1 **From pathogens to microbiota: how *Drosophila* intestinal stem cells**
2 **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 needs to adapt to changes in metabolism and nutrition (Buchon et al., 2013a; Gerseemann 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 hours (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 (Gerseemann 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 Lerna 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). *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: ISC, enteroblasts (EB), EC, and enteroendocrine cells (EE). 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

139 damage due to the associated microbial population and food ingestion. In this section we will review the
140 bacterial species in the *Dmel* gut that can influence ISC activity either through changes in nutrition or
141 damage.

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143 2.1. The *Dmel* microbiota

144 The role of *Dmel* associated microbes in the midgut is hard to understand with only conventional
145 reared (CR) *Dmel*, which are reared without any manipulation or alteration of the microbiota. Fortunately, a
146 number of studies using germ free (GF) and with gnotobiotic *Dmel*, which are GF *Dmel* re-associated with
147 selected microbial species, have examined their impact on *Dmel* physiology. In this regard, *Dmel* provides a
148 simpler system than mammals, thus making it a powerful model to understand gut-microbe interactions
149 (Broderick et al., 2014). Gut microbes are not required for the survival of *Dmel* itself, since GF flies can be
150 maintained on sterile food for generations (Bakula, 1969). Moreover, the association between the microbial
151 community and its host is not stably maintained and is shaped through constant ingestion of microbes (Blum
152 et al., 2013). Most flies have a low bacterial count of a few thousand bacteria per midgut. This number is
153 proportional to the amount of food present in the lumen, suggesting that the bulk of bacteria are associated
154 with food passing through the midgut. Despite this apparent instability, the *Dmel* microbiome includes 5
155 bacterial species that are frequently found in both laboratory-raised and wild-caught flies (Chandler et al.,
156 2011). These species comprise *Acetobacter pomorum*, *A. tropicalis*, *Lactobacillus brevis*, *L. fructivorans* and
157 *L. plantarum*. Flies captured in the wild have a more diverse microbiome compared to flies reared in the lab,
158 including Proteobacteria and species previously identified as pathogens, such as *Providencia*, *Serratia*,
159 *Erwinia*, *Pantoea*, *Enterococcaceae* and *Enterobacteriaceae* and *Pseudomonas*. However, even in wild-
160 caught *Dmel*, *Acetobacter* and *Lactobacillus* species are still the most commonly associated bacteria (Brewer
161 et al., 1981; Broderick and Lemaitre, 2012; Chandler et al., 2011; Cox and Gilmore, 2007; Flyg et al., 1980;
162 Galac and Lazzaro, 2011; Juneja and Lazzaro, 2009; Kloepper et al., 1981; Molina et al., 1974; Nmorsi et al.,
163 2007; Vodovar et al., 2005). In lab-reared adult flies, initial reports suggested that *L. fructivorans* is
164 dominant in young individuals, though the community shifts towards an abundance of *A. pomorum* at 3 to 5
165 weeks of age (Wong et al., 2011). However, a more recent report showed *Acetobacter* to be already abundant
166 in young *Dmel*. This effect depends on the amount of eggs deposited in each vial (Wong et al., 2015),
167 reinforcing the idea that transmission of microbes from generation to generation occurs through the
168 deposition of microbes on the eggs (Bakula, 1969). Regardless of the potential shift from *Lactobacillus* to
169 *Acetobacter*, aging flies show both higher bacterial titers, increased microbial diversity (Broderick et al.,
170 2014) and increased intestinal barrier permeability (Clark et al., 2015). This change may depend on the
171 alteration of the immune response in old flies, called immuno-senescence, and ultimately results in increased
172 ISC proliferation and differentiation, a conserved feature of dysbiosis (Buchon et al., 2009a; Guo et al.,
173 2014).

174 Recently, comparative studies of the transcriptome of GF and CR *Dmel* guts have provided a good
175 description of the effect of microbiota on gut physiology: 152 genes are differentially regulated between GF
176 and CR guts, and gut microbes affect gut physiology by stimulating NF- κ B-regulated immune responses
177 through the immune deficiency (Imd) pathway, altering tissue homeostasis and ISC activity, and modulating
178 gut metabolism and the expression of digestive enzymes (Broderick et al., 2014; Combe et al., 2014). A first
179 effect of the microbiota is to stimulate intestinal turnover and ISC proliferation (Broderick et al., 2014;
180 Buchon et al., 2009a). This results from a combination of the immune response against the microbiota
181 (Buchon et al., 2009a; Ha et al., 2005) and metabolic changes induced in the midgut (Shin et al., 2011). The
182 damaging effect of the microbiota is more evident in old flies, as the gut tissues of GF flies show less over-
183 proliferation and miss-differentiation than CR flies. Consistent with this, GF flies showed an increased
184 lifespan compared to CR flies in two reports (Clark et al., 2015; Guo et al., 2014), although no lifespan
185 extension was detected in Ren et al., (2007); it is possible that this discrepancy was due to differences in
186 culture conditions or microbial composition. One consequence of the aberrant epithelium renewal induced in
187 old flies is disruption of gut barrier integrity, which triggers a systemic activation of the immune response.
188 Accordingly, loss of gut barrier function seems to be a strong predictor of imminent death (Clark et al., 2015;
189 Rera et al., 2012). A recent study also suggests that administration of the drug Rapamycin extends lifespan,
190 despite the decrease of gut barrier functionality, through alteration of microbial density in the midgut (Fan et
191 al., 2015). Altogether, these reports endorse a model by which loss of immune competence in aged flies
192 affects the gut microbes that in turn trigger aberrant renewal, barrier dysfunction, and the death of the fly. In
193 this framework, the loss of the immune capability to maintain a healthy microbiota and the proper regulation
194 of ISCs are the two major drivers of age related gut dysfunction.

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

210 dependent Cdk9-dependent signaling is also required, thus suggesting that sensing of specific microbes or
211 bacteria prepares the immune system for antiviral response (Sansone et al., In press; Xu et al., 2013).

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

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

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239 2.2. Pathogenic bacteria

240 Unlike the normal microbiota, pathogenic bacteria trigger a violent response that leads to EC loss
241 and shortening of the gut, and greatly increase ISC proliferation as described in sections 3 and 4. Due to this
242 effect, pathogens have become an essential tool to study not only the immune response, but also proliferation
243 and differentiation mechanisms in ISCs. There are several bacterial species that have been shown to be
244 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, schematized in Figure 1 and summarized in Figure 4.

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).

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 figure 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

316 components of the Imd pathway are more susceptible to enteric infections by *Sm* and *Pe*, demonstrating a
317 key role of the Imd pathway in intestinal immunity (Basset et al., 2000; Liehl et al., 2006). In the gut, two
318 receptors, PGRP-LC (transmembrane-type; Peptidoglycan recognition protein LC) and PGRP-LE
319 (intracellular-type; Peptidoglycan recognition protein LE), bind diaminopimelic acid (DAP)-type
320 peptidoglycan that composes the cell wall of Gram-negative bacteria and of certain *Lactobacilli* (Kaneko et
321 al., 2004; Leulier et al., 2003; Takehana et al., 2002). During the activation of the Imd pathway, the bacteria-
322 sensors PGRP-LC and/or PGRP-LE recruit the adaptor molecule Imd and form a complex with dFADD
323 (Fas-associated death domain protein) and Dredd (Death-related ced-3/NEDD2-like protein). This complex
324 activates the Mitogen Activated Protein Kinase Kinase (MAPKK) TAK1 (TGF beta activated kinase 1) and
325 eventually leads to the activation of the IKK complex, IKK β /IRD5 and IKK γ /Key. This in turn activates the
326 NF- κ B family member Relish (Rel), which is cleaved by the caspase Dredd allowing for nuclear
327 translocation of the Rel homology domain. The two dominant members of the *Dmel* microbiota, *Acetobacter*
328 and *Lactobacillus* spp., both contain DAP-type peptidoglycan, and can therefore activate AMP production.
329 In agreement with this, it has been shown that GF flies exhibit lower basal expression levels of AMPs in the
330 gut (Buchon et al., 2009a; Lhocine et al., 2008; Ryu et al., 2008) and that old flies show higher levels of Imd
331 pathway activity, consistent with their increased bacterial loads of older flies.

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

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

365

366 3.2. Reactive oxygen species in gut immunity

367 Another key conserved immune mechanism in the gut is the production of ROS. In addition to their
368 microbicidal activity, ROS also act as secondary messengers or as signaling modulators in tissue repair,
369 wound healing and hematopoiesis in both *Dmel* and mammals (Anh et al., 2011; Juarez et al., 2011; Razzell
370 et al., 2013). Two major enzymes generate ROS in response to microbes: Duox catalyzes the formation of
371 hydrogen peroxide (H₂O₂) and hypochlorous acid, while NADPH oxidase (Nox) synthesizes H₂O₂ (Ha et al.,
372 2005; Jones et al., 2013). Dual oxidase 2 (Duox2) regulates interactions between the intestinal microbiota
373 and the mucosa to maintain immune homeostasis in mice and is upregulated in patients with inflammatory
374 bowel disease (IBD) before the onset of inflammation (Grasberger et al., 2015). ROS generated by Duox2
375 selectively mediate the induction of mucins by epidermal growth factor in ECs (Damiano et al., 2015;
376 Grasberger et al., 2015).

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

410

411 **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).

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 β s (TGF- β s), 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 Rämetsä, 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,

462 JAK/STAT signaling plays a major role in stem cell differentiation by interacting with Notch signaling
463 (Buchon et al., 2009b; Jiang and Edgar, 2009). After the proliferative response, JAK/STAT signaling is
464 switched off through Windpipe, which promotes Dome endocytosis and lysosomal degradation (Ren et al.,
465 2015).

466 As mentioned, the EGFR signaling pathway is another regulator of dMyc activity. EGFR is a
467 receptor tyrosine kinase that activates the classical MAPK kinase cascade of Ras, MEK and ERK. EGFR
468 binds several different ligands in *Dmel*: Gurken, Spitz (Spi), Keren (Krn) and Vein (Vn). Except for Vn,
469 which is secreted constitutively, the other ligands first need to be processed by the protease Rhomboid (Rho)
470 for proper maturation (reviewed in Shilo, 2014). EGFR signaling is central to normal ISC proliferation (Jiang
471 and Edgar, 2009; Xu et al., 2011) and crucial for midgut responses to stress. Inactivation of EGFR signaling
472 in ISCs renders them unable to proliferate in response to different stimuli: *Ecc15* infection (Buchon et al.,
473 2010), *Pe* infection (Jiang et al., 2011) or paraquat ingestion (Biteau and Jasper, 2011). EGFR signaling is
474 also required for ISC proliferation in *Notch* tumors (Biteau and Jasper, 2011). In addition, activation of the
475 EGFR pathway is sufficient to trigger ISC proliferation. EGFR pathway activity is partly mediated by
476 several transcription factors including the HMG/box transcriptional regulator *Capicua*, which regulates
477 genes such as *string*, *Cyclin E*, *Ets21C* and *pointed* (Jin et al., 2015). Altogether, these data suggest that the
478 EGFR pathway is the core regulatory mechanism for ISC proliferation. As previously mentioned, the
479 regulatory pathway activities are complementary: in ISCs, the EGFR pathway acts either downstream or
480 parallel to JAK/STAT signaling (Buchon et al., 2010). JAK/STAT and EGF ligand regulation is complex,
481 and the two pathways intersect in different cell types to obtain appropriate outcomes as required for each cell
482 type in response to immune challenge.

483 JAK/STAT and EGFR are also implicated in the regulation of dMyc through the Wg signaling
484 pathway: Wg secreted from EBs is required for dMyc-dependent ISC proliferation in regenerating midguts
485 (Cordero et al., 2012b). Wg is the secreted ligand of the homonymous signaling pathway. Briefly, Wg
486 binding to the membrane receptors Frizzled2 (Fz2) and Arrow (Ar) leads to activation of Dishevelled (Dsh),
487 which blocks the constitutive degradation of Armadillo (Arm) allowing it to translocate to the nucleus where
488 it binds Tcf to regulate gene expression (reviewed in Lento et al., 2013). Accordingly, Wg signaling
489 repressor Apc1, but not Apc2, is also required in ISCs to dampen proliferation, acting through control of
490 dMyc and its transcriptional partner Max (Cordero et al., 2012a). Absence of Apc1 in ISCs leads to tumor
491 formation, loss of gut homeostasis and ultimately the overexpression of the JAK-STAT cytokine Upd3 in
492 ECs, a strong stimulator of ISC proliferation. However, it is not clear whether Upd3 induction in the EC is
493 cell autonomous or a consequence of epithelial stress induced by the tumor. Apc1 depletion also results in
494 upregulation of Spi in ISCs in a dMyc dependent manner (Cordero et al., 2012a; Tian et al., 2015), leading to
495 up-regulation of both the JAK-STAT and EGFR pathways. Downstream components of Wg pathway, such
496 as Fz, Dsh and Arm, are all required cell autonomously in ISCs for self-renewal (Lin et al., 2008).

497 dMyc expression is also regulated through the activation of conserved stress responsive kinases such
498 as Jun-N-Terminal Kinase (JNK), whose activation in progenitor cells leads to over proliferation of ISCs

499 (Cordero et al., 2012b). JNK is an evolutionarily conserved stress sensor that is induced by a variety of
500 challenges and triggers many genes involved in cytoprotection, regeneration, apoptosis and growth (Biteau et
501 al., 2011). In *Dmel* the pathway is simple: Basket is the only JNK, while Hemipterous (Hep) and dMKK4 are
502 JNK kinases. Among the targets of JNK are the AP-1 transcription factors Jun and Fos (Kayak in *Dmel*), and
503 the Forkhead Box O (FOXO) transcription factor. One transcriptional target of AP-1 is *puc*, which encodes a
504 phosphatase that acts as a negative regulator of JNK. The downstream transcription factor Fos is required in
505 ISCs for their proliferation following JNK and EGFR signaling (Biteau and Jasper, 2011).

506 Finally, the insulin pathway, which is another growth pathway, is required for ISC proliferation
507 through the control of dMyc and the TORC1 complex. Binding of *Dmel* Insulin ligand peptides (dIlp) to the
508 *Dmel* Insulin receptor (dInR) leads to the activation of the Phosphoinositide-3 kinase (Pi3k) pathway that in
509 turn activates another kinase, Akt. Activation of the InR pathway prevents the nuclear translocation of
510 FOXO, which occurs when InR pathway activity is low (reviewed in Piper et al., 2011). Loss of TSC
511 (Tuberous Sclerosis Complex), a negative regulator of the TOR pathway, blocks insulin induced
512 proliferation and increases ISC size (Amcheslavsky et al., 2011). Blocking dMyc in flies lacking TSC
513 rescues the phenotype (Amcheslavsky et al., 2011), arguing that dMyc is a major integrator of growth
514 pathway inputs controlling ISC proliferation. Proper regulation of dMyc is central to maintaining gut
515 homeostasis, and the upregulation of dMyc in the aging midgut contributes to the instance of aberrant ISC
516 proliferation (Cordero et al., 2012b). Additionally, the InR pathway is not only required for ISC proliferation
517 in basal conditions, but also in response to damage inflicted by bleomycin treatment (Amcheslavsky et al.,
518 2009).

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

536 Finally, the TGF- β and Hedgehog (Hh) family growth factors act on ISCs to regulate their
537 proliferation. In *Dmel*, TGF- β s include Decapentaplegic (Dpp), Glass bottom boat (Gbb) and Screw (Scw).
538 TGF- β receptors can be of type I or type II. Both are transmembrane receptors that need to form tetramers
539 composed of two type I and two type II receptors. In *Dmel*, there are two type I receptors, either Thickveins
540 (Tkv) or Saxophone (Sax), while the type II receptors are Punt (Put) and Wishful Thinking (Wit). Receptor
541 complexes with two Tkv receptors preferentially bind Dpp, while tetramers with two Sax receptors
542 preferentially bind Gbb. The activated complexes can then phosphorylate and activate the downstream
543 transcription factors Mothers against Dpp (Mad) or Smad, depending on the specific tetramer composition
544 and ligands present. A common output of TGF- β signaling is Daughters against Dpp (Dad) (reviewed in
545 Peterson and O'Connor, 2014). Following damage or *Ecc15* infection, TGF- β signaling is upregulated in the
546 ISCs situated in the anterior and posterior midgut (Guo et al., 2013). However, the function of this pathway
547 in ISCs is described differently by separate groups: some suggested it inhibited proliferation (Guo et al.,
548 2013; Zhou et al., 2015), others found that it stimulated it (Tian and Jiang, 2014). A recent study suggests a
549 complex role of TGF- β signaling on ISC behavior: early in infection, Dpp is secreted from hemocytes to
550 induce ISC proliferation through the Sax-mediated activation of the transcription factor Smox. In the later
551 phase of the regenerative response, ISCs express another receptor, Tkv, diverting the signal to Mad and thus
552 restoring ISC quiescence (Ayyaz et al., 2015). Another paracrine factor stimulating ISC proliferation is the
553 morphogen Hh, which acts as the soluble ligand of the homonymous signaling pathway. Hh binds to the
554 membrane receptor Patched (Ptc), inhibiting Ptc repression of Smoothened (Smo), and thus activating the
555 transcriptional mediator Cubitus interruptus (Ci) (reviewed in Briscoe and Théron, 2013). In homeostatic
556 conditions Hh is expressed in both ECs and ISCs, but only ISC derived Hh is required to respond to DSS
557 challenges (Lin et al., 2008).

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

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

570

571 4.2. Enterocytes initiate a conserved homeostatic response to microbes

572 As discussed earlier, in response to enteric infection a massive proportion of ECs are lost through
573 delamination and possibly other mechanisms, and these must be replaced to maintain gut functions (Buchon
574 et al., 2009b). ECs are the first cells responding to infection and the ECs themselves initiate regeneration by
575 signaling to the rest of the gut. The response to infection is quick: infection with *Pe* leads to increased ISC
576 proliferation within 4 hours and the induction of *puc*, *upd*, *Socs36E* and *Delta* within 2 hours (Jiang et al.,
577 2009). The main signal from ECs triggering ISCs proliferation is the secretion of the JAK/STAT ligand
578 Upd3. Moreover, overexpression of Upd1 and Upd3 in the midgut leads to increased ISC proliferation even
579 in homeostatic conditions (Buchon et al., 2009a). Upd2 is produced not only by ECs but also from
580 progenitors and has an additive effect to Upd3 following *Ecc15* infection (Osman et al., 2012). Upd3 is
581 strongly induced in ECs upon infection with most bacteria and is required for proper ISC proliferation
582 (Buchon et al., 2009a; Jiang et al., 2009). The secretion of different Upds from different cells in the gut
583 activates the JAK/STAT signaling in EBs and the VM, where they induce growth factor release and
584 coordinate ISC differentiation. We will consider the cell specific effects of STAT signaling for each cell
585 type.

586 How is the secretion of Upd3 from ECs regulated? Despite the lack of direct evidence, both the JNK
587 and Hippo signaling pathways have been implicated in the induction of Upd3 expression in ECs. JNK, a
588 stress-activated-protein kinase, is activated in the gut soon after microbial infection. Furthermore, ectopic
589 activation of JNK in ECs through either knock down of the negative regulator *puc*, or expression of a
590 constitutively active form of Hep leads to increased ISC mitosis (Buchon et al., 2009a; Jiang et al., 2009).
591 The mechanisms through which JNK signaling influences ISC proliferation still remain to be elucidated. It
592 has been proposed that JNK may control Upd3 expression by modulation of the Hippo pathway in ECs. The
593 Hippo signaling pathway plays a major role in how ECs control ISC proliferation. In homeostatic conditions,
594 loss of Hippo signaling in ECs leads to the secretion of Upd cytokines and the subsequent activation of
595 JAK/STAT in EBs and VM and EGFR signaling in ISCs (Ren et al., 2010; Staley and Irvine, 2010). JNK
596 stimulates the activation of Yki in ECs in response to damage (Staley and Irvine, 2010). Pez, an
597 evolutionarily conserved protein tyrosine phosphatase, in conjunction with Kibra, an adaptor protein with a
598 tumor suppression role in the Hippo pathway, are also required in ECs to suppress Yki activity (Poernbacher
599 et al., 2012).

600 ECs not only induce EGF Vn secretion from the VM, but also directly secrete EGFs: Krn was found
601 to be induced in ECs following *Pe* infection and to affect ISC proliferation (Jiang et al., 2011). Under basal
602 conditions, ECs also secrete two TGF- β ligands: Dpp and Gbb. Upon challenge, both Dpp and Gbb are
603 induced in the gut; however, which ligand is important for response to damage and which cell type expresses
604 those ligands remains in debate. ECs are a major source of Dpp according to some (Tian and Jiang, 2014),
605 while other groups show that just Gbb, but not Dpp, is required from ECs for the response to infection
606 (Ayyaz et al., 2015; Li et al., 2013; Zhou et al., 2015). TGF- β seems to have a direct role in the ECs
607 themselves: knock down by RNAi in ECs of TGF- β receptors Tkv or Punt resulted in increased apoptosis in

608 ECs and decreased mitotic indexes in ISCs. In guts with TGF- β deficient ECs, an increase of JAK/STAT and
609 EGFR signaling was found in the VM (Li et al., 2013). These results suggest that the TGF- β pathway
610 impacts ISC proliferation both through direct regulation and by modulation of adult cell survival (Li et al.,
611 2013).

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

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618 4.3. Feedback from the enteroblasts modulates ISC activity

619 After division, ISCs give rise to EBs, which are transiently differentiating progenitor cells. EBs are
620 often observed adjacent to ISCs in the midgut with their differentiation halted until reactivated in response to
621 damage or differentiative cues (Antonello et al., 2015). EBs are not only involved in differentiation, but they
622 also influence ISC proliferation, notably through the secretion of cytokines and growth factors. EBs secrete
623 the morphogen Wg, as well as JAK/STAT cytokines, and the EGF ligand Spitz, all of which can stimulate
624 ISC proliferation. In homeostatic conditions, Wg is mainly expressed in the VM (Lin et al., 2010, 2008).
625 However, upon challenge either by *Pe*, DSS or bleomycin, EBs induce Wg production, which is required for
626 ISC proliferation. This suggests that two cell types, the EBs and the VM, are the major regulators of ISC
627 proliferation through their secretion of growth factors (Cordero et al., 2012b). EBs also express two Upd
628 cytokines: Upd1 in basal conditions and Upd3 upon infection with *Ecc15* or *Pe* (Liu et al., 2010; Zhou et al.,
629 2013). Upd1 expression is down-regulated by the Notch pathway (Liu et al., 2010), a key pathway that
630 promotes EB differentiation. This suggests that the proliferative signals released by EBs are coupled to their
631 differentiation.

632 The pathways that control the expression and secretion of growth factors by EBs have just begun to
633 be elucidated. The Hippo pathway plays a central role in EBs, as its inactivation promotes ISC proliferation.
634 Oral infection with *Pe* upregulates Hippo pathway target genes *expanded* and *diap1* in progenitor cells
635 (Shaw et al., 2010). The upstream regulators and downstream targets of Hippo signaling in EBs that control
636 ISC proliferation differ from those acting in ECs, suggesting that the Hippo pathway is a central regulator of
637 midgut proliferation in all epithelial cells. A downstream target of Yki is *bantam*, a microRNA required for
638 the induction of ISC proliferation following DSS-induced damage (Huang et al., 2014). However, it remains
639 unclear whether *bantam* functions in EBs or ISCs. Considering recent data showing a role for Yki in EBs (Li
640 et al., 2014), it is possible that *bantam* is not required cell autonomously in ISCs for their proliferation, but
641 rather in EBs to control ISC proliferation. In EBs, the activation of Hippo signaling also depends on a
642 germinal center kinase, Misshapen (Msn). Msn physically interacts with and modifies Wts to inactivate Yki
643 in a Hippo independent manner, leading to a decrease in the paracrine function of EBs. Loss of *msn* in EBs
644 results in increased ISC proliferation, and is associated with a rise in Upd3 expression (Li et al., 2014). Loss

645 of TGF- β signaling in EBs results in their miss-differentiation, which in turn activates a canonical stress
646 response that leads to the production of Upds and EGFs (Zhou et al., 2015). Finally, Hh signaling is also
647 required in the EBs to promote ISC proliferation. Inhibition of Hh signaling within progenitor cells results in
648 lack of proliferation following bleomycin or DSS challenge, and induction of Hh signaling results in the
649 upregulation of Upd2 in EBs. Accordingly, knock-down of Upd2 specifically in EBs leads to decrease in ISC
650 proliferation (Tian et al., 2015). Altogether, it seems that EBs share similar ligands as ECs in controlling ISC
651 proliferation, including Upds and EGFs. However, as is the case for the Hippo pathway, the regulation of
652 secretion of those factors may differ within the two cell types. Future work should elucidate how and the
653 same signaling pathways can be used to regulate ISCs activity through different mechanisms.

654

655 4.4 Visceral Muscles act as a niche for ISCs

656 VM, together with the EBs, is another cell type that produces the key growth factors that trigger ISC
657 proliferation both in basal conditions and upon damage or bacterial infection. It is also the only cell type that
658 can be considered a true ISC niche by strict definition due to its stability, while ECs and EBs are more
659 transitory and differentiate from the ISCs themselves. As such, the VM integrates multiple signaling
660 pathways to control the release of EGF Vn, the morphogens Wg and Dpp, and the *Dmel* insulin like peptide
661 (dIlp) 3 (Cordero et al., 2012b; Lin et al., 2008; O'Brien et al., 2011). EGF Vn expression in the VM is
662 required for ISC proliferation following exposure to *Ecc15*, *Pe* and paraquat, while the two other EGFs, Krn
663 and Spi, are required in ECs and EBs respectively (Biteau and Jasper, 2011; Buchon et al., 2010; Jiang et al.,
664 2011). The EGFs may be interchangeable, as RNAi knock-down of any single EGF is insufficient to
665 completely block ISC proliferation induced by *Ecc15* infection (Buchon et al., 2010). Furthermore,
666 overexpression of secreted Spi in the VM can rescue *vn* RNAi (Xu et al., 2011), demonstrating that multiple
667 cell types, the ECs, EBs and VM, all act in synergy by producing different EGFs with overlapping activities.
668 In the VM, *vn* is regulated by two pathways; JAK/STAT and Bursicon. Knocking down the transcription
669 factor Stat in VM (Buchon et al., 2010; Jiang et al., 2011) or the cytokine Upd3 in ECs down-regulates *vn*
670 expression following infection (Buchon et al., 2010). In addition, the hormone Bursicon is secreted from EEs
671 and binds to its receptor, Rickets (Rk, also called DLGR2), in the VM, leading to suppression of *vn*
672 expression through activation of cAMP (Scopelliti et al., 2014). In basal conditions, the VM also secretes
673 Wg to promote ISC proliferation and differentiation (Lin et al., 2008), though the EB is the primary source of
674 Wg upon bacterial infection (Cordero et al., 2012b).

675 The TGF- β ligand Dpp is also induced in VM by treatment with bleomycin or paraquat and its
676 depletion from the VM results in a reduction of TGF- β signaling in ISCs (Guo et al., 2013; Zhou et al.,
677 2015). JAK/STAT is required for Dpp expression in the VM in response to bleomycin (Guo et al., 2013).
678 The VM thus acts both as a regulatory niche to influence ISC activity under a variety of conditions, and as an
679 integrator, relaying signals from other cell types. It will be interesting to investigate whether there is also
680 feedback signaling from ISCs to VM that can inhibit the expression of growth factors in VM in a post
681 proliferative stage.

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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 *dIlp3* 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. *dIlp2* itself is responsible for the control of ISC proliferation and is not expressed in the gut (Amcheslavsky et al., 2009). In intestinal neurons, *dIlp7* 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.

718 5.1. Effects of different types of pathogens on ISC regulation

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

736

737 5.2. Role of different microbiota in ISC renewal

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

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

754 should clarify the molecular mechanisms in both the bacteria and the host underlying *Lactobacilli* promotion
755 of epithelial homeostasis.

756 The microbiota also influence ISC proliferation through effects on the host metabolism. For instance,
757 *A. pomorum* modulates insulin through its pyrroloquinoline quinone-dependent alcohol dehydrogenase
758 activity that generates acetic acid and, in turn, regulates body size, energy metabolism and ISC activity (Shin
759 et al., 2011). Interestingly, the insulin pathway has been demonstrated to allow the *Dmel* midgut to grow in
760 response to nutrients (O'Brien et al., 2011). These data together suggest that the impact of the microbiota on
761 the *Dmel* midgut could be more complex than pathogens, as they can modulate responses to both damage
762 and nutrition.

763

764 5.3. Are stresses converging through the same mechanisms?

765 Multiple pathogens and multiple stresses converge on one single network of ISC proliferation. One
766 possibility would be that all stresses stimulate the secretion of growth factors by the epithelium through
767 multiple means. Stress through the studied microbiota and chemicals such as bleomycin and paraquat
768 converge on EC damage and consequent loss, which acts as a trigger to induce ISC proliferation through the
769 same regenerative pathways. Inorganic mercury (HgCl₂) has a similar effect on ISC proliferation through
770 damage to EC, although its effect on signaling pathways has not been studied yet (Chen et al., 2015).
771 However, DSS has a different mechanism of action: DSS disrupts the organization of the basement
772 membrane without damaging ECs, while still causing ISC proliferation. After treatment with DSS, newly
773 produced EBs do not differentiate into ECs and accumulate, while bleomycin treatment and bacterial
774 challenge both induce EB differentiation (Amcheslavsky et al., 2009). Accordingly, bleomycin and *Pe* but
775 not DSS activate JAK/STAT in ISCs, (Ren et al., 2010). To induce ISC proliferation in response to DSS, Yki
776 is required in progenitor cells but not in ECs. However, Yki is necessary in both cell types in response to
777 bleomycin and *Pe* (Ren et al., 2010). Altogether, these results suggest that with the exception of DSS, the
778 response to different stressors all use the same small set of pathways and secreted factors to promote ISC
779 proliferation in a common manner. It would be interesting to explore if DSS is a rare exception and if certain
780 microbiota can also act through disruption of the basal membrane but not EC damage in order to elude the
781 immune response and gain access to the fly. Figure 4 summarizes the response of the gut to different
782 challenges.

783

784 **6. Control of ISC differentiation in response to microbes**

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

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

803

804 **7. Future perspectives and Conclusion**

805 There are still many points to further clarify to understand the immune and regenerative response of
806 the *Dmel* midgut. While the control of proliferation in response to pathogenic infection has been widely
807 studied, the control of differentiation has been mostly studied in homeostatic conditions. However,
808 differentiation plays a major role in gut physiology, and it would be interesting to elucidate the long-term
809 consequences of infections on gut homeostasis. Furthermore, it has recently been demonstrated that stem
810 cells are regionalized and show region specific behavior in the midgut (Buchon et al., 2013b; Dutta et al.,
811 2015; Marianes and Spradling, 2013). Different midgut regions assume different functions, and respond to
812 bacteria at different levels (Bosco-Drayon et al., 2012; Buchon and Osman, 2015; Combe et al., 2014; Neyen
813 et al., 2012). In addition, the gut express different AMPs in each region, which may reflect underlying
814 regionalization of the ISCs themselves. Future work will try to elucidate how stem cell behavior, stress, and
815 bacterial activity are coordinated together in different regions. Regionalization of stem cells is a conserved
816 feature in insects as well as mammals, where differences in ISCs are observed between large and small
817 intestine (Cramer et al., 2015). Moreover, while several different organs, such as the brain and hemocytes,
818 are found to be influencing the gut during the immune response, how the gut response interacts with those
819 organs, and how the dialogue between tissues is established remain intriguing questions. Future work will
820 elucidate whether the gut itself signals these organs, or if other mechanisms allow distant organs to sense
821 changes in gut homeostasis. While in the *Dmel* midgut the recognition of all stresses seems to flow through a
822 consolidated and common response, how different types of damage and bacteria alter gut homeostasis and
823 ISC proliferation is still not clear. Microbiota play an important and conserved role in many diseases, both in
824 *Dmel* and in humans. Clarification of the mechanisms through which gut microbes influence ISC activity
825 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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Bibliography

- Aggarwal, K., Rus, F., Vriesema-Magnuson, C., Ertürk-Hasdemir, D., Paquette, N., Silverman, N., 2008. Rudra Interrupts Receptor Signaling Complexes to Negatively Regulate the IMD Pathway. *PLoS Pathog.* 4, e1000120. doi:10.1371/journal.ppat.1000120
- Aggarwal, K., Silverman, N., 2008. Positive and negative regulation of the *Drosophila* immune response. *BMB Rep.* 41, 267–277. doi:10.5483/BMBRep.2008.41.4.267
- Allen, V.W., O'Connor, R.M., Ulgherait, M., Zhou, C.G., Stone, E.F., Hill, V.M., Murphy, K.R., Canman, J.C., Ja, W.W., Shirasu-Hiza, M.M., 2015. period-Regulated Feeding Behavior and TOR Signaling Modulate Survival of Infection. *Curr. Biol.* doi:10.1016/j.cub.2015.11.051
- Amcheslavsky, A., Ito, N., Jiang, J., Ip, Y.T., Tony Ip, Y., 2011. Tuberous sclerosis complex and Myc coordinate the growth and division of *Drosophila* intestinal stem cells. *J. Cell Biol.* 193, 695–710. doi:10.1083/jcb.201103018
- Amcheslavsky, A., Jiang, J., Ip, Y.T., 2009. Tissue damage-induced intestinal stem cell division in *Drosophila*. *Cell Stem Cell* 4, 49–61. doi:10.1016/j.stem.2008.10.016
- Amcheslavsky, A., Song, W., Li, Q., Nie, Y., Bragatto, I., Ferrandon, D., Perrimon, N., Ip, Y.T.T., 2014. Enteroendocrine cells support intestinal stem-cell-mediated homeostasis in *Drosophila*. *Cell Rep.* 9, 32–9. doi:10.1016/j.celrep.2014.08.052
- Anagnostou, C., Dorsch, M., Rohlf, M., 2010. Influence of dietary yeasts on *Drosophila melanogaster* life-history traits. *Entomol. Exp. Appl.* 136, 1–11. doi:10.1111/j.1570-7458.2010.00997.x
- Anh, N.T.T., Nishitani, M., Harada, S., Yamaguchi, M., Kamei, K., 2011. Essential role of duox in stabilization of *Drosophila* wing. *J. Biol. Chem.* 286, 33244–33251. doi:10.1074/jbc.M111.263178
- Antonello, Z.A., Reiff, T., Ballesta-Illan, E., Dominguez, M., 2015. Robust intestinal homeostasis relies on cellular plasticity in enteroblasts mediated by miR-8-Escargot switch. *EMBO J.* 1–17. doi:10.15252/emboj.201591517
- Apidianakis, Y., Pitsouli, C., Perrimon, N., Rahme, L., 2009. Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U. S. A.* 106, 20883–20888. doi:10.1073/pnas.0911797106
- Apidianakis, Y., Rahme, L.G., 2011. *Drosophila melanogaster* as a model for human intestinal infection and pathology. *Dis. Model. Mech.* 4, 21–30. doi:10.1242/dmm.003970
- Atkinson, W., Shorrocks, B., 1977. Breeding site specificity in the domestic species of *Drosophila*. *Oecologia* 29, 223–232. doi:10.1007/BF00345697

872 Ayyaz, A., Li, H., Jasper, H., 2015. Haemocytes control stem cell activity in the *Drosophila* intestine. *Nat. Cell Biol.* 17,
873 736–748. doi:10.1038/ncb3174

874 Bakula, M., 1969. The persistence of a microbial flora during postembryogenesis of *Drosophila melanogaster*. *J.*
875 *Invertebr. Pathol.* 14, 365–374. doi:10.1016/0022-2011(69)90163-3

876 Basbous, N., Coste, F., Leone, P., Vincentelli, R., Royet, J., Kellenberger, C., Roussel, A., 2011. The *Drosophila*
877 peptidoglycan-recognition protein LF interacts with peptidoglycan-recognition protein LC to downregulate the
878 Imd pathway. *EMBO Rep.* 12, 327–333. doi:10.1038/embor.2011.19

879 Basset, a, Khush, R.S., Braun, a, Gardan, L., Boccard, F., Hoffmann, J. a, Lemaitre, B., 2000. The phytopathogenic
880 bacteria *Erwinia carotovora* infects *Drosophila* and activates an immune response. *Proc. Natl. Acad. Sci. U. S. A.*
881 97, 3376–3381. doi:10.1073/pnas.97.7.3376

882 Beebe, K., Lee, W.-C.C., Micchelli, C. a., 2010. JAK/STAT signaling coordinates stem cell proliferation and
883 multilineage differentiation in the *Drosophila* intestinal stem cell lineage. *Dev. Biol.* 338, 28–37.
884 doi:10.1016/j.ydbio.2009.10.045

885 Beebe, K., Park, D., Taghert, P.H., Micchelli, C. a., 2015. The *Drosophila* Prosecretory Transcription Factor dimmed Is
886 Dynamically Regulated in Adult Enteroendocrine Cells and Protects Against Gram-Negative Infection.
887 *G3: Genes|Genomes|Genetics* 5, 1517–1524. doi:10.1534/g3.115.019117

888 Begon, M., 1974. *Drosophila* and “dead” laboratory medium. *Dros. Inf Serv* 51:106.

889 Begon, M., 1917. The role of yeast in the nutrition of an insect. *J. Biol. Chem.* 30, 122–126.

890 Bischoff, V., Vignal, C., Duvic, B., Boneca, I.G., Hoffmann, J.A., Royet, J., 2006. Downregulation of the *Drosophila*
891 immune response by peptidoglycan- recognition proteins SC1 and SC2. *PLoS Pathog.* 2, 0139–0147.
892 doi:10.1371/journal.ppat.0020014

893 Biteau, B., Hochmuth, C.E., Jasper, H., 2008. JNK activity in somatic stem cells causes loss of tissue homeostasis in the
894 aging *Drosophila* gut. *Cell Stem Cell* 3, 442–55. doi:10.1016/j.stem.2008.07.024

895 Biteau, B., Jasper, H., 2014. Slit/Robo signaling regulates cell fate decisions in the intestinal stem cell lineage of
896 *Drosophila*. *Cell Rep.* 7, 1867–75. doi:10.1016/j.celrep.2014.05.024

897 Biteau, B., Jasper, H., 2011. EGF signaling regulates the proliferation of intestinal stem cells in *Drosophila*.
898 *Development* 138, 1045–55. doi:10.1242/dev.056671

899 Biteau, B., Karpac, J., Hwangbo, D., Jasper, H., 2011. Regulation of *Drosophila* lifespan by JNK signaling. *Exp.*
900 *Gerontol.* 46, 349–354. doi:10.1016/j.exger.2010.11.003

901 Blum, J.E., Fischer, C.N., Miles, J., Handelsman, J., 2013. Frequent replenishment sustains the beneficial microbiome
902 of *Drosophila melanogaster*. *MBio* 4, 1–8. doi:10.1128/mBio.00860-13

903 Bosco-Drayon, V., Poidevin, M., Boneca, I.G., Narbonne-Reveau, K., Royet, J., Charroux, B., 2012. Peptidoglycan
904 sensing by the receptor PGRP-LE in the *Drosophila* gut induces immune responses to infectious bacteria and
905 tolerance to microbiota. *Cell Host Microbe* 12, 153–165. doi:10.1016/j.chom.2012.06.002

906 Brewer, J.W., Harrison, M.D., Winston, J.A., 1981. Survival of two varieties of *Erwinia carotovora* on *Drosophila*
907 *melanogaster* meigen and *Drosophila busckii* Coquillett, (Diptera: *Drosophilidae*) vectors of potato blackleg in
908 Colorado. *Am. Potato J.* 58, 439–449. doi:10.1007/BF02874541

909 Briscoe, J., Théron, P.P., 2013. The mechanisms of Hedgehog signalling and its roles in development and disease. *Nat.*
910 *Rev. Mol. Cell Biol.* 14, 416–29. doi:10.1038/nrm3598

911 Broderick, N. a, Buchon, N., Lemaitre, B., 2014. Microbiota-induced changes in *drosophila melanogaster* host gene
912 expression and gut morphology. *MBio* 5, e01117–14. doi:10.1128/mBio.01117-14

913 Broderick, N. a, Lemaitre, B., 2012. Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 3, 307–321.
914 doi:10.4161/gmic.19896

915 Buchon, N., Broderick, N. a, Chakrabarti, S., Lemaitre, B., 2009a. Invasive and indigenous microbiota impact intestinal
916 stem cell activity through multiple pathways in *Drosophila*. *Genes Dev.* 23, 2333–44. doi:10.1101/gad.1827009

917 Buchon, N., Broderick, N. a, Kuraishi, T., Lemaitre, B., 2010. *Drosophila* EGFR pathway coordinates stem cell
918 proliferation and gut remodeling following infection. *BMC Biol.* 8, 152. doi:10.1186/1741-7007-8-152

919 Buchon, N., Broderick, N. a, Lemaitre, B., 2013a. Gut homeostasis in a microbial world: insights from *Drosophila*

920 *melanogaster*. *Nat. Rev. Microbiol.* 11, 615–26. doi:10.1038/nrmicro3074

921 Buchon, N., Broderick, N. a, Poidevin, M., Pradervand, S., Lemaitre, B., 2009b. *Drosophila* intestinal response to
 922 bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe* 5, 200–11.
 923 doi:10.1016/j.chom.2009.01.003

924 Buchon, N., Osman, D., 2015. All for one and one for all: regionalization of the *Drosophila* intestine. *Insect Biochem.*
 925 *Mol. Biol.* 1–7. doi:10.1016/j.ibmb.2015.05.015

926 Buchon, N., Osman, D., David, F.P. a, Yu Fang, H., Boquete, J.-P.P., Deplancke, B., Lemaitre, B., Fang, H.Y.,
 927 Boquete, J.-P.P., Deplancke, B., Lemaitre, B., 2013b. Morphological and molecular characterization of adult
 928 midgut compartmentalization in *Drosophila*. *Cell Rep.* 3, 1725–38. doi:10.1016/j.celrep.2013.04.001

929 Buchon, N., Silverman, N., Cherry, S., 2014. Immunity in *Drosophila melanogaster* — from microbial recognition to
 930 whole-organism physiology. *Nat. Rev. Immunol.* 14, 796–810. doi:10.1038/nri3763

931 Chakrabarti, S., Liehl, P., Buchon, N., Lemaitre, B., 2012. Infection-induced host translational blockage inhibits
 932 immune responses and epithelial renewal in the *Drosophila* gut. *Cell Host Microbe* 12, 60–70.
 933 doi:10.1016/j.chom.2012.06.001

934 Chakrabarti, S., Poidevin, M., Lemaitre, B., 2014. The *Drosophila* MAPK p38c Regulates Oxidative Stress and Lipid
 935 Homeostasis in the Intestine. *PLoS Genet.* 10, e1004659. doi:10.1371/journal.pgen.1004659

936 Chandler, J.A., Morgan Lang, J., Bhatnagar, S., Eisen, J.A., Kopp, A., 2011. Bacterial Communities of Diverse
 937 *Drosophila* Species: Ecological Context of a Host–Microbe Model System. *PLoS Genet.* 7, e1002272.
 938 doi:10.1371/journal.pgen.1002272

939 Chaston, J.M., Newell, P.D., Douglas, A.E., 2014. Metagenome-Wide Association of Microbial Determinants of Host
 940 Phenotype in *Drosophila melanogaster*. *MBio* 5, e01631–14–e01631–14. doi:10.1128/mBio.01631-14

941 Chen, Z., Wu, X., Luo, H., Zhao, L., Ji, X., Qiao, X., Jin, Y., Liu, W., 2015. Acute exposure of mercury chloride
 942 stimulates the tissue regeneration program and reactive oxygen species production in the *Drosophila* midgut.
 943 *Environ. Toxicol. Pharmacol.* 41, 32–38. doi:10.1016/j.etap.2015.11.009

944 Clark, R.I., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana, A., Rera, M., Pellegrini, M., Ja,
 945 W.W., Walker, D.W., 2015. Distinct Shifts in Microbiota Composition during *Drosophila* Aging Impair Intestinal
 946 Function and Drive Mortality. *Cell Rep.* 12, 1656–1667. doi:10.1016/j.celrep.2015.08.004

947 Clarke, M.F., Fuller, M., 2006. Stem cells and cancer: two faces of eve. *Cell* 124, 1111–5.
 948 doi:10.1016/j.cell.2006.03.011

949 Cognigni, P., Bailey, A.P., Miguel-Aliaga, I., 2011. Enteric neurons and systemic signals couple nutritional and
 950 reproductive status with intestinal homeostasis. *Cell Metab.* 13, 92–104. doi:10.1016/j.cmet.2010.12.010

951 Coluccio, A.E., Rodriguez, R.K., Kernan, M.J., Neiman, A.M., 2008. The Yeast Spore Wall Enables Spores to Survive
 952 Passage through the Digestive Tract of *Drosophila*. *PLoS One* 3, e2873. doi:10.1371/journal.pone.0002873

953 Combe, B.E., Defaye, A., Bozonnet, N., Puthier, D., Royet, J., Leulier, F., 2014. *Drosophila* Microbiota Modulates Host
 954 Metabolic Gene Expression via IMD/NF-κB Signaling. *PLoS One* 9, e94729. doi:10.1371/journal.pone.0094729

955 Cordero, J.B., Stefanatos, R.K., Myant, K., Vidal, M., Sansom, O.J., 2012a. Non-autonomous crosstalk between the
 956 Jak/Stat and Egfr pathways mediates Apc1-driven intestinal stem cell hyperplasia in the *Drosophila* adult midgut.
 957 *Development* 139, 4524–35. doi:10.1242/dev.078261

958 Cordero, J.B., Stefanatos, R.K., Scopelliti, A., Vidal, M., Sansom, O.J., 2012b. Inducible progenitor-derived wingless
 959 regulates adult midgut regeneration in *drosophila*. *EMBO J.* 31, 3901–3917. doi:10.1038/emboj.2012.248

960 Cox, C.R., Gilmore, M.S., 2007. Native microbial colonization of *Drosophila melanogaster* and its use as a model of
 961 *Enterococcus faecalis* pathogenesis. *Infect. Immun.* 75, 1565–1576. doi:10.1128/IAI.01496-06

962 Cramer, J.M., Thompson, T., Geskin, A., LaFramboise, W., Lagasse, E., 2015. Distinct Human Stem Cell Populations
 963 in Small and Large Intestine. *PLoS One* 10, e0118792. doi:10.1371/journal.pone.0118792

964 Damiano, S., Morano, A., Ucci, V., Accetta, R., Mondola, P., Paternò, R., Avvedimento, V.E., Santillo, M., 2015. Dual
 965 oxidase 2 generated reactive oxygen species selectively mediate the induction of mucins by epidermal growth
 966 factor in enterocytes. *Int. J. Biochem. Cell Biol.* 60, 8–18. doi:10.1016/j.biocel.2014.12.014

967 Dantoft, W., Davis, M.M., Lindvall, J.M., Tang, X., Uvell, H., Junell, A., Beskow, A., Engström, Y., 2013. The Oct1
 968 homolog Nubbin is a repressor of NF-κB-dependent immune gene expression that increases the tolerance to gut

969 microbiota. BMC Biol. 11, 99. doi:10.1186/1741-7007-11-99

970 De Lerma Barbaro, a., Perletti, G., Bonapace, I.M., Monti, E., 2014. Inflammatory cues acting on the adult intestinal
971 stem cells and the early onset of cancer (Review). Int. J. Oncol. 45, 959–968. doi:10.3892/ijo.2014.2490

972 Deng, H., Gerencser, A.A., Jasper, H., 2015. Signal integration by Ca²⁺ regulates intestinal stem-cell activity. Nature
973 1–26. doi:10.1038/nature16170

974 Dobson, A.J., Chaston, J.M., Newell, P.D., Donahue, L., Hermann, S.L., Sannino, D.R., Westmiller, S., Wong, A.C.-N.,
975 Clark, A.G., Lazzaro, B.P., Douglas, A.E., 2015. Host genetic determinants of microbiota-dependent nutrition
976 revealed by genome-wide analysis of *Drosophila melanogaster*. Nat. Commun. 6, 6312.
977 doi:10.1038/ncomms7312

978 Dutta, D., Dobson, A.J.J., Houtz, P.L.L., Patel, P.H., Edgar, B.A.A., Buchon, N., Revah, J., Korzeliuss, J., Gläßer, C.,
979 Revah, J., Korzeliuss, J., Patel, P.H., Edgar, B.A.A., Buchon, N., 2015. Regional Cell-Specific Transcriptome
980 Mapping Reveals Regulatory Complexity in the Adult *Drosophila* Midgut. Cell Rep. 12, 346–358.
981 doi:10.1016/j.celrep.2015.06.009

982 Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., Relman,
983 D.A., 2005. Diversity of the human intestinal microbial flora. Science 308, 1635–1638.
984 doi:10.1126/science.1110591

985 Erkosar, B., Storelli, G., Mitchell, M., Bozonnet, L., Bozonnet, N., Leulier, F., 2015. Pathogen Virulence Impedes
986 Mutualist-Mediated Enhancement of Host Juvenile Growth via Inhibition of Protein Digestion. Cell Host Microbe
987 445–455. doi:10.1016/j.chom.2015.09.001

988 Fan, X., Liang, Q., Lian, T., Wu, Q., Gaur, U., Li, D., Yang, D., Mao, X., Jin, Z., Li, Y., Yang, M., 2015. Rapamycin
989 preserves gut homeostasis during *Drosophila* aging. Oncotarget 1–10.

990 Ferrandon, D., 2013. The complementary facets of epithelial host defenses in the genetic model organism *Drosophila*
991 melanogaster: From resistance to resilience. Curr. Opin. Immunol. 25, 59–70. doi:10.1016/j.coi.2012.11.008

992 Flyg, C., Kenne, K., Boman, H.G., 1980. Insect pathogenic properties of *Serratia marcescens*: phage-resistant mutants
993 with a decreased resistance to *Cecropia* immunity and a decreased virulence to *Drosophila*. J. Gen. Microbiol.
994 120, 173–181. doi:10.1099/00221287-120-1-173

995 Galac, M.R., Lazzaro, B.P., 2011. Comparative pathology of bacteria in the genus *Providencia* to a natural host,
996 *Drosophila melanogaster*. Microbes Infect. 13, 673–683. doi:10.1016/j.micinf.2011.02.005

997 Ganter, P.F., 2006. Yeast and Invertebrate Associations, in: Biodiversity and Ecophysiology of Yeasts. Springer-Verlag,
998 Berlin/Heidelberg, pp. 303–370. doi:10.1007/3-540-30985-3_14

999 Gao, Z., Guo, B., Gao, R., Zhu, Q., Qin, H., 2015. Microbiota disbiosis is associated with colorectal cancer. Front.
1000 Microbiol. 6, 1–9. doi:10.3389/fmicb.2015.00020

1001 Gersemann, M., Stange, E.F., Wehkamp, J., 2011. From intestinal stem cells to inflammatory bowel diseases. World J.
1002 Gastroenterol. 17, 3198–203. doi:10.3748/wjg.v17.i27.3198

1003 Gersemann, M., Wehkamp, J., Stange, E.F., 2012. Innate immune dysfunction in inflammatory bowel disease. J. Intern.
1004 Med. 271, 421–8. doi:10.1111/j.1365-2796.2012.02515.x

1005 Gilbert, D.G., 1980. Dispersal of yeasts and bacteria by *Drosophila* in a temperate forest. Oecologia 46, 135–137.
1006 doi:10.1007/BF00346979

1007 Gilboa, L., Lehmann, R., 2007. Changing places: a novel type of niche and stem cell coordination in the *Drosophila*
1008 ovary. Cell Stem Cell 1, 239–40. doi:10.1016/j.stem.2007.08.013

1009 Glittenberg, M.T., Kounatidis, I., Christensen, D., Kostov, M., Kimber, S., Roberts, I., Ligoxygakis, P., 2011. Pathogen
1010 and host factors are needed to provoke a systemic host response to gastrointestinal infection of *Drosophila* larvae
1011 by *Candida albicans*. Dis. Model. Mech. 4, 515–525. doi:10.1242/dmm.006627

1012 Grasberger, H., Gao, J., Nagao-Kitamoto, H., Kitamoto, S., Zhang, M., Kamada, N., Eaton, K. a., El-Zaatari, M.,
1013 Shreiner, A.B., Merchant, J.L., Owyang, C., Kao, J.Y., 2015. Increased Expression of DUOX2 is an Epithelial
1014 Response to Mucosal Dysbiosis Required for Immune Homeostasis in Mouse Intestine. Gastroenterology.
1015 doi:10.1053/j.gastro.2015.07.062

1016 Grimont, P.A.D., Grimont, F., 1978. The Genus *Serratia*. Annu. Rev. Microbiol. 32, 221–248.
1017 doi:10.1146/annurev.mi.32.100178.001253

- 1018 Guo, L., Karpac, J., Tran, S.L., Jasper, H., 2014. PGRP-SC2 promotes gut immune homeostasis to limit commensal
1019 dysbiosis and extend lifespan. *Cell* 156, 109–22. doi:10.1016/j.cell.2013.12.018
- 1020 Guo, Z., Driver, I., Ohlstein, B., 2013. Injury-induced BMP signaling negatively regulates *Drosophila* midgut
1021 homeostasis. *J. Cell Biol.* 201, 945–961. doi:10.1083/jcb.201302049
- 1022 Ha, E.-M., Lee, K.-A., Park, S.H., Kim, S.-H., Nam, H.-J., Lee, H.-Y., Kang, D., Lee, W.-J., 2009a. Regulation of
1023 DUOX by the Gαq-Phospholipase Cβ-Ca²⁺ Pathway in *Drosophila* Gut Immunity. *Dev. Cell* 16, 386–397.
1024 doi:10.1016/j.devcel.2008.12.015
- 1025 Ha, E.-M., Lee, K.-A., Seo, Y.Y., Kim, S.-H., Lim, J.-H., Oh, B.-H., Kim, J., Lee, W.-J., 2009b. Coordination of
1026 multiple dual oxidase-regulatory pathways in responses to commensal and infectious microbes in *drosophila* gut.
1027 *Nat. Immunol.* 10, 949–57. doi:10.1038/ni.1765
- 1028 Ha, E.-M., Oh, C.-T., Bae, Y.S., Lee, W.-J., 2005. A direct role for dual oxidase in *Drosophila* gut immunity. *Science*
1029 310, 847–850. doi:10.1126/science.1117311
- 1030 Huang, H., Li, J., Hu, L., Ge, L., Ji, H., Zhao, Y., Zhang, L., 2014. Bantam is essential for *Drosophila* intestinal stem
1031 cell proliferation in response to Hippo signaling. *Dev. Biol.* 385, 211–219. doi:10.1016/j.ydbio.2013.11.008
- 1032 J. P. Baumberger, 1916. The Food of *Drosophila Melanogaster* Meigen. *Proc. Natl Acad. Sci. USA* 3, 122–126.
- 1033 Jalanka-Tuovinen, J., Salonen, A., Nikkilä, J., Immonen, O., Kekkonen, R., Lahti, L., Palva, A., de Vos, W.M., 2011.
1034 Intestinal microbiota in healthy adults: Temporal analysis reveals individual and common core and relation to
1035 intestinal symptoms. *PLoS One* 6. doi:10.1371/journal.pone.0023035
- 1036 Jiang, H., Edgar, B. a, 2009. EGFR signaling regulates the proliferation of *Drosophila* adult midgut progenitors.
1037 *Development* 136, 483–93. doi:10.1242/dev.026955
- 1038 Jiang, H., Grenley, M.O., Bravo, M.J., Blumhagen, R.Z., Edgar, B.A., 2011. EGFR/Ras/MAPK signaling mediates
1039 adult midgut epithelial homeostasis and regeneration in *drosophila*. *Cell Stem Cell* 8, 84–95.
1040 doi:10.1016/j.stem.2010.11.026
- 1041 Jiang, H., Patel, P.H., Kohlmaier, A., Grenley, M.O., McEwen, D.G., Edgar, B.A., 2009. Cytokine/Jak/Stat signaling
1042 mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell* 137, 1343–55.
1043 doi:10.1016/j.cell.2009.05.014
- 1044 Jin, Y., Ha, N., Forés, M., Xiang, J., Gläber, C., Maldera, J., Jiménez, G., Edgar, B.A., 2015. EGFR/Ras Signaling
1045 Controls *Drosophila* Intestinal Stem Cell Proliferation via Capicua-Regulated Genes. *PLOS Genet.* 11, e1005634.
1046 doi:10.1371/journal.pgen.1005634
- 1047 Jin, Y., Xu, J., Yin, M.-X., Lu, Y., Hu, L., Li, P., Zhang, P., Yuan, Z., Ho, M.S., Ji, H., Zhao, Y., Zhang, L., 2013.
1048 Brahma is essential for *Drosophila* intestinal stem cell proliferation and regulated by Hippo signaling. *Elife* 2,
1049 e00999. doi:10.7554/eLife.00999
- 1050 Jones, R.M., Desai, C., Darby, T.M., Luo, L., Wolfarth, A.A., Scharer, C.D., Ardita, C.S., Reedy, A.R., Keebaugh, E.S.,
1051 Neish, A.S., 2015. Lactobacilli Modulate Epithelial Cytoprotection through the Nrf2 Pathway. *Cell Rep.* 12,
1052 1217–1225. doi:10.1016/j.celrep.2015.07.042
- 1053 Jones, R.M., Luo, L., Ardita, C.S., Richardson, A.N., Kwon, Y.M., Mercante, J.W., Alam, A., Gates, C.L., Wu, H.,
1054 Swanson, P.A., Lambeth, J.D., Denning, P.W., Neish, A.S., 2013. Symbiotic lactobacilli stimulate gut epithelial
1055 proliferation via Nox-mediated generation of reactive oxygen species. *EMBO J.* 32, 1–12.
1056 doi:10.1038/emboj.2013.224
- 1057 Juarez, M.T., Patterson, R. a., Sandoval-Guillen, E., McGinnis, W., 2011. Duox, Flotillin-2, and Src42A are required to
1058 activate or delimit the spread of the transcriptional response to epidermal wounds in *Drosophila*. *PLoS Genet.* 7.
1059 doi:10.1371/journal.pgen.1002424
- 1060 Juneja, P., Lazzaro, B.P., 2009. *Providencia sneebia* sp. nov. and *Providencia burhodogranariae* sp. nov., isolated from
1061 wild *Drosophila melanogaster*. *Int. J. Syst. Evol. Microbiol.* 59, 1108–1111. doi:10.1099/ijs.0.000117-0
- 1062 Kaneko, T., Goldman, W.E., Mellroth, P., Steiner, H., Fukase, K., Kusumoto, S., Harley, W., Fox, A., Golenbock, D.,
1063 Silverman, N., 2004. Monomeric and Polymeric Gram-Negative Peptidoglycan but Not Purified LPS Stimulate
1064 the *Drosophila* IMD Pathway. *Immunity* 20, 637–649. doi:10.1016/S1074-7613(04)00104-9
- 1065 Kim, S.-H., Lee, W.-J., 2014. Role of DUOX in gut inflammation: lessons from *Drosophila* model of gut-microbiota
1066 interactions. *Front. Cell. Infect. Microbiol.* 3, 116. doi:10.3389/fcimb.2013.00116
- 1067 Kleino, A., Myllymaki, H., Kallio, J., Vanha-aho, L.-M., Oksanen, K., Ulvila, J., Hultmark, D., Valanne, S., Ramet, M.,

- 1068 2008. Pirk Is a Negative Regulator of the *Drosophila* Imd Pathway. *J. Immunol.* 180, 5413–5422.
1069 doi:10.4049/jimmunol.180.8.5413
- 1070 Kloepper, J.W., Brewer, J.W., Harrison, M.D., 1981. Insect transmission of *Erwinia carotovora* var. *carotovora*
1071 and *Erwinia carotovora* var. *atroseptica* to potato plants in the field. *Am. Potato J.* 58, 165–175.
1072 doi:10.1007/BF02854416
- 1073 Krause, K.H., 2007. Aging: A revisited theory based on free radicals generated by NOX family NADPH oxidases. *Exp.*
1074 *Gerontol.* 42, 256–262. doi:10.1016/j.exger.2006.10.011
- 1075 Kuraishi, T., Binggeli, O., Opota, O., Buchon, N., Lemaitre, B., 2011. Genetic evidence for a protective role of the
1076 peritrophic matrix against intestinal bacterial infection in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.*
1077 108, 15966–71. doi:10.1073/pnas.1105994108
- 1078 Kurz, C.L., Chauvet, S., Andrs, E., Aurouze, M., Vallet, I., Michel, G.P.F., Uh, M., Celli, J., Filloux, A., De
1079 Bentzmann, S., Steinmetz, I., Hoffmann, J.A., Finlay, B.B., Gorvel, J.P., Ferrandon, D., Ewbank, J.J., 2003.
1080 Virulence factors of the human opportunistic pathogen *Serratia marcescens* identified by in vivo screening.
1081 *EMBO J.* 22, 1451–1460. doi:10.1093/emboj/cdg159
- 1082 Lane, S.W., Williams, D. a, Watt, F.M., 2014. Modulating the stem cell niche for tissue regeneration. *Nat. Biotechnol.*
1083 32, 795–803. doi:10.1038/nbt.2978
- 1084 Lee, K.-A., Kim, S.-H., Kim, E.-K., Ha, E.-M., You, H., Kim, B., Kim, M.-J., Kwon, Y., Ryu, J.-H., Lee, W.-J., 2013.
1085 Bacterial-Derived Uracil as a Modulator of Mucosal Immunity and Gut-Microbe Homeostasis in *Drosophila*. *Cell*
1086 153, 797–811. doi:10.1016/j.cell.2013.04.009
- 1087 Lee, K.-A., Lee, W.-J., 2014. *Drosophila* as a model for intestinal dysbiosis and chronic inflammatory diseases. *Dev.*
1088 *Comp. Immunol.* 42, 102–110. doi:10.1016/j.dci.2013.05.005
- 1089 Lee, K.-Z., Ferrandon, D., 2011. Negative regulation of immune responses on the fly. *EMBO J.* 30, 988–990.
1090 doi:10.1038/emboj.2011.47
- 1091 Leulier, F., Parquet, C., Pili-Floury, S., Ryu, J.-H., Caroff, M., Lee, W.-J., Mengin-Lecreux, D., Lemaitre, B., 2003.
1092 The *Drosophila* immune system detects bacteria through specific peptidoglycan recognition. *Nat. Immunol.* 4,
1093 478–484. doi:10.1038/ni922
- 1094 Ley, R.E., Turnbaugh, P.J., Klein, S., Gordon, J.I., 2006. Microbial ecology: human gut microbes associated with
1095 obesity. *Nature* 444, 1022–1023. doi:10.1038/4441022a
- 1096 Lhocine, N., Ribeiro, P.S., Buchon, N., Wepf, A., Wilson, R., Tenev, T., Lemaitre, B., Gstaiger, M., Meier, P., Leulier,
1097 F., 2008. PIMS modulates immune tolerance by negatively regulating *Drosophila* innate immune signaling. *Cell*
1098 *Host Microbe* 4, 147–58. doi:10.1016/j.chom.2008.07.004
- 1099 Li, Q., Li, S., Mana-Capelli, S., Roth Flach, R.J., Danai, L. V., Amcheslavsky, A., Nie, Y., Kaneko, S., Yao, X., Chen,
1100 X., Cotton, J.L., Mao, J., McCollum, D., Jiang, J., Czech, M.P., Xu, L., Ip, Y.T., 2014. The Conserved
1101 Misshapen-Warts-Yorkie Pathway Acts in Enteroblasts to Regulate Intestinal Stem Cells in *Drosophila*. *Dev. Cell*
1102 31, 291–304. doi:10.1016/j.devcel.2014.09.012
- 1103 Li, Z., Zhang, Y., Han, L., Shi, L., Lin, X., 2013. Trachea-Derived Dpp Controls Adult Midgut Homeostasis in
1104 *Drosophila*. *Dev. Cell* 24, 133–143. doi:10.1016/j.devcel.2012.12.010
- 1105 Liehl, P., Blight, M., Vodovar, N., Boccard, F., Lemaitre, B., 2006. Prevalence of local immune response against oral
1106 infection in a *Drosophila/Pseudomonas* infection model. *PLoS Pathog.* 2, 0551–0561.
1107 doi:10.1371/journal.ppat.0020056
- 1108 Limmer, S., Haller, S., Drenkard, E., Lee, J., Yu, S., Kocks, C., Ausubel, F.M., Ferrandon, D., 2011. *Pseudomonas*
1109 *aeruginosa* RhlR is required to neutralize the cellular immune response in a *Drosophila melanogaster* oral
1110 infection model. *Proc. Natl. Acad. Sci.* 108, 17378–17383. doi:10.1073/pnas.1114907108
- 1111 Lin, G., Xu, N., Xi, R., 2010. Paracrine unpaired signaling through the JAK/STAT pathway controls self-renewal and
1112 lineage differentiation of *drosophila* intestinal stem cells. *J. Mol. Cell Biol.* 2, 37–49. doi:10.1093/jmcb/mjp028
- 1113 Lin, G., Xu, N., Xi, R., 2008. Paracrine Wingless signalling controls self-renewal of *Drosophila* intestinal stem cells.
1114 *Nature* 455, 1119–23. doi:10.1038/nature07329
- 1115 Liu, W., Singh, S.R., Hou, S.X., 2010. JAK-STAT is restrained by Notch to control cell proliferation of the *drosophila*
1116 intestinal stem cells. *J. Cell. Biochem.* 109, 992–999. doi:10.1002/jcb.22482
- 1117 Lu, Y., Li, Z., 2015. No Intestinal Stem Cell Regeneration after Complete Progenitor Ablation in *Drosophila* Adult

1118 Midgut. *J. Genet. Genomics* 42, 83–86. doi:10.1016/j.jgg.2014.10.002

1119 Maillet, F., Bischoff, V., Vignal, C., Hoffmann, J., Royet, J., 2008. The *Drosophila* Peptidoglycan Recognition Protein
1120 PGRP-LF Blocks PGRP-LC and IMD/JNK Pathway Activation. *Cell Host Microbe* 3, 293–303.
1121 doi:10.1016/j.chom.2008.04.002

1122 Marianes, A., Spradling, A.C., 2013. Physiological and stem cell compartmentalization within the *Drosophila* midgut.
1123 *Elife* 2, e00886. doi:10.7554/eLife.00886

1124 Mellroth, P., Steiner, H., 2006. PGRP-SB1: An N-acetylmuramoyl l-alanine amidase with antibacterial activity.
1125 *Biochem. Biophys. Res. Commun.* 350, 994–999. doi:10.1016/j.bbrc.2006.09.139

1126 Micchelli, C. a, Perrimon, N., 2006. Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature*
1127 439, 475–9. doi:10.1038/nature04371

1128 Molina, J.J., Harrison, M.D., Brewer, J.W., 1974. Transmission of *Erwinia carotovora* var. *atroseptica* by *Drosophila*
1129 *melanogaster* Meig. I. Acquisition and transmission of the bacterium. *Am. Potato J.* 51, 245–250.
1130 doi:10.1007/BF02851435

1131 Myllymäki, H., Rämét, M., 2014. JAK/STAT Pathway in *Drosophila* Immunity. *Scand. J. Immunol.* 79, 377–385.
1132 doi:10.1111/sji.12170

1133 Nehme, N.T., Liégeois, S., Kele, B., Giammarinaro, P., Pradel, E., Hoffmann, J. a., Ewbank, J.J., Ferrandon, D., 2007.
1134 A model of bacterial intestinal infections in *Drosophila melanogaster*. *PLoS Pathog.* 3, 1694–1709.
1135 doi:10.1371/journal.ppat.0030173

1136 Neyen, C., Poidevin, M., Roussel, A., Lemaitre, B., 2012. Tissue- and ligand-specific sensing of gram-negative
1137 infection in *drosophila* by PGRP-LC isoforms and PGRP-LE. *J. Immunol.* 189, 1886–97.
1138 doi:10.4049/jimmunol.1201022

1139 Nmorsi, O.P.G., Agbozele, G., Ukwandu, N.C.D., 2007. Some Aspects of Epidemiology of Filth Flies: *Musca*
1140 *domestica*, *Musca domestica vicina*, *Drosophila melanogaster* and Associated Bacteria Pathogens in Ekpoma,
1141 Nigeria. *Vector-Borne Zoonotic Dis.* 7, 107–117. doi:10.1089/vbz.2006.0539

1142 O'Brien, L.E., Soliman, S.S., Li, X., Bilder, D., 2011. Altered Modes of Stem Cell Division Drive Adaptive Intestinal
1143 Growth. *Cell* 147, 603–614. doi:10.1016/j.cell.2011.08.048

1144 Ohlstein, B., Spradling, A., 2006. The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. *Nature*
1145 439, 470–4. doi:10.1038/nature04333

1146 Opota, O., Vallet-Gély, I., Vincentelli, R., Kellenberger, C., Iacovache, I., Gonzalez, M.R., Roussel, A., van der Goot,
1147 F.G., Lemaitre, B., 2011. Monalysin, a novel β -pore-forming toxin from the *drosophila* pathogen *pseudomonas*
1148 *entomophila*, contributes to host intestinal damage and lethality. *PLoS Pathog.* 7.
1149 doi:10.1371/journal.ppat.1002259

1150 Osman, D., Buchon, N., Chakrabarti, S., Huang, Y.-T., Su, W.-C., Poidevin, M., Tsai, Y.-C., Lemaitre, B., 2012.
1151 Autocrine and paracrine unpaired signaling regulate intestinal stem cell maintenance and division. *J. cell ...* 125,
1152 5944–5949. doi:10.1242/jcs.113100

1153 Paredes, J.C., Welchman, D.P., Poidevin, M., Lemaitre, B., 2011. Negative Regulation by Amidase PGRPs Shapes the
1154 *Drosophila* Antibacterial Response and Protects the Fly from Innocuous Infection. *Immunity* 35, 770–779.
1155 doi:10.1016/j.immuni.2011.09.018

1156 Patel, P.H., Dutta, D., Edgar, B. a., 2015. Niche appropriation by *Drosophila* intestinal stem cell tumours. *Nat. Cell*
1157 *Biol.* doi:10.1038/ncb3214

1158 Peterson, A.J., O'Connor, M.B., 2014. Strategies for exploring TGF- β signaling in *Drosophila*. *Methods* 68, 183–193.
1159 doi:10.1016/j.ymeth.2014.03.016

1160 Piper, M.D.W., Partridge, L., Raubenheimer, D., Simpson, S.J., 2011. Dietary restriction and aging: a unifying
1161 perspective. *Cell Metab.* 14, 154–60. doi:10.1016/j.cmet.2011.06.013

1162 Poernbacher, I., Baumgartner, R., Marada, S.K., Edwards, K., Stocker, H., 2012. *Drosophila* *Pez* acts in Hippo signaling
1163 to restrict intestinal stem cell proliferation. *Curr. Biol.* 22, 389–96. doi:10.1016/j.cub.2012.01.019

1164 Radtke, F., Clevers, H., 2005. Self-renewal and cancer of the gut: two sides of a coin. *Science* 307, 1904–1909.
1165 doi:10.1126/science.1104815

1166 Rajilić-Stojanović, M., Heilig, H.G.H.J., Tims, S., Zoetendal, E.G., De Vos, W.M., 2013. Long-term monitoring of the

human intestinal microbiota composition. *Environ. Microbiol.* 15, 1146–1159. doi:10.1111/1462-2920.12023

Razzell, W., Evans, I.R., Martin, P., Wood, W., 2013. Calcium flashes orchestrate the wound inflammatory response through duox activation and hydrogen peroxide release. *Curr. Biol.* 23, 424–429. doi:10.1016/j.cub.2013.01.058

Ren, C., Webster, P., Finkel, S.E., Tower, J., 2007. Increased Internal and External Bacterial Load during *Drosophila* Aging without Life-Span Trade-Off. *Cell Metab.* 6, 144–152. doi:10.1016/j.cmet.2007.06.006

Ren, F., Shi, Q., Chen, Y., Jiang, A., Ip, Y.T., Jiang, H., Jiang, J., 2013. *Drosophila* Myc integrates multiple signaling pathways to regulate intestinal stem cell proliferation during midgut regeneration. *Cell Res.* 23, 1133–46. doi:10.1038/cr.2013.101

Ren, F., Wang, B., Yue, T., Yun, E.-Y., Ip, Y.T., Jiang, J., 2010. Hippo signaling regulates *Drosophila* intestine stem cell proliferation through multiple pathways. *Proc. Natl. Acad. Sci. U. S. A.* 107, 21064–21069. doi:10.1073/pnas.1012759107

Ren, W., Zhang, Y., Li, M., Wu, L., Wang, G., Baeg, G.-H., You, J., Li, Z., Lin, X., 2015. Windpipe Controls *Drosophila* Intestinal Homeostasis by Regulating JAK/STAT Pathway via Promoting Receptor Endocytosis and Lysosomal Degradation. *PLOS Genet.* 11, e1005180. doi:10.1371/journal.pgen.1005180

Rera, M., Clark, R.I., Walker, D.W., 2012. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 109, 21528–33. doi:10.1073/pnas.1215849110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1215849110

Reuter, M., Bell, G., Greig, D., 2007. Increased outbreeding in yeast in response to dispersal by an insect vector. *Curr. Biol.* 17, R81–R83. doi:10.1016/j.cub.2006.11.059

Roeder, A., Kirschning, C.J., Rupec, R.A., Schaller, M., Weindl, G., Korting, H.C., 2004. Toll-like receptors as key mediators in innate antifungal immunity. *Med. Mycol.* 42, 485–498. doi:10.1080/13693780400011112

Ryu, J.-H., Ha, E.-M., Oh, C.-T., Seol, J.-H., Brey, P.T., Jin, I., Lee, D.G., Kim, J., Lee, D., Lee, W.-J., 2006. An essential complementary role of NF-kappaB pathway to microbicidal oxidants in *Drosophila* gut immunity. *EMBO J.* 25, 3693–3701. doi:10.1038/sj.emboj.7601233

Ryu, J.-H., Kim, S.-H., Lee, H.-Y., Bai, J.Y., Nam, Y.-D., Bae, J.-W., Lee, D.G., Shin, S.C., Ha, E.-M., Lee, W.-J., 2008. Innate Immune Homeostasis by the Homeobox Gene Caudal and Commensal-Gut Mutualism in *Drosophila*. *Science (80-.)*. 319, 777–782. doi:10.1126/science.1149357

Sang, J.H., Sang, B.Y.J.H., 1956. The Quantitative Nutritional Requirements of *Drosophila Melanogaster*. *J. Exp. Biol.* 33, 45–72.

Sansone, C.L., Cohen, J., Yasunaga, A., Xu, J., Osborn, G., Subramanian, H., Gold, B., Buchon, N., Cherry, S., n.d. Microbiota-dependent priming of antiviral intestinal immunity in *Drosophila*. *Cell Host Microbe*.

Scadden, D.T., 2006. The stem-cell niche as an entity of action. *Nature* 441, 1075–9. doi:10.1038/nature04957

Scopelliti, A., Cordero, J.B., Diao, F., Strathdee, K., White, B.H., Sansom, O.J., Vidal, M., 2014. Local control of intestinal stem cell homeostasis by enteroendocrine cells in the adult *Drosophila* midgut. *Curr. Biol.* 24, 1199–1211. doi:10.1016/j.cub.2014.04.007

Shaw, R.L., Kohlmaier, A., Polesello, C., Veelken, C., Edgar, B. a, Tapon, N., 2010. The Hippo pathway regulates intestinal stem cell proliferation during *Drosophila* adult midgut regeneration. *Development* 137, 4147–58. doi:10.1242/dev.052506

Shihata, A.M.E.-T.A., Mrak, E.M., 1951. The Fate of Yeast in the Digestive Tract of *Drosophila*. *Am. Nat.* 85, 381. doi:10.1086/281692

Shilo, B.Z., 2014. The regulation and functions of MAPK pathways in *Drosophila*. *Methods* 68, 151–159. doi:10.1016/j.ymeth.2014.01.020

Shin, S.C., Kim, S.-H.S.-H.S.-H., You, H., Kim, B., Kim, A.C., Lee, K.-A.K.-A., Yoon, J.-H.J.-H., Ryu, J.-H., Lee, W.-J.W.-J., 2011. *Drosophila* Microbiome Modulates Host Developmental and Metabolic Homeostasis via Insulin Signaling. *Science (80-.)*. 334, 670–4. doi:10.1126/science.1212782

Staley, B.K., Irvine, K.D., 2010. Warts and yorkie mediate intestinal regeneration by influencing stem cell proliferation. *Curr. Biol.* 20, 1580–1587. doi:10.1016/j.cub.2010.07.041

Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., Leulier, F., 2011. *Lactobacillus plantarum* Promotes *Drosophila* Systemic Growth by Modulating Hormonal Signals through TOR-Dependent Nutrient Sensing. *Cell Metab.* 14,

1216 403–414. doi:10.1016/j.cmet.2011.07.012

1217 Swarup, S., Verheyen, E.M., 2012. Wnt/Wingless Signaling in *Drosophila*. *Cold Spring Harb. Perspect. Biol.* 4,
1218 a007930–a007930. doi:10.1101/cshperspect.a007930

1219 Takehana, A., Katsuyama, T., Yano, T., Oshima, Y., Takada, H., Aigaki, T., Kurata, S., 2002. Overexpression of a
1220 pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates imd/relish-mediated antibacterial
1221 defense and the prophenoloxidase cascade in *Drosophila* larvae. *Proc. Natl. Acad. Sci.* 99, 13705–13710.
1222 doi:10.1073/pnas.212301199

1223 Tian, a., Shi, Q., Jiang, A., Li, S., Wang, B., Jiang, J., 2015. Injury-stimulated Hedgehog signaling promotes
1224 regenerative proliferation of *Drosophila* intestinal stem cells. *J. Cell Biol.* 208, 807–819.
1225 doi:10.1083/jcb.201409025

1226 Tian, A., Jiang, J., 2014. Intestinal epithelium-derived BMP controls stem cell self-renewal in *Drosophila* adult midgut.
1227 *Elife* 3, e01857. doi:10.7554/eLife.01857

1228 Tremaroli, V., Bäckhed, F., 2012. Functional interactions between the gut microbiota and host metabolism. *Nature* 489,
1229 242–249. doi:10.1038/nature11552

1230 Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J.M., Lemaitre, B., Hoffmann, J. a, Imler, J.L., 2000.
1231 Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. *Immunity* 13,
1232 737–748. doi:10.1016/S1074-7613(00)00072-8

1233 Vacek, D.C., Starmer, W.T., Heed, W.B., 1979. Relevance of the ecology of *Citrus* yeasts to the diet of *Drosophila*.
1234 *Microb. Ecol.* 5, 43–49. doi:10.1007/BF02010577

1235 Vallet-Gely, I., Opota, O., Boniface, A., Novikov, A., Lemaitre, B., 2010. A secondary metabolite acting as a signalling
1236 molecule controls *Pseudomonas entomophila* virulence. *Cell. Microbiol.* 12, 1666–1679. doi:10.1111/j.1462-
1237 5822.2010.01501.x

1238 Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P., Boccard, F., Lemaitre, B., 2005. *Drosophila*
1239 host defense after oral infection by an entomopathogenic *Pseudomonas* species. *Proc. Natl. Acad. Sci. U. S. A.*
1240 102, 11414–11419. doi:10.1073/pnas.0502240102

1241 Wong, A.C.-N., Dobson, A.J., Douglas, A.E., 2014. Gut microbiota dictates the metabolic response of *Drosophila* to
1242 diet. *J. Exp. Biol.* 217, 1894–901. doi:10.1242/jeb.101725

1243 Wong, A.C.-N., Luo, Y., Jing, X., Franzenburg, S., Bost, A., Douglas, A.E., 2015. The Host as the Driver of the
1244 Microbiota in the Gut and External Environment of *Drosophila melanogaster*. *Appl. Environ. Microbiol.* 81,
1245 6232–6240. doi:10.1128/AEM.01442-15

1246 Wong, C.N.A., Ng, P., Douglas, A.E., 2011. Low-diversity bacterial community in the gut of the fruitfly *Drosophila*
1247 *melanogaster*. *Environ. Microbiol.* 13, 1889–1900. doi:10.1111/j.1462-2920.2011.02511.x

1248 Xu, J., Hopkins, K., Sabin, L., Yasunaga, A., Subramanian, H., Lamborn, I., Gordesky-Gold, B., Cherry, S., 2013. ERK
1249 signaling couples nutrient status to antiviral defense in the insect gut. *Proc. Natl. Acad. Sci. U. S. A.* 110, 15025–
1250 30. doi:10.1073/pnas.1303193110

1251 Xu, N., Wang, S.Q., Tan, D., Gao, Y., Lin, G., Xi, R., 2011. EGFR, Wingless and JAK/STAT signaling cooperatively
1252 maintain *Drosophila* intestinal stem cells. *Dev. Biol.* 354, 31–43. doi:10.1016/j.ydbio.2011.03.018

1253 Yamada, R., Deshpande, S.A., Bruce, K.D., Mak, E.M., Ja, W.W., 2015. Microbes Promote Amino Acid Harvest to
1254 Rescue Undernutrition in *Drosophila*. *Cell Rep.* 10, 865–872. doi:10.1016/j.celrep.2015.01.018

1255 Zaidman-Rémy, A., Hervé, M., Poidevin, M., Pili-Floury, S., Kim, M.S., Blanot, D., Oh, B.H., Ueda, R., Mengin-
1256 Lecreulx, D., Lemaitre, B., 2006. The *Drosophila* Amidase PGRP-LB Modulates the Immune Response to
1257 Bacterial Infection. *Immunity* 24, 463–473. doi:10.1016/j.immuni.2006.02.012

1258 Zaidman-Rémy, A., Poidevin, M., Hervé, M., Welchman, D.P., Paredes, J.C., Fahlander, C., Steiner, H., Mengin-
1259 Lecreulx, D., Lemaitre, B., 2011. *Drosophila* immunity: analysis of PGRP-SB1 expression, enzymatic activity
1260 and function. *PLoS One* 6, e17231. doi:10.1371/journal.pone.0017231

1261 Zeng, X., Han, L., Singh, S.R.R.R., Liu, H., Neumüller, R.A.A.A., Yan, D., Hu, Y., Liu, Y., Liu, W., Lin, X., Hou,
1262 S.X.X.X., 2015. Genome-wide RNAi Screen Identifies Networks Involved in Intestinal Stem Cell Regulation in
1263 *Drosophila*. *Cell Rep.* 1–13. doi:10.1016/j.celrep.2015.01.051

1264 Zeng, X., Hou, S.X., 2015. Enteroendocrine cells are generated from stem cells through a distinct progenitor in the adult
1265 *Drosophila* posterior midgut. *Development* 142, 644–653. doi:10.1242/dev.113357

1266 Zhao, B., Tumaneng, K., Guan, K.-L., 2011. The Hippo pathway in organ size control, tissue regeneration and stem cell
1267 self-renewal. *Nat. Cell Biol.* 13, 877–83. doi:10.1038/ncb2303

1268 Zhou, F., Rasmussen, A., Lee, S., Agaisse, H., 2013. The UPD3 cytokine couples environmental challenge and
1269 intestinal stem cell division through modulation of JAK/STAT signaling in the stem cell microenvironment. *Dev.*
1270 *Biol.* 373, 383–93. doi:10.1016/j.ydbio.2012.10.023

1271 Zhou, J., Florescu, S., Boettcher, A., Luo, L., Dutta, D., Kerr, G., Cai, Y., Edgar, B.A., Boutros, M., 2015. Dpp/Gbb
1272 signaling is required for normal intestinal regeneration during infection. *Dev. Biol.* 399, 189–203.
1273 doi:10.1016/j.ydbio.2014.12.017

1274 Zoetendal, E.G., Akkermans, A.D.L., De Vos, W.M., 1998. Temperature gradient gel electrophoresis analysis of 16S
1275 rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl. Environ.*
1276 *Microbiol.* 64, 3854–3859.

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Pathway name	Ligands	Receptors	Key components	Target genes	Signaling mechanism
JAK/STAT	Unpaired (Upd1), Upd2, Upd3	Domeless (Dome)	Hopscotch (Hop), STAT (TF)	<i>Suppressor of cytokine signaling at 36E</i> (<i>Socs36E</i>)	Upon binding, Dome dimerizes and activates Hop, which phosphorylates the TF STAT.
Hippo		Adhesion proteins	Hippo (Hpo), Salvador (Sav), Warts (Wts), Yorkie (Yki, TF)	<i>diap1</i> and <i>cyclin E</i>	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 <i>Puc</i>	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 interruptus (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	<i>Dmel</i> Insulin ligand peptides (dIlps) 1-7	<i>Dmel</i> 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 Chicho. This leads to activation of Pdk1, which activates Akt and thus blocks FOXO.

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.

Figure1: 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.

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1315 **Figure 2: The *Drosophila* gut immune response.** Two main antimicrobial mechanisms act in the *Drosophila* midgut.
 1316 First the gut is able to secrete antimicrobial peptides (AMPs) controlled mostly by the Imd pathway (in green shade).
 1317 Peptidoglycan from microbes is recognized by PGRP-LC and PGRP-LE, and further activates the Imd pathway leading
 1318 to transcriptional induction of AMPs. Multiple negative regulators control the proper level of Imd activation. Some
 1319 regulators are also targets of the Imd pathway (PGRP-LB, Pirk) while others are regionally expressed in the midgut
 1320 (Caudal). Second, NADPH oxidases (Duox and Nox) produce reactive oxygen species (ROS) that have a bactericidal
 1321 effect (in red shade). Two bacterial patterns activate Duox expression and activity: Peptidoglycan and microbial derived
 1322 Uracil.

Figure 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 dLIP 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.

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1347 **Figure 4: Effect of the microbiota, pathogenic microbes, and abiotic damage on ISC activity.** Abbreviations:
 1348 Intestinal stem cell (ISC), Enterocyte (EC), Enteroendocrine cell (EE), Visceral muscle (VM), Basal membrane (BM).
 1349 In the green shading on the left is summarized the response to the *Dmel* gut microbiota. Microbiota trigger mild
 1350 immune responses that in turn damage ECs, thus inducing low levels of homeostatic proliferation. However, they also
 1351 have a positive influence on ISC activity by modulating nutrient availability and modifying host physiology and insulin
 1352 pathway. In the central red shading is described the response to Pathogens. Virulent bacteria inflict damage to the ECs
 1353 either as a consequence of their virulence or of the strong immune response they induce. This leads to a massive loss of
 1354 ECs, which need to be replaced through a sustained proliferation of ISCs. Some pathogens, such as *Serratia marcescens*
 1355 (Sm) and *Pseudomonas aeruginosa* (Pa) have the ability to cross the intestinal barrier. In the blue shading on the right
 1356 is summarized the response to abiotic challenges. Chemicals such as bleomycin and paraquat induce a strong stress
 1357 response that damages ECs and activates the same pathways as the response to pathogens. DSS seems to have a
 1358 different mechanism of action, acting through disruption of the Basal Membrane.

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