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Resource

Drosophila Intestinal Response to Bacterial Infection: Activation of Host Defense and Stem Cell Proliferation

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SUMMARY

Although *Drosophila* systemic immunity is extensively studied, little is known about the fly's intestine-specific responses to bacterial infection. Global gene expression analysis of *Drosophila* intestinal tissue to oral infection with the Gram-negative bacterium *Erwinia carotovora* revealed that immune responses in the gut are regulated by the Imd and JAK-STAT pathways, but not the Toll pathway. Ingestion of bacteria had a dramatic impact on the physiology of the gut that included modulation of stress response and increased stem cell proliferation and epithelial renewal. Our data suggest that gut homeostasis is maintained through a balance between cell damage due to the collateral effects of bacteria killing and epithelial repair by stem cell division. The *Drosophila* gut provides a powerful model to study the integration of stress and immunity with pathways associated with stem cell control, and this study should prove to be a useful resource for such further studies.

INTRODUCTION

In addition to its digestive functions, the intestinal epithelium is a barrier between the internal and external environment (Sansonetti, 2004). This barrier protects the host against invasion and systemic dissemination of both pathogenic and commensal microorganisms. Studies identifying the mechanisms that regulate gut mucosal immunity in mammals have revealed a central role of innate immunity in these processes, although the complex mechanisms underlying gut immune homeostasis are not fully understood. In evolutionary terms, interactions between bacteria and gut cells are a conserved feature among phyla, and ingestion of potential pathogens and microbes has important implications in nature. This is particularly relevant of insects that feed on microbe-enriched food and since ingestion is the entry route of many human pathogens to their insect vector host. Surprisingly, few studies have analyzed the immune response of the *Drosophila* gut, despite the value of this model organism.

Drosophila is devoid of an adaptive immune system and relies solely on innate reactions for its immune defense (Aggarwal and Silverman, 2008; Lemaitre and Hoffmann, 2007). An attractive feature of *Drosophila* immunity is the existence of multiple defense reactions shared with higher organisms. Epithelia, such as in the alimentary tract and tracheae, are the first lines of defense against pathogens and produce both antimicrobial peptides (AMPs) and reactive oxygen species (ROS). Additionally, specialized hemocytes participate in phagocytosis and encapsulation of foreign invaders. Finally, the fat body, a functional analog of the mammalian liver, is the main site of the humoral (or systemic) response. One of the best-characterized facets of the *Drosophila* systemic immune response is the synthesis and secretion by the fat body of several AMPs with distinct but overlapping specificities. AMP genes are regulated by the Toll and Imd pathways, which share many features with the mammalian Toll-like receptor (TLR) and tumor necrosis factor (TNF-α) signaling cascades that regulate NF-κB transcription factors. The Toll pathway is triggered by the cleavage of the Toll ligand, Spätzle (Spz), and leads to the activation of the NF-κB-like proteins Dif and Dorsal. This pathway is activated by both Gram-positive bacteria and fungi via secreted pattern-recognition receptors and controls, to a large extent, the expression of AMPs active against fungi (e.g., *Drosomycin*). In contrast, the Imd pathway mainly responds to Gram-negative bacterial infection and controls antibacterial peptide genes (e.g., *Diptericin*) via the activation of the NF-κB-like protein Relish (Rel). PGRP-LC acts as a transmembrane receptor upstream of the Imd pathway and is activated by DAP-type peptidoglycan of Gram-negative bacteria (Aggarwal and Silverman, 2008; Lemaitre and Hoffmann, 2007).

The *Drosophila* gut lumen is considered hostile to transient microbial colonization due to physical (acidity) and physiological (peristalsis of the gut) properties and the presence of lysozymes (Hultmark, 1996). Two complementary effector mechanisms are key to controlling bacterial infection in the gut: generation of ROS and local production of AMPs. In *Drosophila,* production of ROS in the gut by the NADPH oxidase enzyme Duox provides an efficient barrier against most ingested microbes (Ha et al., 2005; Ryu et al., 2006). The second line of defense is the induction of AMPs (e.g., *Diptericin* and *Attacin)* in the gut upon oral infection by Gram-negative bacteria (Basset et al., 2000; Liehl et al., 2006; Nehme et al., 2007; Tzou et al., 2000). Like the systemic response, the local production of AMPs is triggered by the Imd

pathway through recognition of Gram-negative peptidoglycan by PGRP-LC (Zaidman-Remy et al., 2006). Recent studies in *Drosophila* have revealed that multiple levels of regulation are employed to limit Imd pathway activity in the gut and prevent excessive or prolonged immune activation (Lhocine et al., 2008; Maillet et al., 2008; Ryu et al., 2008).

However, despite growing interest in gut mucosal immunity, very little is known about the *Drosophila* gut host defense in comparison to our knowledge of the systemic immune response. In this paper, we describe how gut cells respond to infection by *Erwinia carotovora carotovora 15* (*Ecc15*) and define some of the regulatory networks controlling gut immune responses.

RESULTS

Ingestion of *Ecc15* Significantly Modulates the Gut Transcriptome

Previous transcriptome analyses of the *Drosophila* response to immune challenge have focused mainly on septic injury, which results in a systemic response (Boutros et al., 2002; De Gregorio et al., 2001; Irving et al., 2001). More recently, a study following oral bacterial infection of larvae analyzed the global response in whole organisms, encompassing both the gut and systemic immune responses. However, the reaction of the gut was masked by the intensity of the fat body response (Vodovar et al., 2005). To determine the genes specifically induced in the gut, we investigated transcriptome variations in dissected adult guts (minus the Malpighian tubules) after oral infection with the Gram-negative bacterium *Ecc15*. We chose *Ecc15* as ingestion of this bacterium strongly induces the Imd pathway in the gut (Figures S1 and S2) but does not kill the host. Transcriptome data were generated using Affymetrix GeneChip Drosophila Genome 2.0 Array for wild-type and *Rel* mutant flies fed with either sucrose or *Ecc15* and sampled 4 and 16 hr postinfection. Our analysis identified 990 genes whose expression varied in response to *Ecc15* ingestion by at least a 2-fold change over unchallenged. This comprises 988 of the 13,600 present on the array and *Cecropin A1/A2* and *Diptericin A*, which are not present in the Affymetrix GeneChip Drosophila Genome 2.0 Array but were shown to be induced in the gut by RT-qPCR (Figure S1 and data not shown). The 990 genes encode 576 induced and 414 repressed transcripts, 17% of which vary more than 4-fold (see Figures 1A and 2 for a selection of upregulated genes, see Tables S6 and S7 for complete data set). Comparison of our data with a microarray data set of whole flies in response to septic injury (De Gregorio et al., 2001) identified 75 genes that were upregulated in both conditions, including most genes with immune function (Figure 1B). This analysis also revealed a large set of previously unidentified genes that are specific to the gut. Using a global classification, nearly half of these gut-regulated genes were assigned to four functional categories: antimicrobial defense, stress response, cell survival and renewal, and gut physiology (digestive enzymes, transporters, and components of the peritrophic matrix) (Figure 1D). Genes involved in antimicrobial defense and epithelial renewal were enriched among upregulated genes, while genes encoding factors involved in digestion were repressed. In addition, infection induced new sets of stress response genes while others were downregulated. Thus, oral infection with *Ecc15* alters the physiology of the gut with a reduction of digestive function and an increase of immune and cell renewal functions.

Ecc15 Ingestion Induces Immune, Stress, and Developmental Signaling Pathways

To identify candidate regulatory networks that control the gut immune response to *Ecc15*, we determined the signaling components that are transcriptionally upregulated in the gut upon ingestion (Figure 1E). *Ecc15* ingestion induces genes encoding two positive (Kenny and Rel) and four negative (Pirk/Pims, PGRP-LF, PGRP-LB, and PGRP-SC) regulators of the Imd pathway. In the Toll pathway, only the genes encoding Spz, the Toll ligand, and PGRP-SA were moderately induced in the gut, whereas most of the genes of the pathway were significantly upregulated in response to septic injury in whole animals (notably *Toll*, *pelle*, *tube*, *cactus*, *Dif*, *Dorsal*, *SPE*, and *necrotic*) (De Gregorio et al., 2001). Interestingly, three genes encoding components of the JAK-STAT pathway, particularly the receptor Domeless and one of its ligands, Unpaired 3 (Upd3), were strongly induced in the gut in response to ingestion of *Ecc15*. P38c and Mkp3 were the only components of the MAPK pathway to be transcriptionally induced in the gut. Interestingly, P38c was recently implicated in the epithelial immune response of *Drosophila* (Davis et al., 2008). Genes encoding components of the JNK pathway were noticeably absent in our microarray data set. However, the JNK pathway is reportedly activated 1–1.5 hr postinfection following septic injury, suggesting that its activation was not detected, since gene expression was monitored only at 4 and 16 hr. To test if JNK is active in the gut, we used a *lacZ* reporter gene for JNK activity (puc^{E69}, referred to as *puc-lacZ*). We found that *puc-lacZ* expression significantly increased in the gut upon *Ecc15* ingestion (Figure S3), demonstrating that the JNK pathway is also activated in response to infection.

Surprisingly, many genes encoding morphogens and components of developmental pathways were induced in the gut by *Ecc15* ingestion (Figure 1E and Table S6). These include the morphogen gene *Hedgehog* and the Notch pathway genes *Suppressor of Deltex*, *Fringe,* and *Delta*. Importantly, the transcripts of eight components of the EGF-R pathway, including four EGF-R ligands (Vein, Keren, Spitz2, and Argos), two serine proteases participating in the maturation of the EGF ligand (Rhomboid and Rhomboid 4), one transcription factor (Pointed), and its regulator (Edl) were induced. Collectively, our analysis provides evidence that the Imd, JAK-STAT, JNK, and other signaling pathways usually associated with development are activated in the gut in response to bacterial infection.

The Imd Pathway Is a Major Regulator of the Gut Immune Response

To determine the contribution of the Imd pathway to antimicrobial defense in the gut, we examined the effect of the *Rel* mutation on gene expression. The expression of 138 upregulated genes and 78 downregulated genes was altered at least 2-fold in a *Rel* background compared to wild-type. Most of these genes were induced at both the early and late time points (Figure 1C). A clear enrichment of Rel target genes was observed among the most strongly induced genes (Figure 1A). Of these, 108 were positively regulated by *Rel* and may represent immune targets of the Imd pathway (Figure 2

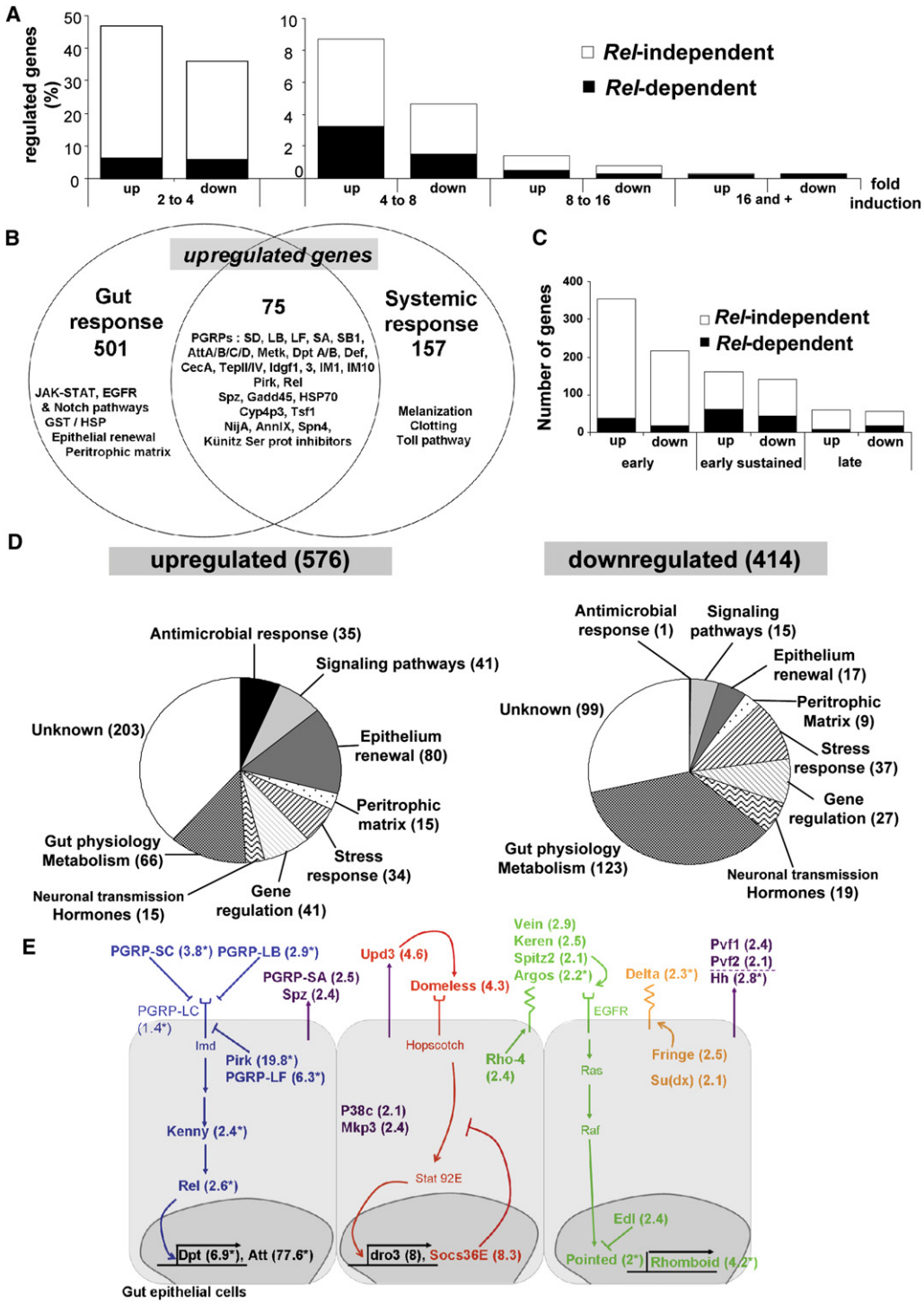

Figure 1. General Statistics on the *Drosophila* Genes Regulated by *Ecc15* in the Gut

(A) Distribution and regulation of genes, based on their fold change. The percentage of genes regulated by *Rel* is indicated in black.

(B) Comparison of the distribution of genes upregulated in the gut upon *Ecc15* ingestion to that of genes induced in whole flies upon septic injury (De Gregorio et al., 2001).

(C) Distribution of induced and repressed genes according to their time course after infection. Genes regulated by *Rel* (indicated in black) are enriched in the early-sustained category.

(D) Repartition of induced (left) and repressed (right) genes in defined categories of gene ontology.

(E) Schematic representation of signaling pathways or signaling components upregulated at the transcriptional level in the gut upon *Ecc15* ingestion (induced genes indicated in bold). Numbers in parentheses correspond to the peak of activation (fold change compared to flies infected with sucrose). Genes denoted by * were affected in the *Rel* mutant background.

	Gene name	Function	*Ecc15* / UC Wild-type 4 hr	Wild-type 16 hr	*Rel* 4 hr	*Rel* 16 hr
	ANTIMICROBIAL RESPONSE					
	PGRP-SD#*	recognition	32.6	11.8	-1.3	-1.8
	PGRP-LF#*	recognition	6.3	2.6	-1	-1.4
$	**TepIV***	recognition	4.8	2.2	3.1	1.7
	PGRP-SB1#*	recognition	3.6	4.9	1	1.3
	PGRP-SC1#*	recognition	3	3.8	-14.1	-11.2
$	**PGRP-LB#***	recognition	2.6	2.9	-2.2	-1.5
	PGRP-SA*	recognition	2.5	1.4	2.1	-1.2
	Idgf3*	recognition	2.5	1.8	2	1.4
	TepII*	recognition	2.5	1.2	1.4	-1.2
	Idgf1*	recognition	2.1	1.2	2.3	1.1
	CG13422*	recognition	1.9	2.7	1.7	1.4
	AttD#*	AMP	77.6	43.7	-4.9	-6.2
$	**AttA#***	AMP	22.1	15.2	-1.7	-1.7
	AttA/B#*	AMP	9.3	7.1	-1.3	-1.5
	Mtk#*	AMP	7.4	29.2	-1	-1.6
	dro2	AMP	6.8	3.2	5.8	2.7
	Def#*	AMP	6.7	23.7	-9.3	-5.1
$	**dro3**	AMP	6.6	8	5.6	5.9
	dro4	AMP	3.6	1.7	2.8	1.2
$	**Drs***	AMP	2.9	1.9	1.1	-2.2
	DptB#*	AMP	2.8	6.9	-1.3	-1.2
	AttC#*	AMP	2.3	4.3	-1	-1.2
	LysX	AMP	1.9	2.3	1.8	1.5
$	**IM3#**	DIM	2.7	2.1	-2.9	-2.7
	CG16836#*	DIM	2.1	-1.1	-1	-1.6
$	**IM1#***	DIM	1.3	2.9	-1	-1
	IM10#*	DIM	1.3	2.2	1.1	-1.1
$	**Spn4#***	serpin	5.1	2.3	2.4	1.2
$	**CG3604#***	Künitz inhibitor	4.2	3.1	1.9	1.1
	CG16713#*	Künitz inhibitor	4.1	3	2	-1.2
	CG16712#	Künitz inhibitor	3	3	1.5	-1.2
	CG31704#	Kazal inhibitor	2.9	2.3	-1.2	-2.3
	Tsf1#*	iron sequestration	3.4	4.5	-1.6	1
	color legend		**<16**	**<8**	**<2**	

	Gene name	Function	*Ecc15* / UC Wild-type 4 hr	Wild-type 16 hr	*Rel* 4 hr	*Rel* 16 hr
	STRESS RESPONSE / DETOXIFICATION					
$	**Hsp70A**	Heat shock protein	7.4	-1.6	9.3	1.8
	Hsp70B*	Heat shock protein	3.3	-2	7	1.6
	Cyp6d2	cytochrome P450	7	4.6	7.3	8.4
	Cyp305a1	cytochrome P450	3.8	1.5	2.4	1.1
$	**GstD8**	Gst δ	48.2	48.9	63.5	70.7
$	**GstD5**	Gst δ	5.3	-1.3	22.6	6.2
	CG2065	oxidative stress	11.2	1.8	10.3	5.1
	CG12224#	oxidative stress	6.2	1.5	1.2	-1.1
$	**TotC**	stress peptide	2.7	2	3.6	3.8
	Fst*	cold response	18.3	4.9	17.2	6.2
	Peritrophic matrix					
$	**CG32302#**	peritrophic matrix	13.4	13.6	1.9	3.2
	CG32284	peritrophic matrix	11.1	9.6	9.1	7.8
$	**CG6933#**	peritrophic matrix	4	10.2	1.5	1.3
	EPITHELIUM RENEWAL					
	p53	gatekeeper	3.2	1.5	3.8	1.8
	His3	cell cycle	1.6	2.2	1.4	1.8
	CycE	cell cycle	-1.1	3.1	-1.4	1.9
	betaTub60D	cytoskeleton	7.8	3.2	4.5	2
	act57b	cytoskeleton	4.9	3.1	5.2	3.5
	squ	cytoskeleton	4.8	2.6	2.6	1.8
	Gadd45*	wounding/stress	5	1.8	2.7	1.5
	NijA*	wounding	4.9	2	3.9	1.5
	CG5550*	wounding	4.7	2.1	3.5	1.1
	UNKNOWN GENES					
	CG8620	unknown	20.5	4	13.5	6.5
$	**CG4367#**	unknown	19.5	1.1	1.1	1.1
$	**CG8317#**	unknown	18.7	10.3	-1.1	1
$	**CG9080#**	unknown	18.5	48.3	-2.1	-2
$	**CG15282#**	unknown	17.2	29.8	-1	-1.2
	CG13325*	unknown	14.7	1.5	40.8	4.1
	CG14219*	unknown	12.6	2.7	10.8	4.2
$	**CG4269#***					
			>2	**>4**	**>8**	**>16**

Figure 2. A Selection of Genes Upregulated in the Gut upon *Ecc15* Ingestion

Names, functions, and folds of induction (compared to sucrose-fed flies) in wild-type and *Rel* flies are indicated. Genes denoted by * were selected as *Drosophila* immune-regulated genes by De Gregorio data sets (De Gregorio et al., 2001). Genes denoted with # are positively regulated by *Rel*. The expression profile of genes indicated with $ were confirmed by RT-qPCR using independent gut samples.

and Table S3). To extend our microarray results and verify that *Rel*-dependent genes were regulated by the Imd pathway, we monitored the expression of ten putative Rel target genes (*Diptericin*, *PGRP-LB*, *Rel*, *IM3, Att,* CG4367, CG8317, CG9080, CG6933, and CG15282) by RT-qPCR in the gut of *Rel*- and *PGRP-LC*^{E12}-deficient flies or of flies expressing *dFADD* or *PGRP-LC RNAi* constructs. Transcript levels of the ten candidate *Rel*-dependent genes were all affected in flies lacking a functional Imd pathway, irrespective of genetic background (Figure 3 and data not shown). In addition, these ten genes were expressed at high levels in unchallenged flies overexpressing *imd* in the midgut (genotype: *NP1-Gal4*; *UAS-imd*) (Figure 3B). This demonstrates that the Imd pathway is both necessary and sufficient for their expression.

We found that 36 of the 75 upregulated genes that were also induced in whole flies in response to septic injury were affected in the *Rel* mutant background in the gut (De Gregorio et al., 2001) (Figure 2 and Table S3). This includes all *PGRPs* (with the exception of *PGRP-SA*), most AMP genes, and genes coding for Imd pathway components. In addition, a Transferrin (Tsf1) and four protease inhibitors of the Serpin or Kunitz families were also induced in the gut in a *Rel*-dependent manner. Among Imd target genes in the gut, *PGRP-SD* and *Attacin A1* and *D* were upregulated more than 20-fold (Figure 2). Interestingly, *Rel* affected a set of genes not previously associated with the immune response, including genes encoding members of the EGF-R pathway and Hedgehog. The most prominent family of this set was a group of six genes encoding proteins with chitin-binding domains that are annotated as putative components of the peritrophic matrix (Figure 2 and Table S3). The peritrophic matrix forms an important physical barrier separating the alimentary bolus from the intestine, preventing direct contact between bacteria and epithelial cells (Lehane, 1997). Our results suggest that the Imd pathway directly participates in the remodeling of this barrier, an as yet poorly characterized process that could be important in the defense against bacteria in the gut. It should be noted that nine additional genes encoding proteins with peritrophin domains were upregulated upon *Ecc15* infection in a *Rel*-independent manner. We also identified a number of genes encoding protease inhibitors or metabolic enzymes (e.g., with guanylate cyclase, oxidoreductase, or glucuronosyl transferase activities) that were induced in a *Rel*-dependent manner in the gut. Finally, 22 genes encoding proteins (e.g., *CG4367*, *CG3703*, *CG11470*) or peptides (e.g., *CG8317*, *CG31789*) with no characterized domains were strongly induced in the gut in a fully *Rel*-dependent manner (Figure 2 and Table S3). Those genes were not induced in response to systemic infection and thus potentially

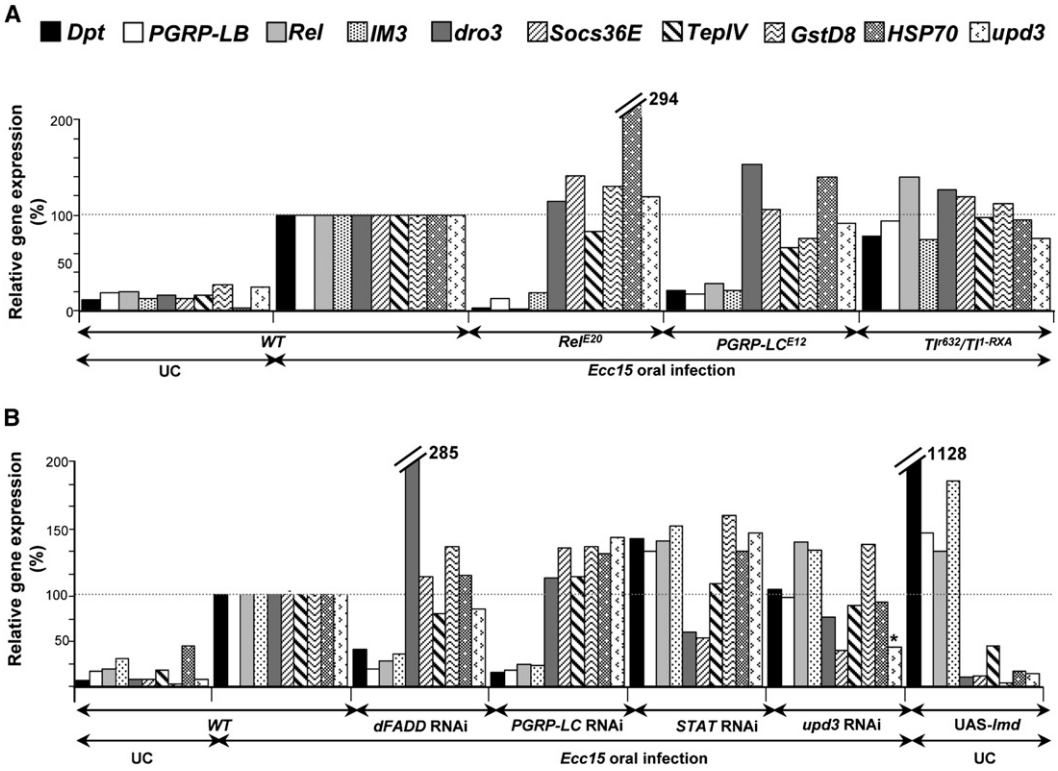

Figure 3. Contribution of Imd, Toll, and JAK-STAT Pathways to the Induction of a Subset of Gut Immune-Regulated Genes

(A) RT-qPCR analysis of gut extracts from unchallenged wild-type flies (UC) or wild-type, Rel^{E20}, $PGRP\text{-}LC^{E12}$, and $Tl^{r632}/Tl^{1\text{-}RXA}$ adult females collected 4 hr after *Ecc15* ingestion.

(B) RT-qPCR analysis of gut extracts from NP1/+ (wild-type), *dFADD, PGRP-LC, STAT RNAi*, and *upd-3 RNAi* flies (genotype: *NP1-Gal4*; *UAS-gene-IR*) that were sampled 4 hr after *Ecc15* ingestion, from unchallenged wild-type flies and unchallenged flies overexpressing *imd* (genotype: *NP1-Gal4; UAS-Imd*). (A) and (B) show the amount of each transcript (normalized to *rp49*) relative to the levels measured in wild-type flies at 4 hr. Expression levels were monitored for genes involved in the antimicrobial response: *Diptericin A* (*Dpt*), *PGRP-LB, Rel, IM3, dro3, and TepIV*, in the JAK-STAT pathway (*upd-3*, *Socs36E*) or in the stress response (*hsp70, GstD8*). * indicates the decrease of *upd3* expression in *upd3 RNAi* flies. Complete results, including statistics and values in some unchallenged mutant flies for experiments (A) and (B), are provided in Table S1.

encode important immune effectors specific to the gut host defense.

Some AMP Genes Are Induced in the Gut in an Imd- and Toll-Independent Manner

The *Drosophila* genome harbors a large number of AMP genes, and those induced during the systemic immune response are all regulated by the Toll and/or Imd pathways. Likewise, our analysis revealed that the four *Attacin* (*A*, *B*, *C*, and *D*), two *Diptericin* (*A* and *B*), *Cecropin A1/A2*, *Defensin*, and *Mechtnikowin* genes are induced in the gut in a *Rel*-dependent manner (Figures 2 and S1; data not shown for *CecropinA1/A2*). Interestingly though, a subset of AMPs that are induced during the systemic immune response, namely *Drosocin* and *Cecropins B* and *C*, were not expressed or regulated at the transcriptional level in the gut. In addition, the antifungal peptide gene *Drosomycin* was only slightly induced in the gut. Instead, we identified three genes encoding uncharacterized Drosomycin-like peptides (dro2, dro3, and dro4, all sharing more than 55% identities with *Drosomycin*) and one lysozyme (*LysX*) that were induced in the gut of wild-type flies upon *Ecc15* ingestion (Figures 2 and 3). This group of AMPs is not known to be induced in the fat body and appears specific to the gut immune response. To confirm this finding, we compared the levels of *Drosomycin* and *dro3* expression by RT-qPCR in guts and abdominal carcasses (reflecting fat body expression) of adult flies following oral infection or septic injury with *Ecc15*. *Drosomycin* was induced only in the fat body in response to systemic infection while *dro3* was induced in the gut upon oral infection (Figure 4A). Although the antimicrobial activity of these Drosomycin-like peptides is not known, this observation suggests that the gut antifungal defense is mediated by a specific set of Drosomycins distinct from those involved in the systemic immune response. Additionally, *dro3* was induced to wild-type levels in the guts of flies lacking a functional Imd pathway (Figure 3). During a systemic infection, antifungal defense is mediated in the fat body by the Toll pathway. However, there was no effect on the inducibility of *dro3* in *Toll* (*Tl*)-deficient flies after ingestion of *Ecc15* (Figure 3A). The absence of an effect of the Toll pathway might be explained by the use of *Ecc15* as an inducer, since the Toll pathway is activated by Gram-positive bacteria or fungi, rather than Gram-negative bacteria. However, *dro3* was still upregulated in the gut of *Tl*-deficient flies orally infected with the fungus *C. albicans* (Figure 4B). In addition,

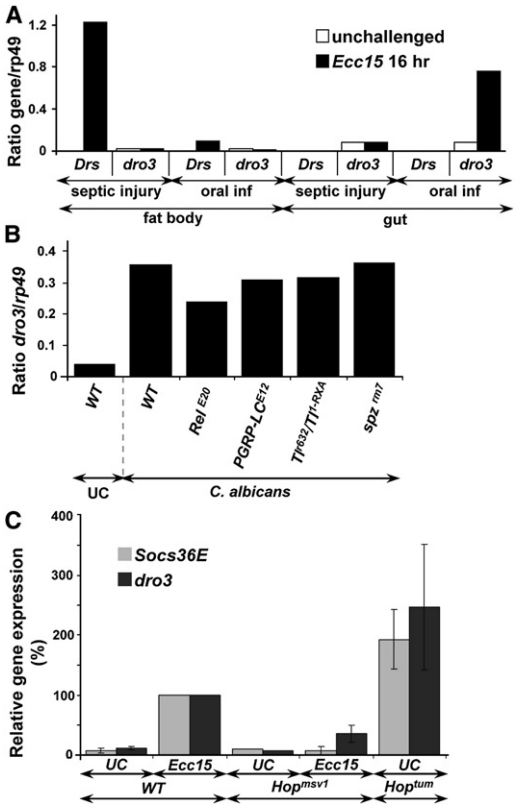

Figure 4. Expression Profile and Regulation of *dro3*

(A) *dro3* and *Drosomycin (Drs)* expressions were monitored by RT-qPCR in gut and carcasses of unchallenged and wild-type flies collected 16 hr after oral infection or septic injury with *Ecc15*. *dro3* was strongly induced in the gut upon *Ecc15* ingestion while *Drs* was expressed in the fat body upon septic injury. Relative expression ratios of *Drs*/*rp49* or *dro3*/*rp49* are shown.

(B) RT-qPCR analysis of *dro3* induction in gut extracts of unchallenged wild-type flies (UC) or wild-type, *Rel*, *PGRP-LC*^{E12}, *Tl*^{r632}/*Tl*$^{1\text{-}RXA}$, and *spz*^{rm7} adult females 16 hr after ingestion of *C. albicans*.

(C) RT-qPCR analysis of gut extracts from unchallenged wild-type and *hop*^{Tum} flies (UC) or wild-type and *hop*^{msv1} adult females 16 hr after ingestion of *Ecc15*.

The figure was based on three independent repeats. Error bars indicate SD.

the *Tl* mutation did not affect any of the gut-regulated genes as analyzed by RT-qPCR (Figure 3).

The JAK-STAT Pathway Contributes to Antimicrobial Defense in the Gut

The induction of immune genes in the gut independent of Toll and Imd suggested the involvement of another immune pathway. Our microarray analysis identified many transcripts of the JAK-STAT pathway, particularly those of the receptor Domeless and of one of its ligands, Upd3, that are upregulated in the gut, suggesting a role of this pathway in host defense. This observation also suggested that oral infection induces the release by gut cells of Upd3, which then activates the JAK-STAT pathway. To test this hypothesis, we monitored the expression level of *Socs36E,* an established target of the JAK-STAT pathway (Bach et al., 2007), in the gut of flies expressing either a *STAT-RNAi* or an *upd3-RNAi* construct under the control of a midgut-specific Gal4 driver (genotype *NP1-Gal4; UAS-gene-IR*). Silencing of *STAT* and *upd3* expression in the gut reduced *Socs36E* expression, confirming that Upd3 activates the JAK-STAT pathway in the gut (Figure 3B). Furthermore, we found that *dro3* expression in response to *Ecc15* infection was reduced in the gut of *STAT-RNAi* and *upd3-RNAi* flies (Figure 3B). To confirm these data, we used fly lines carrying either a loss-of-function (*hop*^{msv1}) or a gain-of-function mutation (*hop*^{Tum}) in the *Drosophila* JAK kinase, Hopscotch (Hop). As expected, *dro3* expression was weakly induced in *hop*^{msv1}-deficient flies after ingestion of *Ecc15* but was strongly expressed in the *hop*^{Tum} mutant in the absence of challenge (Figure 4C). Additionally, the promoter region of *dro3* contains STAT-binding sites (data not shown). We conclude that the JAK-STAT pathway contributes to the expression of AMP genes in the gut.

Having shown that the JAK-STAT pathway functions in gut immunity, we next investigated the pattern of its transcriptional activity along the gut and the nature of the stimuli that activate its expression. To monitor JAK-STAT activity, we used a fly line carrying a reporter construct comprising ten repeats of the Stat92E binding sites of the *Soc36E* gene, upstream of *GFP* (referred to as *STAT-GFP*) (Bach et al., 2007). In unchallenged flies, GFP was detected in only a small population of basal cells (Figure 5Aa). However, ingestion of *Ecc15* induced a strong activation of the GFP reporter gene (Figure 5Ab). Fluorescence was detectable in most cells all along the midgut 4 hr postinfection and remained high at 16 hr. *upd3-Gal4; UAS-GFP* flies were used to monitor the expression pattern of *upd3* following *Ecc15* ingestion. In control flies, a weak GFP signal was present in the cardia and in a few scattered cells along the midgut (Figure 5Ba). In contrast, high levels of GFP expression were detected in patches of cells along the midgut 4 hr after *Ecc15* ingestion (Figure 5Bb). Analysis of the JAK-STAT pathway using RT-qPCR and the Upd3 and STAT reporter genes revealed that, while strongly induced by ingestion of *Ecc15* and, to a lesser extent, *E. coli,* it was only weakly induced by other bacterial strains or peptidoglycan from Gram-negative or Gram-positive bacteria (Figure 5C and Table S2). This contrasts with the activation of the Imd pathway by Gram-negative peptidoglycan (Figure S1) (Zaidman-Remy et al., 2006) and suggests that JAK-STAT pathway activation results from an indirect consequence of bacterial infection. It was recently shown that tissue damage in both tumors and wounds activates expression of the cytokine Upd3 (Pastor-Pareja et al., 2008). Similarly, ingestion of sodium dodecyl sulfate (SDS) or paraquat, two treatments that damage cells, induced *STAT-GFP* and *upd3* expression levels in the gut (Figure 5C and Table S2). Additionally, *upd3* expression upon *Ecc15* ingestion was not affected in flies carrying mutations in the Toll, Imd, or JAK-STAT pathways (Figure 3). Collectively, these results suggest that cell damage induced by *Ecc15* results in the release of Upd3 and activation of the JAK-STAT pathway.

Ecc15 Activates Genes Involved in Stress Response and Epithelium Renewal

A major finding of our study is that ingestion of *Ecc15* activates the expression of many genes that are not directly related to the immune response. Prominent among them are genes involved in stress, cell repair, and epithelial renewal. Our microarray analysis shows that *Ecc15* oral infection induces the expression of 34 stress-responsive genes (Figure 1D). The

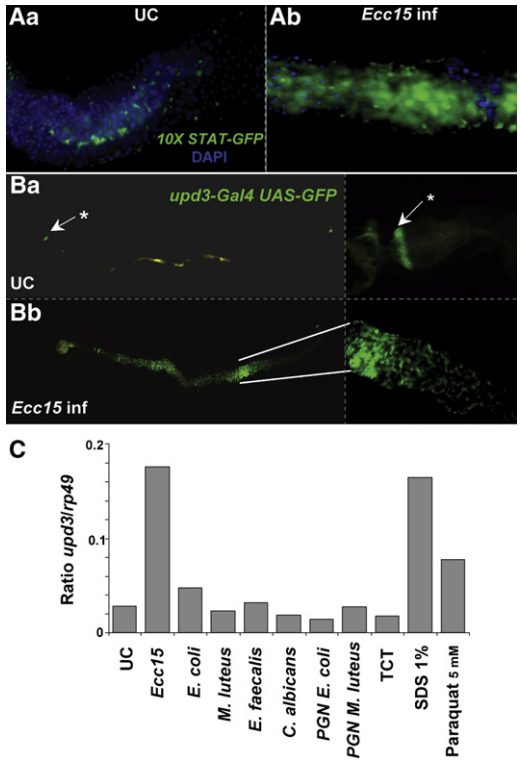

Figure 5. The JAK-STAT Pathway Is Activated in the Gut upon Infection

(A) A *STAT-GFP* in vivo reporter detects the activation of the JAK-STAT pathway in the gut of adult females upon oral infection with *Ecc15*. The STAT-GFP reporter (green) was expressed in a small population of basal cells in unchallenged flies (a). Ingestion of *Ecc15* (16 hr postinfection) induced a strong expression of *STAT-GFP* along the midgut (b). Merge of blue (DAPI) and green (GFP) channels.

(B) Ingestion of *Ecc15* triggers the expression of *upd3* in the gut. Flies carrying *upd3-Gal4* combined with *UAS-GFP* were used to monitor *upd3* expression in the gut. In unchallenged flies (a), the *upd3* reporter gene was expressed in the cardia (right), a subset of dispersed cells in the midgut and the anal pad (data not shown). Oral infection (16 hr postingestion) triggers a strong expression of the reporter gene in groups of cells along the gut (b).

(C) RT-qPCR analysis of *upd3* induction in gut extracts from unchallenged wild-type flies (UC) or wild-type adult females 16 hr postfeeding with various bacterial strains, polymeric peptidoglycan (PGN), monomeric peptidoglycan (TCT), SDS (0.1%), or paraquat (5 mM).

corresponding upregulated transcripts can be assigned to five groups of genes encoding: (1) heat shock proteins, (2) cytochromes P450 (5 genes induced and 20 repressed), (3) glutathione S-transferase of the δ group (all clustered in the genome), (4) proteins regulating ROS activity, and (5) stress peptides (including 3 *Turandot* genes and *frost*) (Figure 2 and Table S4). It is likely that many of these genes, especially those encoding GSTs, cytochromes P450, and ROS enzymes, participate in the detoxification of ROS produced during microbial killing to protect the gut epithelium (Ha et al., 2005). The expression of stress genes such as *GstD8* and *hsp70* was not affected by a reduction in the activity of the JAK-STAT or Imd pathways (Figure 3). However, many of these stress-responsive genes were expressed at higher levels in *Rel*-deficient flies in both unchallenged and challenged conditions (Figure 2 and Tables S1 and S4). This suggests that impairment of AMP-mediated defense could enhance the gut stress response.

Our microarray analysis further identified the induction of many genes involved in cell repair and renewal upon *Ecc15* oral infection. These include genes encoding proteins involved in cell shape and polarity (cytoskeleton components, annexins), cell cycle (histones, helicases, cyclins), cell death, cell survival and wound healing (Gadd45, NijA, p53), and DNA repair (DNA helicase, ligase) (Figure 2 and Table S5). The majority of these genes were not affected by the *Rel* mutation, indicating they are not directly regulated by the Imd pathway. Interestingly, the majority of these cell-cycle-related genes were induced mainly at the late time point (16 hr). These data suggest that infection triggers the expression of repair mechanisms necessary to resolve damage caused by infection.

Epithelium Renewal in Response to Bacterial Infection

Recently, it was shown that the adult *Drosophila* midgut contains multipotent intestinal stem cells (ISCs) scattered along its basement membrane. Upon cell division, each ISC produces one daughter cell that retains the ISC fate and a postmitotic enteroblast that differentiates into either an absorptive enterocyte or a secretory enteroendocrine cell (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). Given the effect of *Ecc15* infection on genes involved in cell repair and renewal, it was plausible to consider that infection could lead to ISC-derived production of new cells to replace damaged epithelial cells. Supporting this hypothesis, *Ecc15* ingestion led to increased cell death in the gut as evidenced by acridine orange staining (Figure 6A). Detection of caspase-3-like activity and TUNEL staining revealed apoptotic cells clustered in patches along the infected gut (Figure 6B and data not shown).

Two methods were used to monitor the impact of *Ecc15* on stem cell proliferation. First, we used genetically marked wild-type cell lineages to identify dividing cells and their progeny (Harrison and Perrimon, 1993). In the absence of infection, small-sized *lacZ*-marked clones were only rarely detected in the midgut. In contrast, large *lacZ*-marked clones were observed in flies collected 3 days after *Ecc15* infection, indicative of cell proliferation (Figure 6C). To extend this finding, guts were stained with an anti-phosphohistone H3 (anti-PH3) antibody that marks dividing cells. Careful examination revealed a very low number of PH3-positive cells in the gut of unchallenged flies (Figures 6Da and 6Dc). These cells were small and basally located, corresponding to ISCs. Strikingly, a high number of PH3-positive cells were detected in gut epithelia of *Ecc15* orally infected flies (Figures 6Db and Dd). Up to 80% of the PH3-positive cells in these guts were restricted to the midgut area, and in some cases, PH3 stainings were clearly indicative of mitosis (Figure 6Dd, inset). Quantification of PH3-positive cells indicated a 10-fold increase in the number of dividing cells in the guts of infected flies compared to uninfected (Figure 7A).

We further characterized the nature of these dividing cells using Escargot (Esg), a specific marker of stem cells and enteroblasts (Micchelli and Perrimon, 2006). In the absence of infection, the *esg-Gal4; UAS-GFP* reporter gene was only rarely expressed in a small, dispersed subset of rounded cells with small nuclei corresponding to the *Drosophila* ISCs (Figures 6Ea and 6Ec). In addition, very few epithelial cells recently derived

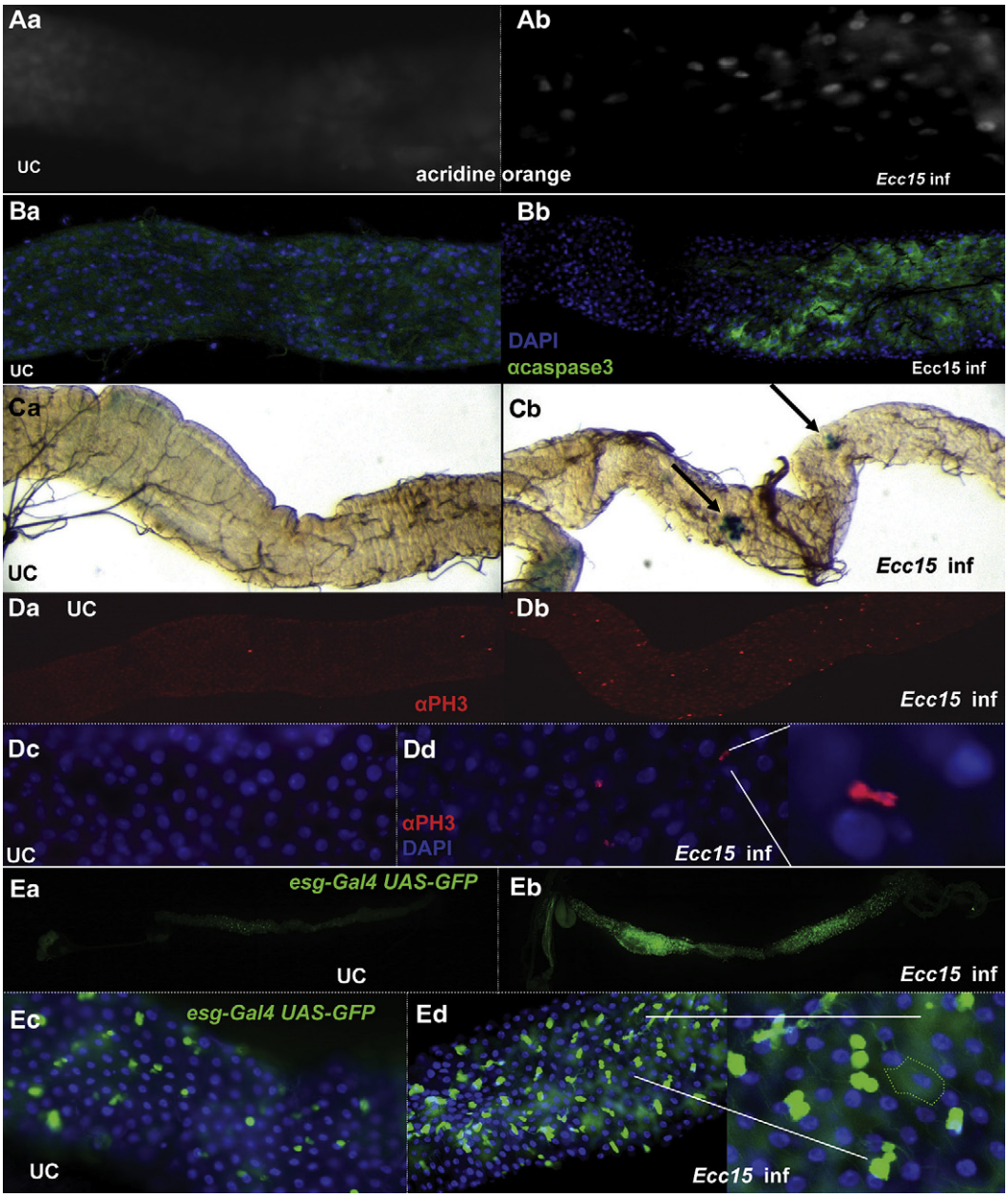

Figure 6. *Ecc15* Ingestion Induces Cell Death and Promotes Cell Proliferation in the Adult Gut

(A and B) Guts of unchallenged and *Ecc15*-challenged wild-type flies, respectively, stained with acridine orange to detect dead cells (a, b) or anti-caspase 3 (a, b) to detect apoptotic cells.

(C) 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* (b) compared to guts from flies fed on sucrose (a). The size of the clone is a direct measure of the rate of cell division of the adult midgut (see Experimental Procedures).

(D) Guts of flies orally infected with *Ecc15* exhibit a higher number of dividing cells. Guts from unchallenged flies (a and c) or flies sampled 16 hr after *Ecc15* ingestion (b and d) are shown. Mitosis is detected in small cells corresponding to ISCs by immunostaining of PH3 (red). DAPI: blue.

(E) Domains of cell proliferation are revealed by the expression of an *esg-Gal4; UAS-GFP* reporter. Guts from unchallenged flies (a and c) and 16 hr *Ecc15*-infected *esg-Gal4; UAS-GFP* flies (b and d) are shown. *Ecc15* ingestion induces a marked increase in the number of GFP-positive cells. In unchallenged flies, most of the GFP signal corresponds to ISCs (identified by their small nuclei). In infected flies, GFP signal was observed in both ISCs and ISC-derived daughter cells. Most GFP-containing cells from infected guts were characteristic of enterocytes (d, far right panel). *esg-Gal4; UAS-GFP,* green; DAPI, blue.

from these stem cells, as indicated by GFP-positive cells with large nuclei, were detected in uninfected flies. In contrast, *Ecc15* ingestion led to a strong increase in GFP signal in cells all along the gut, indicating a recent and extensive increase in the quantity of stem cell-derived cells (Figures 6Eb and 6Ed). This signal was not due to an increase in the number of ISCs

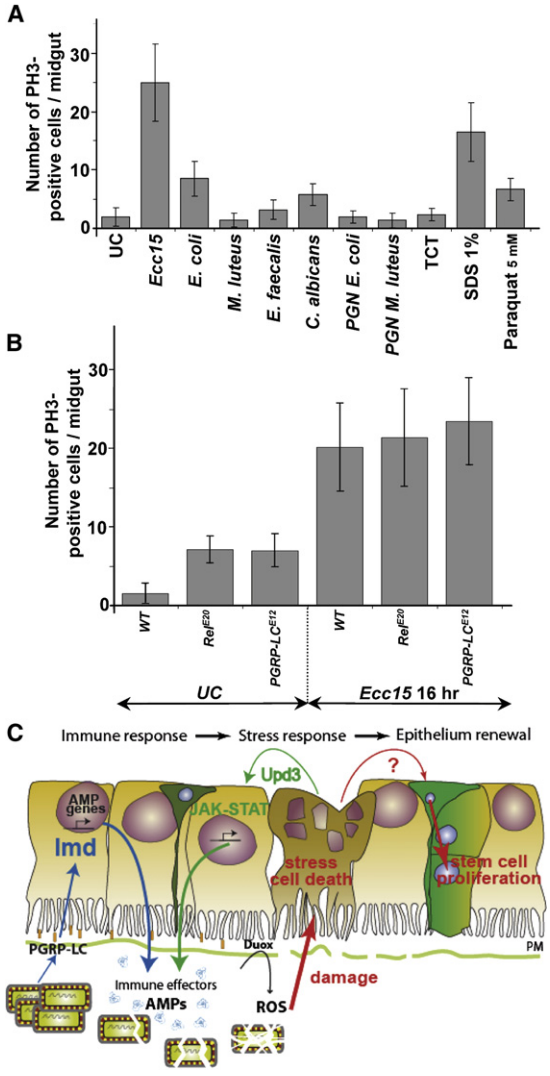

Figure 7. Cell Damage Stimulates ISC Turnover

(A) Quantification of PH3-positive cells per midgut (n = 19) of wild-type unchallenged flies (UC) or flies collected 16 hr after feeding with various bacterial strains, polymeric peptidoglycan (PGN), monomeric peptidoglycan (TCT), paraquat, and SDS.

(B) Quantification of PH3-positive cells per midgut of unchallenged flies or flies 16 hr after ingestion of *Ecc15*.

(C) A model of the gut immune response to oral bacterial infection (see text). Error bars indicate SD.

(identified as intense GFP cells with small nuclei) but rather an increased number of GFP-positive cells with large nuclei derived from these ISCs (Figures 6Ed and S4). This indicates that *Ecc15* infection does not increase the number of ISCs, but instead stimulates their division, promoting a rapid turnover of the epithelium.

Stem Cell Proliferation Does Not Require the Imd Pathway

Our observations suggest that signals arising either from damaged enterocytes or bacterial infection regulate stem cell proliferation. To better understand this regulation, we investigated the nature of the stimuli that activate stem cell proliferation. Stem cell proliferation in the midgut, as monitored by the number of PH3-positive cells or by *esg-GFP* reporter, was induced by ingestion of *Ecc15* and, to a lesser extent, *E. coli*, *C. albicans,* SDS, and paraquat, but was not induced by other bacterial strains or peptidoglycans (Figure 7A and Table S2). We conclude that stem cell proliferation is regulated by the same stimuli that activate the JAK-STAT pathway and results from a signal from stressed/damaged cells rather than bacteria themselves. Furthermore, ingestion of *Ecc15* resulted in similar numbers of dividing cells in the guts of *Rel*, *PGRP-LC*, and wild-type flies (Figure 7B), indicating that the Imd pathway does not directly regulate stem cell proliferation. Interestingly, higher numbers of dividing cells were detected in the guts of Imd immune-deficient flies in the absence of infection. The higher levels of stem cell division observed in flies lacking a functional Imd pathway likely reflects cellular stress due to imbalance in gut homeostasis. In support of this, higher levels of stress-related genes were detected in *Rel*-uninfected flies (Table S3).

DISCUSSION

The regulation of the systemic immune response has been studied extensively in *Drosophila,* providing a paradigm of insect immunity based on the differential activation of Toll and Imd pathways. These studies profoundly impacted both our conception of how insects fight microbial infection and our general comprehension of the metazoan innate immune response. The initial focus on this aspect of immunity was largely historical, due to the discovery of inducible AMPs, which are produced massively by the fat body of *Drosophila*, and also the ease of triggering this response by septic injury. Here, we have used genomic and genetic approaches to decipher the gut antibacterial response of *Drosophila* and analyze the signaling networks that orchestrate this response at the gene level. Our study provides an initial characterization of the complex events that occur during the gut epithelial response to bacteria. These results establish a basis for further analyses of the gut immune response in *Drosophila* and identify mechanisms likely to impact innate immunity in general.

Complexity of the *Drosophila* Gut Response to Bacteria

Using microarray analysis, we identified the suite of genes whose expression is modulated in the gut in response to oral bacterial infection. Our study reveals that the gut immune response is complex both in terms of gene number and intensity of expression. Comparison of our microarray data set with the genes upregulated during systemic infection identified 75 common genes, including most genes with immune signatures such as AMPs, PGRPs, and transferrin. This group of genes constitutes the core of the *Drosophila* antimicrobial response. The Imd pathway regulates the majority of these genes, highlighting the importance of Imd pathway-mediated defense in the gut. We also identified a group of gut-specific immune-regulated genes that are regulated by *Rel*. These new targets of the Imd pathway likely constitute gut-specific host defense mechanisms and are promising targets for further functional characterization. Included in this group are peritrophin-encoding genes, indicating a role of the Imd pathway in the remodeling and reinforcement of the peritrophic matrix.

The gut is a compartmentalized organ with distinct immunoreactive domains (Figure S2). While the Imd pathway is activated all along the gut, AMP genes such as *Diptericin* are expressed with a complex and distinctive expression pattern, indicating that additional levels of regulation restrict AMP expression to some gut segments. This is in agreement with the observation that the homeobox gene *caudal* represses AMP expression in the distal midgut (Ryu et al., 2008). The complexity of the digestive tract, in terms of organization and cell types, likely contributes to the complexity of the gut response to bacteria. The existence of distinct immune-responsive domains along the gut is a feature shared with vertebrates and may contribute to homeostasis. Analyzing the mechanisms that restrict AMP expression to specific domains and the physiological role of this compartmentalization is important to better characterize the gut immune response.

No Role for Toll Signaling in Gut Immunity

In contrast to the systemic response, we did not detect a role for the Toll pathway in the gut immune response. Many of the previously identified Toll target genes that participate in hemolymph reactions such as melanization and clotting (Lemaitre and Hoffmann, 2007) were not induced by *Ecc15* ingestion. In *Drosophila*, Toll is activated by the binding of the cytokine Spz, which is processed in the hemolymph by complex cascades of serine proteases. The absence of a role for Toll in the gut could be explained by the incompatibility of such proteolytic cascades with the acidic conditions of the gut and the presence of digestive trypsins. Thus, Toll signaling appears to be restricted to the fat body and hemocytes during the systemic response, suggesting that the Toll pathway emerged in *Drosophila* as an immune sensor specific to the hemolymph compartment.

In contrast, the Imd pathway regulates immune responses in epithelia, hemocytes, and the fat body, supporting an important and ancestral role of this pathway in antimicrobial defenses (Tzou et al., 2000). To date, host defense is the sole function attributed to the *Drosophila* Imd pathway, whereas other immune pathways, namely the JAK-STAT, JNK, and Toll pathways, have roles in developmental processes as well. These multifunctional roles have likely imposed evolutionary constraints on the latter pathways. In contrast, the unique function of the Imd pathway in immunity makes it compatible with a rapid adaptation to the emergence of new pathogens.

The JAK-STAT Pathway Participates in the Antimicrobial Response of the Gut

Our microarray analysis identified a subset of genes with gut immune functions that are upregulated in an Imd-independent manner. Of particular interest was the induction of three genes encoding Drosomycin-like peptides, which had not been previously characterized. The expression of this specific subset of AMPs could constitute an optimal adaptation to the features of gut, as well as to the nature of pathogens encountered by this tissue. An unexpected result of our study is the observation that *dro3* is regulated by the JAK-STAT pathway. To date, the role of the JAK-STAT pathway in immunity has been limited to antiviral defense and stress response (Lemaitre and Hoffmann, 2007). Our study reveals that, along with the Imd pathway, it plays an important role in the regulation of gut antimicrobial response. Our observations that (1) *upd3* and *Domeless* were both induced in the gut upon *Ecc15* infection and (2) *dro3* and *Soc36E* expression were affected in both *upd3* and *STAT RNAi* flies indicate that bacterial infection induces the expression in gut cells of the cytokine Upd3, which then activates the JAK-STAT pathway in enterocytes. Similar to observations of the hemocyte response to wounds and tumors (Pastor-Pareja et al., 2008), our results show that *Upd3* is induced in the gut by a stimulus associated with damage and stress and not a microbial product. The next step will be to identify other target genes of the JAK-STAT pathway in the gut and to evaluate the relevance of this pathway in gut host defense.

Stress Response and Perturbation of the Digestive Process

Our data demonstrate that ingestion of *Ecc15* triggers an immediate stress response that includes the production of enzymes involved in ROS detoxification. This response is probably a direct consequence of the ROS burst that peaks 1 hr after *Ecc15* infection and is consistent with a previous study demonstrating that ROS production by the NADPH oxidase Duox is essential for elimination of ingested bacteria and requires tight regulation to prevent damage to gut cells (Ha et al., 2005). This stress response is largely independent of the Imd and JAK-STAT pathways, and thus could be a direct consequence of cell damage caused by ROS.

Finally, our microarray analysis reveals that oral bacterial infection impacts gut physiology through the modulation of metabolic enzymes and repression of many digestive enzymes. This digestive arrest may be a direct consequence of gut damage. Interestingly, interruption of feeding is commonly associated with bacterial infection in insects (Dunn et al., 1994; Vallet-Gely et al., 2008). It is not yet clear whether this interruption is a host adaptation to limit bacterial ingestion or a strategy used by entomopathogenic bacteria to counteract peristalsis and persist in the gut. Determining the mechanisms that link bacterial infection to changes in gut physiology will help ascertain whether it is an indirect consequence of tissue damage or requires specific pathway regulation. From this perspective, the induction of numerous genes with neural and hormonal activity, capable of modulating gut physiology upon infection, provides starting points for further investigation.

Epithelial Renewal as a Major Response to Bacterial Infection

Human intestinal cells are continuously replenished by stem cells, the misregulation of which has been implicated in a number of common digestive diseases and cancers (Barker et al., 2008). The adult *Drosophila* midgut also contains multipotent ISCs, and it was suggested that ISCs respond to signals emitted by surrounding epithelial cells to produce appropriate daughter cells (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). To date, conditions triggering cell proliferation in the *Drosophila* gut have not been described, although increased epithelial turnover is reported to occur with aging (Choi et al., 2008; Biteau et al., 2008). Here, we observed that ingestion of *Ecc15* provokes a massive increase in epithelial renewal, as evidenced by clonal analysis and an increased number of dividing cells. Use of specific cell markers shows that this renewal occurs

via increased ISC proliferation and differentiation. These results highlight an unexpected link between oral bacterial infection and epithelial renewal.

Our study demonstrates that the activity of ISCs can be modulated by infection to coordinate cell production. The data suggest that gut homeostasis is maintained through a balance between cell damage, as a result of the collateral effects of bacteria killing (e.g., ROS), and epithelial repair by ISC division. In this regard, epithelial renewal can be considered part of gut host defense, essential for the resolution of infection. Recently, it was demonstrated that some human pathogens employ mechanisms to disrupt epithelial renewal and promote their own colonization (Iwai et al., 2007; Mimuro et al., 2007). In addition, it was reported that exposing lepidopteran midgut cell cultures to *Bacillus thuringiensis* toxin resulted in extensive enterocyte death and a transient increase in stem cell division and differentiation (Loeb et al., 2000). These papers and our study all point to an important function of epithelial renewal in host-pathogen interactions.

Our results show that stem cell proliferation is not regulated by the Imd pathway and occurs as a result of a signal from damaged cells rather than bacteria themselves. We hypothesize that stem cell proliferation is induced by the JAK-STAT pathway through the release of Upd3 from enterocytes damaged by ingestion of *Ecc15* (Figure 7C). This is supported by the strong correlation we observed between stimuli activating stem cell proliferation and those inducing JAK-STAT activity. The induction of many genes encoding components of signaling pathways associated with stem cell differentiation and maintenance (EGF-R, hedgehog, Notch, and JAK-STAT) (Choi et al., 2008; Ohlstein and Spradling, 2007) points to their role in the regulation of epithelial renewal upon infection. This link between infection and stem cell proliferation also provides a framework to further decipher the relationships between chronic infections and cancers of epithelial origin.

In conclusion, our microarray analysis captures the sequence of events defining *Drosophila* gut response to bacterial infection and the resolution of this immune response. It also demonstrates that the *Drosophila* gut provides a powerful model system to study the integration of stress and host immunity with pathways traditionally associated with development and stem cell division.

EXPERIMENTAL PROCEDURES

Fly Stocks

Oregon^R flies or flies carrying one copy of the *NP1-Gal4* transgene were used as wild-type controls. For RNAi, adult flies carrying one copy of the *UAS-RNAi* construct combined with one copy of the *NP1-Gal4* driver were used. The F1 progeny carrying both the *UAS-RNAi* and the *Gal4* driver were transferred to 29°C at late pupal stage for optimal GAL4 and RNAi efficiency. *UAS-RNAi* transgenic fly lines of *dFADD (R1)*, *PGRP-LC (R1)*, and *STAT (R2)* were obtained from Ryu Ueda (Mishima, Japan). *upd3-IR, upd3-Gal4, UAS-GFP;* hop^{msv1}, and hop^{Tum} are described in Agaisse et al., 2003. The transgenic strains *Dpt-lacZ*, *Dpt-GFP*, and *UAS-imd* and the mutant lines $PGRP\text{-}LC^{E12}$, Rel^{E20}, $Tl^{1\text{-}RXA}$, and Tl^{r632} have been described elsewhere (Zaidman-Remy et al., 2006). A *10XSTAT92E-eGFP* transgene driving expression of enhanced *GFP* was used to monitor JAK-STAT pathway activity (Bach et al., 2007). The *esg-Gal4*; *UAS-mCD8GFP* line is described in Micchelli and Perrimon, 2006. Additional fly stocks are described in Supplemental Data.

Bacterial Strains and Infection Experiments

E. carotovora carotovora 15 is a Gram-negative bacterium that induces a systemic immune response after oral infection in larvae but not in adults (Basset et al., 2000). Flies were infected, following a 2 hr starvation, by applying a 1:1 mixture of 5% sucrose and concentrated bacteria (OD 200), peptidoglycan (5 mg/ml), or TCT (tracheal cytotoxin) (0.046 mM) to a filter disk that completely covered the surface of standard fly medium. Flies were maintained at 29°C and guts were dissected 4 and 16 hr after oral contact with infected food.

RT-qPCR

RNA was extracted from 50 dissected guts (without Malpighian tubules) using TRIzol (Invitrogen). RT-qPCR was performed using SYBR Green I (Roche) on a Lightcycler 2.0 (Roche). The amount of mRNA detected was normalized to control *rp49* mRNA values. Relative gene expression is a percentage expression of the ratio value obtained in wild-type infected guts from the same experiment.

Microarray Analysis

RNA pools from 60 guts of 5-day-old females were isolated, purified with RNA clean-up purification kits (Macherey Nagel), and DNase treated. RNA was quantified by NanoDrop ND-1000 and RNA quality was controlled on Agilent 2100 Bioanalyzer chips. For each sample, 1 μg of total RNA was amplified and labeled using the GeneChip IVT Labeling Kit according to the protocol provided by the supplier. Affymetrix *Drosophila* Genome 2.0 arrays were hybridized with 30 μg of labeled cRNA, washed, stained, and scanned according to the protocol described in Affymetrix Manual. Three independent repeats were performed for each time point and gene expression profiles from challenged flies were normalized to the sucrose-fed adults of the same time point. Statistical analyses were performed using the R and Bioconductor statistical packages. Raw data and processed files of the microarray analysis can be found at http://lemaitrelab.epfl.ch/page26728-en.html (Resources).

Live Imaging and Immunofluorescence

For live imaging, guts were dissected in PBS and immediately mounted in the antifading agent Citifluor AF1 (Citifluor). For immunofluorescence, guts were dissected in PBS, fixed for 20 min in 0.1% Tween 20 PBS (PBT) with 4% paraformaldehyde, rinsed in PBT, and then incubated with primary antibodies (dilution 1/150 anti-LB, 1/1000 anti-PH3, 1/500 anti-cleaved Caspase-3, or 1/1000 anti-GFP) in PBT + 1% BSA. Anti-LB or anti-GFP was revealed with Alexa488- or Alexa594-coupled anti-mouse antibodies (Invitrogen), and nuclei were stained by DAPI (Sigma). Apoptosis was detected in dissected guts by acridine orange (Invitrogen) and TUNEL (Roche) stainings, according to the manufacturers' instructions.

Clonal Analysis

The marked lineage system of Harrison and Perrimon was used to generate clones of *lacZ*-expressing cells (Harrison and Perrimon, 1993).

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Results, Supplemental Experimental Procedures, Supplemental References, five figures, and seven tables and can be found online at http://www.cell.com/cellhostandmicrobe/supplemental/S1931-3128(09)00028-6.

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