

Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*

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Gut homeostasis is controlled by both immune and developmental mechanisms, and its disruption can lead to inflammatory disorders or cancerous lesions of the intestine. While the impact of bacteria on the mucosal immune system is beginning to be precisely understood, little is known about the effects of bacteria on gut epithelium renewal. Here, we addressed how both infectious and indigenous bacteria modulate stem cell activity in *Drosophila*. We show that the increased epithelium renewal observed upon some bacterial infections is a consequence of the oxidative burst, a major defense of the *Drosophila* gut. Additionally, we provide evidence that the JAK–STAT (Janus kinase–signal transducers and activators of transcription) and JNK (c-Jun NH₂ terminal kinase) pathways are both required for bacteria-induced stem cell proliferation. Similarly, we demonstrate that indigenous gut microbiota activate the same, albeit reduced, program at basal levels. Altered control of gut microbiota in immune-deficient or aged flies correlates with increased epithelium renewal. Finally, we show that epithelium renewal is an essential component of *Drosophila* defense against oral bacterial infection. Altogether, these results indicate that gut homeostasis is achieved by a complex interregulation of the immune response, gut microbiota, and stem cell activity.

[**Keywords:** Intestinal stem cell; proliferation; gut microbiota; JAK–STAT; JNK; pathogenic bacteria]

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The gut of all organisms is in constant exposure to environmental and microbial aggressions (Sansonetti 2004). It is an active barrier, limiting invasion by pathogenic microorganisms and promoting tolerance to indigenous microbiota (Pedron and Sansonetti 2008). To maintain homeostasis, the gut epithelium is constantly renewed throughout an organism's life by the division and differentiation of intestinal stem cells (ISCs). In *Drosophila*, ISCs are scattered along the basement membrane of the midgut (Micchelli and Perrimon 2006; Ohlstein and Spradling 2006). The division of an ISC generates both a new ISC and a post-mitotic enteroblast, which differentiates into either an absorptive enterocyte or a secretory enteroendocrine cell. In basal conditions, the adult gut epithelium is renewed in ~1–2 wk (Micchelli and Perrimon 2006).

We recently reported a global analysis of the changes in gene expression that occur in the *Drosophila* gut in response to infection by a Gram-negative bacterium, *Erwinia carotovora carotovora 15* (*Ecc15*) (Buchon et al. 2009). This study demonstrated that bacterial infec-

tion triggers expression of a combination of immune, stress, and developmental signaling pathways. Most immune genes—notably, antibacterial peptide genes (e.g., *Diptericin*)—are induced in the gut by the Imd pathway. This pathway is activated upon recognition of peptidoglycan of Gram-negative bacteria by the transmembrane receptor PGRP-LC, and leads to activation of the NF- κ B protein Relish (Zaidman-Remy et al. 2006). Subsequent analysis showed that the JAK–STAT (Janus kinase–signal transducers and activators of transcription) signaling pathway was activated by damage in the gut of infected flies and regulates the production of a subset of antimicrobial peptides. Surprisingly, it was further observed that ingestion of *Ecc15* strongly stimulates ISC division, promoting a rapid turnover of the gut epithelium (Buchon et al. 2009). These results highlighted an unexpected link between oral bacterial infection and epithelial renewal. These data also suggested that, following infection, gut homeostasis is maintained by the balance between cell damage induced by infection and epithelial repair by ISC activation. However, the precise causes of ISC proliferation, as well as the signaling events regulating this induction, were unclear, as was the effect of indigenous or lethal bacteria on this mechanism.

Here we use the genetically tractable model *Drosophila melanogaster* to study the link between gut bacteria, the

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gut immune response, and epithelium renewal. We show that the oxidative burst, induced by the host consecutive to *Ecc15* infection, is a major inducer of ISC activation. Infection with this bacterium activates both JAK-STAT and JNK (c-Jun NH₂ terminal kinase) pathways in ISCs to promote proliferation. Indigenous bacteria induce the same program and lead to a basal level of proliferation. Inversely, *Pseudomonas entomophila*, a lethal entomopathogenic bacterium, interferes with ISC activation, thereby disrupting gut homeostasis.

Results

Infection-induced oxidative burst is a major inducer of epithelium renewal

The gut immune response of *Drosophila* has two main components: an initial oxidative burst by the NADPH oxidase enzyme Duox (Ha et al. 2005a), followed by the synthesis of antimicrobial peptides controlled by the Imd pathway. Previously, we reported that oral infection with *Ecc15* stimulates epithelium turnover in the intestine and strongly activates the transcription of genes known to respond to oxidative stress (Buchon et al. 2009). Since *Ecc15* is known to induce Duox activity (Ha et al. 2005a), we hypothesized that the oxidative burst induced upon ingestion of *Ecc15* could be a major inducer of both gut damage and epithelium renewal. To test this, we monitored epithelium renewal following ingestion of *Ecc15* in flies in which the level of reactive oxygen species (ROS) had been artificially reduced. Intestinal epithelium renewal can be monitored easily in *Drosophila* by (1) counting the number of dividing cells along the midgut using an anti-phosphohistone H3 (anti-PH3) antibody as an indicator of mitotic activity; (2) using the *esgGal4*, *UAS-GFP* marker, which strongly labels ISCs, enteroblasts, and newly synthesized enterocytes; or (3) by cell lineage analysis revealing the expansion of *lacZ*-marked clones; with the two latter monitoring not only cell division, but also epithelium differentiation (Micchelli and Perrimon 2006; Ohlstein and Spradling 2006). As shown previously, we detected a massive epithelium renewal following ingestion of *Ecc15* by both clonal analysis and the stem cell marker *esgGal4*, *UAS-GFP*. In contrast, epithelium renewal did not increase in flies fed *Ecc15* in combination with the antioxidants N-acetyl cysteine (NAC) or glutathione (Fig. 1A,B; quantification in Supplemental Fig. S1C), although these two compounds did not affect *Ecc15* viability (Supplemental Fig. S1A,B). Conversely, ingestion of paraquat, a potent inducer of ROS, induced epithelium renewal without infection (Fig. 1A,B), in agreement with previous studies (Biteau et al. 2008; Buchon et al. 2009; Chatterjee and Ip 2009).

While increased ISC proliferation was observed in Imd-deficient flies infected orally with *Ecc15* (Buchon et al. 2009), silencing of the *Duox* gene by RNAi significantly reduced the number of mitotic ISCs detected with anti-PH3 (Fig. 1C). Similarly, ISC proliferation was also reduced in flies carrying either a mutation in *Gαq*, which

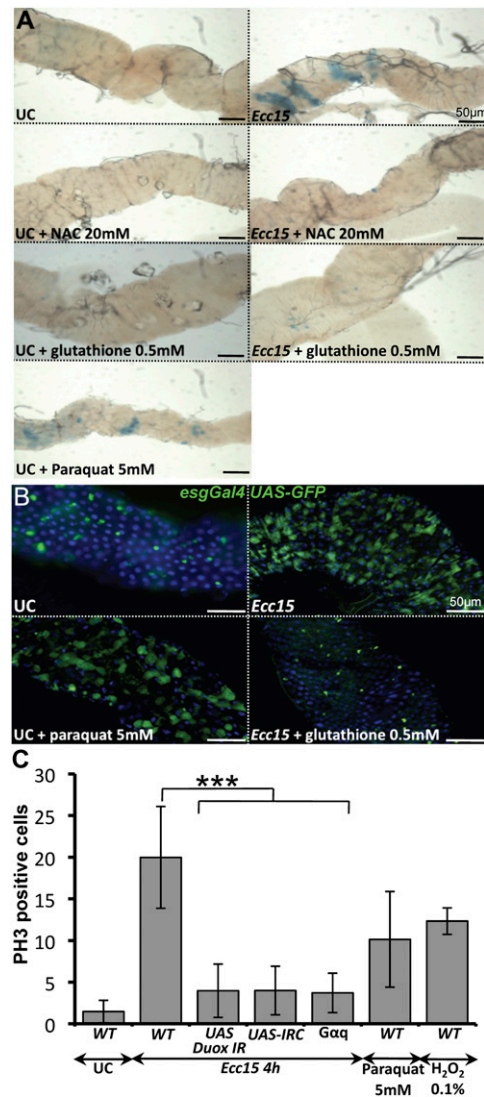


Figure 1. Immune-stimulated oxidative burst induces stem cell activation in the gut. (A) Large *lacZ*-marked clones containing the *tubulin* promoter-*lacZ* fusion due to mitotic recombination are observed in the guts of flies orally infected with *Ecc15* or fed with paraquat. No expansion of *lacZ* clones was detected in the gut of flies fed with both *Ecc15* and antioxidants (NAC or glutathione). Quantification of *lacZ* clones is shown in Supplemental Figure S1C. (B) Ingestion of *Ecc15* or paraquat induces a marked increase in the number of *esgGal4*, *UAS-GFP*-positive cells. In contrast, coingestion of *Ecc15* and glutathione did not induce epithelium renewal. In unchallenged flies, most of the GFP signal corresponds to ISCs. In *Ecc15*-infected flies, the GFP signal was observed in both ISCs and ISC-derived daughter cells (see Supplemental Fig. S6 for distribution of escargot-positive cells along the midgut). Flies were collected 16 h post-infection. (C) Quantification of PH3-positive cells per midgut of unchallenged (UC) flies or flies collected 4 h after feeding with *Ecc15*, paraquat, or H₂O₂. Guts of *Duox* RNAi flies (*daGal4*, *UAS-Duox-IR*), *Gαq*-deficient flies, or flies overexpressing a catalase (*daGal4*, *UAS-IRC*) orally infected with *Ecc15* exhibit a lower number of dividing ISCs compared with wild type. Mean ± standard deviation (SD) are shown. Values significantly different in a Student's *t*-test ($P < 0.001$) are denoted by three asterisks (***).

encodes a heterodimeric G protein responsible for Duox enzymatic activation (Ha et al. 2009), or overexpressing IRC, a catalase involved in ROS detoxification (Fig. 1C; Ha et al. 2005b). Collectively, our results indicate that the increased epithelium renewal observed upon infection with *Ecc15* is largely a consequence of the oxidative burst resulting from Duox activation in response to infection. Reduced ISC proliferation in flies with reduced Duox activity or coinfecting with *Ecc15* and antioxidants was correlated with reduced levels of cell death in the gut (data not shown). This suggests that the increase in epithelium renewal is not a direct effect of ROS, but rather an effect of the damage caused by the oxidative burst. In agreement with this, the ingestion of toxic compounds such as SDS, DSS, or bleomycin is sufficient to induce epithelium renewal (Amcheslavsky et al. 2009; Buchon et al. 2009).

Activation of the JAK–STAT pathway in ISCs by the cytokine Upd3 stimulates epithelium renewal upon Ecc15 infection

We next investigated the nature of the signaling pathways stimulating epithelium renewal upon *Ecc15* infection. The JAK–STAT pathway has been implicated in stem cell control in Malpighian tubules, testis, and ovaries (Gregory et al. 2008). We demonstrated previously that three genes encoding components of the JAK–STAT pathway—the receptor *Domeless*, one of its ligands, Unpaired 3 (*Upd3*), and a repressor of the receptor/JAK complex, *Socs36E*—were strongly induced in the gut following ingestion of *Ecc15* and paraquat (Buchon et al. 2009). Interestingly, the level of JAK–STAT signaling activity in *Ecc15*-infected guts was reduced in *Duox* RNAi flies or flies fed with antioxidants, as revealed by the decreased induction of the target genes *upd3* and *Socs36E*, and of the JAK–STAT reporter gene *STAT-GFP* (Fig. 2A; Bach et al. 2007; data not shown). Moreover, use of the *STAT-GFP* reporter gene revealed that the JAK–STAT pathway is already active at a basal level in ISCs and enteroblasts of unchallenged flies (Supplemental Fig. S2A). The strong correlation between stimuli activating stem cell proliferation and those inducing JAK–STAT activity prompted us to examine the role of the JAK–STAT pathway in controlling ISC proliferation upon bacterial infection.

In this aim, we expressed RNAi or dominant-negative constructs targeting JAK–STAT pathway components in either ISCs (using *esgGal4*) or enterocytes (using *NP1Gal4*) and monitored stem cell activity. Stem cell proliferation in the midgut, as monitored by the number of PH3-positive cells, was still observed in flies with reduced STAT activity in enterocytes (*NP1Gal4,UAS-STAT92E-IR*) (Fig. 2B). The mitotic index was even higher than in wild-type flies. Since the expression of a *STAT92E-IR* construct driven by *esgGal4* resulted in lethality, we generated conditional *STAT92E-IR* individuals by combining the *esgGal4* transgene and a temperature-sensitive version of the Gal4 inhibitor, *tubGal80^{TS}* (referred to as *esgGal4^{TS}*). When reared at a restrictive temperature (18°C, Gal80^{TS} on, Gal4 system off) during

their entire development, adult flies displayed a wild-type number and distribution of ISCs. However, shifting flies to a permissive temperature (29°C, Gal80^{TS} off, Gal4 system on) for 3 d to deplete STAT in ISCs changed the pattern of escargot-positive cells along the midgut (Fig. 2C; Supplemental Fig. S2B). In the anterior midgut, we observed a reduction in the number of escargot GFP-positive cells. However, small aggregates of escargot GFP-positive cells were observed occasionally in the anterior midgut of *esgGal4^{TS} STAT92E-IR* flies (indicated by an arrow in Fig. 2C3). In contrast, a high number of GFP-positive cells were consistently observed in the posterior region of the midgut. These cells were small-nucleated and did not express the ISC marker Delta or the enteroendocrine marker Prospero, raising the possibility that they are enteroblasts (Supplemental Fig. S2C,D). This suggests that the JAK–STAT pathway is not required for ISC proliferation, but rather for the proper differentiation of ISC progeny in basal turnover conditions. In contrast to wild-type flies, no increase in the number of mitotic cells (PH3-positive) nor change in the pattern and distribution of escargot GFP-positive cells was observed in the gut of *esgGal4^{TS} STAT92E-IR* flies upon *Ecc15* infection (Fig. 2B,C), indicating a lack of epithelium renewal induction. Similar results were obtained with the use of the dominant-negative version of the JAK–STAT pathway receptor *Domeless* (Fig. 2B; Brown et al. 2001; data not shown).

We further tested the effect of reducing JAK–STAT signaling by generating mosaic animals with clones that are mutant for the *STAT92E* transcription factor (Lee and Luo 2001; Krezmien et al. 2007). Upon *Ecc15* infection of the mosaic flies, *STAT92E* mutant clones were rarely observed, but when present were composed of only small-nucleated cells. In contrast, control clones contained both small diploid progenitors and large polyploid enterocytes (Supplemental Fig. S2E). This indicates that a reduction in JAK–STAT signaling in ISCs blocks epithelium renewal induced upon *Ecc15* infection. Finally, overexpressing a gain-of-function form of the JAK kinase Hop in ISCs (*UAS-hop^{Tum}*) triggered epithelium renewal without infection (Fig. 2B,D1). We conclude that the JAK–STAT pathway is required in ISCs for the increased epithelium renewal induced upon bacterial infection with *Ecc15*.

We reported previously that the JAK–STAT ligand *upd3* is transcriptionally induced in the gut upon ingestion of both *Ecc15* and paraquat (Buchon et al. 2009). Use of an *upd3-Gal4; UAS-GFP* reporter transgene (Agaisse et al. 2003) revealed that *upd3* was expressed mostly in enterocytes (Fig. 2E). In agreement, *upd3* expression was decreased in flies when *upd3-IR* was expressed in enterocytes, but not when it was expressed in ISCs (Supplemental Fig. S2F). Interestingly, physical injury of the gut with forceps rapidly induced a localized expression of *upd3* (Fig. 2F), demonstrating that *Upd3* is induced in the vicinity of damaged enterocytes and supporting its role in the activation of the JAK–STAT pathway in ISCs. Accordingly, specific silencing of *upd3* expression in enterocytes, but not in ISCs, strongly decreased the number of proliferative ISCs in response to infection (Fig. 2B). Conversely, the ectopic expression of *upd3* in ISCs was

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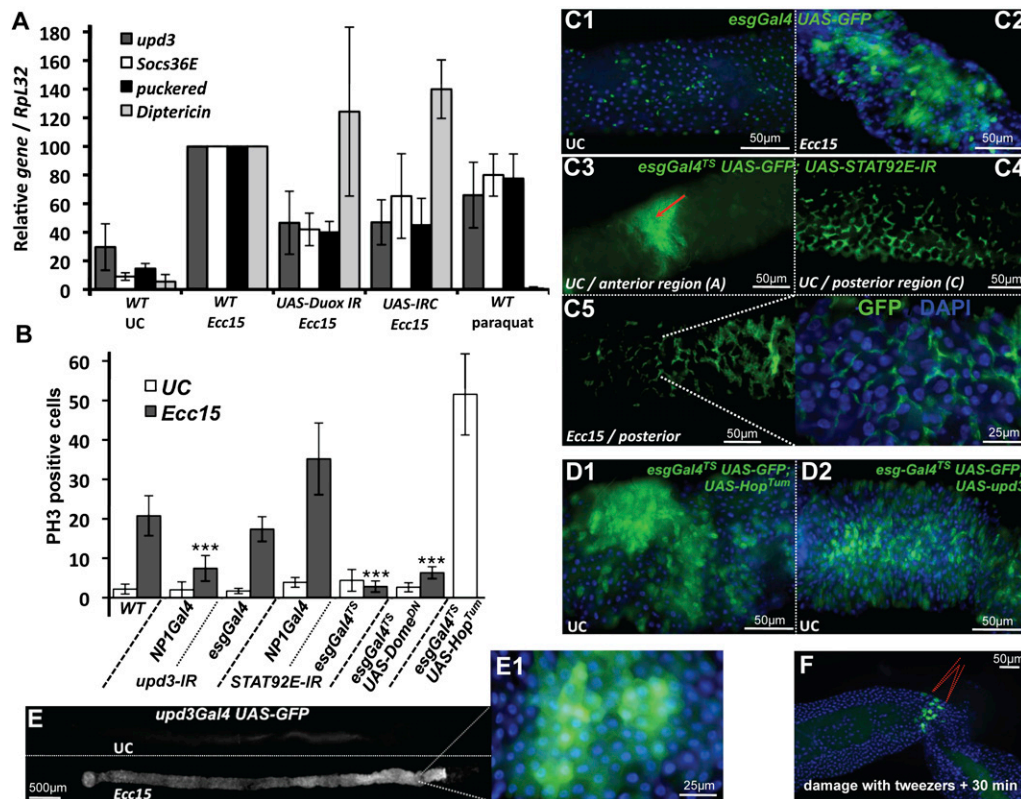


Figure 2. The JAK–STAT pathway is required for ISC proliferation upon *Ecc15* infection. (A) Oxidative stress modulates the activation of JAK–STAT and JNK pathways upon *Ecc15* infection. RT–qPCR analysis of gut extracts shows that the expression levels of JAK–STAT (*upd3*, *Socs36E*, monitored 4 h post-infection) and JNK (*puckered*, monitored 30 min post-infection) target genes were reduced upon *Ecc15* infection in the gut of *Duox RNAi* flies or flies expressing a catalase (*daGal4*; *UAS-IRC*). Ingestion of paraquat was sufficient to induce *upd3*, *Socs36E*, or *puckered*. In contrast, the level of *Diptericin* (a gene tightly regulated by the Imd pathway) was not affected by conditions modulating ROS. Mean of four independent experiments \pm SD are shown. (B) Quantification of PH3-positive cells per midgut shows an increase in the number of mitotic cells upon *Ecc15* infection in both wild-type flies and flies with reduced JAK–STAT activity in enterocytes (*NP1Gal4*, *UAS-STAT92E-IR*), but not in ISCs (*esgGal4^{TS}*, *UAS-STAT92E-IR*). Silencing of *upd3* in enterocytes (*NP1Gal4*, *UAS-upd3-IR*), but not in ISCs (*esgGal4*, *UAS-upd3-IR*), reduced the number of mitotic ISCs in the guts of flies collected 4 h after feeding with *Ecc15*. Overexpression of a gain-of-function allele of *hop* in ISCs increases the mitotic index (*esgGal4^{TS}*, *UAS-hop^{um}*). Mean \pm SD are shown. Values significantly different from infected wild-type flies in a *t*-test at $P < 0.001$ are denoted by three asterisks (***). (C1–C5) A loss of stem cells was observed in the anterior region of *esgGal4^{TS}*, *UAS-STAT92E-IR* flies raised at 29°C. In some flies, accumulation of small-nucleated escargot-positive cells were observed in the anterior part of the midgut, as indicated with a red arrow in C3. (C4) Accumulation of small-nucleated escargot-positive cells was observed in the posterior regions of the gut of all *esgGal4^{TS}*, *UAS-STAT92E-IR* flies raised at 29°C, in the absence of infection. (C5) No increase in epithelium renewal was observed in these flies upon infection with *Ecc15*, as indicated by the lack of large nuclei cells expressing GFP. Expression of the *esgGal4*, *UAS-GFP* reporter gene was monitored 16 h after infection with *Ecc15*. (D1, D2) Overexpression of *hop^{um}* (D1) or *upd3* (D2) in ISCs is sufficient to induce a high level of epithelium renewal in the absence of infection. (E) *upd3* expression is induced along the gut following *Ecc15* ingestion. (E1) *upd3* was expressed mostly in enterocytes 4 h after *Ecc15* infection, as revealed by the GFP signal (green) of *upd3Gal4* *UAS-GFP* flies. (F) Physical damage to the gut with tweezers induced local expression of the *upd3Gal4*, *UAS-GFP* reporter within 30 min following injury.

sufficient to induce a high level of epithelium renewal in the absence of infection (Fig. 2D2). Altogether, these results indicate that upon *Ecc15* infection, enterocytes release Upd3, which activates the JAK–STAT pathway in ISCs to promote proliferation and to repair damage to the gut.

The JNK pathway is required for ISC maintenance and proliferation in the gut of infected flies

The JNK pathway is activated by a variety of environmental challenges, including oxidative stress, and was

demonstrated recently to be required in ISCs to maintain gut homeostasis in aging flies (Wang et al. 2003; Biteau et al. 2008). We reported previously that the JNK pathway is activated in the gut of flies soon after ingestion of *Ecc15* (Buchon et al. 2009). Furthermore, coimmunostaining of the ISC marker *esg-GFP* and of *puckered* (*puc-lacZ*), a reporter of JNK pathway activity (Martín-Blanco et al. 1998), revealed that the JNK pathway was strongly activated in both enterocytes and ISCs upon *Ecc15* infection (Fig. 3A). Interestingly, RT–qPCR analysis revealed that JNK induction was dampened in both *Duox*

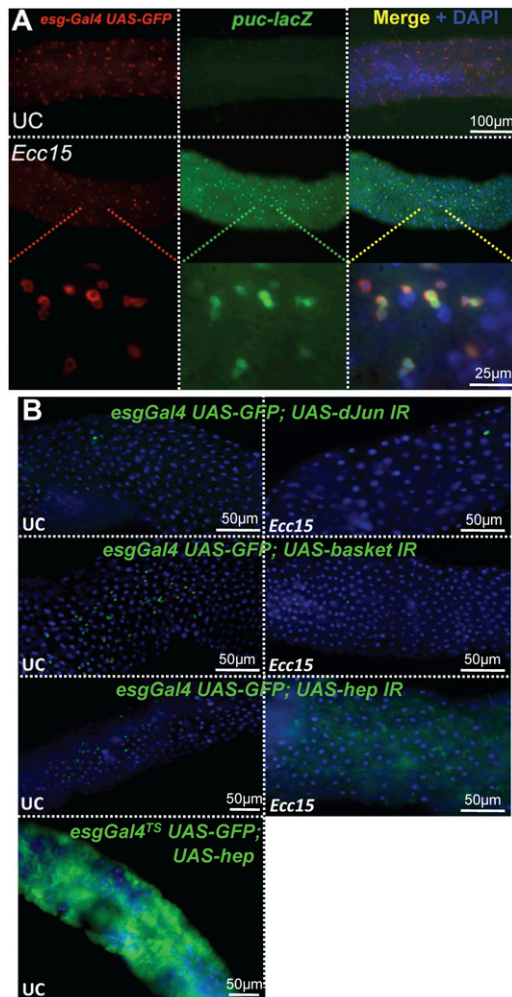


Figure 3. The JNK pathway is required for ISC maintenance and proliferation upon *Ecc15* infection. (A) Immunostaining with antibodies against GFP (red) or LacZ (green) of *esgGal4*, *UAS-GFP*, *puc^{E69}* (*puc-lacZ*) flies collected 30 min after *Ecc15* infection shows that the level of JNK signaling activity increases (top center panel, compared with middle center panel) upon *Ecc15* infection in both enterocytes and ISCs, the staining being more intense in ISCs (merge, right panels); higher magnification is shown in the bottom panels. (B) Loss of ISCs and the absence of epithelium renewal were observed in the guts of flies with reduced JNK activity in ISCs. Guts of flies carrying the *esgGal4*, *UAS-GFP* combined with *UAS-dJun-IR*, *UAS-basket IR*, or *UAS-hep-IR* were examined 16 h post-infection with *Ecc15*. Silencing of *dJun-IR* in ISCs reduced the number of ISC in both unchallenged and challenged animals. In contrast, silencing of the genes encoding the JNK Basket and JNKK Hep in ISCs leads to a loss of ISCs only after infection with *Ecc15*. Ectopic expression of *UAS-hep* in ISCs induces epithelium renewal in the absence of infection.

RNAi flies and flies coinfecting with *Ecc15* and antioxidants, as monitored by a decrease in expression of *puckered*, a target gene of the JNK pathway (Fig. 2A). These results prompted us to investigate the involvement of the JNK pathway in ISC proliferation during bacterial infection. Silencing the transcription factor *dJun* in ISCs

by RNAi resulted in the loss of ISCs along the gut of unchallenged flies, as indicated by the absence of escargot GFP-positive cells (Fig. 3B). Immunostaining with the ISC-specific marker Delta confirmed that the absence of escargot GFP-positive cells corresponded with the loss of ISCs (data not shown). Similar results were obtained when the inducible *esg-Gal4 tubGAL80^{ts}* system was used to drive the *dJun-IR* construct, indicating that the loss of ISCs does not result from an effect of JNK during development (data not shown). Interestingly, knockdown of the JNK *basket* or the JNK kinase (JNKK) *hemipterous* (*hep*) in ISCs or overexpression of a negative regulator of JNK signaling (*UAS-puckered*) did not alter the number of ISCs in unchallenged flies (Fig. 3B; data not shown). However, upon *Ecc15* infection, the number of ISCs was decreased dramatically in the gut of flies with reduced JNK activity, and no epithelium renewal was observed (Fig. 3B). Moreover, immunostaining for the apoptotic marker caspase3 revealed that, upon *Ecc15* infection, some remaining ISCs were undergoing apoptosis when depleted for the JNK Basket (Supplemental Fig. S3A). Importantly, no increase in the number of dividing cells was observed upon *Ecc15* infection in the gut of flies with reduced JNK activity in ISCs, as monitored by the number of PH3-positive cells (Supplemental Fig. S3B). In contrast, overexpression of *hep* with the *esg-Gal4^{TS}* driver led to increased epithelium renewal in the absence of infection (Fig. 3B; Supplemental Fig. S3B).

We conclude that the lack of JNK pathway activity in ISCs results in a premature loss of ISCs and the consequent absence of epithelium renewal upon bacterial infection with *Ecc15*.

Epithelium renewal is an essential component of Drosophila defense to Ecc15

Both wild-type and Imd-deficient flies survive ingestion of *Ecc15* (Basset et al. 2000), indicating that even without a functional Imd-dependent immune response, this bacterium is not lethal. Importantly, we observed no difference in the level of epithelium renewal between wild-type and Imd-deficient flies in response to *Ecc15* (Buchon et al. 2009). In contrast, reduction of JNK or JAK-STAT signaling in ISCs abolishes the epithelium renewal that is induced upon infection with *Ecc15*, but does not affect the Imd-dependent immune response (data not shown). To analyze the extent to which epithelium renewal is important in the resistance to *Ecc15* infection, we compared the survival of flies in which the activity of the JNK (*UAS-basket-IR*, *UAS-dJun-IR*), JAK-STAT (*UAS-upd3-IR* or *UAS-STAT92E-IR*), and Imd (*UAS-dFadd-IR*) pathways had been selectively reduced in either enterocytes (Fig. 4A) or ISCs (Fig. 4B). Strikingly, flies impaired in their capacity to renew their epithelium—due to the inhibition of either JAK-STAT or JNK signaling in ISCs, or the silencing of *upd3* in enterocytes—succumbed 4–8 d following ingestion of *Ecc15* (Fig. 4A,B). Examination of flies impaired in epithelium renewal at 4 d revealed that their guts were morphologically altered, with regions devoid of enterocytes (Fig. 4C) or displaying aberrant cell

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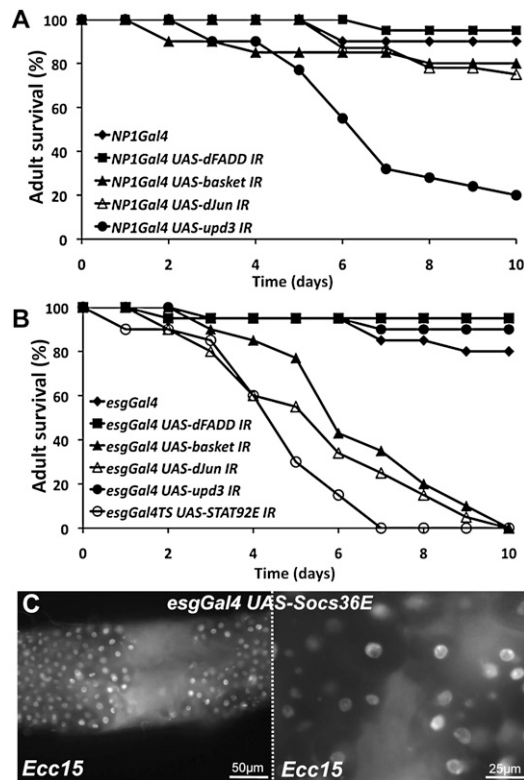


Figure 4. Epithelium renewal is required for proper resistance to *Ecc15* infection. (A,B) Survival analysis at 29°C shows that flies impaired in epithelium renewal succumbed 4–8 d following ingestion of *Ecc15*. *UAS-dFADD-IR*, *UAS-basket-IR*, *UAS-dJun-IR*, or *UAS-upd3-IR* constructs were expressed in either enterocytes (*NP1Gal4*; A) or ISCs (*esgGal4* or *esgGal4^{TS}*; B). (C) Guts of flies impaired in epithelium renewal displayed altered gut morphology as revealed by the lack of DAPI nuclear staining. Flies were observed 4 d post-infection.

shapes, suggesting a disruption in the continuity of the gut and a lack of tissue integrity. The corresponding increase in susceptibility demonstrates that epithelium renewal is necessary for the proper recovery of flies after infection with *Ecc15* and is more critical than the Imd pathway in the response to this bacterium.

Indigenous microbiota impact epithelium renewal through ISC stimulation

Even in basal conditions, the gut is permanently in contact with indigenous microorganisms (Hooper 2004). We used axenically raised flies to investigate how gut microbiota impact epithelium renewal. Both the number of mitotic ISCs and the rate of epithelium renewal were reduced in axenic flies as compared with conventionally raised (CR) flies (Fig. 5A; Supplemental Fig. S4A,B). This reduction in epithelium renewal was not due to an inability of axenic flies to activate renewal, as infection with *Ecc15* and reintroduction of culturable indigenous bacteria increased mitotic activity in these flies (data not shown). Moreover, RT-qPCR analysis demonstrated that

the gut microbiota regulate, to some extent, the basal levels of JAK–STAT, JNK, and Imd pathways (Fig. 5B). Use of the *upd3Gal4*, *UAS-GFP* reporter gene revealed that *upd3* was expressed at low levels in a few cells scattered along the gut of CR flies. In sharp contrast, no expression of *upd3* was observed in the gut of axenic flies (Fig. 5C). Taken together, these data suggest that, under normal conditions, gut microbiota stimulate these pathways, promoting a basal level of epithelium renewal. Interestingly, higher numbers of dividing cells were detected in the absence of infection in the guts of *Relish^{E20}* or *PGRP-LC^{E12}* flies, which are deficient for the Imd pathway (Fig. 5A). These flies harbor 10-fold higher counts of indigenous bacteria than wild-type (Supplemental Fig. S4C), suggesting that an abnormally abundant microbiota could increase epithelium renewal. To test this hypothesis, we raised *Relish^{E20}* and *PGRP-LC^{E12}* flies in axenic conditions and monitored ISC proliferation by anti-PH3 staining. Increased ISC proliferation was no longer observed in *Relish^{E20}* and *PGRP-LC^{E12}* axenic flies (Fig. 5A). These data indicate that indigenous bacteria condition the basal level of epithelium renewal by stimulating ISC division, probably through an increase of JAK–STAT and JNK activity.

Recent studies have revealed that age-related deterioration of the intestinal epithelium is associated with excessive ISC proliferation and aberrant differentiation (Biteau et al. 2008; YJ Choi et al. 2008; Park et al. 2009). In agreement with this, we observed a 10-fold higher mitotic index in the midguts of 30-d-old flies as compared with 3-d-old flies by anti-PH3 staining (Fig. 5A). Moreover, increased *esgGal4*, *UAS-GFP* signal was observed in the guts of 30-d-old flies (Fig. 5D1), at a similar level to that observed with *Ecc15* infection. Higher magnification revealed the accumulation of large, rounded escargot-positive cells, described previously as a hallmark of gut disorganization upon aging (Fig. 5D2; Biteau et al. 2008). This deregulation of epithelium renewal correlated with increased expression levels of target genes of the JNK, JAK–STAT, and Imd pathways (Fig. 5B). These age-dependant symptoms were exacerbated in the *Relish^{E20}* flies (Fig. 5A), whose guts were morphologically altered with regions devoid of enterocytes (Supplemental Fig. S4D). Interestingly, guts from both wild-type and *Relish^{E20}* 30-d-old flies contain higher counts of indigenous bacteria than their younger counterparts (Supplemental Fig. S4C; Ren et al. 2007), raising the possibility that the increased epithelium renewal in older flies could be caused by the abundant microbiota. We examined axenically raised flies to investigate how gut microbiota impact age degeneration of the gut. Strikingly, guts from 30-d-old flies raised axenically have levels of epithelium renewal more similar to that of young flies, as indicated by the mitotic index and use of the *esgGal4*, *UAS-GFP* marker (Fig. 5A,D). This correlates with reduced levels of JNK and JAK–STAT activity (Fig. 5B). Moreover, the guts of old *Relish^{E20}* axenic flies do not exhibit alterations in intestinal integrity (Supplemental Fig. S4D). These data indicate that age-related defects in the gut are largely reverted in the absence of indigenous bacteria.

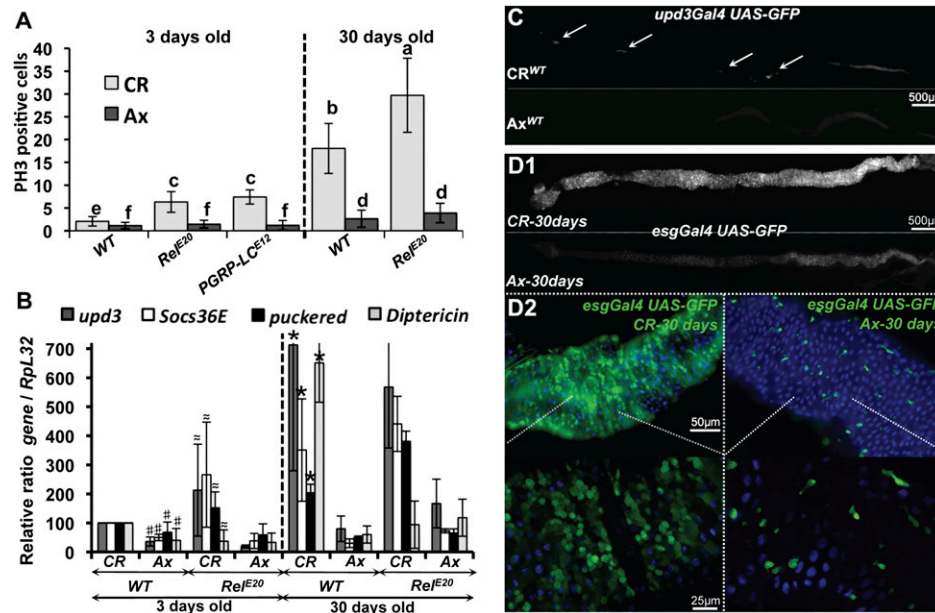


Figure 5. Indigenous gut microbiota activate a basal level of ISC activity. (A) Axenic (Ax) flies display a level of ISC proliferation lower than CR flies, as measured by the number of mitotic cells along the gut of unchallenged flies (PH3-positive cells). Higher numbers of dividing cells were detected in the guts of CR flies, but not axenic *Rel^{E20}* or *PGRP-LC^{E12}* flies, in the absence of infection. Both young (2–4 d) and old (30 d) flies were examined. Means \pm SD are shown. Means were analyzed by ANOVA and separated for significance according to Fisher's protected LSD at $P = 0.05$. Means designated with the same letter are not significantly different. (B) RT-qPCR analysis shows that axenic flies express lower levels of *Diptericin*, *upd3*, *Socs36E*, and *puckered* compared with CR flies in the absence of infection. Expression of these genes increased in old CR flies, but not old axenic flies. Means \pm SD are shown. Values significantly different from the wild-type CR flies in a *t*-test at $P < 0.001$ are denoted for axenic wild type versus CR wild type (#), CR *Rel^{E20}* versus CR wild type (\approx), and old CR wild type versus young CR wild type (*). (C) Basal *upd3* expression was not detected in axenic flies, as revealed by the GFP signal of *upd3Gal4 UAS-GFP* flies. (D1) The guts of old (30 d) CR, but not axenic, *esgGal4 UAS-GFP* flies display increased epithelium renewal all along the gut. (D2) Large rounded escargot-positive cells accumulate in the guts of old CR, but not axenic, wild-type flies.

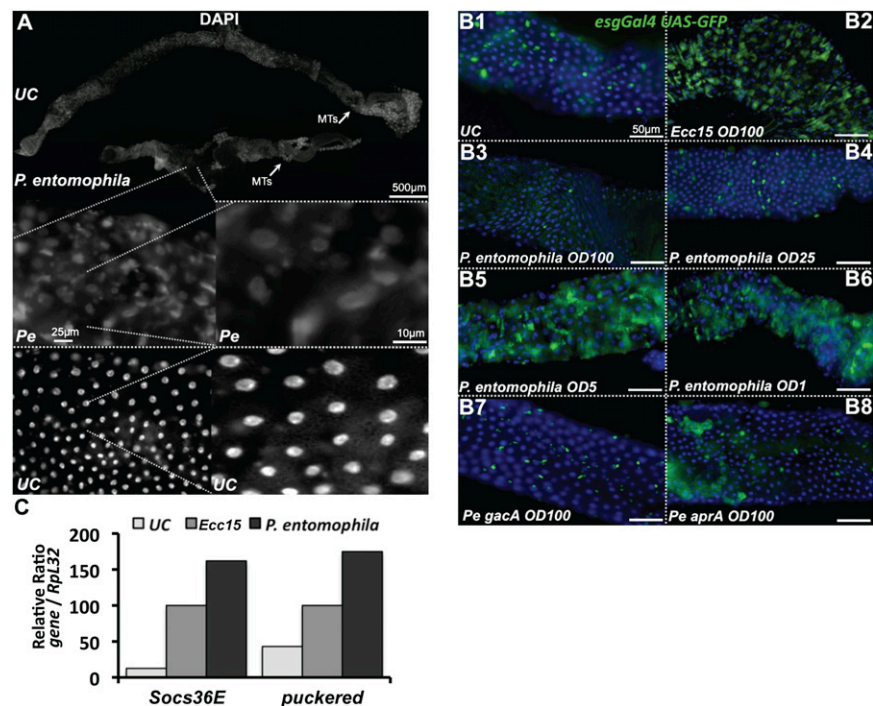
Disruption of gut epithelium renewal by an entomopathogenic bacterium

P. entomophila is an entomopathogenic bacterium that, unlike *Ecc15*, is lethal to flies within 1–2 d of ingestion at high dose (Vodovar et al. 2005). We investigated the impact of *P. entomophila* on gut homeostasis and observed that, following ingestion, the length of the gut was reduced compared with uninfected flies (Fig. 6A). Closer examination of these guts revealed that enterocytes were missing along the gut or severely damaged, as evidenced by the loss of nuclear localization of DAPI signal (Fig. 6A, magnification). Use of *esgGal4, UAS-GFP* demonstrated that, in contrast to *Ecc15*, epithelium renewal was not detected following ingestion of *P. entomophila*. Instead, the number of ISCs was reduced, suggesting that infection with *P. entomophila* provoked a loss of ISCs along the gut, particularly in the anterior midgut (Fig. 6B). Since ingestion of *P. entomophila* still induces expression of both *Socs36E* and *puckered* in the gut (Fig. 6C), we conclude that this bacterium disrupts epithelium renewal despite proper activation of the required pathways. Furthermore, this impact on epithelium renewal appears to be linked to its lethality. Mutations in the genes encoding the GacA:GacS two-component system render *P. entomophila* fully avirulent, and ingestion of a *gacA*

mutant has no significant effect on the immune response (Liehl et al. 2006). In a similar manner, *gacA* derivatives did not affect the level of epithelium renewal (Fig. 6B7). A mutation in *aprA*, a gene encoding a metalloprotease abundantly produced by *P. entomophila*, attenuates its pathogenicity (Liehl et al. 2006). Interestingly, ingestion of *P. entomophila aprA* induced a significant level of epithelium renewal without dramatically decreasing the number of ISCs (Fig. 6B8), thus supporting a correlation between pathogenicity and absence of epithelium renewal. To further test this hypothesis, we performed a dose response assay with the fully virulent *P. entomophila* strain. At concentrations of $OD_{600} = 5$ or lower, ingestion of *P. entomophila* increased gut epithelium renewal to a level similar to that observed with a high dose of *Ecc15* (Fig. 6B; Supplemental Fig. S5A). Epithelium renewal was not observed at higher doses ($OD_{600} > 12.5$), and loss of ISCs was apparent at concentrations higher than $OD_{600} = 50$ (Fig. 6B; Supplemental Fig. S5A). This graded effect of *P. entomophila* on epithelium renewal correlates to the severity of gut damage and the level of fly mortality (Supplemental Fig. S5B). Interestingly, epithelium renewal was also disrupted when flies ingested a high dose of paraquat or SDS (Supplemental Fig. S5A). Collectively, these results confirm that increased epithelium renewal is primarily a response to

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Figure 6. *P. entomophila* (*Pe*) disrupts infection-induced epithelium renewal. (A) DAPI staining revealed that *P. entomophila*-infected guts are shorter than unchallenged guts 16 h after ingestion. The gut of *P. entomophila*-infected flies is composed of regions devoid of enterocytes and remaining enterocytes display abnormalities, as indicated by the loss of nuclear staining for DAPI. (B1–B8) Epithelium renewal was not observed in the gut of *esgGal4*, *UAS-GFP* flies infected with high doses of *P. entomophila* (OD₆₀₀ > 25, B3, B4). (B3) Lack of ISCs was observed when flies were fed a high concentration of *P. entomophila* (OD₆₀₀ = 100). (B5, B6) In contrast, infection with lower doses of *P. entomophila* (OD₆₀₀ < 5) stimulated epithelium renewal. The avirulent *P. entomophila* mutant *gacA* induced neither epithelium renewal nor the loss of ISCs (B7), while the *aprA* derivative induced a slight increase of epithelium renewal (B8). Guts of *esgGal4*, *UAS-GFP* were examined 16 h after infection. (C) Infection with *P. entomophila* induced both JAK–STAT (*Socs36E*, monitored 4 h post-infection) and JNK (*puckered*, monitored 30 min post-infection) pathways in the gut, as revealed by RT-qPCR.



damage and that this response is inhibited above a certain threshold of damage.

Discussion

Our study demonstrates that both invasive and indigeneous bacteria influence the level of epithelium turnover of the adult gut. Our results support a model that links the damage resulting from exposure to bacteria to ISC proliferation through JNK and JAK–STAT activation (see model in Fig. 7). The resulting increase in epithelium renewal is required to maintain gut homeostasis and survive bacterial infection, which demonstrates its essential role in host defense.

Previous studies have shown that the NADPH oxidase Duox plays an essential role in *Drosophila* gut immunity by generating microbicidal effectors such as ROS to eliminate both invasive and dietary microbes (Ha et al. 2005a, 2009). *Ecc15* is a potent activator of Duox, which in turn is important in the clearance of this bacterium (Ha et al. 2005a). This oxidative burst is coordinated with the induction of many genes involved in ROS detoxification upon *Ecc15* ingestion (Buchon et al. 2009). Here, we provide evidence that the observed increase in epithelium renewal upon *Ecc15* infection is a compensatory mechanism that repairs the damage inflicted to the gut by this oxidative burst. This is supported by our observation that reducing ROS levels by either the ingestion of antioxidants or silencing the *Duox* gene reduces epithelium renewal. Although ISC proliferation could be directly triggered by ROS, it is more likely a consequence of

signals produced by stressed enterocytes. A number of data support this hypothesis: (1) Ingestion of corrosive agents can also induce ISC proliferation (Biteau et al. 2008; Amcheslavsky et al. 2009; Buchon et al. 2009), and (2) physical injury is sufficient to induce local activation of the cytokine Upd3, which promotes epithelium renewal. Interestingly, a significant increase in epithelium renewal was observed in *Duox RNAi* flies at late time points following infection (Supplemental Fig. S1D), correlating with damage attributed to the proliferation of *Ecc15* in the guts of *Duox*-deficient flies (Ha et al. 2005a). While the increase in epithelium renewal observed with *Ecc15* is clearly linked to the damage induced by the host immune response, it is likely that effects on epithelium renewal by other pathogens could be more direct and mediated by virulence factors, such as the production of cytolytic toxins.

Our data indicate that the JAK–STAT and JNK pathways synergize to promote ISC proliferation and epithelium renewal in response to the damage induced by infection. The JAK–STAT pathway is implicated in the regulation of stem cells in multiple tissues and is proposed to be a common regulator of stem cell proliferation (Gregory et al. 2008). Our data extend this observation by showing that the JAK–STAT pathway is also involved in ISC activation upon bacterial infection. We demonstrate that the cytokine Upd3 is produced locally by damaged enterocytes and subsequently stimulates the JAK–STAT pathway in ISCs to promote their proliferation. Our results globally agree with a recent study showing that the JAK–STAT pathway is involved in ISC proliferation

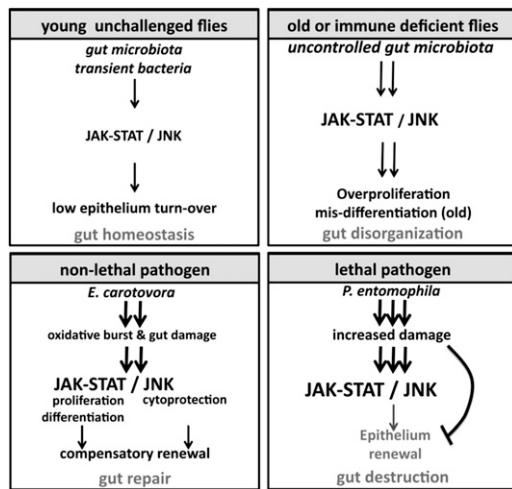


Figure 7. Modulation of gut epithelium renewal by invasive pathogens and indigenous gut microbiota. The JAK-STAT and JNK signaling pathways are required to maintain gut homeostasis upon exposure to a broad range of bacteria. In normal conditions, low levels of the indigenous gut microbiota and transient environmental microbes maintain a basal level of epithelium renewal. The increase in gut microbes in old or Imd-deficient flies is associated with a chronic activation of the JNK and JAK-STAT pathways, leading to an increase in ISC proliferation and gut disorganization. The impact of pathogenic bacteria can have different outcomes on gut homeostasis, depending on the degree of damage they inflict on the host. Damage to the gut caused by infection with *E. carotovora* is compensated for by an increase in epithelium renewal. Infection with a high dose of *P. entomophila* disrupts the homeostasis normally maintained by epithelium renewal and damage is not repaired, contributing to the death of the fly.

upon infection with a low dose of *P. entomophila* (Jiang et al. 2009). This work and our study clearly demonstrate that the JAK-STAT pathway adjusts the level of epithelium renewal to ensure proper tissue homeostasis by linking enterocyte damage to ISC proliferation. In the study by Jiang et al. (2009), they also uncover an additional role of this pathway in the differentiation of enteroblasts during basal gut epithelium turnover. The implication of the JAK-STAT pathway in differentiation could explain the accumulation of the small-nucleated escargot-positive cells we observed in the gut of flies with reduced JAK-STAT signaling in ISCs (Fig. 2C). The JAK-STAT pathway was also shown previously to control the expression of some antimicrobial peptides such as Drosomycin 3 (Dro3) (Buchon et al. 2009). Therefore, the JAK-STAT pathway has a dual role in the gut upon infection, controlling both the immune response and epithelium renewal.

Our data show that the lack of JNK pathway activity in ISCs results in the loss of ISCs in guts infected with *Ecc15*, thus preventing epithelium renewal. Our findings are consistent with the attributed function of JNK at the center of a signal transduction network that coordinates the induction of protective genes in response to oxidative challenge (Wang et al. 2003; Biteau et al. 2008). This

cytoprotective role against ROS would protect ISCs from the oxidative burst induced upon *Ecc15* infection, explaining why ISCs die by apoptosis when JNK activity is reduced. As suggested by Biteau et al. (2008), it is likely that JNK signaling is required not only to protect ISCs from oxidative stress, but also to induce stem cell proliferation to replace damaged differentiated cells. This is supported by the observation that overexpression of the JNKK Hep in ISCs is sufficient to trigger an epithelium renewal in the absence of infection. In addition, increased JNK activity in ISCs of old flies has been linked to hyperproliferative states and age-related deterioration of the intestinal epithelium (Biteau et al. 2008). Our study extends this observation by showing that JNK signaling is also required for epithelium renewal upon *Ecc15* infection. Thus, infection with *Ecc15* recapitulates in an accelerated time frame the impacts of increased stress observed in guts of aging flies

We observed that the inhibition of the dJun transcription factor in ISCs leads to a loss of stem cells in the absence of infection, suggesting that this transcription factor plays a critical role in ISC maintenance in the gut. We have no definitive explanation for why the *dJun-IR* construct behaves differently than the *basket* and *hep-IR* constructs. We speculate that this could be due to (1) differences in the basal activity of the JNK pathway, which would be blocked only with the *dJun-IR* that targets a terminal component of the pathway; (2) effects of Jun in ISCs independent of the JNK pathway, as reported in other systems (Bates et al. 2008); or (3) side effects of the *dJun-IR* construct.

In contrast to the requirement of the JNK pathway upon *Ecc15* infection, Jiang et al. (2009) reported that oral ingestion with a low dose of *P. entomophila* still induced mitosis in the JNK-defective mutant *hep¹*. In agreement, we found that inhibiting the JNK pathway in ISCs did not block the induction of epithelium renewal by a low dose of *P. entomophila* (Supplemental Fig. 3C). This difference in the requirement of the JNK pathway may be explained by the nature of these two pathogens. Whereas *Ecc15* damages the gut through an oxidative burst that activates the JNK pathway, the stimulation of epithelium renewal by *P. entomophila* could be due to a more direct effect of this bacterium on the gut. Altogether, Jiang et al. (2009) and our study point to an essential role of the JAK-STAT pathway in modulation of epithelium renewal activity, while the role of JNK may be dependent on the infectious agent and any associated oxidative stress. While it is known that the JNK pathway is activated by a variety of environmental challenges including ROS, the precise mechanism of activation of this pathway has not been elucidated. Similarly, the molecular basis of *upd3* induction in damaged enterocytes is not known. Future work should decipher the nature of the signals that activate these pathways in both ISCs and enterocytes, as well as the possible cross-talk between the JNK and JAK-STAT pathways in ISC control.

The observation that flies unable to renew their gut epithelium eventually succumb to *Ecc15* infection highlights the importance of this process in the gut immune

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response. It is striking that defects in epithelium renewal are more detrimental to host survival than deficiency in the Imd pathway, even though this pathway controls most of the intestinal immune-regulated genes induced by *Ecc15* (Buchon et al. 2009). Our results are in agreement with a previous study indicating that, in the *Drosophila* gastrointestinal tract, the Imd-dependent immune response is normally dispensable to most transient bacteria, but is provisionally crucial in the event that the host encounters ROS-resistant microbes (Ryu et al. 2006). However, our study demonstrates that efficient and rapid clearance of bacteria in the gut by *Duox* is possible only when coordinated with epithelium renewal to repair damage caused by ROS. This finely tuned balance between bacterial elimination by *Duox* activity and gut resistance to collateral damage induced by ROS is likely the reason why flies normally survive infection by *Ecc15*. Yet, this calibration also exposes a vulnerability that could easily be manipulated or subverted by other pathogens. Along this line, our work also exposes the range of impact different bacteria can have on stem cell activation. We observed that infection with high doses of *P. entomophila* led to a loss of gut integrity, including the loss of stem cells. Moreover, the ability of *P. entomophila* to disrupt epithelium renewal correlates with damage to the gut and the death of the host. Since both JNK and JAK–STAT pathways are activated upon infection with *P. entomophila*, this suggests that this bacterium activates the appropriate pathways necessary to repair the gut, but ISCs are unable to respond accordingly. Interestingly, a completely avirulent *P. entomophila* mutant (*gacA*) does not persist in the gut and does not induce epithelium renewal. In contrast, an attenuated mutant (*aprA*) somewhat restores epithelium renewal. These observations, along with the dose response analysis using *P. entomophila* and corrosive agents, suggest that the virulence factors of this entomopathogen disrupt epithelium renewal through excessive damage to the gut. Of note, recent studies suggest that both *Helicobacter pylori* and *Shigella flexneri*, two bacterial pathogens of the human digestive tract, interfere with epithelium renewal to exert their pathological effects (Iwai et al. 2007; Mimuro et al. 2007). This suggests that epithelium renewal could be a common target for bacteria that infect through the gut (Chatterjee and Ip 2009; Cronin et al. 2009; Jiang et al. 2009). In this respect, the host defense to oral bacterial infection could be considered as a bimodular response, composed of both immune and homeostatic processes that require strict coordination. Disruption of either process results in the failure to resolve the infection and impedes the return to homeostasis.

In contrast to the acute invasion by pathogenic bacteria, indigenous gut microbiota are in constant association with the gut epithelium, and thus may impact gut homeostasis. Using axenically raised flies, we established that indigenous microbiota stimulate a basal level of epithelium renewal that correlates with the level of activation of the JAK–STAT and JNK pathways. This raises the possibility that both indigenous and invasive bacteria, such as *Ecc15*, are capable of triggering epithe-

lium renewal by the same process. Additionally, our data support a novel homeostatic mechanism in which the density of indigenous bacteria is coupled to the level of epithelium renewal. This is the first report that gut microbiota affect stem cell activation and epithelium renewal, concepts proposed previously in mammalian systems (Hooper 2004) but never fully demonstrated. This also implies that variations in the level of epithelium renewal observed in different laboratory contexts could actually be due to impacts from gut microbes.

Importantly, in this context, we show that lack of indigenous microbiota reverts most age-related deterioration of the gut. Aging of the gut is usually marked by both hyperproliferation of ISCs and differentiation defaults that lead to disorganization of the gut epithelium (Biteau et al. 2008; N Choi et al. 2008). These alterations have been shown to be associated with activation of the PDGF- and VEGF-related factor 2 (Pvf2)/Pvr and JNK signaling pathways directly in ISCs (Biteau et al. 2008; N Choi et al. 2008). Accordingly, inhibition of the JNK pathway in ISCs fully reverts the epithelium alterations that occur with aging (Biteau et al. 2008). This raises the possibility that gut microbiota could exert their effect through prolonged activation of the JNK pathway. Interestingly, immune-deficient flies, lacking the Imd pathway, also display hyperproliferative guts and have higher basal levels of activation of the JNK and JAK–STAT pathways. The observation that these flies also harbor higher numbers of indigenous bacteria further supports a model in which failure to control gut microbiota leads to an imbalance in gut epithelium turnover. Future work should analyze the mechanisms by which gut microbiota affect epithelium renewal and whether this is due to a direct impact of bacteria on the gut or is mediated indirectly through changes in fly physiology. Moreover, the correlation between higher numbers of indigenous bacteria and increased disorganization of the gut upon aging in flies lacking the Imd pathway raises the possibility that a main function of this pathway is to control gut microbiota. This is in agreement with concepts emerging in mammals that support an essential role of the gut immune response in maintaining the beneficial nature of the host–microbiota association (Hooper 2009). This function also parallels the theory of “controlled inflammation” described in mammals, where a low level of immune activation is proposed to maintain gut barrier integrity (Sansonetti 2004; Pedron and Sansonetti 2008).

In conclusion, this study unravels some of the complex interconnections between the immune response, invasive and indigenous microbiota, and stem cell homeostasis in the gut of *Drosophila*. Based on the evolutionary conservation of transduction pathways such as JNK and JAK–STAT between *Drosophila* and mammals, it is likely that similar processes occur in the gut of mammals during infection. Interestingly, stimulation of stem cell activity by invasive bacteria is proposed to favor the development of hyperproliferative states found in precancerous lesions (Macdonald and Monteleone 2005; Radtke and Clevers 2005). Thus, *Drosophila* may provide a more accessible model to elucidate host mechanisms to

maintain homeostasis and the impact of bacteria on this process.

Materials and methods

Fly stocks and rearing

For description of the stock genotypes, specificities of the Gal4 drivers, and efficiency of RNAi lines used in this study, see the Supplemental Material. *Drosophila* stocks were maintained at 23°C using standard fly medium (maize flour, dead yeast, agar, and fruit juice) devoid of living yeast. RNAi constructs were validated by RT-qPCR (Supplemental Fig. S7). Axenic Canton, Oregon, *esgGal4*, *UAS-GFP*, *Rel^{E20}*, and *PGRP-LC^{E12}* stocks were generated by bleaching embryos and maintaining embryos and emerging flies on autoclaved fly medium. The presence of bacteria in gut homogenates was examined by PCR amplification of 16S rRNA genes using eubacterial primers (27F and 1492R), and by culturing the homogenates on mannitol agar or 1/10-strength tryptic soy agar.

Bacterial strains

Ecc15 is a Gram-negative bacterium that induces a strong local immune response (Basset et al. 2000; Buchon et al. 2009). *P. entomophila* is a Gram-negative bacterium that is lethal to *Drosophila* when ingested (Vodovar et al. 2005; Liehl et al. 2006).

Infection and compound feeding

Bacterial infections were performed as described elsewhere (Buchon et al. 2009). For oral infection, flies were incubated 2 h at 29°C in an empty vial and then placed in a fly vial with food solution and maintained at 29°C. The food solution was obtained by mixing a pellet of an overnight culture of bacteria (OD₆₀₀ = 200), 10 mM paraquat (Promega), or 0.2% H₂O₂ (Sigma) with a solution of 5% sucrose (1:1), added to a filter disk that completely covered the surface of standard fly medium. Flies were incubated for 2 d at 29°C on the contaminated filter, or 1 d for survival analysis, after which they were transferred to fresh vials. For antioxidant treatments, flies were fed a mixture of bacteria (final OD₆₀₀ = 100) and sucrose (2.5% final), to which glutathione (0.5 mM final; Sigma) or NAC (20 mM final; Sigma) was added; control flies were fed bacteria and sucrose or sucrose alone.

Clonal analysis

The marked lineage system developed by Harrison and Perrimon (1993) was used to generate clones of *lacZ*-expressing cells (Ohlstein and Spradling 2006). See the Supplemental Material for details. Adults of the genotype *yw, hs-FLP; X-15-33/X-15-29* were produced by standard crosses. To induce somatic recombination, 3-d-old adult flies were heat-shocked for 60 min at 37°C on three consecutive days. A day after, flies were orally infected with *Ecc15* or fed on sucrose. Guts were dissected 10 d after infection and stained with X-gal (Romeo and Lemaitre 2008).

Live imaging and immunofluorescence

For live imaging, guts were dissected at room temperature in 1× phosphate-buffered saline (PBS) and immediately mounted in the anti-fading agent AF1 (Citifluor). Samples were observed for fluorescence with an Axioplan imager Z1 and Axiocam mRM camera (Zeiss). For immunofluorescence, guts were dissected in

1× PBS; fixed for 20 min in PBS, 0.1% Tween 20 (PBT), and 4% paraformaldehyde; and then stained with primary antibody (anti-PH3 [1/1000; Upstate Biotechnologies/Millipore], anti-cleaved Caspase-3 [1/500; Asp175, Cell signal], anti-GFP [1/1000; Roche Diagnostics], anti-β-galactosidase [1/500; Sigma], anti-Delta [1/5000], anti-Prospero [1/500] [Developmental Studies Hybridoma Bank]) in PBT + BSA. Secondary staining was performed with Alexa488 or Alexa594-coupled anti-mouse or anti-rabbit antibodies (Invitrogen). DNA was stained with DAPI (Sigma).

RT-qPCR

Total RNA was extracted from 40 dissected guts using Trizol (Invitrogen). RT-qPCR was performed using SYBR Green I (Roche) on a LightCycler 2.0 as described previously (Buchon et al. 2009). The amount of mRNA detected was normalized to control *RpL32* values. Primers used to monitor mRNA quantification can be obtained on request.

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References

- Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N. 2003. Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev Cell* 5: 441–450.
- Amcheslavsky A, Jiang J, Ip YT. 2009. Tissue damage-induced intestinal stem cell division in *Drosophila*. *Cell Stem Cell* 4: 49–61.
- Bach EA, Ekas LA, Ayala-Camargo A, Flaherty MS, Lee H, Perrimon N, Baeg GH. 2007. GFP reporters detect the activation of the *Drosophila* JAK/STAT pathway *in vivo*. *Gene Expr Patterns* 7: 323–331.
- Basset A, Khush RS, Braun A, Gardan L, Boccard F, Hoffmann JA, Lemaitre B. 2000. The phytopathogenic bacteria *Erwinia carotovora* infects *Drosophila* and activates an immune response. *Proc Natl Acad Sci* 97: 3376–3381.
- Bates KL, Higley M, Letsou A. 2008. Raw mediates antagonism of AP-1 activity in *Drosophila*. *Genetics* 178: 1989–2002.
- Biteau B, Hochmuth CE, Jasper H. 2008. JNK Activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut. *Cell Stem Cell* 3: 442–455.
- Brown S, Hu N, Hombria JC. 2001. Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene *domeless*. *Curr Biol* 11: 1700–1705.
- Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B. 2009. *Drosophila* intestinal response to bacterial infection: Activation of host defense and stem cell proliferation. *Cell Host Microbe* 5: 200–211.
- Chatterjee M, Ip YT. 2009. Pathogenic stimulation of intestinal stem cell response in *Drosophila*. *J Cell Physiol* 220: 664–671.
- Choi N, Kim J, Yang D, Kim Y, Yoo M. 2008. Age-related changes in *Drosophila* midgut are associated with PVF2, a PDGF/VEGF-like growth factor. *Aging Cell* 7: 318–334.
- Choi YJ, Hwang MS, Park JS, Bae SK, Kim YS, Yoo MA. 2008. Age-related upregulation of *Drosophila* caudal gene via

Buchon et al.

- NF- κ B in the adult posterior midgut. *Biochim Biophys Acta* **1780**: 1093–1100.
- Cronin SJ, Nehme NT, Limmer S, Liegeois S, Pospisilik JA, Schramek D, Leibbrandt A, Simoes RD, Gruber S, Puc U, et al. 2009. Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. *Science* **325**: 340–343.
- Gregory L, Came PJ, Brown S. 2008. Stem cell regulation by JAK/STAT signaling in *Drosophila*. *Semin Cell Dev Biol* **19**: 407–413.
- Ha E, Oh C, Bae YS, Lee WJ. 2005a. A direct role for dual oxidase in *Drosophila* gut immunity. *Science* **310**: 847–850.
- Ha E, Oh C, Ryu J, Bae YS, Kang SW, Jang IH, Brey P, Lee WJ. 2005b. An antioxidant system required for host protection against gut infection in *Drosophila*. *Dev Cell* **8**: 125–132.
- Ha EM, Lee K, Park SH, Kim SH, Nam HJ, Lee HY, Kang D, Lee WJ. 2009. Regulation of DUOX by the G α q-phospholipase C β -Ca²⁺ pathway in *Drosophila* gut immunity. *Dev Cell* **16**: 386–397.
- Harrison DA, Perrimon N. 1993. Simple and efficient generation of marked clones in *Drosophila*. *Curr Biol* **3**: 424–433.
- Hooper LV. 2004. Bacterial contributions to mammalian gut development. *Trends Microbiol* **12**: 129–134.
- Hooper LV. 2009. Do symbiotic bacteria subvert host immunity? *Nat Rev Microbiol* **7**: 367–374.
- Iwai H, Kim M, Yoshikawa Y, Ashida H, Ogawa M, Fujita Y, Muller D, Kirikae T, Jackson PK, Kotani S, et al. 2007. A bacterial effector targets Mad2L2, an APC inhibitor, to modulate host cell cycling. *Cell* **130**: 611–623.
- Jiang H, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, Edgar BA. 2009. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell* **137**: 1343–1355.
- Krzemień J, Dubois L, Makki R, Meister M, Vincent A, Crozatier M. 2007. Control of blood cell homeostasis in *Drosophila* larvae by the posterior signaling centre. *Nature* **446**: 325–328.
- Lee T, Luo L. 2001. Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development. *Trends Neurosci* **24**: 251–254.
- Liehl P, Blight M, Vodovar N, Boccard F, Lemaitre B. 2006. Prevalence of local immune response against oral infection in a *Drosophila/Pseudomonas* infection model. *PLoS Pathog* **2**: e56. doi: 10.1371/journal.ppat.0020056.
- Macdonald TT, Monteleone G. 2005. Immunity, inflammation, and allergy in the gut. *Science* **307**: 1920–1925.
- Martín-Blanco E, Gampel A, Ring J, Virdee K, Kirov N, Tolkovsky AM, Martínez-Arias A. 1998. *puckered* encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in *Drosophila*. *Genes & Dev* **12**: 557–570.
- Micchelli CA, Perrimon N. 2006. Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature* **439**: 475–479.
- Mimuro H, Suzuki T, Nagai S, Rieder G, Suzuki M, Nagai T, Fujita Y, Nagamatsu K, Ishijima N, Koyasu S, et al. 2007. *Helicobacter pylori* dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. *Cell Host Microbe* **2**: 250–263.
- Ohlstein B, Spradling AC. 2006. The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. *Nature* **439**: 470–474.
- Park JS, Kim YS, Yoo MA. 2009. The role of p38b MAPK in age-related modulation of intestinal stem cell proliferation and differentiation in *Drosophila*. *Aging* **1**: 637–651.
- Pedron T, Sansonetti PJ. 2008. Commensals, bacterial pathogens and intestinal inflammation: An intriguing ménage à trois. *Cell Host Microbe* **3**: 344–347.
- Radtke F, Clevers H. 2005. Self-renewal and cancer of the gut: Two sides of a coin. *Science* **307**: 1904–1909.
- Ren C, Webster P, Finkel SE, Tower J. 2007. Increased internal and external bacterial load during *Drosophila* aging without life-span trade-off. *Cell Metab* **6**: 144–152.
- Romeo Y, Lemaitre B. 2008. *Drosophila* immunity: Methods for monitoring the activity of toll and imd signaling pathways. *Methods Mol Biol* **415**: 379–394.
- Ryu J, Ha E, Oh C, Seol J, Brey P, Jin I, Lee D, Kim J, Lee D, Lee WJ. 2006. An essential complementary role of NF- κ B pathway to microbicidal oxidants in *Drosophila* gut immunity. *EMBO J* **25**: 3693–3701.
- Sansonetti PJ. 2004. War and peace at mucosal surfaces. *Nat Rev Immunol* **4**: 953–964.
- Vodovar N, Vinals M, Liehl P, Basset A, Degrouard J, Spellman P, Boccard F, Lemaitre B. 2005. *Drosophila* host defense after oral infection by an entomopathogenic *Pseudomonas* species. *Proc Natl Acad Sci* **102**: 11414–11419.
- Wang MC, Bohmann D, Jasper H. 2003. JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. *Dev Cell* **5**: 811–816.
- Zaidman-Remy A, Herve M, Poidevin M, Pilifloury S, Kim MO, Blanot D, Oh BH, Ueda R, Mengin-Lecreulx D, Lemaitre B. 2006. The *Drosophila* amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity* **24**: 463–473.



Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*

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