Downregulation of the Drosophila immune response by peptidoglycan-recognition proteins SC1 and SC2
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Peptidoglycan-recognition proteins (PGRPs) are evolutionarily conserved molecules that are structurally related to bacterial amidases. Several Drosophila PGRPs have lost this enzymatic activity and serve as microbe sensors through peptidoglycan recognition. Other PGRP family members, such as Drosophila PGRP-SC1 or mammalian PGRP-L, have conserved the amidase function and are able to cleave peptidoglycan in vitro. However, the contribution of these amidase PGRPs to host defense in vivo has remained elusive so far. Using an RNA-interference approach, we addressed the function of two PGRPs with amidase activity in the Drosophila immune response. We observed that PGRP-SC1/2–depleted flies present a specific over-activation of the IMD (immune deficiency) signaling pathway after bacterial challenge. Our data suggest that these proteins act in the larval gut to prevent activation of this pathway following bacterial ingestion. We further show that a strict control of IMD-pathway activation is essential to prevent bacteria-induced developmental defects and larval death.

Introduction

The antimicrobial host defense of Drosophila involves rapid synthesis of small-sized cationic peptides by the fat body [1,2]. These antimicrobial peptides are released into the open circulatory system where they attack invading microorganisms. The transcription of the genes encoding these peptides is under the control of two distinct signaling pathways. The Toll pathway, which is primarily activated after gram-positive bacterial and fungal infections, controls the expression of drosomycin, an antifungal peptide, together with many other genes via the NF-κB-family member DIF (dorsal-related immune factor) [3,4]. The second cascade, known as the IMD (immune deficiency) signaling pathway, is predominantly triggered after gram-negative infection and regulates, via the NF-κB protein Relish, the synthesis of some antibacterial peptides and many other genes [5,6]. An efficient Toll-pathway activation after gram-positive bacterial infection requires the function of at least three soluble proteins, namely peptidoglycan-recognition protein-SA (PGRP-SA) [7], PGRP-SD [8], and gram-negative binding protein-1 [9,10]. On the other hand, sensing of gram-negative bacterial infection has been shown to be dependent on two other PGRP family members, PGRP-LC [11–13] and PGRP-LE [14,15].

The ability of Drosophila to discriminate between gram-positive and gram-negative bacteria relies on the specific recognition of different forms of peptidoglycan (PGN) [16,17]. Bacterial PGN consists of long carbohydrate chains of alternating N-acetylglucosamine and N-acetylmuramic acid connected via stem peptides [18] (Figure S1). Most PGNs from gram-positive bacteria contain L-lysine in the third position of the stem peptide (Lys-PGN) and are recognized by PGRP-SA and PGRP-SD [7–9]. In PGN of gram-negative bacteria and in that of gram-positive bacilli, the lysine residue is replaced by meso-diaminopimelic acid (m-DAP) (Figure S1). This second type of PGN (m-DAP-PGN) is sensed by PGRP-LC and PGRP-LE receptors, leading to the activation of the IMD pathway [14–17,19].

PGRPs form a large group of proteins present in insects and mammals [20–25], which have in common a 160-amino acid–domain with striking sequence similarity to N-acetylmuramyl-L-alanine amidases (NAMLLA) [26]. These bacterial enzymes hydrolyze the bond formed between the lactyl group in N-acetylmuramic acid and the L-alanine in the stem peptide of PGN. In some of these PGRP molecules, the amidase function is conserved, as documented for Drosophila PGRP-SC1 [27] and PGRP-LB [28], and for mouse and human PGRP-L [29,30]. In others, such as in PGRP-SA, SD, LE, or LC, the replacement of a critical cysteine residue within the PGRP domain abolishes this enzymatic function [27]. On the basis of genetic experiments, it is assumed that PGRPs without amidase activity serve as recognition receptors for...
Synopsis

It has long been known that the mammalian immune response needs to be kept under tight control. Responses that are delayed or of insufficient vigor can lead to a failure to control infection. However, excessive or inappropriate inflammation can be harmful or event fatal. Using the fruit fly as a model, evidence is presented that such an immuno-modulation is also essential in invertebrates and is mediated by peptidoglycan-recognition proteins (PGRPs). PGRPs are evolutionarily conserved molecules derived from enzymes that cleave bacterial peptidoglycan. It has been shown previously that some PGRPs have lost this enzymatic activity and function as sensors of bacteria upstream of the Drosophila immune pathways. The contribution of PGRPs which have maintained enzymatic activity to host defense has remained elusive so far. Here, the authors investigate in vivo data on the role of Drosophila PGRPs with enzymatic activity. Their results suggest that these proteins are required in the larval gut to negatively regulate the immune response, thus preventing bacterially induced developmental defects and larval death.

microbial PGN. However, the in vivo function of PGRPs with amidase activity remains unclear, and PGRP-L mutant mice show no immune phenotype. On the basis of in vitro experiments, it has been proposed that amidase PGRPs could act as scavenging molecules. Indeed, degradation of PGN by Drosophila PGRP-SC1b markedly reduces its immuno-stimulatory potency in cell-culture assays [27]. We report here an in vivo study on the role of Drosophila PGRPs with amidase activity. We show that two PGRPs with described amidase activity, namely PGRP-SC1 and PGRP-SC2, control the intensity of the Drosophila immune response. We also present evidence that in the absence of such a control, infection-induced IMD-pathway over-activation can cause developmental defects and larval death.

Results

Loss-of-Function Mutants for PGRP-SC1/2 Are Generated by RNA Interference

In order to address the function of NAMLLA PGRPs under in vivo conditions, we analyzed the immune response of Drosophila with reduced PGRP-SC1 and PGRP-SC2 levels. The Drosophila genome contains a cluster of two tandemly arranged PGRP-SC1 loci (named a and b) and a single PGRP-SC2 locus [25] (Figure S2A). The two PGRP-SC1 mRNAs differ by only three nucleotides and translate into a unique protein that is 70% identical to the PGRP-SC2 polypeptide (Figure S2B and S2C). Furthermore, PGRP-SC1 and PGRP-SC2 proteins form a separate cluster in the PGRP phylogenetic tree (Figure S2D). To eliminate potential problems of functional redundancy between these two homologous enzymes, we decided to simultaneously knockdown the PGRP-SC1 and the PGRP-SC2 genes in vivo. The presence of long stretches of identical sequences in their transcripts prompted us to take advantage of the RNA-interference method (Figure S2B). Using PGRP-SC1- and PGRP-SC2-specific primers, we could demonstrate that adult flies carrying a UAS iPGRP-SC construct (see Materials and Methods) together with a ubiquitous Gal4 driver (DaloGal4) exhibited a 90% reduction of both PGRP-SC1 and PGRP-SC2 mRNA levels (Figure 1). The transcript levels of PGRP-SA and PGRP-SD, two closely related family members, were unaffected in these flies, demonstrating the specificity of the designed UAS iPGRP-SC construct (Figure 1).

IMD Pathway in PGRP-SC1/2–Depleted Flies Is Over-Activated

We first analyzed the potential role of PGRP-SC in adult flies, which are more amenable than larvae to pricking and survival experiments. For this, we infected UAS iPGRP-SC and DaloGal4; UAS iPGRP-SC flies (DaloGal4 is a ubiquitous driver)
with gram-negative bacteria and measured diptericin transcript levels as a conventional readout for IMD-pathway activation. Six hours after infection with Enterobacter cloacae or Escherichia coli (a time-point that corresponds to the peak of diptericin mRNA kinetics in wild-type flies), no differences were noted between the levels of diptericin mRNA in UAS iPGRP-SC and DaGal4;UAS iPGRP-SC flies (Figure 2A). However, whereas the diptericin mRNA level dropped significantly at 24 and 48 h in UAS iPGRP-SC flies (as it usually does in the wild-type condition), it remained high in DaGal4;UAS iPGRP-SC flies. Similar results were obtained with another m-DAP-PGN–containing bacteria (Bacillus subtilis), although the differences between UAS iPGRP-SC and DaGal4;UAS iPGRP-SC could be detected as soon as 6 h after infection (Figure 2A).

Experiments performed with another ubiquitous driver (ActinGal4) and an independent UAS iPGRP-SC insertion generated identical results (data not shown). When gram-positive bacteria were used as inducers, two of them (Micrococcus luteus and Enterococcus faecalis) did not activate the IMD pathway above clean injury levels. A third one, Staphylococcus aureus, triggered slightly higher diptericin-transcription levels in PGRP-SC–depleted flies than in controls, although the induction was mild, as expected from gram-positive bacteria (Figure 2A).

These results indicate that depletion of PGRP-SC induces an over-activation of the IMD pathway after bacterial infection. This phenotype was not observed in noninfected DaGal4;UAS iPGRP-SC flies or after pricking with a clean needle (Figure 2A). Altogether, this indicates that the observed effects are dependent on the presence of bacteria and do not correspond to a constitutive activation of the IMD pathway in PGRP-SC–depleted flies. They also demonstrate that the other putative secreted Drosophila amidases (PGRP-SB1 and PGRP-SB2) are not able to compensate for the absence of PGRP-SCs in vivo. In the absence of loss-of-function mutants for each of the three PGRP-SC genes, we can obviously not rule out the possibility that one or two of them are responsible for the observed phenotype. To obtain additional proof that the effects observed in PGRP-SC–depleted flies were specific, we performed similar experiments in flies in which the non-amidase bacterial receptor PGRP-SA had been depleted by RNA interference. Whereas DaGal4;UAS iPGRP-SA flies showed an expected reduced ability to respond to infection by the gram-positive M. luteus, their response to E. coli remained wild-type (Figure 2B).

Therefore, DaGal4;UAS iPGRP-SA flies behave as classical PGRP-SA<sup>null</sup> loss-of-function mutants [7] and not like flies with reduced PGRP-SC levels.

PGRP-SC1/2–Depleted Flies Are Able to Clear Bacteria

Since PGRP-SC1 has been proposed to act as a scavenger molecule [27], we tested whether the IMD-pathway over-activation observed in PGRP-SC-depleted flies could reflect the inability of these flies to clear bacteria. If this were to be proved the case, accumulation of bacteria in the body cavity of DaGal4;UAS iPGRP-SC flies could explain the over-activation of the IMD pathway. To test this hypothesis, we compared bacterial loads and survival curves of IKK<sup>bey1</sup>, UAS iPGRP-SC, and DaGal4;UAS iPGRP-SC flies infected with E. cloacae or with E. coli (Figure 2C and 2D). Whereas IKK<sup>bey1</sup> mutant flies showed a very high bacterial load and a strong susceptibility to these bacteria, no such phenotypes were observed in DaGal4;UAS iPGRP-SC flies. Similar results were obtained with B. subtilis (unpublished data). This suggests that the over-response observed in PGRP-SC–depleted flies did not result from an uncontrolled bacterial growth in the hemolymph, and that the role of PGRP-SC proteins in vivo is not to scavenge bacteria from the circulating hemolymph.

Toll-Pathway Activation Is Wild-Type in PGRP-SC1/2 Mutant Flies

It is notable that non-enzymatic PGRPs, such as PGRP-SA, PGRP-LC, or PGRP-LE are able to discriminate between Lys-type and m-DAP-type PGN [14–17] (Figure 1S1). Recent experiments have nevertheless demonstrated that PGRP-SC1b can act as a cleaving enzyme for both gram-positive and gram-negative bacterial PGN in vitro. We therefore asked whether reducing the endogenous levels of PGRP-SC1/2 could also have an effect on Toll-pathway activation by gram-positive bacteria. As illustrated in Figure 3, the effects were IMD-pathway–specific since Toll-dependent activation of diptericin by gram-positive or gram-negative bacteria (M. luteus, E. faecalis, S. aureus, E. cloacae, E. coli, B. subtilis) were similar in UAS iPGRP-SC and DaGal4;UAS iPGRP-SC flies. This difference between the role of PGRP-SC1/2 on Toll- and IMD-pathway activation could reflect functional redundancy between amidases for Lys-type PGN which might not exist for m-DAP-type PGN-cleaving enzyme. Alternatively, this could pertain to the difference in the mode of activation of the transmembrane receptors upstream of each pathway.

PGRP-SC1/2 Function in Larval Immune Response

We next addressed the role of PGRP-SC proteins in larvae. Previous qualitative analyses indicated that PGRP-SC1 and PGRP-SC2 genes are transcribed in almost identical patterns and mostly in the gut cells [25]. Using quantitative RT-PCR, we confirmed that the larval gut is strongly enriched in PGRP-SC1 and PGRP-SC2 mRNA and represents the main site of PGRP-SC amidase synthesis at this developmental stage (Figure 4A). In a previously established model of infection by ingestion [31], it was observed that larvae fed with the gram-negative bacteria Erwinia carotovora carotovora induce diptericin transcription in the fat body. Surprisingly, most of the other gram-negative bacterial species tested in this assay failed to do so. We reasoned that the gut PGRP-SC amidases might act to reduce the PGN immunogenic potential of these bacteria, preventing them from activating a systemic immune response.

To test this hypothesis, UAS iPGRP-SC control larvae and DaGal4;UAS iPGRP-SC larvae, which exhibit a strong reduction of PGRP-SC1 and PGRP-SC2 mRNA levels in their gut (Figure 4A), were fed with various bacterial species. We then monitored diptericin expression in whole larvae or, more specifically, in the fat body. As previously reported [31], we found that ingested E. carotovora carotovora, but not E. coli, was able to activate the IMD pathway in UAS iPGRP-SC control larvae (Figure 4B). Strikingly, reducing the PGRP-SC levels induced a strong increase in the expression level of the diptericin mRNA at 6 and 24 h after feeding on E. carotovora carotovora, as compared to controls (Figure 4B). Under these conditions, E. coli now became a good inducer of diptericin expression. This increase in the level of diptericin transcription was totally blocked in a PGRP-LC mutant background, demonstrating that, in this process, the PGRP-SC
Figure 2. IMD-Pathway Activation Is Downregulated by PGRP-SC1/2

(A) Kinetics of diptericin mRNA induction (Dipt/RpL32) after infection by various bacteria. Each histogram corresponds to the mean value of five independent experiments (± standard deviation). Asterisks indicate that the difference between DaGal4;UAS iPGRP-SC and control UAS iPGRP-SC values is statistically significant ($p < 0.05$). One hundred percent corresponds to the level of activation at 6 h in control flies. In the lower panel, diptericin
amidases act upstream of the IMD-pathway transmembrane receptor (Figure 4B). As shown above for the immune response in adults, reducing the PGRP-SC levels had no effect on Toll-pathway activation in larvae (Figure 4C).

PGRP-SC1/2 Are Required in the Larval Gut to Dampen the Immune Response

We tested whether similar results could be obtained by reducing PGRP-SC levels specifically in the larval gut using a tissue-specific driver (CadGal4) [32]. Consistent with previous reports indicating that CadGal4 is not a very strong driver [32], we noted that the reduction of PGRP SC1/2 mRNA levels in the gut were not as pronounced in CadGal4;UAS iPGRP-SC than in DaGal4;UAS iPGRP-SC larvae (Figure 4A). However, this reduction was sufficient to trigger an activation of the IMD pathway after feeding on E. coli (Figure 4B). Using a DiptlacZ reporter transgene, we could show that up to 40% of the CadGal4;UAS iPGRP-SC larvae activated the IMD pathway in the fat body after feeding on E. coli (Figure 4D and E). In wild-type control larvae, this percentage was only 5% (Figure 4E). In parallel experiments, we tested the effects of over-expressing the PGRP-SC1b protein in the larval gut. Whereas 80% of the diptLacZ;UAS PGRP-SC1b control larvae fed with E. carotovora activated diptericin transcription in the fat body, this percentage dropped to 10% in larvae which specifically over-expressed PGRP-SC1b in the gut (Figure 4E). Altogether, these results are compatible with the hypothesis that an essential role of gut PGRP-SC1/2 amidases in larvae is to modulate activation of the IMD pathway. We propose that this modulation is achieved by lowering the amount of immunogenic PGN, most probably via an amidase-dependent degradation. However, we cannot rule out that it is also partly due to sequestration of the PGN.

Larvae with Reduced PGRP-SC1/2 Levels Are Highly Susceptible to Infection

To evaluate the consequences of the reduction of this immuno-modulatory function, we followed the fate of naturally infected wild-type and PGRP-SC–depleted larvae. We observed that mortality was three to four times higher in depleted larvae fed with E. coli or E. carotovora than in controls (Figure 5A). This increase in larval lethality was expressing the PGRP-SC1b protein in the larval gut. Whereas 80% of the diptLacZ;UAS PGRP-SC1b control larvae fed with E. carotovora activated diptericin transcription in the fat body, this percentage dropped to 10% in larvae which specifically over-expressed PGRP-SC1b in the gut (Figure 4E). Altogether, these results are compatible with the hypothesis that an essential role of gut PGRP-SC1/2 amidases in larvae is to modulate activation of the IMD pathway. We propose that this modulation is achieved by lowering the amount of immunogenic PGN, most probably via an amidase-dependent degradation. However, we cannot rule out that it is also partly due to sequestration of the PGN.

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![Figure 3. Toll-Pathway Activation Is Wild-Type in DaGal4;UAS iPGRP-SC Flies](image-url)

*Figure 3. Toll-Pathway Activation Is Wild-Type in DaGal4;UAS iPGRP-SC Flies*

Kinetics of drosomycin mRNA induction (Drs/RpL32) after infection by gram-positive (upper panel) and gram-negative (lower panel) bacteria. Each histogram corresponds to the mean value of six independent experiments (± standard deviation). Asterisk indicates that the difference between DaGal4;UAS iPGRP-SC and control UAS iPGRP-SC values is statistically significant (p < 0.05). One hundred percent corresponds to the level of activation 24 h after infection in control flies. In the lower panel, drosomycin induction after gram-negative bacterial infections is compared to that of S. aureus infection which is set to 100%. RpL32 is used as an internal control.

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![Figure 4. PGRP-SC1/2 Activity in Larval Gut Activates the IMD Pathway](image-url)

*Figure 4. PGRP-SC1/2 Activity in Larval Gut Activates the IMD Pathway*

(A) Amidase activity in gut extracts was measured with N-succinimidyl succinate (N-succ). Analyses were performed with 50% SP-EP and 10% SP-EP. N-succ is used as an internal control.

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![Figure 5. Larvae with Reduced PGRP-SC1/2 Levels Are Highly Susceptible to Infection](image-url)

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totally suppressed in a PGRP-LC mutant background, demonstrating that over-activation of the IMD pathway was indeed the cause of larval death. Interestingly, a small percentage of the DaGal4;UAS iPGRP-SC larvae that pupariated and eclosed as pharate adults presented developmental defects such as wing notching (Figure 5B–5D). These phenotypes were never observed in control larvae fed with bacteria. To test whether this wing phenotype could be due to...
increased cell death during larval development, imaginal discs from infected larvae were stained with acridine orange. Wing discs from PGRP-SC–depleted, bacteria-fed larvae showed higher levels of cell death than wing discs from control larvae fed with normal levels of PGRP-SC (Figure 5E and 5F).

Discussion

The need for a tight balance between initiation and resolution in the control of inflammation in vertebrates has been documented for a long time. Recent reports have reviewed the molecular mechanisms that are put in place to dampen inflammation and to prevent damaging effects associated with a prolonged immune response [33,34]. The data presented here suggest that immune response needs to be tightly regulated also in invertebrates.

Taken together, our data provide novel insights into the physiological roles of PGRPs in Drosophila. They show that in addition to the function as a pattern-recognition receptor of some PGRP family members, others can specifically control the level of activation of the IMD signaling pathway. Flies deficient for PGRP-SC1a, PGRP-SC1b, and PGRP-SC2 present a specific over-activation of the IMD pathway. A recent report described an effect of a PGRP-SC1 mutant (pick) on Toll-pathway activation [35], a phenotype that we did not observe in PGRP-SC1/2–depleted flies (see Figure 3). This discrepancy is not yet fully understood but could be explained by the fact that picky flies are mutant only for PGRP-SC1a and PGRP-SC1b, whereas PGRP-SC2 is also affected in our PGRP-SC–depleted flies.

Our results indicate that the gut is the main tissue in which the regulation by PGRP-SC proteins is taking place. However, the fact that IMD-pathway over-activation was also detected when bacteria were introduced directly into the circulating hemolymph suggests that these secreted proteins could also be present in the blood or in the circulating hemocytes. We further show that in the absence of a control of the immune response, infection can lead to developmental defects or death by over-activation of the immune pathway. Interestingly, recent reports indicate that other immune-induced pathways can have a harmful effect on fly survival. Salmonella typhimurium–infected flies produce a tumor necrosis factor (TNF)–like cytokine which has been shown to be damaging for the host [36]. In addition, flies in which the gut catalase level is experimentally reduced show high mortality rates after ingestion of microbe-contaminated foods. This has been interpreted as evidence that infection-mediated induction of reactive oxygen species (such as H2O2) must be tightly balanced to avoid larval lethality [32]. In this respect, our data indicate that PGRP-SC1/2 may act as detoxifying proteins for bacterial PGN in flies. Although we did not demonstrate that the amidase function of these PGRPs is required for this effect, in vitro biochemical data strongly suggest that it is the case. Similar functions have recently

Figure 5. Reduction of PGRP-SC1/2 Levels Sensitizes Larvae to Bacterial Infection

(A) The percentage of dead larvae is measured 24 h after natural infection. Numbers in parentheses correspond to the total number of infected larvae. Each histogram corresponds to the mean value of six independent experiments (± standard deviation) for E. coli and five for E. carotovora carotovora. Asterisks indicate that the difference between UAS iPGRP-SC and DaGal4;UAS iPGRP-SC values is statistically significant (p < 0.05).

(B) The percentage of adults showing wing notching is measured 7 d after natural infection. Numbers in parentheses correspond to the total number of infected larvae.

(C–F) Natural infection with E. carotovora carotovora and E. coli triggers increased cell death and developmental defects in DaGal4;UAS iPGRP-SC flies. Wing imaginal discs dissected from DaGal4;UAS iPGRP-SC larvae (F) show higher levels of cell death after natural infection than discs from infected UAS iPGRP-SC control larvae (E). Consistently, some DaGal4;UAS iPGRP-SC adults derived from infected larvae exhibit wing notching (indicated by arrowheads) (D), which was never observed in infected controls (C).

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been attributed to enzymes which reduce the immunogenic potential of lipopolysaccharide during vertebrate immune response [37].

The results presented here are consistent with previous data showing that over-expression of some components of the IMD pathway are larval lethal. However, the molecular mechanisms by which over-activation of the IMD pathway leads to lethality remain unknown. A number of observations may provide clues about this issue: (i) several components of the IMD pathway are homologous to mammalian proteins involved in signaling through the TNF receptor, a pathway known to trigger apoptosis [38]; and (ii) the MAP3 kinase TAK1, which is an essential component of the IMD pathway, has been shown to function both as an IKK kinase (regulating diptericin expression) and as a JNK (c-Jun N-terminal kinase) kinase [39]. It is significant in this context that inappropriate activation of the JNK signaling cascade in the wing disc leads to apoptotic pathway-dependent morphological defects [40]. Further investigations will be needed to clarify the molecular links existing between the activation of the *Drosophila* IMD pathway and the developmental defects which we observed; in particular, a role of the apoptosis pathways in this process should be considered. Finally, it will be of interest to investigate whether amidases or other PGN-modifying enzymes are involved in modulating bacteria-induced immune response in mammals. In this respect, it is intriguing that one human PGRP family member (PGRP-II) is expressed in the esophagus [21], which evokes the gut expression of PGRP-SC.

Materials and Methods

**Bacterial strains.** The following microorganisms were used: *E. coli*, *M. luteus*, *E. carotovora carotovora* 15, *B. subtilis*, *E. cloacae*, *E. faecalis*, *S. aureus*, and *E. coli* AmpR.

*Drosophila strains.* IKK(Δα) is a loss-of-function mutation allele. PGRP-LCΔα is a complete deletion of the PGRP-LC locus. Flies carrying either of these mutations are unable to activate the IMD pathway. PGRP-SαM8 is a point-mutation null allele which prevents Toll-pathway activation by some bacteria. All these alleles have been previously described [8]. Daughterless Gal4 (DaGal4) and Caudal Gal4 (CaudGal4) are transgenic strains in which all (DaGal4) or only the gut (CaudGal4) cells express the yeast Gal4 transcription factor. In HGal4 flies, the production of the Gal4 protein is inducible by a heat pulse. DaGal4/UAS iPGRP-SC are strains in which all the cells produced PGRP-SC dsRNA targeting the endogenous PGRP-SC transcript to degradation by RNA interference. *Drosophila* strains produced include: PGRP-SA, PGRP-SC1a, PGRP-SC1b, PGRP-SC2, PGRP-SD, PGRP-LB, PGRP-LC, PGRP-LE, Ikky, and RpL32.

**Septic injuries, bacterial growth, and fly survival experiments.** Cells from overnight bacterial cultures were recovered by centrifugation at 3,000 g for 10 min at room temperature. The supernatant was discarded and the pellet was resuspended in fresh Luria-Bertani (LB) media. Cell suspensions were serially diluted in PBS, and the concentration of cells was determined by optical-density measurement. The number of colony-forming units per fly was determined through overnight growth on plates. Survival assays were repeated four times.

**Generation of UAS iPGRP-SC, UAS iPGRP-SA, and UAS PGRP-SC1b constructs.** The UAS iPGRP-SC plasmid was constructed by inserting a 558-bp PCR fragment corresponding to the PGRP-SC1a coding sequence flanked by BamHI and Nhel sites between the *Nhel–BamHI* sites (sense), and the *Xhel–BglII* sites (antisense), respectively, into the RNAi vector [41]; primers were as follows: forward 5’-GGGGGATCC ATGGTTTCTAAAGTGTCCCT-3’, reverse 5’-GGGGCTAAGC TACCCAGACCAGTGGGCA-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence; primers were as follows: forward 5’-GGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’. The same strategy was used for plasmid UAS iPGRP-SA by inserting a 612-bp fragment corresponding to the PGRP-SA coding sequence: primers were as follows: forward 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’, reverse 5’-GGGGGATCCATGGTTTCCAAATGGCTGCTT-3’.
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