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Article

Infection-Induced Host Translational Blockage Inhibits Immune Responses and Epithelial Renewal in the *Drosophila* Gut

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SUMMARY

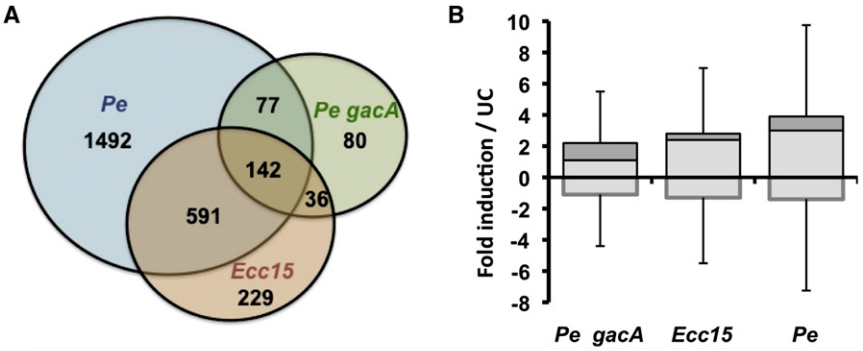
Typically, immune responses control the pathogen, while repair and stress pathways limit damage caused by pathogenesis. The relative contribution of damage to the outcome of pathogenesis and the mechanistic links between the immune and repair pathways are poorly understood. Here, we analyze how the entomopathogenic bacterium *Pseudomonas entomophila* induces irreversible damage to the *Drosophila* gut. We find that *P. entomophila* ingestion induces a global translational blockage that impairs both immune and repair programs in the fly gut. *P. entomophila*-induced translational inhibition is dependent on bacterial pore forming toxins and reactive oxygen species produced by the host in response to infection. Translational arrest is mediated through activation of the GCN2 kinase and inhibition of the TOR pathway as a consequence of host damage. Together, our study draws a model of pathogenesis in which bacterial inhibition of translation by excessive activation of stress responsive pathways inhibits both immune and regenerative epithelial responses.

INTRODUCTION

Pathogenic bacteria are usually defined by their capacity to inflict damage to their host either directly (e.g., through the production of toxins) or indirectly by activating a deleterious immune response. In response to pathogens, both resistance and tolerance mechanisms contribute to maintain host integrity (Schneider and Ayres, 2008). Resistance mechanisms involve the activation of various immune responses that directly restrict microbial growth. Tolerance mechanisms encompass repair and stress pathways that limit damage caused by pathogens. The concerted action of these two mechanisms is illustrated during infection in the *Drosophila* gut where both immune and repair mechanisms are required to limit pathogenesis (Buchon et al., 2009a; Chatterjee and Ip, 2009). In the outcome of disease, the respective contribution of damage caused directly by the pathogen versus collateral damage of the host response is less known. Moreover, the mechanisms accounting for the difference between lethal and nonlethal infections are poorly understood. To address these questions, we are dissecting the interaction between the entomopathogen *P. entomophila* and its host *Drosophila*, an interaction that ultimately disrupts gut integrity and kills the host.

P. entomophila is a bacterial pathogen of *Drosophila* that we originally isolated from flies sampled in Guadeloupe (Vodovar et al., 2005). After ingestion, *P. entomophila* induces the transcription of antimicrobial peptide genes via the Imd pathway, both locally in the intestinal epithelium and systemically in the fat body, an organ analog to the mammalian liver. Despite the induction of strong local and systemic immune responses in *Drosophila*, *P. entomophila* remains highly pathogenic as it rapidly induces a cessation of feeding and gut damage (Liehl et al., 2006; Vodovar et al., 2005). This indicates that *P. entomophila* can in some way subvert the gut immune response.

In *Drosophila*, several mechanisms of defense have been identified to survive microbial infection in the gut (Royet, 2011). Ingestion of bacteria induces the rapid synthesis of microbicidal reactive oxygen species (ROS) in the *Drosophila* gut by a NADPH oxidase called Duox (Ha et al., 2005). Complementary to this ROS response, several antimicrobial peptides (e.g., *Diptericin*) are produced in the gut under the control of the Imd pathway. This local immune response is triggered by the recognition of Gram-negative peptidoglycan by the Imd pathway (Zaidman-Rémy et al., 2006) and was shown to contribute to host survival upon intestinal infection with several pathogenic bacteria (Liehl et al., 2006; Nehme et al., 2007). Additionally, phenotypic analysis of flies with a thinner peritrophic matrix due to the lack of the Drosocrystallin chitin-binding protein suggested that this matrix is required for host defense against enteric pathogens, specifically preventing the damaging action of pore-forming toxins on intestinal cells (Kuraishi et al., 2011). Finally, efficient and rapid recovery from bacterial infection is possible only when bacterial clearance is coordinated with repair through renewal of the epithelium damaged by infection (Buchon et al., 2009a; Chatterjee and Ip, 2009; Jiang et al., 2009). Upon damage, epithelial renewal of the *Drosophila* gut is stimulated by the release of the secreted ligands Upd3, Vein, and Keren, which activate the JAK/STAT and EGFR pathways in intestinal stem cells to promote both their division and differentiation,



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CG number	Gene Title	Gene Symbol	molecular function	Fold change compared to wild type uninfected guts					
				Pe 4h	Pe 16h	gacA 4h	gacA 16h	Ecc15 4h	Ecc15 16h
antimicrobial defense									
CG10146	attacin	AttA	antimicrobial activity	23.1	14.2	8.6	3.4	18.8	13.7
CG8175	metchnikowin	Mtk	antimicrobial activity	11.4	11	4	1.3	7.5	27.2
CG32283	drosomycin-E	dro3	antimicrobial activity	7.4	22.2	1.4	-1.5	7.2	8.1
Stress response									
CG33338	p38c	p38c	SAPK signaling	5.6	5.3	1.2	-1.1	2	1.6
CG2275	Jun oncogene	Jra	Jun (JNK signaling)	4.4	4.5	-1.1	-1.1	2.2	1.2
CG31509	Turandot	TotA	Turandot	10.6	152.8	1.9	1.2	2.1	2
CG6449	ninjurin A	NijA	tissue regeneration	6.1	2.3	1.3	-1.2	4.9	2.1
CG11086	Gadd45	Gadd45	Gadd45	73.2	43.5	1.6	-1.2	5.1	1.9
CG18743	heat shock 70	Hsp70	heat shock protein	2.7	10.5	1.5	-1.9	7.6	-1.5
CG11765	peroxiredoxin 2540	Prx2540-1	oxidative stress	31.1	5.5	26.1	-1.3	1.6	1.5
CG12242	Glutathione S transferase D5	GstD5	oxidative stress	41.9	29.2	3.1	-1.7	5.2	-1.2
CG4423	Glutathione S transferase D6	GstD6	oxidative stress	1.8	36.6	1	1.1	1.8	1.3
CG8523	Multi drug resistance 50	Mdr50	detoxification	1.3	3.1	-1.2	-1.1	-2	-2.2
DNA damage									
CG18801	Ku80	Ku80	DNA damage	2	7.6	1.2	-1.3	4.2	1.6
CG10215	Ercc1	Ercc1	DNA damage	16.5	30.1	1.1	-1	3.6	1.5
CG10873	p53	p53	DNA damage checkpoint	4.3	12.4	-1.3	-1.1	3.1	1.5
Hypoxia									
CG7951	HIF-alpha	sima	transcription factor	1.3	1.8	1.1	1	-1.3	-1.2
CG7590	scylla	scyl	hypoxia induced gene	2.1	1.4	-1.3	1	1.2	1.1
Apoptosis									
CG6829	Apaf-1 related killer	Ark	apoptosis	1.8	4.1	-1.1	1.2	1.7	1.9
CG5370	caspase	Dcp-1	apoptosis	2.6	2.6	-1.2	1.1	-1.2	-1.1
CG12284	Inhibitors of Apoptosis protein	th	apoptosis inhibitor	2.4	2.5	-1	-1	1.5	1.2
epithelium renewal									
CG5963	unpaired 3	upd3	JAK-STAT cytokine	7.5	18.3	1	-1.1	4.1	2.2
CG5988	unpaired 2	upd2	JAK-STAT cytokine	2.3	5.4	1	1	1.5	-1
CG15154	Suppressor of cytokine signaling at 36E	Socs36E	JAK-STAT inhibitor	13	5	1.1	-1.7	8	2
CG10491	defective dorsal discs	vn	EGF	7.2	10.3	1.1	-1.3	3.4	2.3
CG32179	spitz2	Krn	EGF	1.9	7.1	-1.2	-1	2.5	1.6
CG4531	roulette	argos	EGFR inhibitor	1.3	1.2	1	-1.2	2.3	1.9
CG7103	PDGF- and VEGF-related factor 1	Pvf1	PDGF-VEGF related	3.4	2.3	-1.3	-1.2	3.3	2.4
gut structure									
CG16963	drosocrystallin	Cry	peritrophic matrix	19.6	8	2.9	-1.2	5.6	2.1
CG4835	CG4835	CG4835	Mucin	2.7	7.1	1.8	1.2	3.1	3
CG31893	Dm-peritrophin-15b	Peritrophin-15b	peritrophin	-1.6	-9.7	-1.3	-1	-1	-1.5
Metabolism/digestion									
CG18402	insulin receptor	InR	Insulin pathway	3	4	-2.3	-1.2	2.3	1.5
CG4812	Serine Protease 3	Ser8	serine protease	-18.7	-51.5	-1.4	1.3	-17.7	-4.5
CG9564	Trypsin 29F	Try29F	Trypsin	1	-4.8	-1.1	2.3	-1.2	1.7
CG18211	betaTrypsin	betaTry	Trypsin	-1.1	-11	-1.1	1	-1.1	-1.5
CG6467	Jonah 65A	Jon65Aiv	elastase	-1.1	-6.4	-1	1	-1	-1.1
CG9092	beta-galactosidase-1	Gal	galactosidase	-2	-24.2	2	-1.3	-3.3	-3.2
CG8823	Lip3	Lip3	Lipase	-1.6	-4.7	1.3	1.4	-1.8	-3.9

Figure 1. *P. entomophila* Induces Genes Involved in Antimicrobial Response, Stress Response, and Epithelium Renewal

(A) Comparison of the distribution of genes induced in the gut upon *Ecc15*, *P. entomophila*, and *P. entomophila GacA* mutant oral infection.

(B) A Box-plot representation of global gene regulation by *P. entomophila gacA*, *Ecc15*, and *P. entomophila* shows that increased pathogenicity is associated with an increase in the range of transcriptional gene induction. UC, unchallenged control.

(C) A selection of genes differentially regulated upon *P. entomophila* oral infection. Gene symbol, their putative function, and fold of induction (compared to sucrose) in wild-type flies are indicated.

establishing a homeostatic regulatory loop (Buchon et al., 2009a; Jiang et al., 2009).

Infection with a high dose of *P. entomophila* inflicts strong damage to its host, which leads to the rupture of gut integrity associated with a loss of intestinal stem cells and enterocytes (Buchon et al., 2009a; Chatterjee and Ip, 2009; Jiang et al., 2009). The lack of epithelial turnover has suggested that the damage inflicted by *P. entomophila* is too severe to be repaired or that the repair program is blocked. In this paper, we analyze how *P. entomophila* infection disrupts gut homeostasis.

RESULTS

Ingestion of *P. entomophila* Induces the Transcription of Immune, Stress, and Repair Genes

To identify how the gut response to lethal and nonlethal bacterial pathogens differs, we compared the host response to three Gram-negative bacterial strains: *P. entomophila* (lethal), *E. carotovora* (nonlethal, but pathogenic) and *P. entomophila gacA* mutant (avirulent). We performed a transcriptional profiling experiment on fly gut tissue infected with either wild-type *P. entomophila* or the avirulent *P. entomophila gacA* (*Pe gacA*) mutant and compared it to the results previously reported with *Ecc15* (Buchon et al., 2009b). Microarray analysis revealed that 3,097 out of the 17,000 genes encoded by the *Drosophila* genome were changed by a factor of two or more after ingestion of *P. entomophila*. Our analysis revealed that *P. entomophila*, *P. entomophila gacA*, and *Ecc15* regulate together a core of 142 genes involved in antimicrobial and oxidative stress (Glutathion-S-transferase-d, Gstds) responses (Figure 1A). Both *P. entomophila* and *Ecc15* induced an additional pool of 591 genes, implicated in antimicrobial and stress responses (Hsp, Gstds, Turandots), stem cell activation, and epithelium repair (EGFR and JAK-STAT pathways) (Figure 1C). However, induction of most of these genes was higher in flies infected with *P. entomophila* than *Ecc15* (Figure 1B). Finally, the lethal-pathogen *P. entomophila* specifically induced an additional 1,492 genes. Those genes include additional stress-responsive genes (e.g., Gstd6, Mdr), the cytokine Upd2, and genes related to hypoxia (HIF, Scylla), DNA damage (*Ku80*, *Ercc1*, *p53*), and apoptosis (*Ark*, *Dcp-1* or *thread*) (Figure 1C). Genes encoding digestive enzymes tend to be repressed upon bacterial infection; *P. entomophila* having a stronger effect on them than *Ecc15*.

Collectively, our analysis reveals that the amplitude of transcriptome changes correlates with the pathogenicity of bacteria. The observation that stress and damage related pathways are strongly induced by *P. entomophila* is in accordance with its capacity to inflict severe damage.

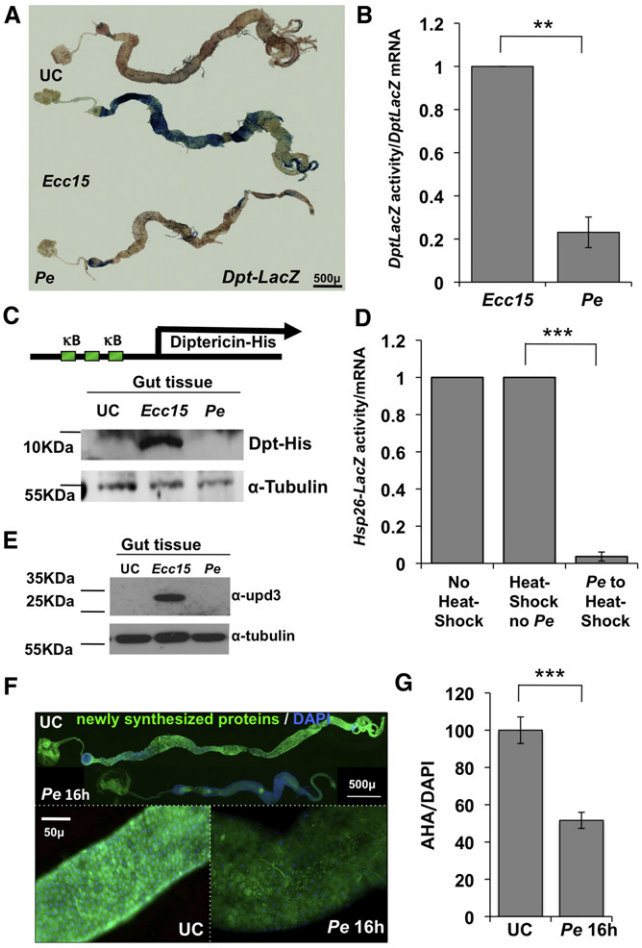

Figure 2. *P. entomophila* Infection Is Associated with a Blockage of Translation

(A) Contrary to flies infected with *Ecc15*, only a low level of *Dpt-lacZ* reporter gene activity was detected in the guts of flies infected 16 hr with *P. entomophila*.

(B) *P. entomophila* infection uncouples *lacZ* transcription and translation as revealed by a decrease in the ratio of β-galactosidase activity over *lacZ* mRNA expression (Dpt-LacZ activity/*Dpt-LacZ* mRNA) in flies collected 16 hr after oral infection with *P. entomophila*.

(C) Western blot analysis with an anti-Histidine HRP antibody reveals that Dpt-His is not synthesized in the gut at 16 hr after *P. entomophila* infection.

(D) The ratio of LacZ activity/*LacZ* mRNA in the gut was monitored in *hsp26-lacZ* flies after heat-shock with or without *P. entomophila* prefeeding. Translation of this inducible *lacZ* transgene is also blocked after *P. entomophila* infection.

(E) Western blot analysis with an anti-Upd3 antibody reveals that Upd3 is not synthesized in the gut at 16 hr after *P. entomophila* infection.

(F) Synthesis of new proteins was monitored in the guts by measurement of the incorporation of a methionine analog, L-azidohomoalanine (AHA, green signal). *P. entomophila* infection dampens global translation in the gut.

(G) Quantification of AHA signal (green)/DAPI signal (blue) from four or more representative images using pixel intensity of gut sections after oral infection with *P. entomophila*, for 16 hr, shows that *P. entomophila* decreases nascent protein synthesis by about 50%. Mean values of three experiments (n = 10 to 20 guts each) ± SE are shown. UC, unchallenged; *Ecc15*, *Erwinia carotovora carotovora 15*; *Pe*, *P. entomophila*.

See also Figure S1.

P. entomophila Infection Induces a Global Translation Blockage in the Gut

The observation that *P. entomophila* infection induces a strong expression of antimicrobial peptide genes while causing death of its host suggests the existence of mechanisms that interfere with the immune response downstream of transcription. To determine how *P. entomophila* is able to subvert the immune response, we compared the expression of a *Diptericin-lacZ* gene fusion (*Dpt-lacZ*) in the gut upon infection with *Ecc15* and *P. entomophila*. Infection with *Ecc15* led to a patterned expression of *Dpt-lacZ* along the gut (Figure 2A) as previously described (Buchon et al., 2009b). In contrast, *Dpt-lacZ* was detectable only in the cardia (a sphincter at the entrance of the midgut) of *P. entomophila*-infected flies (Figure 2A). In order to determine to which extent *Dpt-lacZ* is transcribed and translated, we determined the amount of *Dpt-lacZ* transcript and the resulting β-galactosidase activity. Strikingly, although infection with both *P. entomophila* and *Ecc15* caused a high induction of the *Dpt-lacZ* transcription, the corresponding increase in LacZ activity was low in *P. entomophila* compared to *Ecc15* infected guts. This is illustrated by a decrease in the ratio between β-galactosidase activity and *Dpt-lacZ* transcript levels (Figure 2B). To confirm this result, we used a transgenic line expressing the Dpt peptide tagged with Histidine under the control of its own promoter. We observed only a low amount of Dpt-His protein in the gut after *P. entomophila* infection, while *Ecc15* triggered a strong synthesis of Dpt-His (Figure 2C). Thus, *P. entomophila* ingestion induces a strong transcriptional induction *of Dpt*, but blocks the production of this antimicrobial peptide at the translational level. Similarly, the expression of *Attacin-GFP* reporter gene was also absent after infection with *P. entomophila* (Figure S1A available online).

To determine whether this translation inhibition was specific to antimicrobial peptide genes or a more generic phenomenon, we extended this analysis to genes not related to the immune response. Figure 2D shows that *P. entomophila* suppresses the β-galactosidase activity of the *hsp26-lacZ* line after a heat shock that was performed 2 hr after infection. Similarly, western blot analysis showed that the Hsp70 protein was not detectable in the gut of *Drosophila* infected with *P. entomophila*, although the *hsp70* gene was induced transcriptionally as shown by the microarray analysis (Figures S1B and 1C). After infection, epithelium renewal is stimulated by the release of a secreted ligand, Upd3, from stressed enterocytes, which activates the JAK/STAT pathway in progenitors to promote their division and differentiation, establishing a homeostatic regulatory loop (Buchon et al., 2009a; Jiang et al., 2009). A western blot analysis showed that Upd3 was not produced in *P. entomophila* infected guts despite the strong induction of the *upd3* gene (Figures 2E and 1C). Quantification of *puc*^{E69}, *a P-lacZ* reporter gene inserted in the gene encoding the phosphatase *Puckered* (a negative regulator of the JNK pathway), revealed a lower level of LacZ activity in guts infected with *P. entomophila* compared to *Ecc15* when normalized to the amount of *lacZ* transcript (Figure S1C). The uncoupling between protein and messenger RNA (mRNA) amounts was indeed due to an inhibition of translation and not a general decrease in protein stability since it mainly affected proteins synthesized de novo upon infection (Dpt, Upd3) but not proteins produced prior to infection

(Figures S1D and S1E). To confirm this notion, we directly measured the global translation rate of *Drosophila* guts by quantifying in situ the incorporation of L-azidohomoalanine (AHA), a methionine analog upon infection. We observed that *P. entomophila* infection dampens global translation by about 50% (Figure 2F; quantification in Figure 2G). Taken together, the results show that *P. entomophila* infection is associated with a global inhibition of protein synthesis, thereby uncoupling the transcription and translation of immune, stress and repair responsive genes in the midgut (see also Figure S1F).

The Pore-Forming Toxin Monalysin Contributes to *P. entomophila* Translation Blockage

P. entomophila virulence factors required for *Drosophila* infection include a secreted metalloprotease (AprA) that protects against antimicrobial peptides and Monalysin, a pore-forming toxin, that participates in the damage to intestinal cells (Liehl et al., 2006; Opota et al., 2011). Both AprA and Monalysin production are regulated by the GacS-GacA two-component system, a major regulator that controls the production of secreted proteins and secondary metabolites (Liehl et al., 2006; Opota et al., 2011). We next investigated the implication of these virulence factors in *P. entomophila*-mediated inhibition of translation. Mutations affecting GacA-GacS and to a lesser extent Monalysin, but not AprA, alleviated *P. entomophila* induced translation inhibition, as revealed by increased AHA incorporation (Figure S2A) and Dpt-lacZ enzymatic activity/*Dpt-lacZ* transcript ratio (Figures S2B and S2C). Thus, our analysis establishes a link between pore forming toxins and the ability of *P. entomophila* to induce a translational arrest.

GCN2-Dependent Phosphorylation of eIF2α Is Required for *P. entomophila* Induced Translation Blockage

One established pathway leading to arrest of cap-dependent protein synthesis is the phosphorylation of the eukaryotic initiation factor 2α (eIF2α) (Holcik and Sonenberg, 2005). Under resting conditions, eIF2α is not phosphorylated and is part of the complex that recruits the initiator Methionine-tRNA (transfer RNA)to the start codon. When phosphorylated, however, it acts as an inhibitor of general translation. To elucidate the mechanisms underlying translation inhibition by *P. entomophila*, we analyzed the status of eIF2α phosphorylation in guts of flies after ingestion of *Ecc15* or *P. entomophila*. Consistent with a general inhibition of translation, western blot analysis showed that eIF2α is phosphorylated in gut extracts collected after *P. entomophila*, but not after *Ecc15* infection (Figure 3A). In mammals, a family of kinases (PKR, GCN2, PERK, HRI) that respond to starvation or stresses induce eIF2α phosphorylation (Figure 3B). Two of them, GCN2 (general control nonrepressed 2) and PERK (PKR-like endoplasmic reticulum kinase) are conserved in *Drosophila*. GCN2 is mainly activated by the accumulation of uncharged tRNAs after nutrient starvation, while PERK is activated when unfolded proteins accumulate in the endoplasmic reticulum (Wek and Cavener, 2007). Using the Dpt-lacZ enzymatic activity/*Dpt-lacZ* transcript ratio as readout of *P. entomophila* translation inhibition, we tested the implication of these two kinases in *P. entomophila*-mediated blockage of translation. The Dpt-lacZ activity/transcript ratio upon *P. entomophila* infection was similar in guts of flies deficient for *PERK* or the wild-type

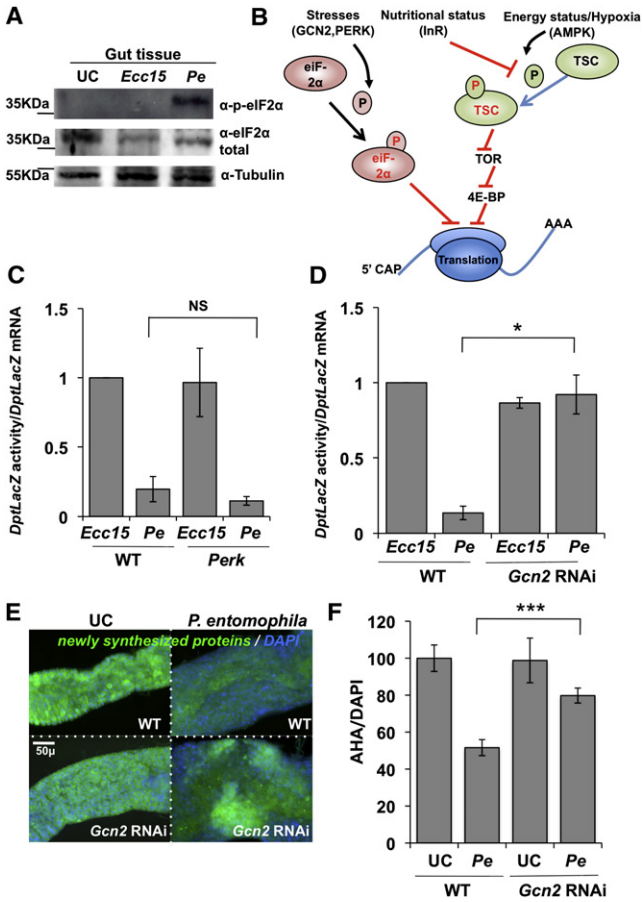

Figure 3. *Gcn2* Regulates the Phosphorylation of eIF2α to Repress Protein Synthesis

(A) Use of specific phospho-eIF2α and eIF2α antibodies revealed that eIF2α is phosphorylated 16 hr following oral infection with *P. entomophila*.

(B) Inhibition of general translation by various stress pathways. Phosphorylation of eIF2α is induced by GCN2, in response to amino acid starvation, and PERK, in response to cell stress during the unfolded protein response. Energy starvation elevates the AMP/ATP ratio activating the AMPK-TSC pathway. This in turn attenuates TOR activity to inhibit phosphorylation of 4E-BP. In addition, insulin production attenuates the TSC repression of TOR activity.

(C and D) Reduction of Dpt-lacZ activity/*Dpt-lacZ* ratio was observed in the *P. entomophila* infected guts of *perk* mutant (*Dpt-lacZ; perk*) and wild-type flies, but not in flies with reduced expression of *Gcn2* (*Gcn2* RNAi: *Dpt-lacZ; Gcn2-IR/Myo1A-Gal4*). Mean values of at least three experiments (n = 10 to 20 guts each) ± SE are shown.

(E) A higher level of newly synthesized protein was observed in the gut of *Gcn2* RNAi flies collected 16 hr after infection with *P. entomophila*, as determined by the incorporation of L-azidohomoalanine AHA. Restoration of translation was observed in patches of cells in *Gcn2* RNAi flies.

(F) Quantification of AHA signal (done as in Figure 2G).

(Figure 3C). In contrast, inactivation of *GCN2* in the gut by RNA interference (RNAi) restored the levels of Dpt-lacZ activity (Figure 3D). Similarly, the level of global translation as measured by AHA incorporation was higher in *GCN2* RNAi guts compared to the wild-type, upon *P. entomophila* infection (Figures 3E and 3F). We conclude that phosphorylation of eIF2α by GCN2 is involved in the bulk arrest of protein synthesis upon *P. entomophila* infection.

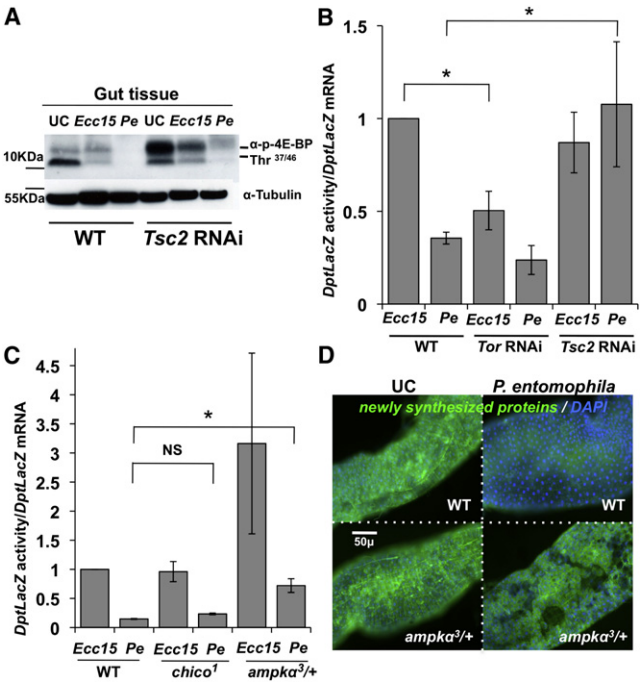

Figure 4. *TOR* Regulates the Phosphorylation of 4E-BP1 to Repress Protein Synthesis

(A) A decrease in the phosphorylated form of 4E-BP1 (detected as two bands) was observed when flies were infected with *P. entomophila* compared to *Ecc15* as revealed by the use of a speciðc phospho-4E-BP1 ($Thr^{37/46}$) antibody. An increased amount of phospho 4E-BP1 was observed in gut extracts from *P. entomophila*-infected *Tsc2-RNAi* flies when compared to the wild-type. As expected, a higher amount of phospho 4E-BP1 was observed in gut extracts from *Tsc2* RNAi compared to wild-type flies in unchallenged condition. Genotypes were as in (B).

(B) Knockdown of *Tsc2* alleviated *P. entomophila* inhibition of translation as revealed by a higher Dpt-lacZ activity/*Dpt-lacZ* mRNA ratio. Knockdown of TOR by RNAi induced inhibition of translation of Dpt-lacZ upon *Ecc15* oral infection. WT: *tub-GAL80^{ts}*; *da-Gal4,Dpt-lacZ*. *Tor*-RNAi: *tub-GAL80^{ts}*; *da-gal4, Dpt-lacZ/UAS-Tor-IR*. *Tsc2*-RNAi: *tub-GAL80^{ts}*; *da-Gal4,Dpt-lacZ/UAS-Tsc2-IR*. Flies were shifted to 29°C 3 days after eclosion and analyzed 1 week later.

(C) Reduction of AMPK but not *chico* activity alleviated *P. entomophila* inhibition of translation. Experiments were performed as in panel B with *ampkα^3*/+ and *chico^1*/*chico^1* flies.

(D) A higher level of newly synthesized proteins was observed in the gut of *ampkα^3*/+ flies after infection with *P. entomophila*, as determined by the incorporation of L-azidohomoalanine AHA. Restoration of translation was observed in patches of cells. See quantification in Figure S3H. Mean values of at least three experiments (n = 10 to 20 guts each) ± SE are shown.

In the all panels, guts were collected 16 hr after oral bacterial infection. See also Figure S3.

The TOR Pathway Mediates *P. entomophila*-Induced Translation Inhibition

To further elucidate the mechanism underlying gut translation inhibition by *P. entomophila*, we examined the ability of the bacteria to modulate the activity of the translational repressor 4E-BP1, another key regulator of translation. 4E-BP1 is a target of the TOR kinase that alleviates its inhibitory activity through its phosphorylation (Hay and Sonenberg, 2004). Under positive-growth conditions, TOR is active and maintains 4E-BP1 in its phosphorylated state, rendering 4E-BP1 incapable of inhibiting translation. However, under nutritional and environmental stress conditions, TOR is inactive, and 4E-BP1 becomes hypophosphorylated and inhibits cap-dependent translation (Figure 3B). At 16 hr postinfection, *P. entomophila* caused a strong reduction in 4E-BP1 phosphorylation, while the total amount of 4E-BP1 remained unaffected (Figure 4A and S4A). This suggested that *P. entomophila* infection also inhibits translation through 4E-BP1. Therefore, we hypothesized that *P. entomophila* infection could inhibit TOR activity and thereby reduce protein synthesis. In animals, the Tuberous sclerosis protein complex (Tsc1/2) is a negative regulator of TOR kinase activity (Hay and Sonenberg, 2004). Interestingly, knockdown by RNAi of the *Tsc2* gene restored the Dpt-lacZ activity in guts infected by *P. entomophila* (Figure 4B). Consistent with this observation, knockdown of TSC by RNAi also increased the amount of phosphorylated 4E-BP1 (Figure 4A). This increase was not only detected in guts infected with *P. entomophila*, but also in both unchallenged and *Ecc15*-infected intestines. Conversely, the knockdown of TOR by RNAi was sufficient to block translation of the *Dpt-lacZ* reporter upon infection with the nonlethal bacterium *Ecc15* (Figure 4B).

The inhibition of the TOR pathway by the TSC complex is determined by several inputs (Figure 3B), the main two being the activation of the AMP kinase (AMPK) that senses low intracellular ATP levels and the decrease of insulin receptor signaling in response to a decrease in systemic growth signals (Mihaylova and Shaw, 2011). This dual regulation ensures an optimal coordination between translation and nutrient/energy availability. We therefore investigated which of the two branches mediates the TSC inhibition of TOR upon *P. entomophila* infection. Figure 4C shows that no inhibition of translation by *Ecc15* was observed in flies deficient for *chico* that encodes an insulin receptor (InR) adaptor protein, or in flies expressing a dominant negative form of InR in the gut (Figure S3A). Additionally, *P. entomophila* was still able to block translation in the gut of flies expressing a constitutively active form of insulin receptor (Figure S3A). Finally, no change in expression of insulin-like peptide genes was detected in flies that ingested *P. entomophila* (Figures S3B–S3G). These experiments indicate that the insulin receptor pathway is not involved in *P. entomophila* repression of host translation. Conversely, inhibition of translation by *P. entomophila* was less marked in flies lacking one copy of *ampkα* (genotype: *ampkα*/+) (Figures 4C, 4D, and S3H for quantification). Silencing of the *ampkα* gene by RNAi in the midgut also partially restored Dpt-lacZ activity in *P. entomophila* infected flies (Figure S3I). Collectively, our results show that at least two mechanisms, eIF2α phosphorylation through GCN2 activation and 4E-BP hypophosphorylation through AMPK-TSC inhibition of TOR activity, repress host translation after *P. entomophila* infection.

Oxidative Stress Is Necessary to Block Translation in the Gut upon Oral Infection

In our microarray analysis, we identified several ROS detoxifying genes (ex. *gstD*) strongly induced after *P. entomophila* infection (Figure 1C) suggesting that gut cells are exposed to an oxidative burst. Consistent with this, ingestion of *P. entomophila* induces a higher level of intestinal ROS compared to *Ecc15* (Figure 5A and 5B). Oxidative stress is often associated with a reduction

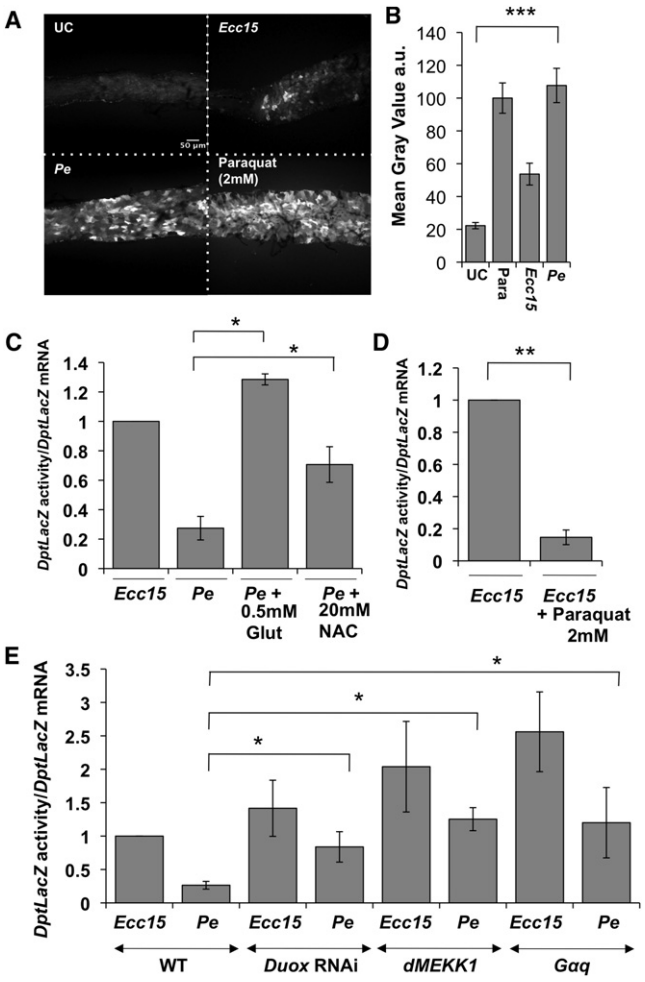

Figure 5. Inhibition of Translation Is a Consequence of Oxidative Burst Associated with *P. entomophila* Oral Infection

(A) Representative image of ROS-induced DCF-DA fluorescence signal in the gut of flies orally infected with *Ecc15*, *P. entomophila* or Paraquat for 30 min at 29°C. The fluorescent signal in the *P. entomophila*-infected gut was significantly higher compared to *Ecc15*.

(B) Quantification of ROS-induced DCF-DA derived from three independent experiments where at least six guts were imaged per condition.

(C) An increased Dpt-LacZ activity/*Dpt-LacZ* mRNA ratio was observed in flies cofed with *P. entomophila* and chemical antioxidants (N-acetyl-Cysteine or glutathione).

(D) Ingestion of paraquat with *Ecc15* is sufficient to provoke a reduction of translation as revealed by a low Dpt-LacZ activity/*Dpt-LacZ* mRNA ratio.

(E) Gut extracts of *P. entomophila* infected flies knocked down for *Duox* (*tub-Gal80^{TS}*; *da-Gal4*, *UAS-Duox-IR*), deficient for *Gαq*, or Mekk1 (*MEKK1^{Ur3}*) display an increased Dpt-LacZ activity/*Dpt-LacZ* mRNA ratio compared to wild-type flies. Mean values of at least three experiments (n = 10 to 20 guts each) ± SE are shown. Guts were collected 16 hr after oral infection.

See also Figure S4.

in global translation (Holcik and Sonenberg, 2005). This led us to hypothesize that protein synthesis arrest could be, in part, a consequence of the *P. entomophila*-induced oxidative burst and the subsequent activation of stress pathways. To this end, flies were fed with *P. entomophila* in combination with the antioxidants N-acetyl cysteine (NAC) or glutathione. Ingestion of both compounds restored a higher Dpt-lacZ activity upon *P. entomophila* infection (Figures 5C and S4B), indicating that reduction of the levels of ROS could alleviate *P. entomophila*-mediated inhibition of translation. In addition, a reduction of Dpt-lacZ activity/*Dpt-lacZ* transcript ratio was observed in flies cofed with *Ecc15* and paraquat, a potent inducer of ROS, compared to flies fed with *Ecc15* alone (Figure 5D). This indicates that increasing the levels of ROS artificially in the midgut of *Ecc15* infected flies is sufficient to block translation of the *Dpt-lacZ* reporter to a similar extent than observed with *P. entomophila*.

In *Drosophila*, *Duox*, a NADPH oxidase, is the main enzyme catalyzing ROS production in the gut upon oral infection (Ha et al., 2005). Silencing of the *Duox* gene by RNAi significantly increased translation of induced genes in the gut of flies upon *P. entomophila* infection as revealed by a higher Dpt-lacZ enzymatic activity/*Dpt-lacZ* transcript ratio (Figure 5E). Similarly, a significant increase of Dpt-lacZ activity was also observed in *P. entomophila*-infected flies carrying either a mutation in *Gαq*, which encodes a heterodimeric G protein responsible for the enzymatic activation of Duox, or mutated in *Mekk1*, that controls *Duox* transcription (Figure 5E). Collectively, these results indicate that the reduction in protein synthesis observed with *P. entomophila* is in part a consequence of the oxidative burst associated with infection.

To clarify the relationship between ROS production by *Duox* and stress pathways mediating translational arrest, we monitored translation inhibition in Duox, GCN2, and AMPK knockdown flies cofed with the ROS-inducing compound paraquat and the nonlethal bacterium *Ecc15*. In this experiment, direct ROS production by paraquat does not require Duox activity, therefore we expected that knockdown of genes involved in the translation blockage, but not ROS production, would alter Dpt-lacZ activity. Consistent with this, silencing of *Duox* by RNAi did not restore Dpt-lacZ activity in response to *Ecc15* infection and paraquat (Figure S4C). Conversely, inhibition of GCN2 or AMPK activity alleviated translation blockage in flies cofed with paraquat and *Ecc15* (Figure S4C). These results suggest that upon infection, GCN2 and TOR pathways regulate translation in response to the production of ROS by Duox.

Translation Blockage Is an Essential Facet of *P. entomophila* Pathogenicity

Our present study raises the possibility that the ability of *P. entomophila* to cause the death of its host is a direct consequence of the translation arrest in the gut that would block not only the immune response, but also tissue repair. Consistent with this notion, neither the JAK-STAT nor the EGFR pathway was fully activated upon infection with *P. entomophila*, despite the observation that ligands activating JAK-STAT and EGFR pathways were strongly induced at the transcriptional level (Figures S1F and 2E).

To test this hypothesis, we monitored epithelium renewal in wild-type or *GCN2* RNAi flies after *P. entomophila* ingestion. Epithelium renewal was monitored in flies both by counting the number of mitotic stem cells along the gut using an anti-phospho-histone H3 (PH3) antibody, and using a lineage tracing system, *esg$^{F/O}$* (Jiang et al., 2009). Epithelium renewal was not detected in wild-type flies after ingestion of *P. entomophila* (Figures 6A and 6B). In contrast, silencing of *GCN2* by RNAi in

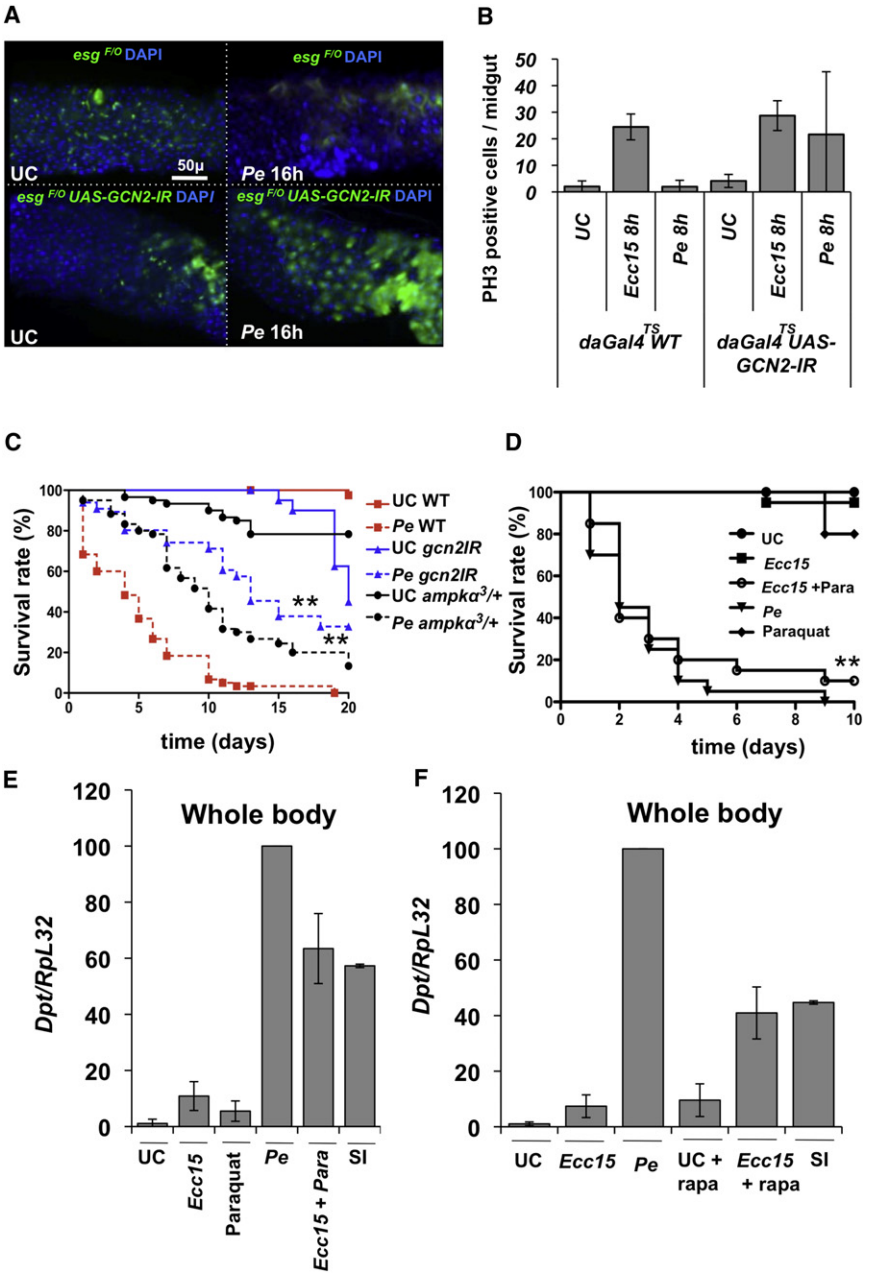

Figure 6. Reducing Stress Signaling Improves Host Survival to *P. entomophila*

(A) The *esg*$^{F/O}$ lineage tracing system was used to monitor epithelium renewal (seen by the expansion of GFP-expressing cells). An increase of epithelium renewal was observed in the gut of *Gcn2* RNAi but not in wild-type flies collected 16 hr after infection.

(B) Quantification of dividing stem cells (pH3-positive) per midgut shows an increase in the number of mitosis in flies with reduced Gcn2 activity upon *P. entomophila* infection compared to wild-type flies.

(C) A survival analysis of wild-type, *Gcn2* RNAi and *ampkα*^{3}/+ flies shows that a reduction in *Gcn2* and *AMPK* signaling increases survivals to oral infection with *P. entomophila*.

(D) Wild-type flies cofed with 2 mM paraquat and *Ecc15* show increased mortality. A Kaplan-Meier log-rank test was used in (C) and (D) to determine statistical significance $^{**}p < 0.001$.

(E) Flies cofed with *Ecc15* and 2 mM paraquat exhibit a strong expression of the *Dpt* gene in whole flies; the levels being similar to that observed in flies collected after septic injury with *Ecc15*. *Dpt* expression was monitored by RT-qPCR in whole flies 16 hr after oral infection with *Ecc15*.

(F) Flies cofed with *Ecc15* and 50 µM rapamycin exhibit a strong expression of the *Dpt* gene. The experiment was done as in (E). Mean values of at least three experiments (n = 10 to 20 flies each) ± SE are shown. SI, septic injury.

See also Figure S4.

P. entomophila-infected flies resulted in an increase in stem cell proliferation and gut repair, as illustrated by the appearance of newly synthesized GFP-positive enterocytes along the gut and a higher PH3 count (Figures 6A and 6B). This suggests that defective gut repair after *P. entomophila* ingestion is caused in part by stress-mediated inhibition of translation. This notion was further supported by the observation that flies with reduced *GCN2* activity or heterozygous for the *ampkα* mutation were more resistant to *P. entomophila* infection (Figure 6C). Interestingly, *GCN2* RNAi flies cofed with compound C, a specific inhibitor of *ampkα* showed a better survival rate than *GCN2* RNAi *or ampkα* /+ flies, suggesting that *GCN2* and AMPK pathways contribute in an additive manner to *P. entomophila*-mediated pathogenesis (Figure S4D). Nevertheless Figure S4E shows that both *Tsc2* RNAi and *GCN2* RNAi flies have a shorter life expectancy in unchallenged condition. Importantly they also show an enhanced susceptibility to *Ecc15* at late time points (10–15 days). Thus, both GCN2 and TOR pathways contribute to host defense in response to nonlethal pathogens. We conclude that stress responsive pathways regulating translation are part of a host response that is beneficial to cope with infection and restore full homeostasis. However, in response to some pathogens such as *P. entomophila*, they can become detrimental and aggravate the pathogenesis by blocking translation of immune and repair effectors.

Inhibition of Translation Results in a Systemic Immune Response

Striking differences distinguish the immune response to lethal bacteria like *P. entomophila* from the non-lethal pathogen *Ecc15*. Notably, oral infection with *P. entomophila* triggers a systemic response (i.e., production of antimicrobial peptides by the fat body) in adult flies, while the response to *Ecc15* remains confined to the gut (Vodovar et al., 2005; Zaidman-Rémy et al., 2006). This prompted us to investigate whether inhibition of translation could explain the specific immune patterns

caused by *P. entomophila*. To test this possibility, we analyzed the immune response to oral infection with *Ecc15* in wild-type flies in which translation was artificially reduced. Flies cofed with *Ecc15* and paraquat exhibited a translation blockage and an increased susceptibility to infection, similar to that observed upon *P. entomophila* infection (Figures 5D and 6D). Of note, neither the dose of paraquat alone nor *Ecc15* alone was sufficient to cause any lethality indicating that both *Ecc15* and translation inhibition synergize to promote pathogenesis. We next monitored *Dpt* expression in whole flies cofed with *Ecc15* and paraquat to measure the systemic expression of *Dpt* by the fat body (the contribution of gut to *Dpt* being negligible in whole flies). Strikingly, flies cofed with *Ecc15* and paraquat (Figure 6E) displayed a strong systemic immune response that was never observed upon feeding of *Ecc15* or paraquat alone. Importantly, translation of immune genes in the fat bodies of flies infected with *P. entomophila* or cofed with *Ecc15* and paraquat was not affected (data not shown). Similar results were obtained when flies were co-fed with *Ecc15* and rapamycin, an inhibitor of TOR (Figures 6F and S4F). Thus, an immune response similar to that observed with *P. entomophila* could be recapitulated upon *Ecc15* infection through the use of chemical compounds that inhibit protein synthesis in the gut. We conclude that inhibition of translation is an important feature shaping the immune response.

DISCUSSION

P. entomophila Disrupts Host Homeostasis by Translational Blockage

P. entomophila is an entomopathogen that kills flies and other insects when fed at high doses (Vodovar et al., 2005). In this paper, we show that *P. entomophila* induces both immune and repair pathways at the transcriptional level, indicating that the bacterium is recognized by its insect host. However, *P. entomophila* infection causes a severe reduction in protein synthesis, thereby inhibiting immune and repair gene programs. We show that this blockage of translation is in large part a consequence of strong ROS activity in the gut. Our data indicate that Duox activation contributes to this oxidative burst, although we cannot exclude other ROS sources. Interestingly, coingestion of *Ecc15* with paraquat (Figure 5D) leads to translation reduction in the midgut, blockage of the immune response, and lethality, thus recapitulating all the traits of *P. entomophila* pathogenesis. Hence, addition of damaging agents to a nonlethal pathogenic bacterium is sufficient to block translation and lead to host mortality. This indicates that inhibition of translation is a consequence of an integrated response to cellular damages. In agreement with this notion, feeding flies with high dose of SDS (without bacteria) is also sufficient to induce a translational arrest (Figure S4G). Moreover, Monalysin, a pore-forming toxin of *P. entomophila*, also contributes to *P. entomophila* translation arrest. We also observed that the arrest in protein synthesis is a consequence of the activation of stress pathways. The observation that a reduction of GCN2, and to a lesser extent AMPK, signaling improved fly survival demonstrates that stress pathways have a detrimental impact on the host defense against *P. entomophila*. Nevertheless, these two stress pathways do contribute to host survival in response to *Ecc15*. A beneficial role of stress pathways in the gut host defense is further supported by recent results showing that the p38 stress pathway also contributes to survival to oral bacterial infection (Chen et al., 2010). Collectively, our data allow us to draw a model of *P. entomophila* pathogenesis in which inhibition of translation by excessive activation of stress pathway plays a central role by paralyzing the global host response to infection. Many diseases are associated with deleterious immune responses (inflammation/autoimmunity). Our studies suggest that overactivation of stress pathways that usually help endure the consequence of an infection could also contribute to pathogenesis.

Multiple Stress Responsive Pathways Mediate *P. entomophila*-Mediated Translation Inhibition

At least two stress responsive pathways, GCN2 and AMPK, are activated in the gut and participate in the translational blockage caused by *P. entomophila*. Since GCN2 is activated upon accumulation of uncharged tRNAs (Hinnebusch, 1994) and AMPK in response to low intracellular ATP (Hay and Sonenberg, 2004), it is possible that epithelial intestinal cells experience a nutrient depletion stress that results in host translation blockage. As observed with other entomopathogens, oral infection with *P. entomophila* results in the cessation of feeding, which could lead to a nutrient stress, resulting in the induction of stress responsive pathways. In opposition to this notion, the level of insulin signaling, a readout of nutrient availability was not affected in *P. entomophila* infected flies. Additionally, protein synthesis arrest occurs in the intestine, but not in other tissues (i.e., fat body). Therefore, the translation inhibition induced by *P. entomophila* is more likely the consequence of a direct stress on the intestinal epithelium than a systemic starvation effect. Our study shows that translation is inhibited in the gut as a consequence of strong ROS production by the host. Interestingly, both GCN2 and AMPK are considered as redox regulators and are activated in response to oxidative stress (Chaveroux et al., 2011; Shin et al., 2011). Our microarray analysis indicates that *P. entomophila* also stimulates genes that are signatures of hypoxia, heat shock, and DNA damage responses (Figure 1C). All these stresses have been associated to AMPK activation and the resulting decrease in translation. An important question is to further differentiate whether ROS induce stress responsive pathways directly or indirectly through the cell damages they inflict. Analyzing the crosstalk and hierarchal position between these pathways and how they synergize to block translation requires further investigation.

Inhibition of Translation in Host-Pathogen Interactions

In the field of host-pathogen interactions, translation blockage has historically been associated with viral infection (Mohr, 2006). Reduction of translation is used as a mechanism to limit virus production and propagation within its host, as viruses rely solely on host protein synthesis for the translation of their mRNAs. In vitro studies also indicated that translational arrest can be induced by bacterial toxins (Passador and Iglewski, 1994). More recently, several reports have highlighted a contribution of inhibition of translation in the pathogenesis of other microbes. The intracellular protozoan parasite *Leishmania major* blocks the translational machinery of macrophages, a step essential for parasite survival and dispersion (Jaramillo et al.,

2011). Protein arrest was induced through the cleavage of mTOR by the *Leishmania* protease GP63 that leads to de-phosphorylation of the translational inhibitor 4E-BP1. Fontana et al. have shown that virulent strains of *Legionella pneumophila* secrete five effectors causing a global decrease of host translation in macrophages (Fontana et al., 2011). Arrest in protein synthesis was also observed when cultivated cells were subjected to a sublethal dose of pore-forming toxins such as Aerolysin and Listeriolysin (Gonzalez et al., 2011). This effect is mediated through a pronounced but transient phosphorylation of eIF2α. Other factors, such as low ATP levels, are also suspected to play an important role in reducing protein synthesis. Gonzalez et al. have proposed that the protein synthesis arrest that occurs in response to pore-forming toxins is part of a cell repair program in which cells enter a quiescent, low energy-consumption state to ensure that plasma membrane integrity and ionic balance are restored (Gonzalez et al., 2011; Kloft et al., 2010). This is reminiscent of the situation we observed in the gut of *Drosophila* in response to *P. entomophila* where the action of a pore-forming toxin is also required. Therefore, we hypothesize that the levels of cellular damage inflicted by *P. entomophila* infection, due to the combined action of ROS and pore-forming toxin, reach a threshold that leads cells to reduce translation and enter a quiescent stage to favor repair. As illustrated by our in vivo study, this adaptive response can be detrimental to the host by inhibiting both tissue repair and the immune response. Thus, one of the differences between nonlethal and lethal infections could be due to the severity of cellular damages determining different ranges of host response. In agreement, we observe that the rate of translation is also slightly lowered by infection with the nonlethal pathogen *Ecc15* compared to unchallenged flies, or flies fed with the completely avirulent *P. entomophila gacA* (Figure S5A, S5B, and S2). This suggests that inhibition of translation is a quantitative readout of the global level of pathogenesis upon infection. It is well established that *Bacillus thuringiensis* damages epithelial cells by the action of the pore-forming toxin Cry contained in its crystals (Soberón et al., 2010). Therefore, we hypothesize that translation arrest could also be involved in other insect-entomopathogenic bacterium interactions.

Inhibition of Translation and the Immune Response to Pathogens

The innate immune response to pathogens differs from that induced by benign microbes. It has recently been suggested that the innate immune recognition of pathogen-encoded activities, which has been termed "patterns of pathogenesis" in metazoans, could act in concert with direct bacterial recognition by pattern recognition receptors (PRRs) to distinguish pathogens from nonpathogens. This multilayered recognition can lead to qualitatively distinct innate immune responses that are commensurate with the potential threat (Vance et al., 2009). From this perspective, the immune response to lethal *P. entomophila* differs from that to nonlethal *Ecc15*. Notably, oral infection with *P. entomophila* triggers both local and systemic responses while the response to *Ecc15* mostly remains confined to the gut of adults. Three mechanisms have been proposed to explain how a gut infection results in a systemic immune response in the fat body: (1) the release of nitric oxide (NO) that acts as a signaling molecule in larvae (Foley and O'Farrell, 2003), (2) the translocation of peptidoglycan from lumen to hemolymph (Gendrin et al., 2009; Zaidman-Rémy et al., 2006) and (3) a rupture in gut integrity. The first hypothesis is unlikely, as the use of a viable null mutation in the *NO synthase* gene did not have any effect on the systemic activation of the immune response by gut pathogens (Figures S6A–S6C). In this study, we observed that flies in which translation levels in the gut has been artificially lowered, mount a systemic immune response to *Ecc15*. Thus, inhibition of translation affects the immune response and might explain the specific immune patterns caused by *P. entomophila*. It is possible that the translation inhibition allows peptidoglycan to diffuse from the gut to the hemolymph as a consequence of epithelial leakiness. Alternatively, inhibition of translation could block the synthesis of negative regulators of the Imd pathway (*PGRP-LB*, *pirk*). Indeed, we found that translation of genes encoding short-lived proteins (Hsp) or requiring de novo synthesis upon infection (*Dpt, Upd3*) was highly affected by the arrest of protein synthesis. Thus, inhibition of translation could phenocopy a *PGRP-LB* deficiency resulting in a systemic immune response to oral infection (Zaidman-Rémy et al., 2006). This hypothesis is in line with a recent study revealing that inhibition of host translation by *L. pneumophila* effectors results in a more potent host immune response from the host (Fontana et al., 2011). It was suggested that *L. pneumophila* virulence effectors cause a global decrease in host translation, thereby preventing synthesis of IκB, an inhibitor of the NF-κB inflammatory response. Thus, the rate of protein synthesis could act as a direct sensor of pathogen-encoded activities, modulating the immune response through short-lived, negative regulators. Such a sensor would reflect the global level of cellular stress and therefore act in response to any type of infectious damage, rather than recognizing a specific pathogen effector.

Concluding Remarks

Our results, together with other recent studies (Dunbar et al., 2012; Fontana et al., 2011; Jaramillo et al., 2011; McEwan et al., 2012; Tattoli et al., 2012), indicate that inhibition of protein synthesis could play a central role in host-pathogen interactions, contributing both to the mechanisms of pathogenesis and shaping the immune response. The effect of translation inhibition could have been overlooked in many host-pathogen interactions for which transcriptional readouts are more often used. Remarkably, the few studies that have integrated global translation in the process of host/pathogen interactions reveal the importance of this mechanism on both pathogenesis and immune response. In conclusion, we propose that inhibition of translation can act as an interaction node between stress and immune pathways shaping host defense.

EXPERIMENTAL PROCEDURES

Fly Stocks and Infection

For description of the fly lines used in this study, see the Supplemental Experimental Procedures. *Drosophila* stocks were maintained at 23°C with standard fly medium. For oral infection, 3- to 5-day-old adult female flies were incubated 2 hr at 29°C in an empty vial before being transferred to a fly vial with infection solution and maintained at 29°C. The infection solution was obtained by mixing an equal volume of 100× concentrated pellet from an overnight culture of

Ecc15 or *P. entomophila* (OD_{600} = 200) with a solution of 5% sucrose (1:1) and deposited on a filter disk that completely covered the surface of standard fly medium. Flies were incubated for one day at 29°C on the contaminated filter, after which they were transferred to fresh vials without living yeast.

Analysis of Whole-Genome mRNA Expression by Affymetrix Droso2.0 Chips

RNA was collected from 60 guts of 5-day-old females. RNA was isolated, purified with RNA clean up purification kits (Macherey Nagel), and DNase treated. RNA quantities were determined with NanoDrop ND-1000 spectrophotometer, and then quality was verified on Agilent 2100 bioanalyzer chips. For each sample, 1 μg total RNA was amplified and labeled with the GeneChip IVT labeling kit according to manufacturer's protocol. Affymetrix *Drosophila* Genome 2.0 arrays were hybridized with 30 μg labeled complementary RNA, washed, stained, and scanned according to the protocol described in Affymetrix GeneChip Expression Analysis Manual (Fluidics protocol EukGeWS2v5_450). Statistical analyses were performed with the R and Bioconductor statistical packages. All the genes integrated in the analysis shown in Figure 1B were differentially expressed by at least 2-fold with a p value < 0.05. Raw data and processed files of the microarray analysis can be found at http://lemaitrelab.epfl.ch/page26728-en.html (Resources).

Monitoring the Level of Translation

Two methods were used to quantify the levels of protein translation in the gut. First, we monitored in gut extracts of *Dpt-lacZ* flies the ratio between Dpt-lacZ (β-galactosidase) activity, normalized on the amount of protein, and *Dpt-lacZ* transcript level, normalized on the amount of RpL32. The ratio obtained with guts collected 16 hr post *Ecc15* infection was set to a value of 1. This ratio monitors the level of translation of neosynthesized transcripts such as *Dpt*, which are induced upon infection. Reduction of this ratio indicates a translation decrease. Second, the Click-iT AHA for Nascent Protein Synthesis kit (Invitrogen) was used to monitor the global level of translation of all transcripts. Flies were infected as described above, except that the infection solution was obtained by mixing an equal volume of 100× concentrated pellet from an overnight culture of *P. entomophila* (OD_{600} = 200) with a solution of 5% sucrose (1:1) and AHA (50 μM final). Control flies were fed a solution of 2.5% sucrose and 50 μM AHA. Guts were dissected in 1X phosphate buffered saline (PBS), fixed for 20 min in PBS, and 4% paraformaldehyde; the reaction was completed according to manufacturer's instructions. Images of the anterior midgut were taken for comparison.

Imaging and Immunohistochemistry

For live imaging, guts were usually collected 16 hr after infection and dissected at room temperature in 1× PBS and immediately mounted in the anti-fading agent AF1 (Citifluor). Samples were observed for fluorescence with an Axioplot imager Z1 and Axiocam mRM camera (Zeiss). For immunofluorescence, guts were dissected in 1× PBS, fixed for 20 min in PBS and 0.1% Tween 20 (PBT), and 4% paraformaldehyde; then stained with primary antibody (1/500 anti-pH3 (Upstate/Millipore) in PBT + BSA). Secondary staining was performed with Alexa594 anti-rabbit antibodies (Invitrogen). DNA was stained with DAPI (Sigma). For immunohistochemistry, guts were dissected in 1× PBS, fixed for 10 min in PBS and 0.5% glutaraldehyde; washed in PBS, then incubated in staining solution (0.3% X-Gal, 2 mM potassium ferricyanide, 2 mM potassium ferrocyanide, 50 mM sodium phosphate [pH 8.0], 25% Ficoll-400) at 37°C. Guts were mounted in 70% Glycerol and anterior midguts were imaged with Axioplot imager Z1 and Axiocam mRM camera (Zeiss). Values of signal intensity are the average of green signal measured on representative fields of ten guts and quantified with Fiji.

ROS Measurement

ROS level in the adult gut was monitored by the addition of 100 μM DCF-DA fluorescent dye (Invitrogen, C400) to freshly dissected gut tissue. The dissections were done in the presence of 20 mM NEM (N-ethyl maleimide, Sigma) and the tissue was preserved in NEM until addition of DCF-DA dye. The tissue was incubated in the dye for 30 min and then mounted in 70% Glycerol. Sections of anterior midguts were imaged immediately after. The DCF-DA fluorescent signal was analyzed with excitation at 488 nm, emission at 529 nm. Images were acquired with a Zeiss LSM700 upright confocal microscope and a 20×/0.8 NA objective. Values of signal intensity are the average signal measured on representative fields of at least six guts and quantified with Fiji.

Statistics

Each experiment was repeated independently a minimum of three times (unless otherwise indicated), error bars represent the standard error of the mean of replicate experiments (unless otherwise indicated). Statistical significance was calculated with a Student's t test or log-rank test, and p values of < 0.05 = *, < 0.01 = **, and < 0.001 = *** were considered significant.

ACCESSION NUMBERS

The ArrayExpress accession number for the microarray data reported in this paper is E-MTAB-1200.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.chom.2012.06.001.

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