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RESEARCH ARTICLE

Gut-derived peptidoglycan remotely inhibits bacteria dependent activation of SREBP by Drosophila adipocytes

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Abstract

Bacteria that colonize eukaryotic gut have profound influences on the physiology of their host. In Drosophila, many of these effects are mediated by adipocytes that combine immune and metabolic functions. We show here that enteric infection with some bacteria species triggers the activation of the SREBP lipogenic protein in surrounding enterocytes but also in remote fat body cells and in ovaries, an effect that requires insulin signaling. We demonstrate that by activating the NF-κB pathway, the cell wall peptidoglycan produced by the same gut bacteria remotely, and cell-autonomously, represses SREBP activation in adipocytes. We finally show that by reducing the level of peptidoglycan, the gut born PGRP-LB amidase balances host immune and metabolic responses of the fat body to gut-associated bacteria. In the absence of such modulation, uncontrolled immune pathway activation prevents SREBP activation and lipid production by the fat body.

Author summary

An increasing body of evidence indicates that microbes, which live closely associated with animals, significantly influence their development, physiology and even their behavior. The mechanisms that underly these mutual interactions are not yet completely understood. Using Drosophila as a model system, we study the impact of gut bacteria on the host physiology. We present here data showing that some bacteria present in the fly gut can stimulate the production of lipids in the remote fat body tissue via gut autophagy and insulin signaling. However, these bacteria produce many compounds and metabolites such as the cell wall peptidoglycan. Our data show that by cell-autonomously activating the NF-κB signaling pathway in the remote fat body, cell wall peptidoglycan antagonizes bacteria-triggered lipogenesis. We finally show that to prevent this antagonistic effect, flies produce an enzyme, called PGRP-LB, that cleaves the peptidoglycan into its inactive form. Our data highlight the multiple layers of interactions that take place between gut-associated bacteria and a eukaryotic host.
Introduction

In order to develop to adulthood and to later survive in their environment, multi-cellular organisms constantly adapt their metabolism needs to the nutrient availability. These nutrients come from food sources that are unavoidably contaminated by microbes on which they proliferate. Some of the microorganisms ingested with food, or those already associated with the digestive tract, directly participate to the host nutrition either by serving as food themselves or by metabolizing ingested aliments. These transient or permanent gut-associated microbes need to be either tolerated by the host if beneficial, or eliminated if detrimental, a function dedicated to the immune system. Hence, metabolism and immunity, that regulate the host’s responses to these environmental inputs, nutrients and microbes, have co-evolved to provide a coordinated output at the organismal level. In mammals, this optimized response benefits from the fact that some immune cells are embedded into the adipose tissue [1–3]. Immune cells act as direct regulators of fat metabolism and innate immune signaling can impact metabolic responses cell-autonomously or via systemic inflammation [4–10]. Beside its role in lipid storage and energy expenditure, the adipose tissue is thus considered as an immune organ able to simultaneously sense nutrient and detect microorganism-derived compounds. Communication between the immune cells and adipocytes is essential to coordinate an ad hoc host metabolic response in physiological conditions and in response to microbial challenges [2].

In Drosophila, the fat body is the major site for lipid depository and combines energy storage, de novo synthesis, and breakdown functions that, in vertebrates, are dedicated to adipose and hepatic tissues [11,12]. In addition, via the production of many immune effectors including antimicrobial peptides, it plays a key role in orchestrating the innate immune responses to microbial infection [13–15]. Hence, Drosophila provides unique advantages to unravel the complex integration and regulation of these two essential physiological systems, before they evolved into more complex organs in vertebrates. Previous work has shown that Drosophila infection with bacteria or with the intracellular parasite Tubulinoessa ratisbonensis leads to a depletion of fat body lipid stores [16]. Other studies, based on gain-of-function approaches, revealed that ectopic activation of the NF-κB pathways either Toll or IMD can result in lipid storage reduction. More precisely, immune signaling activation shifts anabolic lipid metabolism from triglyceride storage to phospholipid synthesis to support immune function [17].

Former results have shown that immune activation in the fat body cells can be triggered by bacteria present in the digestive tract. For that, the bacterial cell wall component peptidoglycan produced by gut-associated bacteria must cross the gut epithelium and reach the circulating hemolymph where it gets in contact with remote tissues. By activating receptors of the PGRP family expressed in adipocytes this gut-born bacterial ligand activates an NF-κB dependent AMP production [18–20]. This effect is buffered by the PGRP-LB amidase that, by cleaving the PGN into non-immunogenic fragments, prevents a diffusion of PGN to the hemolymph and hence a constant deleterious NF-κB activation in fat body cells of orally infected flies [20,21].

In the present study, we analyze the coordinate metabolic and immune responses of Drosophila to the presence of bacteria in the intestine. We show that flies orally fed with some bacteria species including Escherichia coli (E. coli) and Erwinia carotovora carotovora (E.cc) activate SREBP locally in enterocytes and remotely in adipocytes, in an insulin signaling-dependent manner. We also show that by activating the NF-κB/IMD pathway in adipocytes, PGN released by the same bacteria, cell-autonomously antagonizes SREBP-activation in adipocytes. Finally, we demonstrate that by regulating the levels of circulating PGN via the PGRP-LB amidase, flies can adjust their metabolic and immune responses towards gut bacteria.
Results

Specific gut bacteria species activate an SREBP-dependent lipogenesis in adult adipocytes

Our previous data showed that *Drosophila* enteric infection by the phytopathogen *E. cc* affects lipogenesis in adult adipocytes [20]. To further assess the effects of these bacteria of adult lipogenesis we monitored SREBP (Sterol Regulatory Element Binding Protein) activation in gnotobiotic flies orally infected with specific bacterial species (see Material and methods). SREBP is a conserved transcription factor that control lipid synthesis. Produced as a pro-peptide inserted into the endoplasmic reticulum (ER) membrane, it is post-translationally matured upon physiological modifications. In response to cellular lipids needs, SREBP exits the ER and travels to the Golgi apparatus where its active domain is freed by two successive proteolytic cleavages [22]. Upon nuclear translocation, mature SREBP activates the transcription of target genes controlling lipid synthesis [23]. We took advantages of the *Gal4::SREBP* reporter whose transcription relies on the native SREBP promoter and in which the resulting chimeric protein is processed like the endogenous SREBP [24]. We generated a novel *LexA::SREBP* reporter which mimics *Gal4::SREBP* activation (S1 and S2 Figs). Both chimeric fusion proteins are proteolytic cleaved and respectively activate UAS and LexAop fluorescent reporters, in cells wherein SREBP ensures *de novo* lipid synthesis [24–27] (Fig 1A and 1B, S1 and S2 Figs).

In addition to its constitutive activation in oenocytes already reported [24], *Gal4::SREBP* expression was unexpectedly detected in fat bodies of flies orally fed with a mixture of *E.cc* + sucrose compared to flies fed on sucrose only (Fig 1A and 1B). When *E. coli* was used to orally infect flies, an even stronger fat body SREBP activation was observed (Fig 1A and 1B). Other bacteria species such *Lactobacillus plantarum* (L. plantarum), *Acetobacter pomorum* (A. pomorum) or *Enterococcus faecalis* (E. Faecalis) failed to trigger activation of this lipogenic regulator (Fig 1A). These results were confirmed by immuno-histochemistry with an anti-SREBP antibody specific to the transcriptionally active N-terminal domain. While *E. cc* feeding induces a weak SREBP nuclear translocation in adipocyte, a much stronger response was observed in *E. coli* fed adults fat bodies (Fig 1C). These results were further corroborated using the transcription of the SREBP target gene, Acetyl-CoA synthase (ACS) as a readout [24,28]. *ACS* mRNA levels were increased in *E.cc*-fed flies compared to sucrose-fed flies and this increase was even stronger with *E. coli* (Fig 1E).

*E. coli* triggers SREBP activation in enterocytes and ovaries

We then asked whether *E.cc* and *E. coli* would activate lipogenesis in other tissues and organs known to be lipogenic. We first monitored the enterocytes which are in close proximity to the gut bacteria and represent another major source of lipids for the organism. Both *E. coli* and *E. cc*-triggered *Gal4::SREBP* activation in midgut enterocytes (Fig 1D). Gut bacteria also had some influences on female gonads. While ovaries of sucrose-fed females were atrophic, ovaries of females raised on an *E. coli*-contaminated solution resemble those of females fed on regular food, suggesting that *E. coli* represent a source of nutriment (Figs 2A and S3). Ovaries from *E.cc* females show an intermediate phenotype. In addition, SREBP activation was observed in mid-stage follicle of *E. coli* fed females, but not in ovaries from flies fed on sucrose only (Fig 2A and 2B). However, both gut and fat body of *E. coli* fed virgin females displayed a much weaker SREBP activation than mated ones (Fig 2C and 2D). This agrees with previous report showing that SREBP activation in enterocytes and in ovaries support oocyte production in mated females [29,30]. Consistently, males fed with *E. coli* displayed no sign of SREBP activation in enterocyte and only a constitutive no-bacteria dependent activation of SREBP in adipocytes (Fig 2C and 2D). All together our data show that gut-associated *E. coli* and *E.cc* activates SREBP
Fig 1. Ingestion of E. coli or E. cc bacteria activates SREBP both in adipocytes and enterocytes. (A) Pictures of adult flies fed 8 days with either sucrose or on a mixture of sucrose + bacteria such as E. cc or E. coli or A. pomorum or E. faecalis or L. plantarum\textsuperscript{w1118}, and showing Gal4::SREBP activation (green). Flies fed on sucrose show activation of Gal4::SREBP in oenocytes and in flight muscle + oenocytes, respectively. E. coli feeding, and to a less extend E. cc ingestion, promote activation of Gal4::SREBP in fat bodies while ingestion of either A. pomorum or E. faecalis or L. plantarum\textsuperscript{w1118} do not. Flies of the following genotypes were used: w\textsuperscript{1118}/w\textsuperscript{1118}, Gal4::SREBP, UAS-2XEGFP\textsuperscript{+/+}. oe: oenocytes, fm: flight muscles. (B) Confocal images of the dorsal part of adult abdominal carcasses viewed from inside, showing Dipt-Cherry
expression (red) and Gal4::SREBP activation (green) from flies fed on sucrose or on a mixture of sucrose + bacteria (E. cc or E. coli). Both E. coli and E. cc feeding activate Gal4::SREBP in fat body cells, while no expression of Dipt-Cherry is visible, as expected. Constitutive expression of Dipt-Cherry in pericardial cells and activation of Gal4::SREBP in oocytes are indicated. Flies of the following genotypes were used: w^{118}/w^{118}; Gal4::SREBP, UAS-2XEGFP+/+; Dipt-Cherry^{C1}/Dipt-Cherry^{C2}, pc: pericardial cells, oe: oocytes. (C) Confocal images of fat body from adult flies fed 48h on sucrose, or on a mixture of sucrose + bacteria (E. cc or E. coli) showing immunofluorescence of dSREBP (green) and DAPI staining (blue). White arrows are showing nuclear translocation of dSREBP induced by E. cc feeding. Flies of the following genotypes were used: w^{118}/w^{118}; (D) Confocal images of the R4 domain of adult midguts from flies fed with either sucrose or with a mixture of sucrose + bacteria (E. cc or E. coli), showing Dipt-Cherry expression (red) and Gal4::SREBP activation (green). Both E. coli and E. cc feeding activates Gal4::SREBP in enterocytes, while a faint expression of Dipt-Cherry is induced. Flies of the following genotypes were used: w^{118}/w^{118}; Gal4::SREBP, UAS-2XEGFP+/+; Dipt-Cherry^{C1}/Dipt-Cherry^{C2}. (E) Histograms showing the expression of ACS measured by q-RT-PCR and performed with mRNA extracted from adult abdominal carcasses of control or PGRP-LBΔ adults fed 4 days with either sucrose, or with a mixture of sucrose + bacteria (E. coli or E. cc). The mRNA level in non-infected control flies was set to 1 and values obtained with indicated genotypes were expressed as a fold of this value. Histograms correspond to the mean ± SD of 6 experiments (n = 6). *p<0.05, **p<0.001, ***p<0.0001; Kruskal-Wallis test. Flies of the following genotypes were used: w^{118}/w^{118}; Dipt-Cherry^{C1}/Dipt-Cherry^{C2} (Control) and w^{118}/w^{118}; PGRP-LBΔ, Dipt-Cherry^{C1}/
PGRP-LBΔ, Dipt-Cherry^{C1} (PGRP-LBΔ). Scale bar is 0.25 mm (A), 100 μm (B), 5 μm (C) and 50 μm (D).

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locally in enterocytes and remotely in fat body and ovaries suggesting that these bacteria represent a source of food for the flies.

**Bacterial ingestion promotes insulin signaling pathway**

To further demonstrate that bacteria-mediated SREBP activation corresponds to a modification of the nutritional status the fly, we monitored insulin signaling in fat body cells using tGPH as cellular indicator of PI3K activity. Indeed, tGPH is recruited to plasma membranes by the second messenger product of PI3K, PI3P [31]. Flies fed with E.cc or E. coli, or raised on yeast extract as a medium containing AA source, showed tGPH membrane recruitment in adipocytes (Fig 3A). Such an effect was not observed when flies where fed on sucrose only (Fig 3A). These results, suggesting that gut E. coli and E. cc activate insulin signaling in adipocytes, were confirmed using q-RT-PCR on adult’s abdominal carcasses. When compared to sucrose diet, mRNA levels of the negatively regulated insulin pathway target gene 4EBP/Thor were decreased following E. coli and E. cc feeding, in a similar way as flies fed on regular food (Fig 3B). We then wondered whether insulin signaling was required for SREBP activation by gut bacteria using chicoΔ, a loss-of-function allele of the Insulin Receptor Substrate Chico/IRS. We found that, in addition to their expected small size, chicoΔ mutant females did not show any sign of LexA::SREBP activation when fed with E. coli (Fig 3C). The typical signal of LexA::SREBP activation in enterocytes and in adipocytes of E. coli fed flies was absent in chicoΔ mutants (Fig 3D and 3E). Only a weak, bacteria-independent, SREBP activation was observed in fat bodies and midguts from chicoΔ animals (Fig 3D and 3E). Consistently, we found that mutants for Foxo, a catabolic transcription factor negatively regulated by the insulin pathway, displayed a consistent activation of SREBP in adipocytes when using a dose of E. coli (10 times less concentrated), that is normally not sufficient to activate Gal4::SREBP in wild-type flies (Fig 3F). Our results indicate that enteric infection by E. coli or E. cc activates insulin signaling, a prerequisite for SREBP activation in enterocytes and adipocytes. Consistently, overexpression of a dominant negative form of the insulin receptor specifically in the fat body was sufficient to completely block E. coli induced SREBP activation in adipocytes (Fig 3G).

**E. coli-dependent fat body SREBP activation depends on gut autophagy**

Since gut bacteria-derived PGN can activate NF-kB/Relish in fat body cells and that Relish has been shown to restrain the transcription of the ATGL/Brummer lipase in the same cells [10],
Fig 2. *E. coli* ingestion triggers SREBP activation in ovaries and in fat body of mated females. (A) Pictures of adult ovaries from virgin or mated females fed 2 days with sucrose or with a mixture of sucrose + bacteria (*E. cc* or *E. coli*), showing *LexA::SREBP* activation (green). Atrophy of ovaries is obvious in mated female fed with sucrose, not with *E. coli*, and to a less extend with *E. cc*. Stage 10 follicles display activation of *LexA::SREBP* (arrows in A), in females fed with *E.cc* or *E. coli*. (B) Confocal images of ovarioles dissected from females fed 2 days with sucrose or with *E. coli,*
showing LexA::SREBP activation (green). Females fed with *E. coli* display activation of LexA::SREBP both in nurse cells and in follicle cells of stage 10 egg chambers. nc: nurse cells and fc: follicle cells. (C) Confocal images of fat body from virgin or mated females (or males) fed 2 days on sucrose or on a mixture of sucrose + *E. coli* showing Gal4::SREBP activation (green). Activation of Gal4::SREBP in adipocytes is strong in mated female and faint in virgin females. Male fat bodies however show no induction but constitutive activation of Gal4::SREBP in a "salt and pepper" pattern. (D) Confocal images of the R4 domain of adult midguts from virgin or mated females (or males) fed 2 days with a mixture of sucrose + *E. coli*, showing Gal4::SREBP activation (green). *E. coli* feeding does not promote activation of Gal4::SREBP in enterocytes from virgin females or males (virgin and mated). Flies of the following genotypes were used: w^{118f}/w^{118f}, LexA::SREBP, 13XLexAop2-6XGFP/+ (A-B), w^{118f}/w^{118f}, Gal4::SREBP, UAS-2XEGFP/+ (females in C-D), w^{118f}/Y; Gal4::SREBP, UAS-2XEGFP/+ (males in C-D). pc: pericardial cells, oe: oocytes. Scale bar is 200 μm (A), 50 μm (B and D) and 20 μm (C and E).

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we asked whether the bacteria-dependent SREBP activation could be consecutive to Brummer repression. In contrast to what was expected, Brummer transcript levels were increased in the presence of bacteria and followed a regulation that resembles that of ACS (Figs 3H and 1E). This suggests that SREBP activation in fat body of bacteria fed flies is not secondary to a reduced rate of lipid degradation by lipase. Another possibility is that SREBP activation is a direct consequence of an increase of the AA pool generated by bacteria-dependent gut protein catabolism. To test this hypothesis, we analyzed the consequences of blocking autophagy, known to participates to protein degradation, specifically in enterocytes on SREBP activation in adipocytes [32]. In contrast to what was observed in control flies, fat body from adults in which the autophagy effector protein ATG1 was down regulated via RNA interference in enterocytes, displayed a faint activation of SREBP upon *E. coli* feeding (Fig 3I).

**IMD signaling inhibits SREBP cell-autonomously in adipocytes**

Although both *E. coli* and *E. cc* are able to activate fat body lipogenesis, we noticed that the effects were stronger with *E. coli* than with *E. cc*. Interestingly, previous works has shown that gut *E. cc* is a stronger inducer of fat body NF-κB signaling than *E. coli*, a difference attributed to the ability of *E. cc* to release PGN in larger amounts than *E. coli*. We hence hypothesized that bacteria-dependent gut-born PGN could buffer SREBP activation in fat body cells. To test this hypothesis, we analyzed the effects of enteric infection with bacteria in a mutant for PGRP-LB, an enzyme that cleaves PGN into non-immunogenic muropeptides. In such mutants, an excess of gut-born PGN reaches the different immune competent tissues leading to a higher NF-κB pathway activation. Fat body SREBP activation was weaker in *PGRP-LB* mutants than in wild type controls infected by *E. coli* (Figs 4A and 1B). This weaker SREBP activation was paralleled by a stronger NF-κB activation monitored with the *Dipt-mCherry* transgene (Fig 4A and 4C) or by q-RT-PCR (Fig 4B). This was, however, not the case in enterocytes (Fig 4D). These results were confirmed using isoform specific alleles of the PGN cleaving enzyme PGRP-LB. Inactivation of the extracellular isoform (PGRP-LB<sub>PC</sub>, named here PGRP-LB<sup>PC</sup>), which is expected to trigger an increase of circulating PGN levels, lead to an NF-κB signaling upregulation and a reduction of SREBP activation in fat body (Fig 4E). Such effects were not observed in flies carrying mutations in the cytosolic isoform (PGRP-LB<sup>PD</sup> named here PGRP-LB<sup>PD</sup>) which do not affect the levels of circulating PGN. Moreover, the lack of Gal4::SREBP activation observed in *E. cc*-fed PGRP-LB<sup>PD</sup> mutant flies, was reverted by the simultaneous inactivation of the IMD pathway core component *Dredd<sup>F64</sup>* (Fig 5A and 5B) demonstrating that an excessive IMD signaling can antagonize bacteria-dependent SREBP activation. The fact that similar results were obtained following PGRP-LC or Relish inactivation demonstrate that the steps implicating PGN sensing at the membrane and Relish-dependent transcription are involved in the process (S4 Fig).
Fig 3. *E. coli* and *E. cc* ingestion promotes systemic insulin signaling pathway and insulin signaling is required for SREBP activation by *E. coli*. (A) Confocal images of fat body from flies fed 4 days with either sucrose, regular food or a mixture of sucrose + *E. coli* or *E. cc*. The expression of the tGPH marker is shown in green. An intense recruitment of tGPH at the cell surface of adipocytes is observed in flies fed on regular food or orally infected with either *E. coli* or *E. cc*, when compared to a sucrose diet. Flies of the following genotypes were used: *w^{1118} w^{1118} /w^{1118}*, tGPH/tGPH, pc:
To identify the tissue(s) in which NF-κB activation is required for SREBP regulation, we monitored the effects of IMD pathway components over expression, which is sufficient to activate downstream signaling in the absence of bacteria. PGRP-LCa or IMD overexpression, either ubiquitously (daGal4) or specifically in the fat body (r4Gal4), prevented the activation of LexA::SREBP in E. coli fed flies (Fig 5C). Moreover, clonal over expression of IMD or PGRP-LCa cell-autonomously prevented LexA::SREBP activation in fat body of females fed with E. coli (Fig 5D). These data demonstrate that the gut bacteria-dependent SREBP activation in fat body cells is cell-autonomously repressed by a PGN-dependent IMD/NF-κB pathway activation.

**Inhibiting IMD signaling in adipocytes improves survival of E. cc infected PGRP-LB<sup>−</sup> mutant flies**

To test the physiological relevance of this antagonism, we monitored the survival curves and lipid droplet accumulation in various genetic combinations chronically infected with E. cc. As expected, whereas wild-type flies fed with E. cc showed lipid droplet accumulation, this was not the case for PGRP-LB mutants (Fig 6A). In addition, PGRP-LB mutants died much earlier than their wild-type siblings upon E.cc chronic infection (Figs 6B and S6). As shown for SREBP activation (Fig 5A), the reduced lifespan and lipid droplet non-accumulation in adipocytes observed in E.cc-infected PGRP-LB<sup>−</sup> mutants were restored by the simultaneous inactivation of IMD pathway component Dredd (Fig 6A and 6B). Since IMD/NF-κB signaling specifically inhibits SREBP activation and lipogenesis in adipocytes, we tested whether IMD signaling buffering in adipocytes could ameliorate the survival of PGRP-LB<sup>−</sup> mutant flies chronically infected with E.cc. To do so, we took advantage of a UAS-dFadd<sup>D</sup> transgene whose
Fig 4. Bacteria-dependent gut-born PGN antagonizes SREBP activation in adipocytes. (A) Pictures of adult flies, control or PGRP-LBΔ mutants, fed 8 days with a mixture of sucrose + E. coli or E. cc, and showing Dipt-Cherry expression (red) and Gal4::SREBP activation (green). E. coli feeding promotes activation of Gal4::SREBP in fat bodies from control and PGRP-LBΔ mutant flies. Ingestion of E. cc, however, triggers activation of Gal4::SREBP in fat body from control flies, but not from PGRP-LBΔ mutant’s flies. As expected, activation of Dipt-Cherry is observed in fat body from PGRP-LBΔ mutants fed with bacteria. The constitutive activation of Gal4::SREBP in oenocytes is indicated (no arrows). Flies of the following genotypes were used: w^{1115}/w^{1115}, Gal4::SREBP, UAS-2XEGFP/+; Dipt-Cherry^{C1}/Dipt-Cherry^{C2} (Control) and w^{1115}/w^{1115}, Gal4::SREBP, UAS-2XEGFP/+; Dipt-Cherry^{C1}, PGRP-LBΔ/Dipt-Cherry^{C2}, PGRP-LBΔ (PGRP-LBΔ). (B) Histograms showing the expression of Diptericin measured by q-RT-PCR and performed with mRNA extracted from adult abdominal carcasses of control adults fed 4 days with either sucrose, on with a mixture of sucrose + bacteria (E. coli or E. cc). The mRNA level in non-infected control flies was set to 1 and values obtained with indicated genotypes were expressed as a fold of this value. Histograms correspond to the mean ± SD of seven experiments (n = 7). *p<0.05, **p<0.01, ***p<0.001; Kruskal-Wallis test. Flies of the following genotypes were used: w^{1115}/w^{1115}, Gal4::SREBP, UAS-2XEGFP/+; Dipt-Cherry^{C1}/Dipt-Cherry^{C2} (Control) and w^{1115}/w^{1115}, PGRP-LBΔ, Dipt-Cherry^{C1}/PGRP-LBΔ, Dipt-Cherry^{C2} (PGRP-LBΔ). (C-D) Confocal images of the dorsal part of adult abdominal carcasses viewed from inside (C) or of the midgut R4 domain (D) from PGRP-LBΔ mutant flies fed with E. cc, showing Dipt-Cherry expression (red) and Gal4::SREBP activation (green). Adipocytes from PGRP-LBΔ mutant flies display high level of Dipt-Cherry expression but no activation of Gal4::SREBP. Constitutive expression of Dipt-Cherry in pericardial cells and activation Gal4::SREBP in oenocytes are indicated (C). Enterocytes of PGRP-LBΔ mutant flies display activation of both reporters (D). Flies of the following genotypes were used: w^{1115}/w^{1115}, Gal4::SREBP, UAS-2XEGFP/+; Dipt-Cherry^{C1}, PGRP-LBΔ/Dipt-Cherry^{C2}, PGRP-LBΔ (PGRP-LBΔ). (E) Confocal images of adult fat body from CRISPR mutant flies PGRP-LBΔw or PGRP-LBΔw, fed 72h with E. cc (A) or E. coli (B) and showing Dipt-Cherry expression (red) and Gal4::SREBP activation (green). (A) E. cc feeding induces activation of Dipt-Cherry in adipocytes from PGRP-LBΔw animals, but not from PGRP-LBΔw animals. Gal4::SREBP activation is faint in the CRISPR-specific mutant allele flies PGRP-LBΔw and strong in the PGRP-LBΔw one. (B) E. coli feeding induced a comparable activation of Gal4::SREBP in both PGRP-LBΔw or PGRP-LBΔw-adipocyte’s mutant flies, but no activation of Dipt-Cherry. Flies of the following genotypes were used: w^{1115}/w^{1115}, PGRP-LBΔw/Dipt-Cherry^{C1}/PGRP-LBΔw, Dipt-Cherry^{C2}/PGRP-LBΔw-Dipt-Cherry^{C2} (PGRP-LBΔw) and w^{1115}/w^{1115}, PGRP-LBΔw/Dipt-Cherry^{C1}/PGRP-LBΔw, Dipt-Cherry^{C2}/PGRP-LBΔw (PGRP-LBΔw), pc: pericardial cells. Scale bar is 0.25 mm (A), 100 μm (C), 50 μm (D) and 20 μm (E).
Fig 5. The NF-κB signaling pathway inhibits SREBP activation cell-autonomously in adipocytes. (A) Pictures of adult flies fed 7 days with either *E. coli* (A) or *E. cc* (B) showing Dipt-Cherry expression (red) and *Gal4*:SREBP activation (green). Flies mutant for the loss-of-function allele *Dredd*Δ4 activate *Gal4*:SREBP in fat body, when fed with either *E. coli* or *E. cc*. Two copies of the *Dredd*Δ4 allele suppress the faint and the lack of activation of *Gal4*:SREBP observed in *PGRP-LB*Δ mutant flies fed with *E. coli* and *E. cc*, respectively (A). (B) Confocal images of fat body from flies fed 2 days with sucrose or with a mixture of sucrose + the indicated bacteria. Activation of *Gal4*:SREBP (shown in green) absent in sucrose fed flies, is similarly detectable in *Dredd*Δ4 mutant flies fed with either *E. coli* or *E. cc*. Double mutant *Dredd*Δ4; *PGRP-LB*Δ flies activate *Gal4*:SREBP.
SREBP in adipocytes when fed 2 days with E. cc. Flies of the following genotypes were used: w^1118, Dredd^64D;^1118, Dredd^64d; Gal4: SREBP, UAS-2XEGFP/+; Dipt-Cherry^+/+; Dipt-Cherry^+/+ (for Dredd^64D) or w^1118, Dredd^64D/; Gal4: SREBP, UAS-2XEGFP/+; Dipt-Cherry^+/+; PGRP-LB^+/+; PGRP-LB^+/+ or w^1118, Dredd^64D/; Gal4: SREBP, UAS-2XEGFP/+; Dipt-Cherry^+/+; PGRP-LB^+/+ (for Dredd^64D: PGRP-LB^+/+). (C) Pictures of adult flies fed 3 days with sucrose or a mixture of sucrose + E. coli showing LexA::SREBP activation (green in top panels and red in bottom panels). Flies fed on sucrose and overexpressing IMD or PGRP-LCa in fat body cells (with r^4Gal4), do not activate LexA::SREBP. Upon E.coli feeding, the activation of LexA::SREBP is inhibited in fat bodies from flies overexpressing IMD or PGRP-LCa in fat body cells with r^4Gal4, or from flies over expressing IMD ubiquitously with da^4Gal4. Flies of the following genotypes were used: w^1118/r^4Gal4, LexA::SREBP, 13XLexAop-2XEGFP/+; r^4Gal4/+ (Control in top panels) or w^1118/r^4Gal4, LexA::SREBP, 13XLexAop-2XEGFP/+; da^4Gal4 or r^4Gal4/UAS-IMD (da^4Gal4 and r^4Gal4 in top panels), or w^1118/r^4Gal4, LexA::SREBP, LexAop-mCherry.mito/+; r^4Gal4/+ (Control in bottom panels) or w^1118/r^4Gal4, LexA::SREBP, LexAop-mCherry.mito/+; r^4Gal4/UAS-PGRP-LCa (r^4Gal4 in bottom panels). (B) Confocal images of fat body showing clones of adipocytes overexpressing IMD or PGRP-LCa (red) and activation of LexA::SREBP (green), from flies fed 2 days with E. coli. Fat body clones over expressing either IMD or PGRP-LCa inhibits LexA::SREBP activation is strictly autonomous manner. Flies of the following genotypes were used: w^1118. CoinFLP [w^1118; LexA::SREBP, LexAop-CD8-GFP-2A-CD8-GFP, UAS-CD4: Tomato/ hs-FLP.GS, Tub^m63A]; UAS-IMD or UAS-PGRP-LCa/+; Scale bar is 0.25 mm (A and C) and 20 µm (B and D).

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targeted expression can block IMD signaling over activation typically observed in guts and fat bodies of E. cc-infected PGRP-LB^4 mutant (Fig 6C). We then tested the effects of a tissue-specific IMD silencing on the lifespan of chronically E. cc-infected PGRP-LB^4 mutants. We found that blocking IMD signaling in enterocytes with Mex^Gal4 or in muscles using Mef2^Gal4 did not ameliorate the lifespan of E. cc fed PGRP-LB^4 mutants. In contrast, ubiquitous and fat body specific expression of UAS-dFadd^Gal4 significantly improved PGRP-LB^4 mutant resistance to chronic E. cc infection (Figs 6D and S6). These results suggest that by buffering IMD pathway activation in the fat body, the PGRP-LB amidase allows this tissue to generate lipids. This could be a mean for the host to better resist to chronic infection (Fig 7).

Discussion

We showed here that gut-associated bacteria can influence host lipid metabolism by activating SREBP in adipocytes. Axenic flies fed with sucrose displayed phenotypes of undernourished animal, such as ovarian atrophy and reduced systemic insulin signaling. At contrary, E. coli fed animal had fully developed ovaries and displayed local (gut) and systemic (fat body) activation of the insulin signaling, genetically upstream of SREBP activation in these lipid organs. Which bacteria associated metabolites or constituents mediate these effects? Dietary amino-acids are obvious candidates [33,34]. Consistently, heat-killed E. coli remained good inducer of adipocytes SREBP (S5A Fig). In addition, gut specific inhibition of autophagy prevented its activation. These results, which strongly suggest that bacteria derived amino-acids are the triggering signal, are consistently with published data obtained using yeast as a food source [35]. Likewise, fly microbiota contributes to protein processing upstream of the nutrient-sensing Tor and insulin signaling to promote growth [36,37]. In addition, starved adult flies favored a bacteria-contaminated sucrose solution over an axenic one [38]. Together with other reports, our data show that flies can use microbes as amino-acids source leading to the activation of the insulin/Tor signaling and SREBP in fat body [35,39,40]. SREBP activation could be used by the host as a mean to replenish its lipid pools affected by the bacterial infection. It was recently shown that in infected flies, hemolymphatic lipids are removed by the Malpighian tubules and excreted [41]. This mechanism, which provided protection against lipid peroxidation, is a central component of host physiological adaptation to infection, since flies lacking it succumb to infection. Lack of SREBP activation in Ecc infected fat body would prevent lipid pools replacement and hence contribute to host death.

L. plantarum^w^4, A. pomorum, E. faecalis and Saccharomyces cerevisiae were not able to activate SREBP, in agreement with previous results showing that S. cerevisiae and L. plantarum...
Fig 6. Inhibition of the NF-κB signaling pathway in adipocytes ameliorates survival of PGRP-LBΔ mutant flies infected with E. cc. (A) (left panels) Confocal images of fat body from flies fed 8 days with sucrose or with a mixture of sucrose + E. cc, and showing lipid droplets labelled with Bodipy (green). The reduction of lipid storage characteristic of E. cc infected PGRP-LBΔ flies is suppressed in presence of two copies of the loss of function allele DreddΔSS (right). Graph showing the quantification of lipid droplets size performed on confocal images obtained from 3 independent experiments (n = 3). a.u.
arbitrary unit, no significant (n.s.), **p < 0.0001; Kruskal-Wallis test. (B) Survival analysis of flies orally infected with E. cc. The Dredd<sup>ΔDSS</sup> allele suppresses the deleterious effect of PGRP-LB<sup>Δ</sup> mutation on fly's survival. For A and B, flies of the following genotypes were used: w<sup>1118</sup>/w<sup>1118</sup> (Control), w<sup>1118</sup>/w<sup>1118</sup>; PGRP-LB<sup>B</sup>/PGRP-LB<sup>Δ</sup> (PGRP-LB<sup>Δ</sup>), w<sup>1118</sup>, Dredd<sup>ΔDSS</sup>/w<sup>1118</sup>, Dredd<sup>ΔDSS</sup>; w<sup>1118</sup>, Dredd<sup>ΔDSS</sup> (Dredd<sup>ΔDSS</sup>) and w<sup>1118</sup>, Dredd<sup>ΔDSS</sup>/w<sup>1118</sup>, PGRP-LB<sup>Δ</sup>/PGRP-LB<sup>Δ</sup> (Dredd<sup>ΔDSS</sup>; PGRP-LB<sup>Δ</sup>). (C) Confocal images of the R1 domain of adult midgut (left panels) or of adult fat body (right panels) from PGRP-LB<sup>Δ</sup> mutant fed 24h with E. cc, showing Dipt-Cherry (red) expression. Inhibition of the IMD signaling pathway via expression of UAS-dFadd<sup>B</sup> is effective in enterocytes or in adipocytes using Mex<sup>Gal4</sup> or cG<sup>Gal4</sup> respectively. (D) Survival analysis of PGRP-LB<sup>Δ</sup> mutant flies orally infected with E. cc. The expression of UAS-dFadd<sup>B</sup> ubiquitously, using da<sup>Gal4</sup>, or in adipocytes, using either cG<sup>Gal4</sup> or rG<sup>Gal4</sup> decreases the deleterious effect of PGRP-LB<sup>Δ</sup> mutation on fly's survival. These flies have a significantly extended lifespan compared to the corresponding control flies (Log-rank (Mantel-Cox) test, *** p < 0.0001). Adult PGRP-LB<sup>Δ</sup> mutant flies expressing UAS-dFadd<sup>B</sup> in enterocytes, using either Mex<sup>Gal4</sup> or in muscle, using Mex<sup>Gal4</sup> have no significant (n.s.) extended lifespan compared to the corresponding control flies (Log-rank (Mantel-Cox) test). Flies of the following genotypes were used: w<sup>1118</sup>/w<sup>1118</sup>; +/+ or Mex<sup>Gal4</sup>/+; or cG<sup>Gal4</sup>/+; PGRP-LB<sup>Δ</sup>; Dipt-Cherry<sup>ΔC1</sup>/PGRP-LB<sup>Δ</sup>, UAS-dFadd<sup>B</sup> (C-D) and w<sup>1118</sup>/w<sup>1118</sup>; PGRP-LB<sup>Δ</sup>, da<sup>Gal4</sup> or PGRP-LB<sup>Δ</sup>; Mex<sup>Gal4</sup> or PGRP-LB<sup>Δ</sup>; rG<sup>Gal4</sup>/PGRP-LB<sup>Δ</sup>, UAS-dFadd<sup>B</sup> (D). Scale bar is 100 µm (midguts in C) and 20 µm (fat bodies in A and C).

are unable to efficiently rescue adult's lifespan of undernourished flies [35]. Further investigation will be necessary to uncover the strain specific compounds and mechanism(s) responsible for this species-specific regulation of SREBP and, in a broader aspect, to stimulate lipid metabolism.

In Drosophila, immunity and metabolism are linked structurally via the fat body, an organ homologous to the mammalian liver, adipose tissue and immune system, made of a single cell type: the adipocyte [42]. In mammals, immune cells are embedded into the adipose tissue, allowing direct influence of one cell type on the other [7]. Our data indicate that chronic activation of the IMD/NF-κB pathway prevents gut bacteria-dependent SREBP processing and thus lipid metabolism. By restricting the diffusion of PGN to the fly hemolymph, the PGRP-LB<sup>B</sup> enzyme allows gut bacteria-dependent lipogenesis in remote adipocytes and promotes fly survival. In the absence of such brake, lipid storages of orally infected flies are rapidly depleted and life span is reduced. Since E. cc-fed PGRP-LB<sup>Δ</sup> mutant display ovarian atrophy associated with a reduction of vitellogenic stages [43], it is possible that sex-hormones misregulation is contributing to SREBP processing inhibition in this organ.

We found that Foxo is a negative regulator of SREBP processing in adipocytes and that IMD/NF-κB signaling pathway inhibits SREBP processing, without affecting Insulin/P3K signaling (S7 Fig). Thus, we propose that the NF-κB transcription factor Relish and Foxo acts in parallel, or together, to negatively regulates SREBP processing. Interestingly, both transcription factors have common immune and metabolic target genes in Drosophila fat body [6,10,44,45]. One possibility would be that Relish and Foxo negatively regulate the transcription of genes that are essentials for SREBP processing, such as the escort factor SCAP (SREBP Cleavage Activating Protein), and/or the proteases S1P (Site-1 Protease) and S2P (Site-2 Protease) [22,28].

Inhibition of lipid metabolism triggered by bacterial infection have been reported in the past, although in different contexts. When bacteria such as Mycobacterium marinum are injected into Drosophila body cavity, the transcription factor Meff2, which activates transcription of metabolic genes in non-infected individuals, switches its activity to enhance transcription of immune genes [8]. As a result, anabolic transcripts are reduced and energy stores, such as lipids, are lost. Toll and the IMD signaling pathways are acting genetically upstream of Meff2 in this process. Lee and colleagues found that E. cc infection triggers lipid catabolism in enterocytes which, via a TRAF3-AMPK/WTS-ATG1 pathway, contributes to the activation of DUOX, a member of the NADPH oxidase family acting as the first line of host defense in Drosophila gut [46]. Finally, the bacteria produced short chain fatty acid acetate acts as a microbial metabolic signal that activates signaling through the IMD pathway in enteroendocrine cells.
This, in turn, increases transcription of the endocrine peptide tachykinin, which is essential for timely larval development and optimal lipid metabolism and insulin signaling [47].

Our work sheds light on how gut bacteria influences lipid metabolism and contributes to the development of an immune-metabolic disorder, through the action of the highly conserved transcription factors SREBP, NF-κB and Foxo and the universal bacteria cell-wall component PGN. Furthermore, it shows how by buffering gut-born circulating PGN levels, the PGRP-LB amidase, allows the appropriate balance between metabolic and immune responses.
Material and methods

**Drosophila strains and maintenance**

The strains used in this work are: w1118 BL#3605, Gal4::SREBP from BL#38395, UAS-2XEGFP BL#6874, Dipterica- CherryGL [48], PGRP-LB [21], PGRP-LCGL [49], Re[20] BL#9457, Dreddδ[55] and Dreddδ[64] (a gift from François Leulier), daGal4 BL#55851, r4Gal4 and MexGal4 (kindly provided by Yixian Zheng), Mef2Gal4 BL#27390, c8Gal4 BL#7011, UAS-IMD (kindly provided by François Leulier), UAS-PGRP-LCa BL#30917, CoinFLPGal4 BL#59269, hs-FLP,G5 BL#58356, TubGal80ts BL#7108, UAS-CD4::Tomato (kindly provided by Frank Schnorrer), LexA::SREBP (this work, molecular details of the construct under request), 13XLexAop2::6XGFP BL#52265, LexAop-CD8-GFP-2A-CD8-GFP BL#66545, tGPH BL#8163, 13XLex-Aop2-mcd8-GFP BL#32203, chicoι BL#10738, Df(2L)ED729 BL#24134, foxoα494 BL#42220, foxo25 BL#80944, PGRP-LB[Δ60] and PGRP-LB[Δ10A] (Kurz et al., 2017), UAS-dFaddδR ([50]; kindly provided by Pascal Meier), UAS-InrDN BL#8252 and UAS-ATG1IR BL#44034. Flies were grown at 25°C on a yeast/cornmeal medium in 12h/12h light/dark cycle-controlled incubators. For 1liter of food, 8.2g of agar (VWR, cat. #20768.361), 80g of cornmeal flour (Westhove, Farigel maize H1) and 80g of yeast extract (VWR, cat. #24979.413) were cooked for 10min in boiling water. 5.2g of Methylparaben sodium salt (MERCK, cat. #106756) and 4 ml of 99% propionic acid (CARLOERBA, cat. #409553) were added when the food had cooled down. For antibiotic (ATB) treatment, the standard medium was supplemented with Ampicillin, Kanamycin, Tetracycllin and Erythromycin at 50 μg/ml final concentrations.

**Drosophila genetics and analysis**

To generate UAS-IMD and UAS-PGRP-LCa overexpressing clones or to overexpress UAS-InrDN in adult adipocytes or UAS-ATG1IR in adult enterocytes, 5 days old mated females were raised and aged in presence of ATB at 22°C. Adult flies were then transferred into non-ATB media, and placed 24h at 29°C to inactivate Gal80ts, before the infection by bacteria for the following 48h at 29°C. For UAS-IMD and UAS-PGRP-LCa overexpressing clones, no heat shock was required for clone induction. Flies of the following genotype were used: CoinFLPGal4/+; hs-FLP,G5/LexA::SREBP, LexAop-CD8-GFP-2A-CD8-GFP; UAS-IMD, TubGal80ts/+ or PGRP-LCa, TubGal80ts/+ for Fig 5D or CoinFLPGal4/+; hs-FLP,G5/tGPH, UAS-CD4::Tomato; UAS-IMD, TubGal80ts/+ or PGRP-LCa, TubGal80ts/+ for 4B Fig.

**Imaging**

Whole fly imaging was performed on adult females totally immersed in 70% EtOH. Images were captured using a ZEISS SteREO Discovery.V12 microscope. For dissected tissues, adult flies were cut apart in cold PBS, fixed for 20min in 4% paraformaldehyde on ice and rinsed 3 times in PBT (1XPBS + 0.1% Triton X-100). The tissues were mounted in Vectashield (Vector Laboratories) fluorescent mounting medium, with or without DAPI. Images were captured with an LSM 780 ZEISS confocal microscope.

**Bodipy and Nile red staining**

For Bodipy and Nile Red staining, adult tissues were dissected in PBS, fixed for 20min in 4% paraformaldehyde on ice, rinse 3 times in 1XPBS without detergent and stained with Nile red (Cat. No. 72485, Sigma-Aldrich) or Bodipy 493/503 (Cat. No. D3922, ThermoFisher) at respectively 1:10000 and 1:1000, in PBS for 30min.
Immunofluorescence
Adult flies were cut apart in cold PBS, fixed for 20 min in 4% paraformaldehyde on ice and rinsed 3 times in PBT (1XPBS + 0.1% Triton X-100). Dissected tissues were blocked 2h in PBT + 3% BSA and then incubated overnight with the primary anti-dsREBP (3B2, Ref. ATCC-CRL-2693 from ACC) antibody (1:50 in PBT), in a cold room, without shaking. After 3 washes in PBT, the dissected tissues were incubated 2h with a goat anti-mouse Alexa Fluor 488 antibody (1:500 in PBT, Ref. ab150113 from abcam). The tissues were next rinsed three times in PBT and mounted in Vectashield (Vector Laboratories) fluorescent mounting medium, with DAPI. Images were captured with an LSM 780 ZEISS confocal microscope.

Quantification of lipid droplets
Fiji/ImageJ was used for quantification of lipid droplet size in adult adipocytes imaged by confocal microscopy. First, the area was measured for the entire field of view or a region-of-interest (ROI) of defined size. Second, and prior to converting image to binary, a ‘smooth’ function was applied to the image to remove inherent graininess of the lipid stain and allow for more accurate quantification of lipid droplets. Then ‘Watershed’ was performed on binary images, and ‘analyze particles’ was used to quantify LD number and size.

Bacterial strains
The following microorganisms were used: Erwinia carotovora carotovora 15 strain 2141 (grown at 30°C), Lactobacillus plantarum strain WJL (grown at 37°C), Escherichia coli strain DH5α (grown at 37°C), Acetobacter pomorum (grown at 30°C) and Enterococcus faecalis (grown at 37°C). Microorganisms were cultured overnight in Luria-Bertani (for E. cc, E. coli and E. faecalis) or MRS medium (for L.plantarum and A. pomorum). Cultures were centrifuged at 4000 g for 15 min at RT and re-suspended in 1XPBS. Cells were serially diluted in 1XPBS and their concentration was determined by optical density (OD) measurement at 600 nm. For heat killed bacteria, cells were re-suspended in 1XPBS and heated at 95°C for 15 min.

Adult oral infection
We used 4–6 days old female raised at 25°C in presence of ATB in the food. 24h before the infection, female flies were transferred in vials without ATB and then placed in a fly vial with microorganism contaminated food. The food solution was obtained by mixing a pellet of an overnight culture of bacteria or yeast (OD = 200) with a solution of 5% sucrose (50/50) and added to a filter disk that completely covered the agar surface of the fly vial. For E. coli heat inactivation, a solution of E. coli diluted (final OD_{600} = 100) in 2.5% Sucrose was incubated at 96°C for 20 minutes, then cool down before use.

Survival tests with bacterial infection
For oral infections, 15 adult flies were transferred every 2 days in a fresh vial in which 150 microliters of a fresh solution of E. cc (OD = 200)/ 5% sucrose (50/50) has been deposited. Experiments were performed in triplicate.

Quantitative real-time PCR
RNA from whole dissected organs (n = 12) was extracted with RNeasy Mini Kit. Three hundred ng of total-RNA was then reverse transcribed in 10 μl reaction volume using the Superscript III enzyme (Invitrogen) and random hexamer primers. Quantitative real-time PCR was performed on a CFX96 Real-Time PCR Detection System (BIO-RAD) in 96-well plates using
the FastStart Universal SYBR Green Master (Sigma-Aldrich). The amount of mRNA detected
was normalized to control rp49 mRNA values. Normalized data was used to quantify the rela-
tive levels of a given mRNA according to cycling threshold analysis (ΔCt). All datasets were
organized and analyzed in Microsoft Excel 2016.

**Plasmid pP[LexA:: dSREBPg]**

An 8.7 kb fragment (containing the entire dSREBP gene, 2.9 kb upstream and 0.7 kb down-
stream) was amplified by PCR using the High-Fidelity PCR System (Roche) and the [pac-
man] Bac CH322-183B11 as DNA template. The forward primer used for amplification was 5’-
CGGAATTCCGGATCTCCCAGAGATGGCACCTTTGG-3’ and the reverse primer was 5’-
CGGAATTCCACATGTCATCTGAGCGGATACC-3’. EcoR1 linkers were added during amplification and the resultant fragment was ligated into pRIVwhite (a gift from Jean-
Paul Vincent) to obtain pP[dSREBPg]. The open reading frame was sequenced in its entirety.
Restriction sites for Asc1 and Fse1 were inserted into pP[dSREBPg] at the beginning of the
ORF (Asc1, inserted immediately after aa3) and immediately following the bHLH region
(Fse1, inserted immediately preceding aa. 362). The primers used for insertion of the Asc1 site
were 5’-GGACCCGCTCCAGGTGAGGCGGGCTCGAATTCAGCTGGCGGACCTC-3’ and its
reverse complement. Primers used for insertion of the Fse1 site were 5’-GGGATCCGCGCTCC
AAGGATCGAAGCAGGCTTCAGCTGGCGGACCTC-3’ and its reverse complement. The
sites were inserted individually into pP[dSREBPg]. Nar1 (for the Asc1 site) or Nar1-nhe1 frag-
ments (for the Fse1 site) were excised out of the resulting vector and then subcloned together
into Nar1-nhe1 digested pP[dSREBPg]. The resultant vector pP[dSREBPg/F] was sequenced in
the regions that had been subject to PCR. In order to generate pP[LexA::SREBP], a cDNA
fragment encoding a fusion of the LexA DNA binding domain fused to the RelA transactiva-
tion domain was amplified by PCR from pBPnsLexA::p65Uw (Plasmid #26230 from
Addgene). Asc1 and Fse1 linkers were added during amplification. This fragment was then
ligated into pP[dSREBPg/F].

**Statistical analysis**

The Prism software (GraphPad) was used for statistical analyses. For q-RT PCR experiments
we used the nonparametric Kruskal-Wallis test. P value was indicated as follow: * for P<0.05,
** for P<0.01, *** for P<0.001. ns for not significantly different. We used the log-rank test
Mantel-Cox for survival data analyses. **** for P<0.0001. ns for not significantly different.

**Supporting information**

S1 Fig. *Gal4::SREBP and LexA::SREBP transgenes used in this study*. 1, Schematic drawing
of the SREBP genomic locus. 2, In the *Gal4::SREBP* transgene, the transcription factor
domain-encoding sequence was replaced by a *Gal4::VP16*-encoding sequence to report SREBP
activation. 3, In the *LexA::SREBP* transgene the transcription factor domain-encoding
sequence was replaced by a LexA::RelA-encoding sequence to report SREBP activation.
(TIF)

S2 Fig. Gut bacteria stimulate LexA::SREBP activation in adult fat body. (A) Pictures of
adult flies fed 2 days with sucrose, or a mixture of sucrose + *E. coli* or *E. cc*, showing LexA::
SREBP activation (green). Flies fed with sucrose show activation of LexA::SREBP in oenocytes,
noticeable after a longer exposure time (panel with asterisk). Both *E. coli* and *E. cc* feeding pro-
motes activation of LexA::SREBP in fat bodies. (B) Confocal images of fat body from flies fed 2
days with sucrose or with *E. coli* and showing LexA::SREBP activation (green). Flies fed on
sucrose show feeble activation of LexA::SREBP in adipocytes, noticeable after increasing the gain during image acquisition (panel with asterisk). E. coli feeding, however, promotes strong activation of LexA::SREBP in adipocytes. (C) Pictures of adult flies, control or PGRP-LBΔ mutant, fed 2 days with sucrose + E. coli, showing LexA::SREBP activation (green). Ingestion of E. coli triggers activation of LexA::SREBP in fat body from control flies, but not from PGRP-LBΔ mutant's flies. Flies of the following genotypes were used: w1118/ΔΔ, LexA::SREBP, 13XLex-Aop2-6XGFP/+ (Control in A, B and C), and w1118/ΔΔ, LexA::SREBP, 13XLexAop2-6XGFP/+; PGRP-LBΔ/PGRP-LBΔ (PGRP-LBΔ in C). Scale bar is 0.25 mm (A and C) and 20 μm (B). (TIF)

S3 Fig. Gut bacteria sustain oogenesis. Quantification of the different stages of oocytes observed in the female's ovary, after feeding 24h on sucrose, or on a mixture of sucrose + E. coli or E. coli. Apoptotic events were quantified as oocytes with compact and dense nurse cell nuclei, using DAPI staining. Histograms correspond to the mean ± SD of three experiments (n = 3). For each oocyte stage, sucrose values were used as reference for statistical analysis. *p < 0.05; Kruskal-Wallis test. Flies of the following genotypes were used: w1118/ΔΔ, LexA::SREBP, 13XLexAop2-6XGFP/+.

(TIF)

S4 Fig. PGRP-LC and Relish are required for SREBP inhibition observed in E. coli orally infected PGRP-LB mutants. Confocal images of fat body from flies fed 2 days with a mixture of sucrose + E. coli. Double mutant PGRP-LCΔΔ, PGRP-LBΔΔ or RelE20, PGRP-LBΔ flies activate Gal4::SREBP in adipocytes from flies fed 2 days with E. coli, while PGRP-LBΔΔ mutants do not. Flies of the following genotypes were used: w1118/ΔΔ; Gal4::SREBP, UAS-2XEGFP/+; PGRP-LCΔΔ, PGRP-LBΔΔ/ PGRP-LBΔ Δ (Top left panel, PGRP-LBΔΔ) or w1118/ΔΔ; Gal4::SREBP, UAS-2XEGFP/+; PGRP-LCΔΔ, PGRP-LBΔΔ/ PGRP-LCΔΔ, PGRP-LBΔΔ (PGRP-LCΔΔ, PGRP-LBΔΔ) or w1118/ΔΔ; Gal4::SREBP, UAS-2XEGFP/+; RelE20, PGRP-LBΔΔ/ PGRP-LBΔΔ (Top right panel, PGRP-LBΔΔ) or w1118/ΔΔ; Gal4::SREBP, UAS-2XEGFP/+; RelE20, PGRP-LBΔΔ/ PGRP-LBΔΔ, PGRP-LBΔΔ (PGRP-LCΔΔ, PGRP-LBΔΔ). Scale bar is 20 μm.

(TIF)

S5 Fig. Activation of SREBP by heat killed bacteria or by E. coli on regular food. (A) Confocal images of fat body from female flies fed 2 days with a mixture of sucrose + alive or heat killed bacteria (E. coli or E. coli cc). Both heat killed bacteria are efficiently activating SREBP in adipocytes. (B) Confocal images of fat body from female flies fed 2 days with E. coli without sucrose, or with E. coli dropped on regular food, showing Gal4::SREBP activation (green). Absence of sucrose does not impact the strong activation of SREBP by E. coli, while presence of regular food diminishes it. Flies of the following genotypes were used: w1118/ΔΔ, LexA::SREBP, 13XLexAop2-6XGFP/+; Gal4::SREBP, UAS-2XEGFP/+ (A) and w1118/ΔΔ, Gal4::SREBP, UAS-2XEGFP/+ (B). Scale bar is 20 μm.

(TIF)

S6 Fig. Survival curves of PGRP-LB mutants fed with sucrose. Survival analysis of PGRP-LBΔ mutant flies fed with sucrose. The expression of UAS-dFaddΔR ubiquitously using daGal4, or in adipocytes, using either cgGal4 or r4Gal4, or in enterocytes using Mef2Gal4 or in muscle, using Mef2Gal4 have no significant impact on flies’ lifespan, compared to the corresponding control flies. Flies of the following genotypes were used: w1118/ΔΔ; PGRP-LBΔΔ, daGal4 or PGRP-LBΔΔ, Mef2Gal4 or PGRP-LBΔΔ, r4Gal4/PGRP-LBΔΔ, UAS-dFaddΔR. (TIFF)
S7 Fig. Gut bacteria promote cell surface recruitment of tGPH in adipocytes and this is not affected by over expressing IMD or PGRP-LCa cell autonomously. Confocal images of fat body from adult flies fed 2 days with either sucrose, or a mixture of sucrose + *E. faecalis* or heat killed (H.k) *E. coli*, and showing the tGPH marker (green). Ingestion of heat killed *E. coli* promote recruitment of tGPH at the cell surface of adipocytes, compared to a sucrose diet. (B) Confocal images of fat body showing clones of adipocytes overexpressing IMD or PGRP-LCa (red) and the tGPH marker (green), from flies fed 1 day with *E. cc* or 2 days with *E. coli*. Fat body clones over expressing either IMD or PGRP-LCa do not affect tGPH recruitment at the cell surface of adipocytes. Flies of the following genotypes were used: *w^{1118}/w^{1118}*; tGPH/tGPH (A) and *w^{1118}, CoinFLP^Gala^/w^{1118}; tGPH, UAS-CD4:-Tomato/hs-FLP.G5, Tub^Gala^; UAS-IMD or UAS-PGRP-LCa/+*. Scale bar is 20 μm. (TIFF)

**S1 Numerical data. Numerical data underlying the results.**

(XLSX)

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### Author Contributions

**Conceptualization:** Bernard Charroux, Julien Royet.

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**Writing – original draft:** Bernard Charroux.

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