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From pathogens to microbiota: How Drosophila intestinal stem cells react to gut microbes

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ABSTRACT

The intestine acts as one of the interfaces between an organism and its external environment. As the primary digestive organ, it is constantly exposed to a multitude of stresses as it processes and absorbs nutrients. Among these is the recurring damage induced by ingested pathogenic and commensal microorganisms. Both the bacterial activity and immune response itself can result in the loss of epithelial cells, which subsequently requires replacement. In the *Drosophila* midgut, this regenerative role is fulfilled by intestinal stem cells (ISCs). Microbes not only trigger cell loss and replacement, but also modify intestinal and whole organism physiology, thus modulating ISC activity. Regulation of ISCs is integrated through a complex network of signaling pathways initiated by other gut cell populations, including enterocytes, enteroblasts, enteroendocrine and visceral muscles cells. The gut also receives signals from circulating immune cells, the hemocytes, to properly respond against infection. This review summarizes the types of gut microbes found in *Drosophila*, mechanisms for their elimination, and provides an integrated view of the signaling pathways that regulate tissue renewal in the midgut.

1. Introduction

To fulfill its essential role in nutrient digestion and absorption, the intestine has to cope with continuous stress caused by physical, chemical, and biological damage. Damage, either as a result of infection or immune response, results in dramatic loss of enterocytes (ECs), which are the most abundant differentiated cell type in the gut. Moreover, the gut need to adapt to changes in metabolism and nutrition (Buchon et al., 2013a; Gersemann et al., 2011). In response to these changes and stresses, the gut is capable of maintaining epithelial integrity by homeostatic repair, as well as adapting its growth according to nutrient availability (Buchon et al., 2014; O'Brien et al., 2011). This is achieved through the tight regulation of intestinal stem cells (ISCs). Without ISCs, there is no gut regeneration (Lu and Li, 2015). The proliferative capability of ISCs is amazing: *Drosophila melanogaster* (*Dmel*) midguts depleted of ECs are able to completely recover in less than 60 h (Jiang and Edgar, 2009). It has become increasingly clear that gut microbes, both transient pathogens and commensal bacteria, influence the integrity and the physiology of the gut epithelium. As in mammals (Tremaroli and Bäckhed, 2012), efficient control of these microbes is important for proper functioning of the insect midgut. In aged animals, guts show epithelial deterioration associated with dysfunction of the immune response, microbial dysbiosis, and increased oxidative stress leading to excessive ISC proliferation (Biteau et al., 2008; Guo et al., 2014). This identifies the interaction between ISCs and microbes as central to host health (reviewed in Buchon et al., 2013a) both in young and old flies, and suggests a complex network for gut homeostasis that includes gut microbes, stem cell activity, and the immune response.

In humans, dysbiotic conditions are commonly associated with intestinal pathologies such as inflammatory bowel disease, Crohn's disease and ulcerative colitis (Gersemann et al., 2012). In addition, microbial dysbiosis and the associated inflammation unbalances epithelium renewal, potentially leading to cancer (Gao et al., 2015; Radtke and Clevers, 2005), which is increasingly recognized as a stem cell disease (Clarke and Fuller, 2006; De Lerma Barbaro et al., 2014). Despite progress in the field, the study of interactions between ISCs and gut microbes remains complex, especially in the mammalian gut that houses several hundred bacterial species, many of which are non-culturable (Eckburg et al., 2005; Jalanka-Tuovinen et al., 2011; Ley et al., 2006; Rajilić-Stojanović et al., 2013; Zoetendal et al., 1998).

Dmel has recently emerged as an outstanding model to study gut homeostasis in health and disease (Apidianakis and Rahme, 2011; Broderick et al., 2014; Buchon et al., 2013a; Lee and Lee, 2014).

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Dmel and mammalian intestines are remarkably similar in structure and function, but the *Dmel* gut microbiota is primarily composed of only 5 to 10 bacterial species, and thus simpler to characterize (Wong et al., 2011). Similar to mammals, the *Dmel* midgut is not only composed of different cell types, but it also comprises 5 major regions along its length that differ from one another in function, physiology, and homeostasis, and can be further subdivided in as many as 14 sub-regions based on gene expression patterns (Buchon and Osman, 2015; Buchon et al., 2013b; Dutta et al., 2015; Marianes and Spradling, 2013). The *Dmel* midgut epithelium is composed of 4 different cell types: ISCs, enteroblasts (EBs), ECs, and enteroendocrine cells (EEs). ISCs divide and transiently differentiate into non-dividing EBs, which terminally differentiate in either absorptive ECs or secretory EEs (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). It has recently been proposed that only ECs derive from EBs, with the EEs stemming directly from ISCs through distinct post-mitotic progenitors, the pre-EEs (Zeng and Hou, 2015). The muscular sheet that surrounds the gut, called visceral muscle (VM), acts as a niche for ISCs thereby controlling and regulating ISC behavior. The VM is instructed by ECs, EBs and EEs to properly perform its niche role (Biteau and Jasper, 2011; Buchon et al., 2010; Guo et al., 2013). A niche is defined as a specific anatomic location that regulates how stem cells participate in tissue generation, maintenance, and repair (Scadden, 2006). However, recent takes on the subject are re-defining the concept of niche as a more dynamic entity (Gilboa and Lehmann, 2007; Lane et al., 2014).

Our current knowledge of the complex interactions between the gut epithelium and enteric microbes derives from studying the host response against pathogenic bacteria, as this is similar, though more drastic, compared to the response against non-pathogenic microbiota. In order to survive infection, the gut epithelium needs both resistance against the bacteria, which consists in mounting an efficient immune response to eliminate the threat, as well as tolerance/resilience against the associated damage, which is the ability to survive the deleterious consequences of infection (reviewed in Ferrandon, 2013). In brief, upon infection ECs are lost, either through an effect of the bacteria or due to the immune response itself, and need to be replaced through ISC proliferation. Epithelium renewal is thus an essential component of the *Dmel* defense mechanism against pathogens, and flies with impaired renewal succumb to infection that would be otherwise non-lethal (Buchon et al., 2009a). However, the effects of microbiota are more than just chronic damage: microbiota can exert a protective role toward *Dmel* and also act as a nutrient, as described in section 2.

Using *Dmel*, we have started to unravel the complex signaling network regulating ISC behavior in both healthy conditions and in response to stress. In this review, we will first describe the microbes related to the *Dmel* midgut, both commensal and pathogenic, and detail how they affect ISC proliferation and epithelium renewal. We will further discuss the immune response against microbes. Finally, we will discuss the specific signaling pathways involved in regulating ISC behavior in response to microbes.

2. Microbes in the *Drosophila melanogaster* midgut

The *Dmel* midgut, like the digestive tracts of all animals, is populated by various microbial species acquired through food ingestion and transmitted via defecation or regurgitation (Gilbert, 1980). In the wild, *Dmel* feeds mostly on rotten fruits and decaying matter. *Dmel* thus ingests a broad variety of microorganisms, some of which may serve as primary food sources, as is probably the case for yeasts (Begon, 1917; J. P. Baumberger, 1916; Sang and Sang, 1956). Other environmental microbes pass transiently through the midgut and can have pathogenic effects. Finally, a few microbial species are frequently present in the midgut and become part of the gut microbiota, but the exact nature and consistency of these associations with *Dmel* remain unclear (reviewed in Broderick and Lemaitre, 2012). Gut repair through ISCs in *Dmel* occurs not only as a consequence of infection, but is constant in order to counteract damage due to the associated microbial population and food ingestion. In this section we will review the bacterial species in the *Dmel* gut that can influence ISC activity either through changes in nutrition or damage.

2.1. The Dmel microbiota

The role of *Dmel* associated microbes in the midgut is hard to understand with only conventional reared (CR) *Dmel*, which are reared without any manipulation or alteration of the microbiota. Fortunately, a number of studies using germ free (GF) and with gnotobiotic *Dmel*, which are GF *Dmel* re-associated with selected microbial species, have examined their impact on *Dmel* physiology. In this regard, *Dmel* provides a simpler system than mammals, thus making it a powerful model to understand gut–microbe interactions (Broderick et al., 2014). Gut microbes are not required for the survival of *Dmel* itself, since GF flies can be maintained on sterile food for generations (Bakula, 1969). Moreover, the association between the microbial community and its host is not stably maintained and is shaped through constant ingestion of microbes (Blum et al., 2013). Most flies have a low bacterial count of a few thousand bacteria per midgut. This number is proportional to the amount of food present in the lumen, suggesting that the bulk of bacteria are associated with food passing through the midgut. Despite this apparent instability, the *Dmel* microbiome includes 5 bacterial species that are frequently found in both laboratory-raised and wild-caught flies (Chandler et al., 2011). These species comprise *Acetobacter pomorum*, *Acetobacter tropicalis*, *Lactobacillus brevis*, *Lactobacillus fructivorans* and *Lactobacillus plantarum*. Flies captured in the wild have a more diverse microbiome compared to flies reared in the lab, including Proteobacteria and species previously identified as pathogens, such as *Providencia*, *Serratia*, *Erwinia*, *Pantoes, Enterococcaceae* and *Enterobacteriaceae* and *Pseudomonas*. However, even in wild-caught *Dmel, Acetobacter* and *Lactobacillus* species are still the most commonly associated bacteria (Brewer et al., 1981; Broderick and Lemaitre, 2012; Chandler et al., 2011; Cox and Gilmore, 2007; Flyg et al., 1980; Galac and Lazzaro, 2011; Juneja and Lazzaro, 2009; Kloepper et al., 1981; Molina et al., 1974; Nmorsi et al., 2007; Vodovar et al., 2005). In lab-reared adult flies, initial reports suggested that *L. fructivorans* is dominant in young individuals, though the community shifts towards an abundance of *A. pomorum* at 3–5 weeks of age (Wong et al., 2011). However, a more recent report showed *Acetobacter* to be already abundant in young *Dmel*. This effect depends on the amount of eggs deposited in each vial (Wong et al., 2015), reinforcing the idea that transmission of microbes from generation to generation occurs through the deposition of microbes on the eggs (Bakula, 1969). Regardless of the potential shift from *Lactobacillus* to *Acetobacter*, aging flies show both higher bacterial titers, increased microbial diversity (Broderick et al., 2014) and increased intestinal barrier permeability (Clark et al., 2015). This change may depend on the alteration of the immune response in old flies, called immunosenescence, and ultimately results in increased ISC proliferation and differentiation, a conserved feature of dysbiosis (Buchon et al., 2009a; Guo et al., 2014).

Recently, comparative studies of the transcriptome of GF and CR *Dmel* guts have provided a good description of the effect of microbiota on gut physiology: 152 genes are differentially regulated between GF and CR guts, and gut microbes affect gut physiology by stimulating NF-κB-regulated immune responses through

the immune deficiency (Imd) pathway, altering tissue homeostasis and ISC activity, and modulating gut metabolism and the expression of digestive enzymes (Broderick et al., 2014; Combe et al., 2014). A first effect of the microbiota is to stimulate intestinal turnover and ISC proliferation (Broderick et al., 2014; Buchon et al., 2009a). This results from a combination of the immune response against the microbiota (Buchon et al., 2009a; Ha et al., 2005) and metabolic changes induced in the midgut (Shin et al., 2011). The damaging effect of the microbiota is more evident in old flies, as the gut tissues of GF flies show less over-proliferation and miss-differentiation than CR flies. Consistent with this, GF flies showed an increased lifespan compared to CR flies in two reports (Clark et al., 2015; Guo et al., 2014), although no lifespan extension was detected in Ren et al. (2007); it is possible that this discrepancy was due to differences in culture conditions or microbial composition. One consequence of the aberrant epithelium renewal induced in old flies is disruption of gut barrier integrity, which triggers a systemic activation of the immune response. Accordingly, loss of gut barrier function seems to be a strong predictor of imminent death (Clark et al., 2015; Rera et al., 2012). A recent study also suggests that administration of the drug Rapamycin extends lifespan, despite the decrease of gut barrier functionality, through alteration of microbial density in the midgut (Fan et al., 2015). Altogether, these reports endorse a model by which loss of immune competence in aged flies affects the gut microbes that in turn trigger aberrant renewal, barrier dysfunction, and the death of the fly. In this framework, the loss of the immune capability to maintain a healthy microbiota and the proper regulation of ISCs are the two major drivers of age related gut dysfunction.

The gut microbiota not only modulates ISC proliferation through tissue damage, but may also serve a fortifying role for the host immune system. Larvae missing the gut microbiota are more sensitive to oral infection by *Candida albicans* (Glittenberg et al., 2011). Moreover, flies with a normal microbiome are less susceptible to infection by *Serratia marcescens* (*Sm*) and *Pseudomonas aeruginosa* (*Pa*), and further addition of *L. plantarum* increased protection against these infections (Blum et al., 2013). This immune function of the microbiota may be due to either activation of the immune system, resulting in a more efficient immune response, or an improved repair of infectious damage, potentially through ISC modulation. Along those lines, a recent publication suggests that *Lactobacilli* could bolster the gut response to damage and shows that *L. plantarum* promotes cytoprotection against abiotic oxidative stress by upregulating the Keap/Nrf2 pathway in the midgut (Jones et al., 2015). Another possibility is that the established microbiota out-competes incoming pathogenic bacteria for available resources, or that it causes the gut environment to be less favorable for pathogenic bacteria. Another possible function of microbiota is in the defense against viruses: *A. pomorum*, induces the NF-κB dependent secretion of Pvf2, which acts as a ligand for the receptor tyrosine kinase PVR. PVR is necessary and sufficient for induction of ERK signaling, which has a broadly antiviral role in insects. However, microbiota alone is not sufficient for the production of Pvf2: a virus-dependent Cdk9-dependent signaling is also required, thus suggesting that sensing of specific microbes or bacteria prepares the immune system for antiviral response (Sansone et al., 2015; Xu et al., 2013).

Moreover, the gut microbiota modulate ISC activity by affecting host physiology, including changes in nutrition and metabolism (Dobson et al., 2015). Comparison of GF and CR flies suggests a function of the microbiota as a major cofactor to sustain host development and growth. In *Dmel* larvae, the microbiota promotes growth in nutrient scarce conditions: re-association with *L. plantarum* alone promotes intestinal peptidase expression and proteolytic activity, thus leading to increased digestive abilities and increased amino acid levels. The microbiota is also sufficient to induce growth through a TOR-dependent host nutrient sensing system that depends on amino acids (Erkosar et al., 2015; Storelli et al., 2011). Work from another laboratory shows that although *L. plantarum* and other microbes such as *Commensalibacter intestini* and *L. brevis* could enhance larval growth, only *A. pomorum* could fully restore optimal growth on protein poor diet by promoting insulin signaling (Shin et al., 2011). The differences between these two results probably depend on the conditions used to generate nutrient scarcity, which indicates that the microbiota could serve a number of additional roles in different nutritional conditions. Recently, gut microbes have been shown to supplement dietary B vitamins on a low-yeast diet, as well as promote protein nutrition in female flies and reduce lipid/carbohydrate storage on high sugar diets (Wong et al., 2014). The involvement of microbiota in nutrition was also confirmed through a metagenome–wide association approach that demonstrated the importance of gut microbes in modulating nutrient acquisition (Chaston et al., 2014). Of note, nutritional pathways such as TOR have recently been demonstrated to affect resistance to pathogens. Systemic inhibition of the TORC1 complex by rapamycin injection results in decreased resistance to infection with *Burkholderia cepacia*, while inhibition of TORC2 results in increased resistance (Allen et al., 2015). These results suggest an intricate relationship between energy and nutrition related pathways and resistance to infection.

Altogether, despite the fact that the microbiota are not directly necessary for *Dmel* survival and their presence can become detrimental over time, their incidence is also beneficial in terms of nutritional and immune contribution. In addition, the microbiota alters basal ISC activity by inducing low levels of damage, triggering basal immunity and altering host physiology. Future studies will elucidate how those multiple signals from the microbiota converge on regulating ISC activity.

2.2. Pathogenic bacteria

Unlike the normal microbiota, pathogenic bacteria trigger a violent response that leads to EC loss and shortening of the gut, and greatly increase ISC proliferation as described in Sections 3 and 4. Due to this effect, pathogens have become an essential tool to study not only the immune response, but also proliferation and differentiation mechanisms in ISCs. There are several bacterial species that have been shown to be infectious in *Dmel* through the oral route and to induce intestinal pathogenesis. *Enterococcus faecalis* is often found as part of the normal microbiota, however a specific pathogenic strain expressing the toxin cytolysin is able to colonize the midgut and kill *Dmel* within a week (Cox and Gilmore, 2007). Two *Pseudomonades* have been shown to be highly virulent to *Dmel*: *Pseudomonas entomophila* (*Pe*) and *P. aeruginosa* (*Pa*), a human opportunistic pathogen, induce damage to the midgut upon infection by activating c-Jun N-terminal kinase (JNK) pathway and apoptosis in ECs, resulting in ISC over proliferation (Apidianakis et al., 2009; see also Section 4). Orally ingested *Pa* also cross the intestinal barrier and proliferate in the hemolymph, causing infected flies to die of bacteremia (Limmer et al., 2011). *Pe* is highly pathogenic to both adults and larvae, leading to death 1–2 days after ingestion of high doses. *Pe* virulence partially depends on the action of a pore forming toxin, Monalysin, and induces a global translation blockage, which leads to the rapid demise of *Dmel* by inhibiting immune and repair responses in the gut (Chakrabarti et al., 2012; Opota et al., 2011; Vallet-Gely et al., 2010; Vodovar et al., 2005). The highly virulent *Db11* strain of *Sm* is found in natural *Dmel* populations (Cox and Gilmore, 2007; Flyg et al., 1980; Galac and Lazzaro, 2011) and is highly pathogenic to a wide range of hosts including insects, plants, nematodes and mammals (Grimont

and Grimont, 1978; Kurz et al., 2003). *Sm* resists the immune response in the insect gut due to its lipopolysaccharide O-antigen, and crosses the intestinal barrier, killing flies in just 6 days (Nehme et al., 2007). The gram-negative *Erwinia carotovora carotovora 15* (*Ecc15*) is another pathogen widely used in the study of gut immunity. *Ecc15* in the gut triggers a strong Imd-dependent immune response as well as the production of reactive oxygen species (ROS), which leads to a massive loss of ECs. However, the midgut is able to regenerate, and wild-type flies survive *Ecc15* infection (Buchon et al., 2009b). We can speculate that there are 2 classes of bacterial pathogenesis ensuing after oral infection. In the case of *Pe* and *Ecc15*, pathogenesis comes from a direct effect on the midgut, while for *Pa* and for *Sm* the gut is only a transient passage, and lethality originates from later systemic infection. However, all pathogen-induced damage of the gut leads to ISC proliferation by inducing similar mechanisms, as discussed in Section 4 and schematized in Fig. 1.

2.3. Yeasts and other fungi

Yeast is a classical source of nutrition in lab cultures, and yet its interactions with *Dmel* midgut have not been studied extensively. In the wild, flies are found associated with *Kloeckera*, *Pichia* and *Saccharomycodes* species (Atkinson and Shorrocks, 1977; Ganter, 2006; Vacek et al., 1979). The transmission of yeasts is different compared to that of bacteria. While the crop can contain up to 10^5 yeast cells, very few are found in the midgut (Shihata and Mrak, 1951). The immune response against fungi is mainly executed through Toll pathway (reviewed in Roeder et al., 2004). Toll pathway is not one of the main immune response mechanisms found in the midgut itself, as described in section 3, but it is present in the foregut and hindgut (Buchon et al., 2013b). It is thus possible that fungi are not normally in the midgut because the immune response against fungi acts in the foregut. *Dmel* can transmit yeasts to sterile food through defecation and regurgitation (Begon, 1974; Ganter, 2006; Gilbert, 1980), possibly through spores (Coluccio et al., 2008). Yeast can increase the nutritional value of food by funneling and transforming basic elements: for instance, diet composed of sterile banana cannot support the growth of GF larvae unless yeast or additional nutrients such as amino acids, fatty acids, B vitamin and sterols are added (Anagnostou et al., 2010). Moreover, a recent study elucidates the nutritional role of the yeast *Issatchenkia orientalis*: this fungus extracts amino acids directly from nutrient poor diets and increases 20 fold the amount of proteins acquired by *Dmel* (Yamada et al., 2015). This suggests that yeast and *Dmel* function in a mutualistic interaction: *Dmel* spreads yeast, raising its outbreeding (Reuter et al., 2007), and receive in turn a better quality diet (Broderick and Lemaitre, 2012). Recent studies suggest that yeast could also alter *Dmel* physiology beyond its nutritive value. Although yeast is mostly used as a nutrient, flies lacking phospholipase C- β (PLC β), a stimulator of the dual oxidase (Duox) defense system, die from uncontrolled proliferation of *Saccharomyces cerevisiae*, a yeast species commonly used in laboratory fly food (Ha et al., 2009a). This finding demonstrates that a basic form of defense is needed for every microorganism, which, if left unchecked, could multiply enough to induce lethality. While there has been no direct study on the effect of yeast on ISCs, considering their stimulatory effect on both the insulin pathway and Duox mediated ROS production we predict that they may play a major role in controlling ISC activity (Shin et al., 2011).

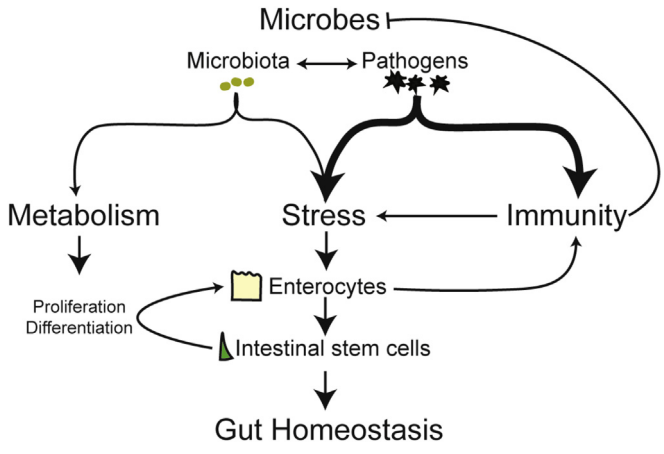

Fig. 1. Host microbe interactions in the Dmel midgut. The gut microbiota and pathogens both trigger stress either directly or through the immune response they induce. As a consequence of this stress, Enterocytes die and delaminate from the midgut. Enterocyte loss is compensated by the activity of intestinal stem cells that regenerate the midgut. The microbiota also influences the proliferation of intestinal stem cell by its impact on host physiology. The immune response in turn controls the microbiota. Therefore, ISCs constantly communicate with gut microbes and integrate stress, immune, and physiological parameters to maintain gut homeostasis.

3. Immune response to gut microbes

Dmel has emerged as a powerful model in which to investigate interactions between pathogens and gut-associated microorganisms in the intestinal tract (Buchon et al., 2013a). In order to maintain a homeostatic equilibrium, the immune response must be tightly regulated to eliminate pathogenic bacteria while allowing for the persistence of beneficial commensals. The gut immune system includes physical and chemical barriers, such as digestive enzymes and pH, which limit the survival of all microbes, as well as several tightly controlled, inducible antimicrobial mechanisms. The physical barriers of the gut include the peritrophic matrix, a chitinous semi-permeable membrane, as well as a thin layer of mucus and epithelial barrier integrity (Kuraishi et al., 2011). The two main inducible antimicrobial effectors in the midgut are: the production of ROS by Duox, a member of the NADPH oxidase family, and the production of anti-microbial peptides (AMPs) via the Imd pathway. In Fig. 2 we summarize the immune response against microbes in *Dmel* midgut.

3.1. Imd signaling and antimicrobial peptide production

The expression of AMPs serves as a major immune response in the gut to control microbes. In *Dmel*, two major signaling pathways, the Imd and Toll pathways, regulate the expression of AMPs in response to systemic infection. In contrast to the systemic response, the immune response in the digestive tract is regionalized: the Toll pathway is utilized in the fore- and hind-gut, whereas the Imd pathway regulates AMP expression in the midgut (Buchon et al., 2009b; Ryu et al., 2006; Tzou et al., 2000). Flies with mutations in components of the Imd pathway are more susceptible to enteric infections by *Sm* and *Pe*, demonstrating a key role of the Imd pathway in intestinal immunity (Basset et al., 2000; Liehl et al., 2006). In the gut, two receptors, PGRP-LC (transmembrane-type; Peptidoglycan recognition protein LC) and PGRP-LE (intracellular-type; Peptidoglycan recognition protein LE), bind diaminopimelic acid (DAP)-type peptidoglycan that composes the cell wall of Gram-negative bacteria and of certain *Lactobacilli* (Kaneko et al., 2004; Leulier et al., 2003; Takehana et al., 2002). During the activation of the Imd pathway, the bacteria-sensors PGRP-LC and/or PGRP-LE recruit the adaptor molecule Imd and form a complex with dFADD (Fas-associated death domain protein) and Dredd (Death-related ced-3/NEDD2-like protein). This complex activates the Mitogen Activated Protein Kinase Kinase (MAPKK) TAK1 (TGF beta activated

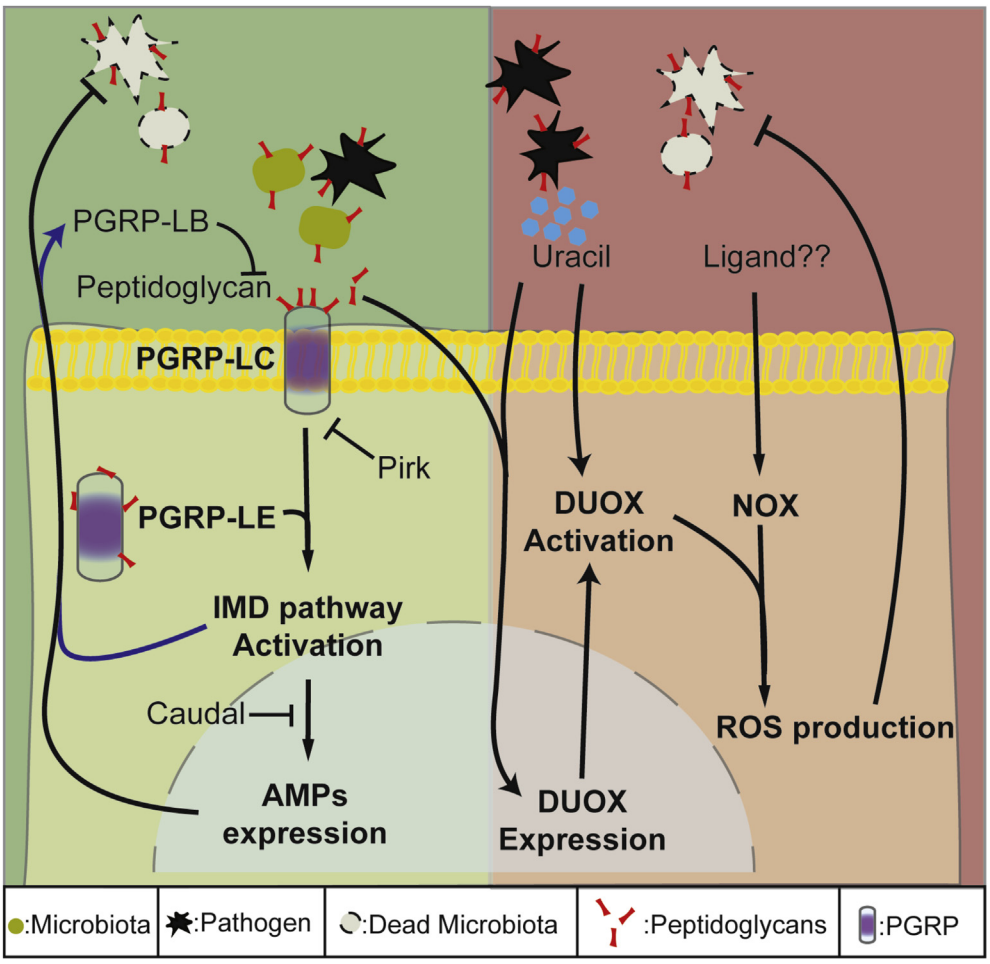

Fig. 2. The Drosophila gut immune response. Two main antimicrobial mechanisms act in the Drosophila midgut. First the gut is able to secrete antimicrobial peptides (AMPs) controlled mostly by the Imd pathway (in green shade). Peptidoglycan from microbes is recognized by PGRP-LC and PGRP-LE, and further activates the Imd pathway leading to transcriptional induction of AMPs. Multiple negative regulators control the proper level of Imd activation. Some regulators are also targets of the Imd pathway (PGRP-LB, Pirk) while others are regionally expressed in the midgut (Caudal). Second, NADPH oxidases (Duox and Nox) produce reactive oxygen species (ROS) that have a bactericidal effect (in red shade). Two bacterial patterns activate Duox expression and activity: Peptidoglycan and microbial derived Uracil. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kinase 1) and eventually leads to the activation of the IKK complex, IKKβ/IRD5 and IKKγ/Key. This in turn activates the NF-κB family member Relish (Rel), which is cleaved by the caspase Dredd allowing for nuclear translocation of the Rel homology domain. The two dominant members of the *Dmel* microbiota, *Acetobacter* and *Lactobacillus* spp., both contain DAP-type peptidoglycan, and can therefore activate AMP production. In agreement with this, it has been shown that GF flies exhibit lower basal expression levels of AMPs in the gut (Buchon et al., 2009a; Lhocine et al., 2008; Ryu et al., 2008) and that old flies show higher levels of Imd pathway activity, consistent with their increased bacterial loads of older flies.

While the host needs to defend against pathogenic microorganisms, it also needs to preserve the positive function of gut commensals. As much as loss of Imd mediated immune response correlates with dysbiosis and aberrant ISC activation, a chronic immune response is also deleterious to the host (Paredes et al., 2011). Conservation of a balanced immune response is achieved through the tight regulation of the Imd pathway by several negative regulators. First of all, sensing bacteria in the gut relies on the recognition of peptidoglycan. As the number of commensal bacteria is significantly lower than that observed during pathogenic infections (Buchon et al., 2010, 2009a; Ren et al., 2007; Ryu et al., 2008), and as the total microbiota does not show uncontrolled growth, the luminal content of peptidoglycan (PGN) remains low under normal conditions. Furthermore, the fact that the intracellular receptor PGRP-LE, serves as a predominant PGN receptor in the posterior midgut, the region most exposed to bacterial components, could ensure keeping a moderate level of Imd pathway activation in normal conditions (Bosco-Drayon et al., 2012; Neyen et al., 2012). In addition, multiple negative regulators of the Imd pathway dampen its reactivity to both the gut microbiota and pathogens. These negative regulators include PGRPs with amidase activity, such as PGRP-LB and PGRP-SC. Those proteins decrease the level of immunostimulatory ligands peptidoglycan in the gut lumen (Bischoff et al., 2006; Mellroth and Steiner, 2006; Paredes et al., 2011; Zaidman-Rémy et al., 2011, 2006). Other negative regulators include the gene *poor Imd response upon knock-in* (*pirk*), which displaces PGRP-LC from the cell membrane to disrupt its interaction with Imd (Aggarwal et al., 2008; Kleino et al., 2008; Lhocine et al., 2008), the transmembrane receptor PGRP-LF (Basbous et al., 2011; Maillet et al., 2008), and multiple additional effectors such as Cullin 1-based ubiquitin ligase complex (SCF), USP36 (also known as Scny), Cylindromatosis, POSH, and Defense repressor 1 (Dnr1) (Aggarwal and Silverman, 2008; Lee and Ferrandon, 2011). The Imd pathway is also specifically downregulated in gut segments where the epithelium is the least sealed, like the posterior midgut; here

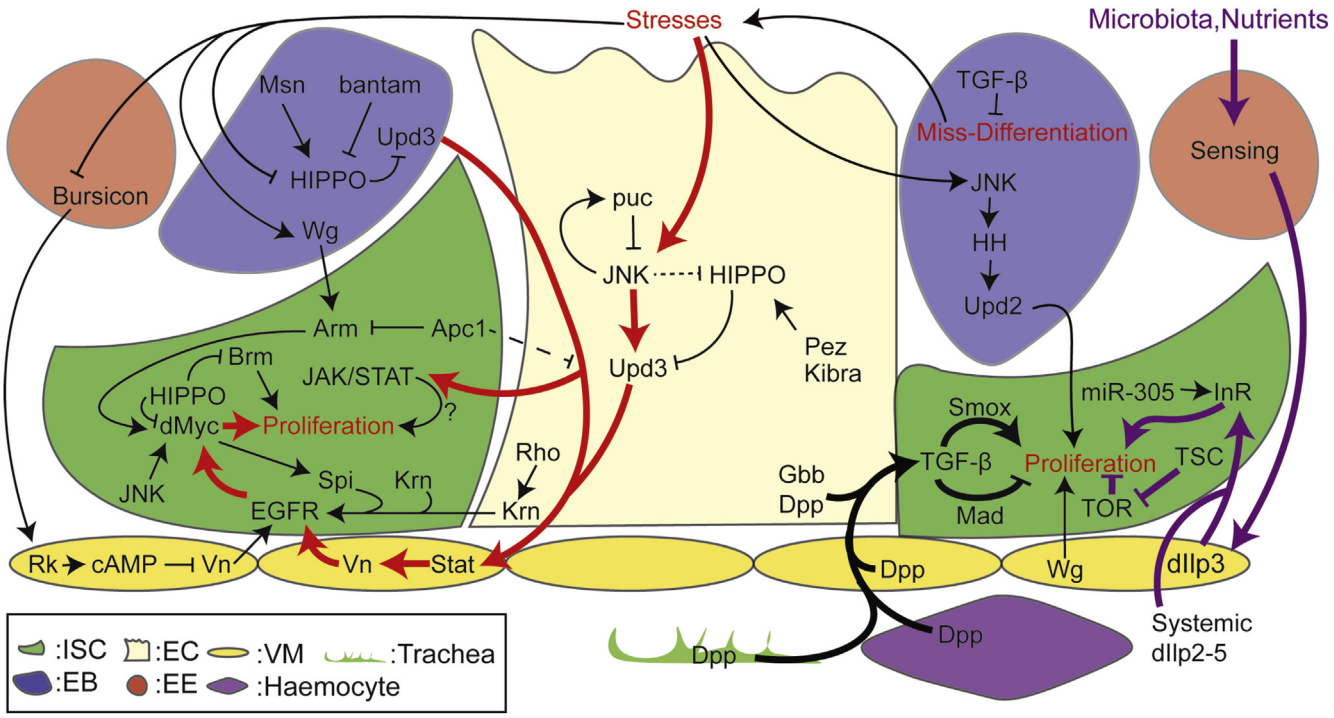

Fig. 3. A comprehensive view of the signaling pathways controlling ISC activity. Signaling pathways are capitalized, while individual proteins follow the normal conventions. Abbreviations: Intestinal stem cell (ISC), Enteroblast (EB), Enterocyte (EC), Entero-endocrine cell (EE), Visceral muscle (VM). Multiple stresses converge in the regulation of JNK and hippo pathway in the midgut, especially in enterocytes. This leads to Upd3 production by the EC that will instruct the proximate ISC environment to secrete growth factors. Activation of JAK/STAT in the VM induces the secretion of the EGF Vein that triggers EGFR in ISCs and promotes proliferation. In addition, two other Upd cytokines (Upd1 and 2) and two additional EGFs (spitz and Keren) participate in this JAK/STAT to EGFR loop (highlighted with bold red arrows). Stresses also cause a JNK – Hh- Upd2 cascade in EBs, which elicit proliferation of ISCs. JNK is important in ISCs themselves. Upd3 is also expressed in EBs following repression of the Hippo signaling pathway. Upon infection, EBs also express the morphogen Wg, which activates dMyc in ISCs leading to proliferation. Finally, Drosophila TGF-βs are secreted by almost every cell type of the midgut as well as by external organs such as tracheae and hemocytes. Early in infection, the TGF-β pathway first promotes ISC proliferation by activating the transcription factor Smox, later on it further inhibits proliferation through a Mad-dependent pathway (highlighted with bold black arrows). EEs are required for the proper regulation of dILP release in the midgut from the VM, a key ligand to control ISC proliferation in response to nutrients, and to the metabolic effect of the microbiota (highlighted with bold purple arrows). EEs also secrete the hormone Bursicon to alter ISC proliferation via control of EGF ligand in the VM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the transcription factor Caudal dampens Imd dependent production of AMPs (Ryu et al., 2008). Another transcription factor, the Oct1 homolog Nubbin represses NF-κB response and thus promotes the tolerance to gut microbiota (Dantoft et al., 2013). Finally, the transcriptional output of the Imd pathway varies in different regions of the gut (Buchon et al., 2009b; Tzou et al., 2000; Vodovar et al., 2005). Alteration of most of these layers of negative regulation induce dysbiosis and are associated with an elevated stress response, loss of enterocytes from the midgut and the triggering of ISC proliferation (Guo et al., 2014; Lhocine et al., 2008). Altogether these data suggest that the multi-faceted regulation of Imd activation prevents intestinal dysbiosis. Failure to maintain an appropriate level of AMPs, either by producing fewer AMPs in an Imd mutant or by making more in a caudal mutant, results in dysbiosis, damage to the epithelium, and ultimately increases stem cell proliferation in the midgut. We hypothesize that one of the key roles of the immune response in the gut is to maintain a tight microbial balance that allows keeping stem cell activity at low levels.

3.2. Reactive oxygen species in gut immunity

Another key conserved immune mechanism in the gut is the production of ROS. In addition to their microbicidal activity, ROS also act as secondary messengers or as signaling modulators in tissue repair, wound healing and hematopoiesis in both *Dmel* and mammals (Anh et al., 2011; Juarez et al., 2011; Razzell et al., 2013). Two major enzymes generate ROS in response to microbes: Duox catalyzes the formation of hydrogen peroxide (H_2O_2) and hypochlorous acid, while NADPH oxidase (Nox) synthesizes H_2O_2 (Ha et al., 2005; Jones et al., 2013). Dual oxidase 2 (Duox2) regulates interactions between the intestinal microbiota and the mucosa to maintain immune homeostasis in mice and is upregulated in patients with inflammatory bowel disease (IBD) before the onset of inflammation (Grasberger et al., 2015). ROS generated by Duox2 selectively mediate the induction of mucins by epidermal growth factor in ECs (Damiano et al., 2015; Grasberger et al., 2015).

In insects, genetic evidence indicates that Duox dependent ROS generation is involved in multiple aspects of gut–microbe interactions, such as microbial elimination, intestinal stem cell activation, redox-dependent regulation of signaling pathways, and cross-linking of the peritrophic matrix (reviewed in Kim and Lee, 2014). Both pathogenic and commensal bacteria have been shown to induce ROS production in the *Dmel* midgut. Duox is activated by oral ingestion of pathogenic microorganisms such as *Ecc15* and *Pe*. Interestingly, impairing ROS production in the fly leads to either a decrease or an increase in the mortality of the fly, depending on the pathogen used. *p38c* mutations decrease ROS production and make *Dmel* more resistant to *Pe*, but this effect of *p38c* is detrimental in the case of *Ecc15* infection (Chakrabarti et al., 2014). The cause of this disparity lies in the level of stress generated by these two pathogens, suggesting that ROS are necessary to eliminate pathogenic bacteria, but are also a source of damage that contributes to the pathogenesis of some bacteria. At high doses, the virulence of *Pe* adds to the stress of immune induced ROS to induce

a generalized translation blockage, impairment of stem cell activity, and ultimately the death of the fly. In line with this finding, sub-lethal doses of *Pe* mimic the effect of infection with *Ecc15*, which triggers moderate EC death and in turn prompts ISC proliferation and repair (Jiang and Edgar, 2009; Vallet-Gely et al., 2010). These data strongly suggest that Duox function has to be regulated to an ideal level to optimize host survival. Accordingly, Duox is regulated at two levels: PLCβ-calcium signaling is responsible for the activation of Duox enzymatic activity whereas the MEKK1-MKK3-p38MAPK-ATF2 pathway is responsible for the induction of Duox gene expression (Chakrabarti et al., 2014; Ha et al., 2009a, 2009b). Under infectious conditions, Duox expression is stimulated by both uracil and PGN, two different bacterial molecules (Ha et al., 2009a; Lee et al., 2013), that lead to maximal ROS production.

Duox expression is kept minimal in response to commensals, and this basal activity prevents yeast overgrowth in the midgut (Ha et al., 2009b). Among gut-associated bacteria, *L. brevis* is able to induce ROS production through Duox (Ha et al., 2009a) and *L. plantarum* induces Nox-mediated ROS generation (Jones et al., 2013). Nox activity consequently promotes ISC proliferation upon initial ingestion of microbes or bacteria in both the murine and *Dmel* intestine (Jones et al., 2015, 2013). This demonstrates that ROS production is not only key to eliminate pathogens, but also to manage commensals. The lifespan of Duox or Nox deficient flies, and that of flies lacking the ROS-detoxifying enzyme immune-regulated catalase is shortened, indicating again that healthy aging can only be achieved when the activity of NADPH oxidases is optimal (Ha et al., 2009a; Jones et al., 2013; Krause, 2007). Duox and Nox show different expression patterns in the various sub-regions and cell types of the midgut, suggesting distinct roles of Duox and Nox in the midgut (Dutta et al., 2015). The current model suggest a direct destructive effect on ECs, which then need to be replaced through ISC dependent regeneration; however, it is also possible that the effect of ROS is indirect, possibly altering bacterial activity and in turn ISC proliferation (Lee and Lee, 2014).

4. Regulation of ISC activity by gut microbes

The gut microbiota, pathogenic bacteria, and the immune response itself all induce stress and damage, and are associated with EC loss. These mechanisms are described below in Section 4.2. ISCs are central to the regeneration of lost tissue, which is achieved through their proliferation following infection/stress and needs to be rigorously regulated to respond correctly to varying levels of damage. Epithelium renewal and ISC activity is regulated by every cell type in the gut and even by neighboring organs. Upon ingestion of pathogens, almost all of the classical developmental signaling pathways are induced to properly regulate ISC proliferation in the effort to rebuild the damaged midgut epithelium. A recent survey of the transcriptome of each gut cell type has shown there are vast changes in gene expression upon infection: 1833 genes were differentially expressed in ISCs, 2646 in EBs, 233 in ECs and 433 in EEs (Dutta et al., 2015). In this section we will review the signaling pathways that regulate *Dmel* ISC activity and discuss how these pathways are coordinated in different cells to converge on the regulation of the proliferative response to infection and stress (Fig. 3, and see Table 1 for a brief description of the signaling pathways). Epithelium renewal is controlled by a number of feedback loops, initiated in ECs, which trigger cell–cell communication that will ultimately reprogram the whole cellular environment of the ISC to promote proliferation. In that regard the VM serves as a niche that integrates signals from the ECs and EBs and modulate ISC behavior through the release of growth factors. This communication network from differentiated cells to the ISCs connects stem cell activity to gut microbes, and is also implicated in tumor initiation (Patel et al., 2015).

Table 1
Signaling pathways found in the Dmel midgut. A brief list of the components and mechanisms of activation of the signaling pathways active in the midgut during ISC regeneration. TF indicates the downstream transcription factor regulating the transcriptional output of the pathway.

Pathway name	Ligands	Receptors	Key components	Target genes	Signaling mechanism
JAK/STAT	Unpaired (Upd1), Upd2, Upd3	Domeless (Dome)	Hopscotch (Hop), STAT (TF)	*Suppressor of cytokine signaling at 36E* (*Socs36E*)	Upon binding, Dome dimerizes and activates Hop, which phosphorylates the TF STAT.
Hippo		Adhesion proteins	Hippo (Hpo), Salvador (Sav), Warts (Wts), Yorkie (Yki, TF)	*diap1* and *cyclin E*	Hpo and Sav complex phosphorylate and activate a Wts–Mats complex. Wts phosphorylates and inhibits the transcriptional co-activator Yki.
JNK			Basket, Hemipterous (Hep), dMKK4, AP-1 (TF including Jun and Fos)	The phosphatase *Puc*	Upstream kinases Hep and dMKK4 can phosphorylate JNK. Jun and Fos are transcriptional activators of target genes.
TGF-β	Decapentaplegic (Dpp), Glass bottom boat (Gbb) and Screw (Scw).	Type I receptors: Thickveins (Tkv), Saxophone (Sax).Type II: Punt (Put), Wishful Thinking (Wit).	Mothers against Dpp (Mad), Smad (TF), Medea (TF)	Daughters against Dpp (Dad)	Ligand binds to tetramers (2x type I, 2x type II). Tetramers with 2 Tkv bind Dpp. Tetramers with 2 Sax receptors bind Gbb. Tetramers phosphorylate and activate the TFs Mad or Smad (depending on tetramers composition and ligand), which will bind to Med to regulate transcription.
Hedgehog	Hedgehog (Hh)	Patched (Ptc)	Smoothened (Smo), Cubitus interrupts (Ci, TF)		Hh binding to Ptc releases repression of Smo, which activates the TF Ci.
Wingless	Wingless (Wg)	Frizzled2 (Fz2), Arrow (Ar)	Dishvelled (Dsh), Armadillo (Arm), Pangolin (TF)		Wg binding to the receptors lead to the activation of Dsh, which inhibits Arm constitutive degradation. Arm and Pangolin complex to regulate gene expression.
EGFR	Gurken, Spitz (Spi), Keren (Krn), Vein (Vn).	Epidermal growth factor receptor (EGFR)	Classical Map Kinase cascade (Ras, MEK, ERK)		Ligand binding to EGFR triggers MAPK cascade. Except for Vn, which is produced as a secreted protein, the ligands need to be processed by the protease Rhomboid (Rho)
Insulin	*Dmel* Insulin ligand peptides (dIlps) 1–7	*Dmel* Insulin Receptor (dInR)	Phosphoinositide-3 kinase (Pi3k) Dp110, Chicho, Pdk1, Akt, FOXO (TF)		dIlps binding to dInr leads to activation of Dp110 through or independent of Chico. This leads to activation of Pdk1, which activates Akt and thus blocks FOXO.

4.1. ISC proliferation is regulated by multiple pathways converging on shared targets

ISC activity is controlled by several signaling pathways including those involved in growth regulation, such as the Hippo, Insulin, and Target of rapamycin (TOR) pathways. ISC behavior is also affected by cytokine and growth factors, including the ligands of the JAK/STAT pathway (Janus kinase (JAK) - Signal Transducer and Activator of Transcription (STAT)), the Epidermal growth factors (EGFs), the Transforming growth factor βs (TGF-βs), and Wingless (Wg). Pathway and growth factor activities intersect and regulate each other to achieve fine regulation of ISCs in both basal conditions and upon immune challenge. While many pathways are involved in ISC regulation, they are funneled toward regulation of shared targets: for example, Hippo, JAK/STAT, JNK, EGF, and Wg pathways are all activated upon bacterial infection to stimulate ISC proliferation, and they all converge on the regulation of the conserved transcription factor dMyc. dMyc is a major transcriptional regulator of growth, and RNAi mediated knockdown of dMyc in ISCs blocks their proliferation autonomously in response to multiple stresses, such as *Pe* infection, or exposure to chemicals such as Dextran Sodium sulfate (DSS) and bleomycin (Ren et al., 2013). It is notable that overexpression of dMyc itself is not sufficient to promote ISC proliferation (Ren et al., 2013). Strikingly, dMyc responds differently depending on the type of stress: for instance dMyc acts downstream or parallel to the Hippo pathway in response to damage by DSS in ISCs and EBs but not in ECs, yet it acts downstream of Hippo, JAK/STAT and EGF receptor (EGFR) signaling in response to bleomycin and *Pe* damage. Indeed, dMyc is a direct transcriptional target of these three pathways (Ren et al., 2010). In *Dmel*, JAK/STAT signaling is activated by three conserved cytokines: Unpaired 1 (Upd1), which is associated with the extracellular matrix, as well as Upd2 and Upd3, which are both diffusible ligands. Binding of these cytokines to the transmembrane receptor Domeless (Dome) triggers dimerization of the receptor and the activation of the Dome associated JAK, which is named Hopscotch (Hop) in *Dmel*. Hop kinases phosphorylate each other and STAT proteins, which then dimerize and travel to the nucleus where they bind to target genes, such as *Suppressor of cytokine signaling at 36E* (*Socs36E*) (reviewed in Myllymäki and Rämet, 2014). Upd1 secreted by stem cells, and Upd2 and Upd3 from ECs and EBs, stimulate JAK/STAT signaling in stem cells. JAK/STAT has an important role in their response to infection. However, it remains unclear whether JAK-STAT is required for proliferation of ISCs due to dMyc regulation, or if it has a cell-non-autonomous role through stimulation of growth factor secretion in the EB (Beebe et al., 2010; Buchon et al., 2009a; Jiang et al., 2009; Liu et al., 2010). Since a role for JAK/STAT in ISCs has only been shown by modulation of the pathway in both ISC and EB simultaneously, rather than by modulation in ISC-only, it remains difficult to clearly identify its role in proliferation. In addition, JAK/STAT signaling plays a major role in stem cell differentiation by interacting with Notch signaling (Buchon et al., 2009b; Jiang and Edgar, 2009). After the proliferative response, JAK/STAT signaling is switched off through Windpipe, which promotes Dome endocytosis and lysosomal degradation (Ren et al., 2015).

As mentioned, the EGFR signaling pathway is another regulator of dMyc activity. EGFR is a receptor tyrosine kinase that activates the classical MAPK kinase cascade of Ras, MEK and ERK. EGFR binds several different ligands in *Dmel*: Gurken, Spitz (Spi), Keren (Krn) and Vein (Vn). Except for Vn, which is secreted constitutively, the other ligands first need to be processed by the protease Rhomboid (Rho) for proper maturation (reviewed in Shilo, 2014). EGFR signaling is central to normal ISC proliferation (Jiang and Edgar, 2009; Xu et al., 2011) and crucial for midgut responses to stress. Inactivation of EGFR signaling in ISCs renders them unable to proliferate in response to different stimuli: *Ecc15* infection (Buchon et al., 2010), *Pe* infection (Jiang et al., 2011) or paraquat ingestion (Biteau and Jasper, 2011). EGFR signaling is also required for ISC proliferation in *Notch* tumors (Biteau and Jasper, 2011). In addition, activation of the EGFR pathway is sufficient to trigger ISC proliferation. EGFR pathway activity is partly mediated by several transcription factors including the HMG/box transcriptional regulator *Capicua*, which regulates genes such as *string*, *Cyclin E*, *Ets21C* and *pointed* (Jin et al., 2015). Altogether, these data suggest that the EGFR pathway is the core regulatory mechanism for ISC proliferation. As previously mentioned, the regulatory pathway activities are complementary: in ISCs, the EGFR pathway acts either downstream or parallel to JAK/STAT signaling (Buchon et al., 2010). JAK/STAT and EGF ligand regulation is complex, and the two pathways intersect in different cell types to obtain appropriate outcomes as required for each cell type in response to immune challenge.

JAK/STAT and EGFR are also implicated in the regulation of dMyc through the Wg signaling pathway: Wg secreted from EBs is required for dMyc-dependent ISC proliferation in regenerating midguts (Cordero et al., 2012b). Wg is the secreted ligand of the homonymous signaling pathway. Briefly, Wg binding to the membrane receptors Frizzled2 (Fz2) and Arrow (Ar) leads to activation of Dishevelled (Dsh), which blocks the constitutive degradation of Armadillo (Arm) allowing it to translocate to the nucleus where it binds Tcf to regulate gene expression (reviewed in Swarup and Verheyen, 2012). Accordingly, Wg signaling repressor Apc1, but not Apc2, is also required in ISCs to dampen proliferation, acting through control of dMyc and its transcriptional partner Max (Cordero et al., 2012a). Absence of Apc1 in ISCs leads to tumor formation, loss of gut homeostasis and ultimately the overexpression of the JAK-STAT cytokine Upd3 in ECs, a strong stimulator of ISC proliferation. However, it is not clear whether Upd3 induction in the EC is cell autonomous or a consequence of epithelial stress induced by the tumor. Apc1 depletion also results in upregulation of Spi in ISCs in a dMyc dependent manner (Cordero et al., 2012a; Tian et al., 2015), leading to up-regulation of both the JAK-STAT and EGFR pathways. Downstream components of Wg pathway, such as Fz, Dsh and Arm, are all required cell autonomously in ISCs for self-renewal (Lin et al., 2008).

dMyc expression is also regulated through the activation of conserved stress responsive kinases such as Jun-N-Terminal Kinase (JNK), whose activation in progenitor cells leads to over proliferation of ISCs (Cordero et al., 2012b). JNK is an evolutionarily conserved stress sensor that is induced by a variety of challenges and triggers many genes involved in cytoprotection, regeneration, apoptosis and growth (Biteau et al., 2011). In *Dmel* the pathway is simple: Basket is the only JNK, while Hemipterous (Hep) and dMKK4 are JNK kinases. Among the targets of JNK are the AP-1 transcription factors Jun and Fos (Kayak in *Dmel*), and the Forkhead Box O (FOXO) transcription factor. One transcriptional target of AP-1 is *puc*, which encodes a phosphatase that acts as a negative regulator of JNK. The downstream transcription factor Fos is required in ISCs for their proliferation following JNK and EGFR signaling (Biteau and Jasper, 2011).

Finally, the insulin pathway, which is another growth pathway, is required for ISC proliferation through the control of dMyc and the TORC1 complex. Binding of *Dmel* Insulin ligand peptides (dIlp) to the *Dmel* Insulin receptor (dInR) leads to the activation of the Phosphoinositide-3 kinase (Pi3k) pathway that in turn activates another kinase, Akt. Activation of the InR pathway prevents the nuclear translocation of FOXO, which occurs when InR pathway activity is low (reviewed in Piper et al., 2011). Loss of TSC (Tuberous Sclerosis Complex), a negative regulator of the TOR pathway, blocks insulin induced proliferation and increases ISC size (Amcheslavsky et al., 2011). Blocking dMyc in flies lacking TSC rescues the

phenotype (Amcheslavsky et al., 2011), arguing that dMyc is a major integrator of growth pathway inputs controlling ISC proliferation. Proper regulation of dMyc is central to maintaining gut homeostasis, and the upregulation of dMyc in the aging midgut contributes to the instance of aberrant ISC proliferation (Cordero et al., 2012b). Additionally, the InR pathway is not only required for ISC proliferation in basal conditions, but also in response to damage inflicted by bleomycin treatment (Amcheslavsky et al., 2009).

Albeit central to proliferation, dMyc is not the sole regulative target of proliferation. Hippo signaling also affects ISC proliferation through chromatin remodeling, thus demonstrating how pathways can have pleiotropic effects altering ISC behavior. The Hippo pathway has a very important role in controlling tissue growth in both *Dmel* and mammals. Specifically, this pathway integrates signals from neighboring cells, including those from cell to cell junctions, and is also thought to respond to physical stimuli. It is regulated by a plethora of different proteins and genes involving cell adhesion molecules and other signaling pathways. A complex composed of Hippo (Hpo), Salvador (Sav) and Warts (Wts) phosphorylates and inhibits the co-activator Yorkie (Yki), thus preventing the activation of genes such as *diap1* and *cyclinE* which delineate organ size (reviewed in Zhao et al., 2011). Brahma (Brm), a central component of the SWI/SFI chromatin remodeling complex, is required in progenitors for ISC proliferation and differentiation in both basal conditions and following DSS exposure. Several components of the Brm complex physically interact with Yki. Loss of Hippo signaling in ISCs, or activation of Yki or Scalloped (Sd) in ISCs leads to increased proliferation, which is blocked by the knockdown of Brm, thus suggesting that Brm acts downstream of Hippo Pathway (Jin et al., 2013). These observations suggest that chromatin remodeling to alter gene accessibility and thus expression is used as a mechanism to regulate ISC proliferation. Further work will be required to elucidate whether chromatin modifications allow ISCs to retain a memory of stress and whether individual ISCs are epigenetically diverse.

Finally, the TGF-β and Hedgehog (Hh) family growth factors act on ISCs to regulate their proliferation. In *Dmel*, TGF-βs include Decapentaplegic (Dpp), Glass bottom boat (Gbb) and Screw (Scw). TGF-β receptors can be of type I or type II. Both are transmembrane receptors that need to form tetramers composed of two type I and two type II receptors. In *Dmel*, there are two type I receptors, either Thickveins (Tkv) or Saxophone (Sax), while the type II receptors are Punt (Put) and Wishful Thinking (Wit). Receptor complexes with two Tkv receptors preferentially bind Dpp, while tetramers with two Sax receptors preferentially bind Gbb. The activated complexes can then phosphorylate and activate the downstream transcription factors Mothers against Dpp (Mad) or Smad, depending on the specific tetramer composition and ligands present. A common output of TGF-β signaling is Daughters against Dpp (Dad) (reviewed in Peterson and O'Connor, 2014). Following damage or *Ecc15* infection, TGF-β signaling is upregulated in the ISCs situated in the anterior and posterior midgut (Guo et al., 2013). However, the function of this pathway in ISCs is described differently by separate groups: some suggested it inhibited proliferation (Guo et al., 2013; Zhou et al., 2015), others found that it stimulated it (Tian and Jiang, 2014). A recent study suggests a complex role of TGF-β signaling on ISC behavior: early in infection, Dpp is secreted from hemocytes to induce ISC proliferation through the Sax-mediated activation of the transcription factor Smox. In the later phase of the regenerative response, ISCs express another receptor, Tkv, diverting the signal to Mad and thus restoring ISC quiescence (Ayyaz et al., 2015). Another paracrine factor stimulating ISC proliferation is the morphogen Hh, which acts as the soluble ligand of the homonymous signaling pathway. Hh binds to the membrane receptor Patched (Ptc), inhibiting Ptc repression of Smoothened (Smo), and thus activating the transcriptional mediator Cubitus interrupts (Ci) (reviewed in Briscoe and Thérond, 2013). In homeostatic conditions Hh is expressed in both ECs and ISCs, but only ISC derived Hh is required to respond to DSS challenges (Lin et al., 2008).

The requirement for so many pathways to control ISC proliferation in response to microbes and other stresses indicates the crucial and preserved role for this process in the re-establishment of homeostasis in the gut. Such tight regulation is necessary considering the vast range of possible damage: from small, tolerable insults due to microbiota, to the life threatening harm caused by pathogens. At the same time, it is interesting to note that these pathways converge on shared targets, like the transcription factor dMyc, to finely regulate ISC proliferation and growth. Recently, another example of integration in ISCs has been proposed, where mitogenic signals originating from Insulin, EGFR and JNK pathways all converge to regulate cytosolic Ca^{2+} levels, which ultimately control ISC activity (Deng et al., 2015).

Fine control is also required from the signal sending cells. As explored in the following sections, many of the pathways found in ISCs are also utilized in signal sending cells. Therefore, signaling pathway integration may not be limited to ISCs, and probably occurs in other cell populations. For example, in ECs the stress response seems to be funneled toward the production of a secreted cytokine, Upd3.

4.2. Enterocytes initiate a conserved homeostatic response to microbes

As discussed earlier, in response to enteric infection a massive proportion of ECs are lost through delamination and possibly other mechanisms, and these must be replaced to maintain gut functions (Buchon et al., 2009b). ECs are the first cells responding to infection and the ECs themselves initiate regeneration by signaling to the rest of the gut. The response to infection is quick: infection with *Pe* leads to increased ISC proliferation within 4 h and the induction of *puc*, *upd*, *Socs36E* and *Delta* within 2 h (Jiang et al., 2009). The main signal from ECs triggering ISCs proliferation is the secretion of the JAK/STAT ligand Upd3. Moreover, overexpression of Upd1 and Upd3 in the midgut leads to increased ISC proliferation even in homeostatic conditions (Buchon et al., 2009a). Upd2 is produced not only by ECs but also from progenitors and has an additive effect to Upd3 following *Ecc15* infection (Osman et al., 2012). Upd3 is strongly induced in ECs upon infection with most bacteria and is required for proper ISC proliferation (Buchon et al., 2009a; Jiang et al., 2009). The secretion of different Upds from different cells in the gut activates the JAK/STAT signaling in EBs and the VM, where they induce growth factor release and coordinate ISC differentiation. We will consider the cell specific effects of STAT signaling for each cell type.

How is the secretion of Upd3 from ECs regulated? Despite the lack of direct evidence, both the JNK and Hippo signaling pathways have been implicated in the induction of Upd3 expression in ECs. JNK, a stress-activated-protein kinase, is activated in the gut soon after microbial infection. Furthermore, ectopic activation of JNK in ECs through either knock down of the negative regulator *puc*, or expression of a constitutively active form of Hep leads to increased ISC mitosis (Buchon et al., 2009a; Jiang et al., 2009). The mechanisms through which JNK signaling influences ISC proliferation still remain to be elucidated. It has been proposed that JNK may control Upd3 expression by modulation of the Hippo pathway in ECs. The Hippo signaling pathway plays a major role in how ECs control ISC proliferation. In homeostatic conditions, loss of Hippo signaling in ECs leads to the secretion of Upd cytokines and the subsequent activation of JAK/STAT in EBs and VM and EGFR signaling in ISCs

(Ren et al., 2010; Staley and Irvine, 2010). JNK stimulates the activation of Yki in ECs in response to damage (Staley and Irvine, 2010). Pez, an evolutionarily conserved protein tyrosine phosphatase, in conjunction with Kibra, an adaptor protein with a tumor suppression role in the Hippo pathway, are also required in ECs to suppress Yki activity (Poernbacher et al., 2012).

ECs not only induce EGF Vn secretion from the VM, but also directly secrete EGFs: Krn was found to be induced in ECs following *Pe* infection and to affect ISC proliferation (Jiang et al., 2011). Under basal conditions, ECs also secrete two TGF-β ligands: Dpp and Gbb. Upon challenge, both Dpp and Gbb are induced in the gut; however, which ligand is important for response to damage and which cell type expresses those ligands remains in debate. ECs are a major source of Dpp according to some (Tian and Jiang, 2014), while other groups show that just Gbb, but not Dpp, is required from ECs for the response to infection (Ayyaz et al., 2015; Li et al., 2013; Zhou et al., 2015). TGF-β seems to have a direct role in the ECs themselves: knock down by RNAi in ECs of TGF-β receptors Tkv or Punt resulted in increased apoptosis in ECs and decreased mitotic indexes in ISCs. In guts with TGF-β deficient ECs, an increase of JAK/STAT and EGFR signaling was found in the VM (Li et al., 2013). These results suggest that the TGF-β pathway impacts ISC proliferation both through direct regulation and by modulation of adult cell survival (Li et al., 2013).

Altogether, these studies demonstrate that the transcriptional regulation of cytokines and growth factors within the ECs is one of the first steps to initiate ISC proliferation in response to stress and immune challenge. Moreover, the current data show that every type of stress triggers Upd3 secretion. Future studies will elucidate how EC secreted factors are regulated and determine how multiple stresses are integrated into the expression of Upd3 to regulate intestinal homeostasis.

4.3. Feedback from the enteroblasts modulates ISC activity

After division, ISCs give rise to EBs, which are transiently differentiating progenitor cells. EBs are often observed adjacent to ISCs in the midgut with their differentiation halted until reactivated in response to damage or differentiative cues (Antonello et al., 2015). EBs are not only involved in differentiation, but they also influence ISC proliferation, notably through the secretion of cytokines and growth factors. EBs secrete the morphogen Wg, as well as JAK/STAT cytokines, and the EGF ligand Spitz, all of which can stimulate ISC proliferation. In homeostatic conditions, Wg is mainly expressed in the VM (Lin et al., 2010, 2008). However, upon challenge either by *Pe*, DSS or bleomycin, EBs induce Wg production, which is required for ISC proliferation. This suggests that two cell types, the EBs and the VM, are the major regulators of ISC proliferation through their secretion of growth factors (Cordero et al., 2012b). EBs also express two Upd cytokines: Upd1 in basal conditions and Upd3 upon infection with *Ecc15* or *Pe* (Liu et al., 2010; Zhou et al., 2013). Upd1 expression is down-regulated by the Notch pathway (Liu et al., 2010), a key pathway that promotes EB differentiation. This suggests that the proliferative signals released by EBs are coupled to their differentiation.

The pathways that control the expression and secretion of growth factors by EBs have just begun to be elucidated. The Hippo pathway plays a central role in EBs, as its inactivation promotes ISC proliferation. Oral infection with *Pe* upregulates Hippo pathway target genes *expanded* and *diap1* in progenitor cells (Shaw et al., 2010). The upstream regulators and downstream targets of Hippo signaling in EBs that control ISC proliferation differ from those acting in ECs, suggesting that the Hippo pathway is a central regulator of midgut proliferation in all epithelial cells. A downstream target of Yki is *bantam*, a microRNA required for the induction of ISC proliferation following DSS-induced damage (Huang et al., 2014). However, it remains unclear whether *bantam* functions in EBs or ISCs. Considering recent data showing a role for Yki in EBs (Li et al., 2014), it is possible that *bantam* is not required cell autonomously in ISCs for their proliferation, but rather in EBs to control ISC proliferation. In EBs, the activation of Hippo signaling also depends on a germinal center kinase, Misshapen (Msn). Msn physically interacts with and modifies Wts to inactivate Yki in a Hippo independent manner, leading to a decrease in the paracrine function of EBs. Loss of *msn* in EBs results in increased ISC proliferation, and is associated with a rise in Upd3 expression (Li et al., 2014). Loss of TGF-β signaling in EBs results in their miss-differentiation, which in turn activates a canonical stress response that leads to the production of Upds and EGFs (Zhou et al., 2015). Finally, Hh signaling is also required in the EBs to promote ISC proliferation. Inhibition of Hh signaling within progenitor cells results in lack of proliferation following bleomycin or DSS challenge, and induction of Hh signaling results in the upregulation of Upd2 in EBs. Accordingly, knock-down of Upd2 specifically in EBs leads to decrease in ISC proliferation (Tian et al., 2015). Altogether, it seems that EBs share similar ligands as ECs in controlling ISC proliferation, including Upds and EGFs. However, as is the case for the Hippo pathway, the regulation of secretion of those factors may differ within the two cell types. Future work should elucidate how and the same signaling pathways can be used to regulate ISCs activity through different mechanisms.

4.4. Visceral muscles act as a niche for ISCs

VM, together with the EBs, is another cell type that produces the key growth factors that trigger ISC proliferation both in basal conditions and upon damage or bacterial infection. It is also the only cell type that can be considered a true ISC niche by strict definition due to its stability, while ECs and EBs are more transitory and differentiate from the ISCs themselves. As such, the VM integrates multiple signaling pathways to control the release of EGF Vn, the morphogens Wg and Dpp, and the *Dmel* insulin like peptide (dIlp) 3 (Cordero et al., 2012b; Lin et al., 2008; O'Brien et al., 2011). EGF Vn expression in the VM is required for ISC proliferation following exposure to *Ecc15*, *Pe* and paraquat, while the two other EGFs, Krn and Spi, are required in ECs and EBs respectively (Biteau and Jasper, 2011; Buchon et al., 2010; Jiang et al., 2011). The EGFs may be interchangeable, as RNAi knock-down of any single EGF is insufficient to completely block ISC proliferation induced by *Ecc15* infection (Buchon et al., 2010). Furthermore, overexpression of secreted Spi in the VM can rescue *vn* RNAi (Xu et al., 2011), demonstrating that multiple cell types, the ECs, EBs and VM, all act in synergy by producing different EGFs with overlapping activities. In the VM, *vn* is regulated by two pathways; JAK/STAT and Bursicon. Knocking down the transcription factor Stat in VM (Buchon et al., 2010; Jiang et al., 2011) or the cytokine Upd3 in ECs down-regulates *vn* expression following infection (Buchon et al., 2010). In addition, the hormone Bursicon is secreted from EEs and binds to its receptor, Rickets (Rk, also called DLGR2), in the VM, leading to suppression of *vn* expression through activation of cAMP (Scopelliti et al., 2014). In basal conditions, the VM also secretes Wg to promote ISC proliferation and differentiation (Lin et al., 2008), though the EB is the primary source of Wg upon bacterial infection (Cordero et al., 2012b).

The TGF-β ligand Dpp is also induced in VM by treatment with bleomycin or paraquat and its depletion from the VM results in a reduction of TGF-β signaling in ISCs (Guo et al., 2013; Zhou et al., 2015). JAK/STAT is required for Dpp expression in the VM in response to bleomycin (Guo et al., 2013). The VM thus acts both as a regulatory niche to influence ISC activity under a variety of

conditions, and as an integrator, relaying signals from other cell types. It will be interesting to investigate whether there is also feedback signaling from ISCs to VM that can inhibit the expression of growth factors in VM in a post proliferative stage.

4.5. Enteroendocrine cells have a minor role in the regulation of ISC proliferation

EEs have an important secretory role during the immune response (Beebe et al., 2015; Dutta et al., 2015), but do not seem to be main regulators of the ISCs in response to those challenges. Instead, EEs seem to regulate ISC proliferation in response to nutrient changes. The expression level of dllp3 correlates to EE number, suggesting that EEs link nutrient sensing to growth (Amcheslavsky et al., 2014). It is thus possible to speculate that EEs have a role in the sensing of microbiota, considering the link between microbiota and Insulin signaling (Shin et al., 2011). EEs also secrete the neuroendocrine hormone Bursicon (Burs), which acts as paracrine factor binding to its receptor Rk in the VM to suppress ISC proliferation through control of *vn* expression. Overexpression of Burs in *Pe* challenged guts is adequate to impede ISC propagation, although *Burs* transcription levels are not affected by infection with *Pe* (Scopelliti et al., 2014).

4.6. Hemocytes and other organs participate in the control of ISC proliferation

Epithelial cells are not the only source of the growth factor cocktail to which ISCs are exposed. A population of hemocytes is found attached to the midgut, and their presence is important to regulate the response to *Ecc15* and *Pe* infections and paraquat exposure. Flies depleted of their hemocytes via apoptosis are more susceptible to oral infection by *Pe* (Ayyaz et al., 2015). During infection or injury, hemocytes secrete the TGF-β Dpp, which is required at early phases of bacterial infection to induce the TGF-β pathway and trigger proliferation in ISCs (Ayyaz et al., 2015). At later stages of infection, ISCs switch their TGF-β pathway to a pro-quiescence state, allowing the midgut to return to homeostasis as described in the ISC Section 4.1. Thus the physiological status of the ISC may alter the ultimate function of the TGF-β pathway, from being an inducer to a repressor of proliferation (Ayyaz et al., 2015; Guo et al., 2013).

ISC proliferation is also affected by systemic signals originating from the brain and by expression of insulin peptides from the tracheae. Notably, Dpp is also expressed in tracheal cells and stimulates TGF-β pathway activity in the intestinal epithelium, subsequently decreasing ISC proliferation (Li et al., 2013). Ablation of the insulin producing cells (IPCs) in the brain by targeted apoptosis impairs the ISC response to ingestion of DSS and bleomycin. dllp2 itself is responsible for the control of ISC proliferation and is not expressed in the gut (Amcheslavsky et al., 2009). In intestinal neurons, dllp7 seems to have a role in regulating intestinal physiology (Cognigni et al., 2011). These results argue that the regulation of stem cells depends on not only multiple local cues, but also key systemic signals.

5. The ISC response to various bacteria is controlled by a single network

In the past few years, multiple examples have been described in which bacteria alter the behavior of ISCs, from the effect of pathogens that induce a strong homeostatic repair loop to the milder effect of the microbiota that modifies basal ISC activity.

5.1. Effects of different types of pathogens on ISC regulation

An interesting question is whether different bacteria causing dramatically distinct infections all influence the immune response and subsequent ISC proliferation through the same/similar mechanisms or through multiple ways. As noted in this review, several oral infection models of *Dmel* have been studied and discussed using multiple bacteria species: *Ecc15*, *Pe*, *Sm* and *Pa*. We have also described in Section 2.2 that these pathogens have different mechanisms of pathogenicity. However, the response of the gut to these pathogens seems to be generalized, including the immune defenses that rely mainly on the induction of AMPs via the Imd pathway and the production of ROS (reviewed in Buchon et al., 2013; You et al., 2014). As a consequence, all types of infections lead to the induction of inflammatory cytokines such as Upd3, which stimulate intestinal stem cell proliferation to restore gut homeostasis. Nevertheless, different infections can have different effects. For instance, *Dmel* survives infection with *Ecc15*, but succumbs to infection with *Pe*, despite the bacteria being eliminated from the gut in both scenarios (Chakrabarti et al., 2012). In this example, the difference resides in the ability of the host to induce ISC-meditated tissue renewal, which is prevented in the case of *Pe* due to translational blockage. This suggests that rather than qualitative differences in the pathways controlling epithelium renewal, combinations of various levels of stress, damage, and repair determine the outcome of different infections. Therefore, it seems that the response to each pathogen involves the same regulatory network of the host, but different levels of virulence will change the ISC response.

5.2. Role of different microbiota in ISC renewal

It has been demonstrated that gut microbiota play an essential role for basal midgut turnover and ISC renewal. Accordingly, CR flies have higher mitotic indexes compared to GF flies (Buchon et al., 2009a; Shin et al., 2011). The microbiota may act by two different mechanisms: like pathogens, though to a lesser extent, the microbiota trigger ISC proliferation by inducing some lesser amount of stress and damage. In addition, the microbiota can alter ISC function by modulating nutrition, and therefore the metabolic properties of the midgut.

Gut microbiota activate ISC division and differentiation at basal levels through the same JNK and JAK/STAT pathways involved in the immune response to pathogenic bacteria, albeit in a reduced manner. Indeed, in immune-deficient or aged flies, altered ability to control the gut microbiota correlates with increased epithelium renewal (Buchon et al., 2009a). In addition, it is increasingly recognized that ROS function as second-messenger signaling that can also influence cellular proliferation and differentiation in a variety of biological systems. Lee et al. demonstrated that bacteria-derived Uracil acts as a ligand for Duox-dependent ROS generation in the *Dmel* gut, subsequently inducing ISC proliferation. Among the five most abundant species of the *Dmel* microbiota, *L. brevis* was identified as a source of Uracil (Lee et al., 2013). However, in another study by Jones and colleagues, exposure to *L. plantarum* induced ROS production dependent on Nox instead of Duox and activated cellular proliferation (Jones et al., 2013). Future studies should clarify the molecular mechanisms in both the bacteria and the host underlying *Lactobacilli* promotion of epithelial homeostasis.

The microbiota also influence ISC proliferation through effects on the host metabolism. For instance, *A. pomorum* modulates insulin through its pyrroloquinoline quinone–dependent alcohol dehydrogenase activity that generates acetic acid and, in turn, regulates body size, energy metabolism and ISC activity (Shin et al.,

2011). Interestingly, the insulin pathway has been demonstrated to allow the *Dmel* midgut to grow in response to nutrients (O'Brien et al., 2011). These data together suggest that the impact of the microbiota on the *Dmel* midgut could be more complex than pathogens, as they can modulate responses to both damage and nutrition.

5.3. Are stresses converging through the same mechanisms?

Multiple pathogens and multiple stresses converge on one single network of ISC proliferation. One possibility would be that all stresses stimulate the secretion of growth factors by the epithelium through multiple means. Stress through the studied microbiota and chemicals such as bleomycin and paraquat converge on EC damage and consequent loss, which acts as a trigger to induce ISC proliferation through the same regenerative pathways. Inorganic mercury ($HgCl_2$) has a similar effect on ISC proliferation through damage to ECs, although its effect on signaling pathways has not been studied yet (Chen et al., 2015). However, DSS has a different mechanism of action: DSS disrupts the organization of the basement membrane without damaging ECs, while still causing ISC proliferation. After treatment with DSS, newly produced EBs do not differentiate into ECs and accumulate, while bleomycin treatment and bacterial challenge both induce EB differentiation (Amcheslavsky et al., 2009). Accordingly, bleomycin and *Pe* but not DSS activate JAK/STAT in ISCs (Ren et al., 2010). To induce ISC proliferation in response to DSS, Yki is required in progenitor cells but not in ECs. However, Yki is necessary in both cell types in response to bleomycin and *Pe* (Ren et al., 2010). Altogether, these results suggest that with the exception of DSS, the response to different stressors all use the same small set of pathways and secreted factors to promote ISC proliferation in a common manner. It would be interesting to explore if DSS is a rare exception and if certain microbiota can also act through disruption of the basal membrane but not EC damage in order to elude the immune response and gain access to the fly. Fig. 4 summarizes the response of the gut to different challenges.

6. Control of ISC differentiation in response to microbes

As previously discussed, EEs serve an important role in the immune response to pathogenic microbes, and depletion of EEs accelerates the death of flies upon *Pe* infection (Dutta et al., 2015). The transcription factor *dimm* has been shown to be responsible for the immune response in EEs by regulating the expression of neuropeptides and AMPs (Beebe et al., 2015), suggesting that the balance of ECs and EEs in the gut is an important parameter for microbial regulation. Little evidence suggests that bacteria could alter this balance by modulating ISC differentiation: Broderick et al. showed that an increased ratio of EE cells over total cells occurs in

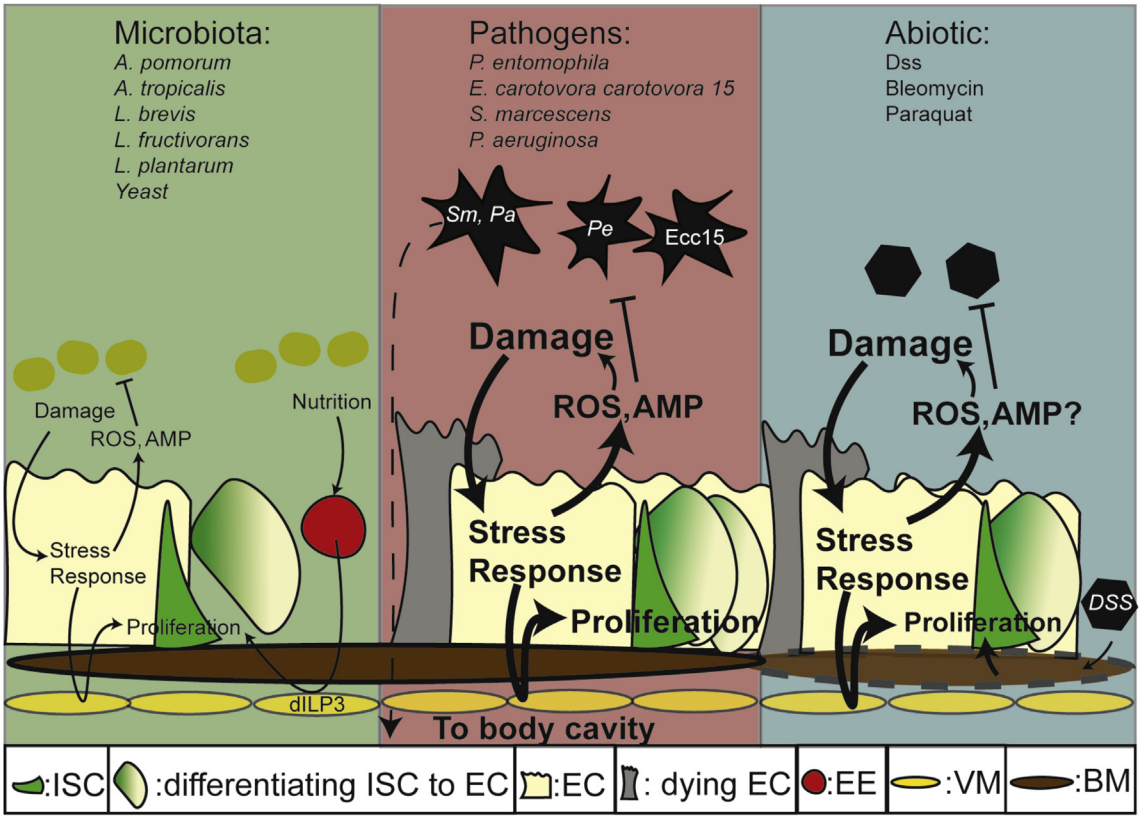

Fig. 4. Effect of the microbiota, pathogenic microbes, and abiotic damage on ISC activity. Abbreviations: Intestinal stem cell (ISC), Enterocyte (EC), Enteroendocrine cell (EE), Visceral muscle (VM), Basal membrane (BM). In the green shading on the left is summarized the response to the Dmel gut microbiota. Microbiota trigger mild immune responses that in turn damage ECs, thus inducing low levels of homeostatic proliferation. However, they also have a positive influence on ISC activity by modulating nutrient availability and modifying host physiology and insulin pathway. In the central red shading is described the response to Pathogens. Virulent bacteria inflict damage to the ECs either as a consequence of their virulence or of the strong immune response they induce. This leads to a massive loss of ECs, which need to be replaced through a sustained proliferation of ISCs. Some pathogens, such as *Serratia marcescens* (Sm) and *Pseudomonas aeruginosa* (Pa) have the ability to cross the intestinal barrier. In the blue shading on the right is summarized the response to abiotic challenges. Chemicals such as bleomycin and paraquat induce a strong stress response that damages ECs and activates the same pathways as the response to pathogens. DSS seems to have a different mechanism of action, acting through disruption of the Basal Membrane. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

GF guts, but the bacteria and mechanism underlying this phenotype have yet to be discovered (Broderick et al., 2014). The main signaling pathways regulating differentiation, and thus EE to total cell ratio, are Notch and JAK/STAT: EBs with high levels of Notch signaling further develop into ECs while EBs with low Notch activity become EE cells. Recent studies proposed a new model for EE cell fate determination in which only ECs are generated through immature progenitor EBs, whereas EEs are generated through a distinct pre-EE progenitor cell (Zeng and Hou, 2015). Other studies also demonstrated a negative feed-back control of EE regeneration through Slit/Robo2 signaling in ISCs but not EBs (Biteau and Jasper, 2014; Zeng et al., 2015). Members of all the pathways regulating differentiation, including JAK-STAT, Notch and Prospero levels are altered upon infection with the pathogen *Pe* (Dutta et al., 2015) or by the gut microbiota (Broderick et al., 2014). This suggests that, in addition to modulating ISC proliferation, bacteria could also regulate cell fate in the gut. Future work should determine to what extent bacteria affect ISC lineage.

7. Future perspectives and conclusion

There are still many points to further clarify to understand the immune and regenerative response of the *Dmel* midgut. While the control of proliferation in response to pathogenic infection has been widely studied, the control of differentiation has been mostly studied in homeostatic conditions. However, differentiation plays a major role in gut physiology, and it would be interesting to elucidate the long-term consequences of infections on gut homeostasis. Furthermore, it has recently been demonstrated that stem cells are regionalized and show region specific behavior in the midgut (Buchon et al., 2013b; Dutta et al., 2015; Marianes and Spradling, 2013). Different midgut regions assume different functions, and respond to bacteria at different levels (Bosco-Drayon et al., 2012; Buchon and Osman, 2015; Combe et al., 2014; Neyen et al., 2012). In addition, the gut express different AMPs in each region, which may reflect underlying regionalization of the ISCs themselves. Future work will try to elucidate how stem cell behavior, stress, and bacterial activity are coordinated together in different regions. Regionalization of stem cells is a conserved feature in insects as well as mammals, where differences in ISCs are observed between large and small intestine (Cramer et al., 2015). Moreover, while several different organs, such as the brain and hemocytes, are found to be influencing the gut during the immune response, how the gut response interacts with those organs, and how the dialogue between tissues is established remain intriguing questions. Future work will elucidate whether the gut itself signals these organs, or if other mechanisms allow distant organs to sense changes in gut homeostasis. While in the *Dmel* midgut the recognition of all stresses seems to flow through a consolidated and common response, how different types of damage and bacteria alter gut homeostasis and ISC proliferation is still not clear. Microbiota play an important and conserved role in many diseases, both in *Dmel* and in humans. Clarification of the mechanisms through which gut microbes influence ISC activity will be vital to identify novel treatments for diseases caused by bacterial unbalance.

Finally, the signaling network controlling ISCs is complex and overlapping to ensure proper response and regeneration in the face of a wide array of challenges. The theme that seems to emerge from recent publications is that this control is funneled toward a few conserved signaling pathways in ISCs that control key transcription factors such as dMyc. This type of funneling pattern is seen also in other midgut cells, such as the ECs, where the response to infection is geared toward the control of Upd3 to properly influence ISC proliferation. ISC regeneration has shown to be vital for the proper functioning of the midgut and many other organs both in physiological and pathological conditions. All in all, the *Drosophila melanogaster* midgut appears to be one of the best models in which to study stem cell regeneration and pathogenesis of infection, thus providing insights that can be used toward the understanding of intestinal physiology and gut-related human diseases.

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