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Review

Drosophila melanogaster innate immunity: an emerging role for peptidoglycan recognition proteins in bacteria detection

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Abstract. Over the past years, parallel studies conducted in mammals and flies have emphasized the existence of common mechanisms regulating the vertebrate and invertebrate innate immune systems. This culminated in the discovery of the central role of the Toll pathway in *Drosophila* immunity and in the implication of Toll-like receptors (TLRs)/interleukin-1(IL-1) in the mammalian innate immune response. In spite of clear similarities, such as shared intracellular pathway components, important divergences are expected between the two groups, whose last common ancestor lived more than half a billion years ago. The most obvious discrepancies lie in the mode of activation of the signalling receptors by microorganisms. In mammals, TLRs are part of protein complexes which directly recognize microbe-associated patterns, whereas *Drosophila* Toll functions like a classical cytokine receptor rather than a pattern recognition receptor. Recent studies demonstrate that members of the evolutionarily conserved peptidoglycan recognition protein family play an essential role in microbial sensing during immune response of *Drosophila*.

Key words. Innate immunity; *Drosophila melanogaster;* Toll; PGRP; antimicrobial peptides; peptidoglycan.

Innate and acquired immunity

The immune system faces two major tasks. First, it must detect all the would-be pathogens which could potentially infect the host, and if they do so, eliminate them. Second, it has to discriminate between the outside world 'non-self' and the constituents of 'self'. In vertebrates, this defense is controlled by two cellular components: the innate and the adaptive immune systems. Innate immunity arose before the separation of invertebrates and vertebrates, and most of the multicellular organisms exclusively depend on it to protect themselves. This system uses germ-line encoded receptors that recognize molecular patterns present on the surface of microorganims. The acquired or adaptive immunity is phylogenetically more recent and has been built atop the innate immune system, by which it is controlled and assisted [1]. In the absence of a functional innate system, the acquired response offers weak protection. The main effectors of adaptive immunity are blood cell receptors (immunoglobulins and T cell receptors), able to recognize any pathogen that the host might ever encounter. Through clonal expansion of lymphocytes, appropriate receptors are produced when necessary. Although both systems were discovered around the same time in the early 1900s, the fascinating discovery of somatic gene rearrangements to generate antibody diversity in the 1980s has drawn much attention to the study of acquired immunity. For the last 10 years, work done mainly in flies, plants and mice has proven how essential and conserved are the defense strategies used by the innate immune system to fight infection. In this review, I will examine the contribution of *Drosophila melanogaster* to our understanding of the mechanisms underlying the innate immune response. In particular, I shall focus on recent progress made on the role of the peptidoglycan

recognition protein (PGRP) family in microbial recognition by the immune system.

Drosophila melanogaster: a model for studying the invertebrate immune response

The *Drosophila* immune response is articulated around two arms which are almost simultaneously activated upon infection and are probably highly interdependent; the humoral and cellular defenses [2–4]. The most immediate response (within a few minutes) to the microbial invasion is the activation of proteolytic cascades that ultimately leads to the formation of black melanin deposits around wounds and foreign entities [5]. One essential step in this process is transformation of the inactive proenzyme prophenoloxidase (ProPO) into an active phenoloxidase enzyme. This enzyme, in turn, oxidizes phenol compounds, resulting in protein cross-linking and quinone polymerization. Proteases represent the largest family of proteins in the *Drosophila* proteome [6–8] and so far the identity of the protein specifically implicated in ProPO activation remains to be found. Although the ProPO zymogens are detectable in the circulating blood (hemolymph in insects), their site of production is in specialized blood cells (crystal cells), highlighting the links between cellular and humoral responses [9]. In addition to the crystal cells, the *Drosophila* bloodstream carries phagocyte-like hemocytes named plasmatocytes, which are reminiscent of the mammalian monocyte/macrophage lineage [9]. An additional cell type consisting of large flat cells called lamellocytes is absent from noninfected *Drosophila* larvae, and only differentiates to ensure encapsulation when parasites too big to be phagocytosed invade the body cavity [9]. These three blood cell types differentiate from pluripotent progenitors' niche in the lymph gland, the larval hematopoietic organ. Through genetic analysis and by analogy with mammalian studies, the gene cassettes which trigger blood cell differentiation into specific lineages are gradually being discovered [10–15]. Although very poorly understood at the molecular level, the role of blood cells in host defense has been shown to be essential. The results obtained with a mutant devoid of blood cells (*Domino*) suggest that hemocytes are not required to mount a systemic immune response after bacterial challenge [3, 16].

Antimicrobial peptides (AMPs) are evolutionarily ancient weapons found throughout the animal and plant kingdoms and are, by far, the best-studied and understood effectors of the *Drosophila* immune response [17, 18]. Absent from the hemolymph of uninfected flies, these amphipathic (hydrophobic and cationic) molecules are rapidly produced by the fat body (the equivalent of the mammalian liver) upon infection, and can reach micromolar concentrations in the blood within hours. The *Drosophila* genome contains at least 30 genes encoding AMPs, which include the broad-spectrum antibiotic peptides cecropins, the more specialized glycine-rich diptericin, which affects Gram-negative bacterial growth, and the antifungal peptide drosomycin [19].

Antimicrobial peptide gene transcription, a molecular read-out to dissect immune pathways

Following the path of developmental biology, *Drosophila* has recently appeared as a favorable model system to study invertebrate immunity. Using AMP gene transcription regulation as a molecular read-out of the humoral immune response, genetic screens carried out in multiple laboratories have provided us with a fairly detailed picture of the molecular cascades which control immune gene regulation in flies [1, 2, 4, 20, 21] (fig. 1). The starting point of this work came from the discovery that all promoters regulating spatiotemporal expression and immune inducibility of AMP genes share well-conserved nucleotidic sequences. Among them were nucleotide sequence motifs similar to mammalian nuclear factor kappa B (NF-κB)/Rel binding sites [22–25]. Work in the mammalian system has shown that in nonstimulated cells, NF-κB/Rel transactivators remain cytoplasmic by forming complexes with inhibitor of NF-κB (I-κB) [26]. Activation of specific upstream signaling receptors, such as interleukin-1R (IL-1R), results in I-κB

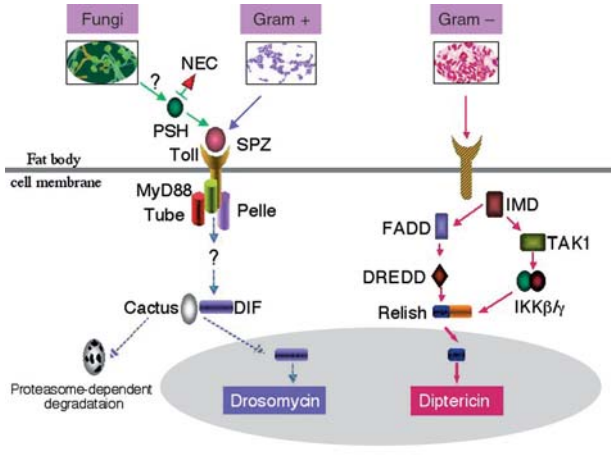

Figure 1. Molecular cascades controlling *Drosophila* innate immunity. This schematic representation illustrates the known components of two signaling pathways activated upon septic injury. The Toll pathway is triggered by Gram-positive bacteria and by fungi. It shows striking homologies with the IL-1R and TLR pathways, which are both key regulators of mammalian innate immune response. The IMD pathway is the main signalling cassette to control resistance to Gram-negative bacterial infection, and is homologous to the TNFα pathway in mammals. It was recently shown that the Toll/IMD dichotomy is not as strict as previously proposed, and that certain bacteria are able to trigger activation of both cascades simultaneously.

phosphorylation, rapidly followed by ubiquitination and proteasome-dependent degradation of the inhibitor [27]. Free from I-*κB*, the NF-κB proteins translocate into the nucleus where they activate target genes [26]. This basic cellular mechanism has been conserved in *Drosophila,* where both NF-κB and I-κB orthologues have been identified.

The Toll pathway

The *Drosophila* genome encodes three Rel proteins, Dorsal, Dif and Relish [6]. The *dorsal* mutant was isolated in studies aimed at identifying genes implicated in embryonic axis polarity [28–30]. Subsequent work led to the characterization of a cascade of genes necessary to regulate NF-κB/dorsal nuclear translocation. In this process, serine proteases present in the perivitelline space surrounding the embryo induce the cleavage of the cytokine nerve growth factor (NGF)-like molecule Spätzle (SPZ) [31–35]. Once activated, SPZ binds to the leucine-rich ectodomain of the transmembrane receptor Toll. This ligand/receptor interaction brings together the intracytoplasmic TIR (Toll/IL-1R) domain of Toll and the death domain-containing proteins DmMYD88, Tube and Pelle [36–40]. Formation of this protein complex ultimately triggers, by a yet unknown mechanism, the phosphorylation and degradation of the *Drosophila* I-κB*,* Cactus, and therefore the nuclear translocation of Dorsal [41]. The similarities with the cytokine-induced NF-κB-dependent activation of acute-phase response genes in mammals [42], prompted studies to probe whether the embryonic dorsoventral signaling cassette was reused, later, at larval and adult stages, to control AMP gene transcription. In an important report, Lemaitre and collaborators showed that a loss-of-function mutation in the *Toll* gene compromises both the survival of flies and the challenge-dependent transcription of the *drosomycin* gene after fungal infection [43]. It was later shown that the Toll pathway is also necessary for resistance to Gram-positive bacteria [44, 45]. Further studies revealed some differences between the Toll pathway that regulates patterning in the embryo and that of the immune response in the adult. Namely, the genes encoding the members of the proteolytic cascade leading to maturation of SPZ in the embryo (*gastrulation defective, snake, easter)* are dispensable for Toll-mediated induction of *drosomycin* in the adult [43]. Also unexpectedly, *drosomycin* remains fully inducible by fungal infection in *dorsal* adult mutant flies [43]. This apparent paradox was resolved by the demonstration that a mutation in another Rel protein, DIF, totally blocks *drosomycin* inducibility. This result indicates that a Dorsal-Cactus complex controls embryonic Toll-dependent gene expression, whereas a DIF-Cactus dimer is needed to regulate activation of immune effector genes after infection [46–49]. In addition to Toll itself, the *Drosophila* Toll family of proteins contains eight members. However, despite intensive efforts, no clear immune function has been attributed so far to any other *Drosophila* Tolls for which in situ expression patterns rather favor a role in development [50, 51].

The IMD pathway

Flies carrying null alleles of Toll are still able to mount a normal response to Gram-negative bacteria, suggesting that *Drosophila* has additional immune genes [43]. Indeed, the so-called IMD pathway is generally considered as the main cascade regulating immune response to Gram-negative bacteria in flies (fig. 1). In contrast to DIF in the Toll pathway, the Rel protein downstream of the IMD pathway is not inhibited by binding to Iκ-B/Cactus. This protein, called Relish, possesses a Rel DNA binding domain as well as ankyrin repeats in its sequence. In the absence of stimuli, the ankyrin repeats prevent nuclear localization of the Rel domain [52, 53]. Soon after infection, Relish is cleaved, allowing the physical dissociation between the ankyrin repeats and the Rel domain which is then free to translocate to the nucleus [54, 55]. Whilst the nature of the protease cleaving Relish remains elusive, genetic experiments led to identification of numerous proteins necessary for Relish cleavage and *diptericin* gene activation. Most of these genes have mammalian orthologues that are components of the tumor necrosis factor alpha (TNF*α)* pathway [56]. The *imd* gene itself codes for a death domain-containing protein sharing sequence homology with mammalian RIP (TNF receptor interacting protein), but devoid of a kinase domain [57]. Yeast two-hybrid screens and genetic epistatis have shown that IMD can form a complex with FADD and DREDD, a member of the caspase family [58–60]. Maturation of Relish after Gram-negative infection is also dependent on a protein complex homologous to the mammalian signalosome, comprising Iκ-B kinase (IKK)*β* and IKK*γ*/NEMO [61–63]. In mammals, the IKK signalling complex phosphorylates I-κB and targets it for proteasome degradation [26]. In flies, Cactus is also phosphorylated in response to Toll activation (41), but this is IKK independent in vivo, since IKK*β* (*ird5*) and IKK*γ* (*key*) mutants are not susceptible to fungal and Gram-positive bacterial infections [61, 62]. As mentioned earlier, the nature of the kinase which phosphorylates Cactus, both in embryogenesis and in immunity, remains to be found. Mutations in another *Drosophila* kinase (TAK1) produce phenotypes which are very similar to that of IKK [64]. In keeping with mammalian data, the easiest model would be that TAK1 is the IKK kinase in the immune deficiency (IMD) pathway [65]. Interestingly, DNA microarrays data indicate that the IMD pathway branches downstream

of TAK1, leading to activation of the Jun kinase (JNK) pathway [66]. It is therefore likely that activation of the IMD pathway could trigger AMP gene expression to fight infection, and the JNK pathway for tissues repair and wound healing. These experiments have been done in cell cultures, and it remains to be shown in which tissues in vivo this JNK pathway activation is taking place.

In conclusion, two signalling pathways converging on Rel-domain-containing proteins control the humoral immune response. In *Drosophila*, the Toll/DIF regulatory cassette responds mainly to fungi and Gram-positive bacteria, and IMD/Relish to Gram-negative bacterial infection. Each cascade presents striking similarities with gene cassettes implicated in mammalian immune response, namely the TLR/IL-1 and TNFα pathways (fig. 1). These similarities point towards evolutionary conservation between vertebrate and invertebrate innate immunity. It should be mentioned that this dichotomous separation associating Toll and Gram-positive bacteria on the one hand and IMD and Gram-negative bacteria on the other is based on data obtained a low number of bacterial strains, mostly *Micrococcus luteus* and *Escherichia coli*. A number of data indicate that both pathways are physiologically needed to efficiently fight Gram-positive or Gram-negative infection, activation of both pathways by *Bacilli* being one of the most convincing demonstrations for cooperation between the pathways [T. Michel and J. Royet, personal communication] [67].

Upstream of the Toll and IMD pathways

The absence of immune phenotypes associated with mutations in Snake, Easter and Gastrulation defective proteases raises the question of the mode of activation of the Toll receptor during the immune response. This issue was partially answered by the phenotypic analysis of flies mutant for a serine protease inhibitor of the serpin family. In this mutant referred to as *necrotic* for the presence of melanotic spots on the cuticle, SPZ is present in its cleaved form and Drosomycin is constitutively produced in a Toll-dependent manner [68]. *nec* mutants exhibit normal embryonic dorso-ventral axis patterning, suggesting that NEC inhibits proteases distinct from EA, SNK or GD. Looking for mutations which would suppress the *nec* gain-of-function phenotype (spontaneous melanization and Drosomycin production), Lygoxigakis and collaborators identified a trypsin-like protease that mediates the fungal-dependent cleavage of SPZ [69]. These results suggest that Toll activation in the adult, as in the embryo, is controlled by a balance between proteases and serpins which ultimately leads to the cleavage of SPZ into a 12-kDa mature protein that binds to the ectodomain of Toll [34, 70]. Specific host proteins, probably on epithelia or in the blood, would then mediate pathogen recognition. This situation contrasts with that of mammalian Toll-like-receptors, which are part of protein complexes which directly interact with microbial motifs [71]. This is illustrated by the binding of the Gram-negative cell wall component lipopolysaccharide (LPS) to a complex made of TLR4, MD-2 and CD-14, or that of peptidoglycan (PGN) and lipotechoic acid (LTA) to TLR2 [72, 73]. Despite numerous genetic screens, the nature of the *Drosophila* proteins that detect microbes when they enter the fly body remains elusive. Recent observations show that, surprisingly, the evolutionarily conserved PGRP family is essential in this recognition step, upstream of both pathways.

The PGRP family

PGRP proteins were discovered almost simultaneously in the silkworm *Bombyx mori* and the mouse *Mus musculus* using two totally unrelated assays. Looking for proteins required for hemolymphatic ProPO activation by peptidoglycan, Ashida and collaborators identified a 19-kDa circulating protein [74, 75]. Based on its restricted affinity for Gram-positive bacterial peptidoglycan (it does not bind lipopolysaccharide or chitine), they named this protein PGRP. Kiselev and collaborators cloned a mouse complementary DNA (cDNA) orthologue by screening a tumor cell line library and called it Tag7 for Tumor-associated gene [76]. Subsequent completion of genome sequencing from multiple organisms has demonstrated that PGRP is an evolutionarily conserved family of proteins. Absent from the *Caenorhabditis elegans* genome, they are found in flies, mosquitoes and humans, although the 'usual' ratio of 1 fly gene to 4 vertebrate orthologues does not apply here [77–80]. The *Drosophila* genome contains 13 PGRP genes. Two genes (PGRP-SC1a and -SC1b) are clustered on the second chromosome and differ only by two nucleotides, suggesting that they originated from a very recent gene duplication event. The *Drosophila* PGRP family can be divided into two subfamilies (fig. 2). The 7 small PGRP-S are ~200-aa-long proteins containing one PGRP domain, a putative signal peptide and no transmembrane domain, which suggests that they are extracellular proteins. Ranging from 200 to 600 aa, the members of the PGRP-L subfamily (6 members) are more heterogeneous. Some are predicted to be membrane-spanning proteins (PGRP-LC, LD, LA), or possibly intracytoplasmic (PGRP-LB, PGRP-LE) for lack of a signal peptide or transmembrane domain [81]. Whereas most of the PGRP-L molecules have only one PGRP domain, computer predictions suggest that some could have two (PGRP-LF). More intriguingly, the gene architecture of a few PGRP genes is compatible with alternative splicing, which could produce proteins with specific PGRP

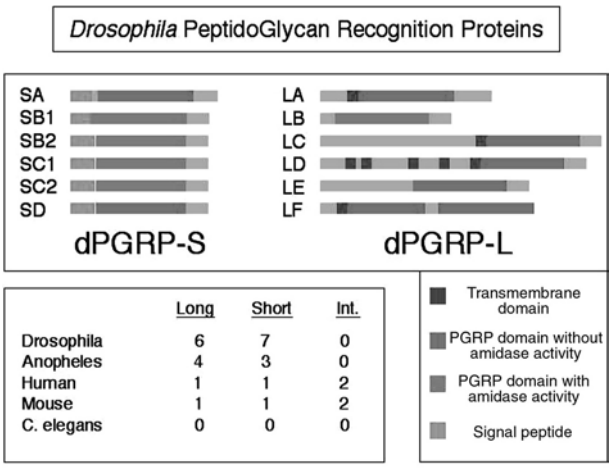

Figure 2. *Drosophila* PGRP family.

domains. Indeed, two isoforms of PGRP-LC (LC-a and LC-x) have been shown to code for proteins sharing intracytoplasmic tail and transmembrane domain, but each having a different PGRP domain [81–83]. Alternative splicing, which seems to be the rule for the PGRP-L members, and absent in the small subfamily, is also a way of creating diversity in *Anopheles* PGRPs [80]. Recent completion of mouse and human genome sequencing led to the identification of 4 PGRP proteins in mammals, 1 PGRP-S, 1 PGRP-L, and 2 PGRP-I (for intermediate), which have no insect homologues [78]. Phylogenetic analyses demonstrate that mammalian PGRP-L originate neither from *Drosophila* PGRP-L nor from mammalian PGRP-S or PGRP-I, but directly from a common ancestor of insect PGRP-S. The common denominator of all PGRP family members is a 160-aa conserved motif known as the PGRP/amidase domain.

The PGRP/amidase domain

The only domain shared by the tens of PGRP proteins encoded in eukaryotic genomes is the 160-aa-long PGRP/amidase protein motif. Homology sequence comparison shows clear similarities between this PGRP domain and an amino acid stretch found in *N*-acetylmuramoyl *L*-alanine amidases [84, 85]. These bacterial enzymes function by hydrolyzing the bond between the *N*-acetylmuramoyl group in the glycan strand and the *L*-alanine in the stem peptide of peptidoglycan. As a consequence, the bacterial cell wall is slowly degraded, allowing its continuous renewal. The prokaryotic amidase showing the highest sequence similarities with eukaryotic PGRP is the bacteriophage T7 lysozyme, which cleaves the PGN in the presence of Zinc [84, 86]. Sequence similarities and analysis of specific residues required for amidase activity seem to indicate that at least half of the *Drosophila* PGRP family members could posess an amidase activity [87] (fig. 2). Indeed, Steiner's group has shown that recombinant PGRP-SC1b protein is as efficient as hen egg white lysozyme for degradation of *Staphylococcus aureus* PGN [87]. As a consequence, the immunostimulatory properties of PGRP-SCIb-treated PGN are significantly lower than those of naive PGN. *PGRP-SCIb* proteins lacking a potential Zn ligand are enzymatically inactive in vitro but retain their ability to bind PGN. The close relationship between PGRPs and amidases has been further confirmed by the demonstration that the soluble form of m-PGRP-L is identical to the previously characterized mammalian serum *N*-acetylmuramoyl *L*-alanine amidase [84, 88].

Some PGRPs can function as pattern recognition receptors

Although not demonstrated in vivo, biochemical characterization of PGRP-SC1b enzymatic activity tends to indicate a possible scavenger function for some PGRPs in *Drosophila* immunity [87]. Once a bacterium is present in the circulation, its PGN is probably degraded by PGRP-SC1b, eventually resulting in bacterial clearance from the hemolymph. While this type of function could be performed by some PGRP family members, others lack essential residues (especially a cysteine at position 130 of the PGRP domain), required for amidase activity, suggesting alternative function for the remaining proteins. In keeping with this hypothesis, recent data attributed a pattern recognition function to two members of the family, PGRP-SA and PGRP-LC. By screening ethyl-methyl-sulfonate (EMS) induced immunocompromised flies, our laboratory isolated the first mutation to inactivate a PGRP molecule in vivo [45]. We named this mutation *Semmelweis*, after Ignaz Philipp Semmelweis, a Hungarian physician who was a pioneer in the field of antiseptic treatments and discovered the cause of puerperal fever. The *semmelweis (seml)* mutation corresponds to a transformation of a well-conserved cysteine residue into a tyrosine in the PGRP-SA protein, a member of the short subfamily of PGRPs. In *seml* mutants, Toll-dependent immune response to Gram-positive bacteria is severely impaired (fig. 3). As a result, *seml* mutant flies show strongly reduced survival rates when challenged by Gram-positive bacteria. Interestingly, the mutation in the *PGRP-SA* gene does not affect either Toll activation by fungi, or the pathway triggered by Gram-negative bacteria. These results suggest the existence of additional pattern recognition receptors (PRRs) specific for fungi or Gram-negative bacteria. Results obtained simultaneously by three laboratories provide support for this hypothesis. Using RNA interference or EMS/transposon-induced mutagenesis, all groups looked for im-

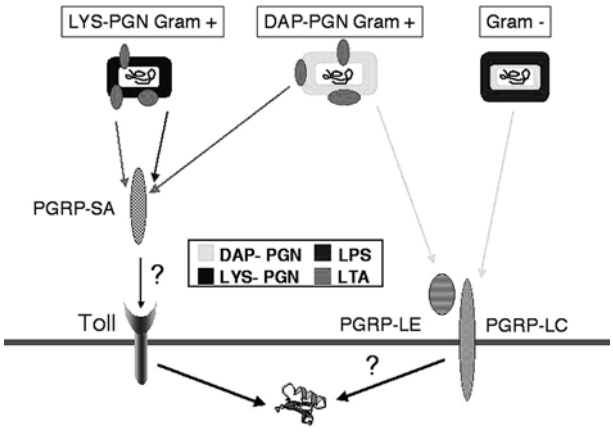

Figure 3. Mode of activation of the Toll and the IMD pathways by bacterial cell wall components. The LYS-PGN and the LTA are both able to activate the Toll pathway. The main activator of the IMD pathway is the DAP-PGN. LPS is a very poor inducer of the immune response in *Drosophila*.

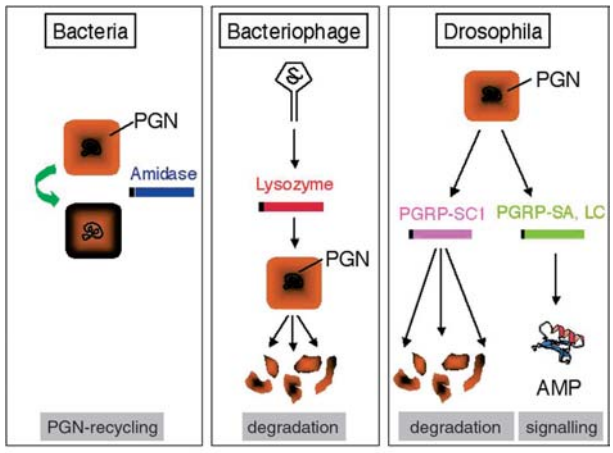

Figure 4. Model illustrating the role of PGRP/amidase domain-containing proteins across evolution. Bacterial cell growth requires continuous PGN remodeling, as 40–50% of the PGN cell wall is degraded at each generation. Amidases belongs to the autolysin family of proteins, which participate in PGN renewal by cleavage at specificic sites. Bacteriophage lysozyme posesses amidase activity required to infect bacteria. In *Drosophila*, two classes of PGRPs are found: the proteins devoid of amidase activity seem to be involved in bacterial sensing and activation of dowstream signalling cascades (PGRP-SA, LC or LE). The other PGRPs have conserved amino acid residues which are essential for amidase activity. It has been shown that one member of this subfamily (PGRP-SC1b) could act as a scavenger protein.

mune phenotypes associated with loss-of-function mutations in the *PGRP-LC* gene [82, 83, 89]. The results indicate that a functional *PGRP-LC* gene is required for normal activation of the pathway by Gram-negative bacteria (fig. 3). In the absence of a known receptor for the pathway, it has been proposed that the transmembrane PGRP-LC protein could function as a receptor for the cassette. However, complete loss-of-function *PGRP-LC* mutants are less susceptible to Gram-negative bacterial infection than mutants which inactivate intracytoplasmic components such as *key* or *dredd* [83]. This could reflect the necessity for a receptor complex upstream of IMD. The PGRP-LE protein whose overexpression triggers IMD pathway activation, could be such a partner for PGRP-LC [90]. Precise characterization of the role of PGRP-LE in the IMD pathway will have to wait for a mutant in this gene. Altogether, these results highlight the central role for PGRP molecules in sensing both Gram-negative and Gram-positive bacteria during *Drosophila* immunity. It is fascinating that in the course of evolution, a prokaryotic enzyme necessary for PGN renewal at the surface of the bacteria has been recruited and turned against bacteria by the eukaryotic immune system. PGRPs seem to play a dual role in the immune system (fig. 4). On the one hand, some PGRP family members (PGRB-SC1B) have conserved this enzymatic activity and probably participate in bacteria blood clearance. On the other hand, other PGRPs, such as PGRP-SA and LC, have kept the ability to bind PGN but have lost the amidase activity, and function by alerting the immune system and by triggering signalling pathways which in turn activate the transcription of antimicrobial effectors (fig. 4).

Which are the bacterial ligands for PGRP?

To better understand the mode of activation of the intracellular pathways in immunocompetent cells, it is necessary to identify the microbial elicitors that trigger an immune response and the host proteins which detect them. In this respect, *Drosophila* research is lagging behind mammalian studies, from which spectacular progress has been achieved as far as non-self recognition by PRR goes [91, 92]. However, it should be emphasized that the search for bacterial elicitors able to activate the immune system has been hindered, in both invertebrate and vertebrate studies, by the difficulty of obtaining totally pure preparations of microbial motifs devoid of any contaminants.

It came as a big surprise that recognition of both Gram-negative and Gram-positive bacteria by the *Drosophila* immune system involved receptors thought to recognize PGN. However, while the PGN binding ability of some PGRP-S from various species have been tested in vitro [81, 87] there is no available information on the microbial motifs recognized by PGRP-L in invertebrates. It should be added that in mammals, the recognition specificity of some TLRs is very broad, ranging from LPS to PGN via lipoarabinomannans [91]. So far, only three major microbe-associated molecular patterns have been tested for their ability to induce the *Drosophila* immune system, namely LPS of Gram-negative bacteria, LTA of Gram-

positive bacteria and peptidoglycan. In mammals, recognition of endotoxin or LPS is an important function of the innate immune system. Failure to control infection can result in Gram-negative sepsis and septic shock as a result of LPS release [72]. Surprisingly, when injected into the fly body cavity, LPS has very limited effects on the activation of the immune response, indicating that LPS is not a major microbial inducer in flies [93]. Recent data point towards an important role of PGN in inducing an immune response in *Drosophila*. Although the structure of PGN is different in every single bacterial strain, bacteria can be subdivided into two large subfamilies. The diaminoacid type peptidoglycan (DAP-PGN) is found in Gram-negative cell walls and in some Gram-positive bacteria of the *Bacilli* genus. The Lysine type peptidoglycan (LYS-PGN) is present exclusively on Gram-positive cell walls. Using highly purified elicitor preparations, Leulier and collaborators showed that DAP-PGN induces the IMD pathway in a PGRP-LC-dependent manner [93]. Injection of LYS-PGN induces the Toll pathway, and this effect is blocked in PGRP-SA mutants. These results indicate that the ability to discriminate between Gram-positive and Gram-negative bacteria could rely on the recognition of specific PGN types by specific PGRP family members (fig. 3). Although performed on limited numbers of bacterial strains, binding experiments between recombinant PGRPs and insoluble PGN seem to confirm the respective affinity of the Toll pathway-associated PGRP-SA for LYS-PGN, and of the pathway PGRP-LE for DAP-PGN [90]. If these results fit well with the ability of Gram-positive *Bacilli* to activate the IMD pathway, they cannot explain how the same bacteria simultaneously stimulate the Toll pathway. Work done in our laboratory shows that *S. aureus* LTA is also an inducer of the Toll pathway, an effect also mediated by PGRP-SA [J. Royet and D. Ferrandon, personal communication] (fig. 3). *Bacilli* could therefore activate the IMD pathway through DAP-PGN, and the Toll pathway through LTA. Another possibility will be that both LTA and DAP-PGN [93] activate the Toll pathway. Further work will be needed to find the minimal structures recognized by these PRRs and to demonstrate a direct binding between PRRs and PAMPs in *Drosophila* [94]. From these recent resuts, we would like to propose that *Drosophila* PGRPs could form a combinatorial repertoire for bacterial detection similar to that described for TLRs in mammalian innate immunity [95]. This could explain why each microorganism which is used to infect *Drosophila* induces a specific profile of antimicrobial peptides [96].

Conclusions

The function of Toll in *Drosophila* host defense inspired the search for orthologues involved in mammalian innate immunity. This culminated in the discovery of a family of TLRs which sense a large spectrum of microbial patterns. The structural and functional similarities between Toll activation of *Drosophila* Rel proteins and TLR-dependent activation of NF-κB has been interpreted as evidence for the existence of a common ancestor. Recent development in both fields, and more specifically in microbial sensing mechanisms, points to important differences. Whereas all known TLRs have an immune function, so far only one out of the 10 *Drosophila* Toll proteins, although not the closest to TLRs in terms of phylogeny, is undoubtedly an immune gene; the others probably play developmental functions. In *Drosophila*, non-self recognition is mediated through recognition of microbial motifs by pattern recognition proteins belonging to the PGRP family, leading, via protease cascades, to the cleavage of SPZ, and therefore to Toll activation. In contrast, TLRs appear to form complexes with coreceptors that directly interact with microbial motifs. Despite extensive progress, the present picture is still fragmentary, and the wealth of data on TLRs could have somewhat overshadowed the role of other molecules in detecting microbes during mammalian innate response. In this respect, it came as a surprise that children with an inherited IL-1 receptor-associated kinase (IRAK) deficiency, and therefore unable to transduce signals from all the TLRs, develop infections to a very limited spectrum of bacteria [97]. Clearly, other PRR molecules are essential for non-self recognition in vertebrates. Among others, one can mention NOD/CARD leucine-rich repeat (LRR) containing proteins and Dectin, which have recently been shown to be essential sensors for microbe-associated motifs [98–102]. In view of these new developments, and after analyzing PGRP mice knockout phenotypes in detail [103], parallels between vertebrate and invertebrate non-self-recognition strategies could be significantly reevaluated.

As far as insect immunity is concerned, the future challenges lie in dissection of the gene cascade which links microbial detection and intracytoplasmic pathway activation. Finding the role of other putative PRRs, such as the 10 remaining PGRPs, the GNBPs [104] or macrophage scavenger receptors [105], will be of great interest to understand the finely tuned regulation of immunity against a large number of microorganisms. In this respect, approaches using broader varieties of microbes, and aimed at identifying *Drosophila* commensal microbes and natural pathogens, will lead to a more physiological understanding of *D. melanogaster* immunity.

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