive. Moreover, the inflammatory response triggered by pathogens and commensals alike has to be carefully contained to prevent excessive stem cell activation and dysplasias. It may not be surprising that these carefully balanced responses are misregulated in aging animals, making the host more susceptible to invading microbes, and promoting inflammatory and dysplastic conditions. A better understanding of the molecular parameters driving these age-related changes, however, promises to provide insight into avenues for therapeutic intervention that may not only be applied to inflammatory diseases and cancers of the gut, but potentially to allay tissue dysfunction in the normally aging human intestine.

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