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Review

General overview on the role of Peptidoglycan Recognition Proteins in insect immunity

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The insect immune system is responsible for maintaining the homeostasis of organisms. If the pathogen is able to breach the defensive barriers of the host, cellular and humoral mechanisms are triggered. Initiation of effective defence response is possible thanks to pathogen-associated molecular patterns, among which peptidoglycan recognition proteins play a prominent role. They recognize pathogen-associated molecular patterns and some of them also have enzymatic activity. The main aim of peptidoglycan recognition proteins is to activate pathways regulating the synthesis of immune peptides. Some of the peptidoglycan recognition proteins are involved in the phagocytosis process, activation of the prophenoloxidase cascade, and regulation of the xenophagy process. The structural diversity and high specificity of peptidoglycan recognition proteins suggests that they can serve many previously unknown functions in insect's systemic response.

Key words: insect immunity, PAMPs, PGRPs, PRRs, peptidoglycan

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Abbreviations: DAP-PGN, diaminopimelic acid type peptidoglycan; GlcNAc, N-acetylglucosamine; GNBPs, Gram-negative binding proteins; Lys-PGN, Lysine-type peptidoglycan; MurNAc, N-acetylmuramic acid; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PAMPs, Pathogen-Associated Molecular Patterns; PGN, peptidoglycan, proPO, prophenoloxidase; PGRPs, Peptidoglycan Recognising proteins; PRRs, pattern recognition receptors; TLR/IL-1R, Toll-like receptor/Interleukin (IL)-1 receptor; TNF, tumor necrosis factor.

INTRODUCTION

Innate immunity is evolutionarily well conserved and relies on the recognition of self and non-self. Pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides (LPS), peptidoglycan, B-glucan, and others are part of the pathogen structure but are not present in the host's organisms. These structures are recognized by host's pattern recognition receptors (PRRs), which detect infection and "transfer this information" to other components of the immune system. This event allows the infected host to induce immune response. Among PRRs, there are peptidoglycan recognition proteins (PGRPs). They have probably evolved from amidases cleaving peptidoglycan between a polymer consisting of N-acetyloglucosamine and N-acetylmuramic acid and peptide bridges. Although many PGRPs have lost their enzymatic properties, they are still able to bind their (former) substrate. These particles have become very important in innate immunity. In this short review article, we describe insect's PGRP and summarize their regulatory role in several aspects of insect immunity.

SHORT DESCRIPTION OF INSECT IMMUNITY

Insects constitute a very good model, which is increasingly being used in scientific research. Despite the lack of acquired immunity characteristic for vertebrates, insects have mechanisms of innate response, which have a series of common features in both vertebrates and invertebrates. Noteworthy is the fact that insects are phylogenetically older organisms; hence, research conducted on insects can be further checked in vertebrates. The fruit fly *Drosophila melanogaster* is one of the most commonly used insect model organisms (Royet, 2004). Research carried out with the use of insects focuses on understanding the mechanisms of innate immunity and its regulatory mechanisms in response to infection with pathogens (Kurata, 2010; Dziarski & Gupta, 2018). The cuticle is the first line of defence in insects, protecting from pathogen entry. Besides the mechanical function, insect epidermis synthesises antimicrobial peptides (Tzou *et al.,* 2000). Overcoming the first line of defence by an intruder triggers a series of cellular and humoral reactions in the insect's body (Kurata, 2010). Cellular reactions include phagocytosis, nodulation, and encapsulation processes. The main reactions representing the group of humoral mechanisms include activation of the phenoloxidase (PO) cascade leading to synthesis of a dark pigment melanin and the hemolymph coagulation process (Ramet *et al.,* 2002; Hultmark, 2003). One of the most important elements of the humoral response is the activation of signalling pathways regulating the production of a number of antimicrobial peptides (Lemaitre & Hoffmann, 2007). Antimicrobial peptides are usually 2-10 kDa amphipathic amino acid chains expressing antimicrobial properties. They play an essential role in the fight against pathogens. The systemic production of antimicrobial peptides takes place in the fat body, which is an analogue of a mammalian liver due to its high metabolic activity. Peptides synthesised in the fat body are secreted to the insect hemolymph. Seven antimicrobial peptide groups have been identified in the fruit fly so far: attacins, cecropins, defensins, diptericins, drosocins, drosomycins, and metchnikowins (Kurata, 2010). The regulation of the expression of genes encoding antimicrobial peptides is mediated by a transcription factor belonging to the *Rel* family. This family also includes the NF-κB factor involved in the immune response of mammals (Akira *et al.,* 2006; Lemaitre & Hoffmann 2007). The activation of *Rel* family transcription factors can occur in two different ways: *via* Toll and/or Imd signalling pathways (Lemaitre *et al.,* 1997; Dziarski & Gupta, 2006).

Both pathways show similarity to the mammalian TLR/IL-1R and the tumour necrosis factor (TNF) alpha receptor signalling pathways (Engstrom, 1997; Khush *et al.,* 2001; Hultmark, 2003). The activation of the Imd pathway occurs when Gram-negative bacteria are detected in the host organism, while the Toll pathway is triggered in the case of infection with Gram-positive bacteria and fungi. It can be concluded that insects must have the ability to identify pathogens and distinguish between infection with Gram-positive and Gram-negative bacteria that infect their bodies (PAMPs) (Charroux *et al.,* 2018; Dziarski & Gupta, 2018).

PATHOGEN-ASSOCIATED MOLECULAR PATTERNS AND PATTERN RECOGNITION RECEPTORS

An effective immune response is possible thanks to its rapid activation. Stimuli for activation of the immune system are provided by so-called pathogen associated molecular patterns (PAMPs), which are elements of the pathogen's structures (Kurata, 2010). Particles representing PAMPs do not occur in the physiological state in the host's organism; therefore, they can serve as a differentiating factor between self and non-self (Matzinger, 2002; Steiner, 2004). Molecules defined as PAMPs have certain repetitive patterns in their structure, which are common for large groups of microorganisms. PAMPs include for example lipopolysaccharide, lipoteichoic acids, zymosan, β-1,3-glucan, flagellin (Hultmark, 2003), and peptidoglycan (Steiner, 2004).

Peptidoglycan (PGN, also referred to as murein or "sacculus") is an essential component of most bacterial cell walls (Vollmer *et al.*, 2013). The peptidoglycan layer is responsible for the species-specific shape of the bacteria and protects the cell from bursting due to its high turgor (Vollmer *et al.*, 2008). Murein is generally made of glycan strands of alternating β-1-4 connected N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNAc) residues, which are cross-linked with short peptides. Each MurNAc is attached to a short peptide composed of 4-5 amino acid residues, which vary between bacterial species. It is worth mentioning that peptide chains are unique and contain D-amino acids (D-Ala, D-Glu, D-Gln) as well as meso-diaminopimelic acid. In almost all Gram-negative bacteria, a pentapeptide built of L-Ala–D-Glu–meso-DAP–D-Ala–D-Ala is the linking peptide (Fig. 1). In Gram-positive bacteria, the variation of the peptide sequence occurs at the second (D-iGlu→D-Gln) and third (meso-DAP→L-Lys) positions (Schleifer and Kandler, 1972; Vollmer, 2015). During peptidoglycan maturation, many pentapeptides are hydrolyzed to tetrapeptides or tripeptides, which results in loss of one or two D-Ala. The stem peptides are mostly cross-linked between D-Ala at position 4 and the meso-DAP at the position 3 (Vollmer, 2013). Pepti-

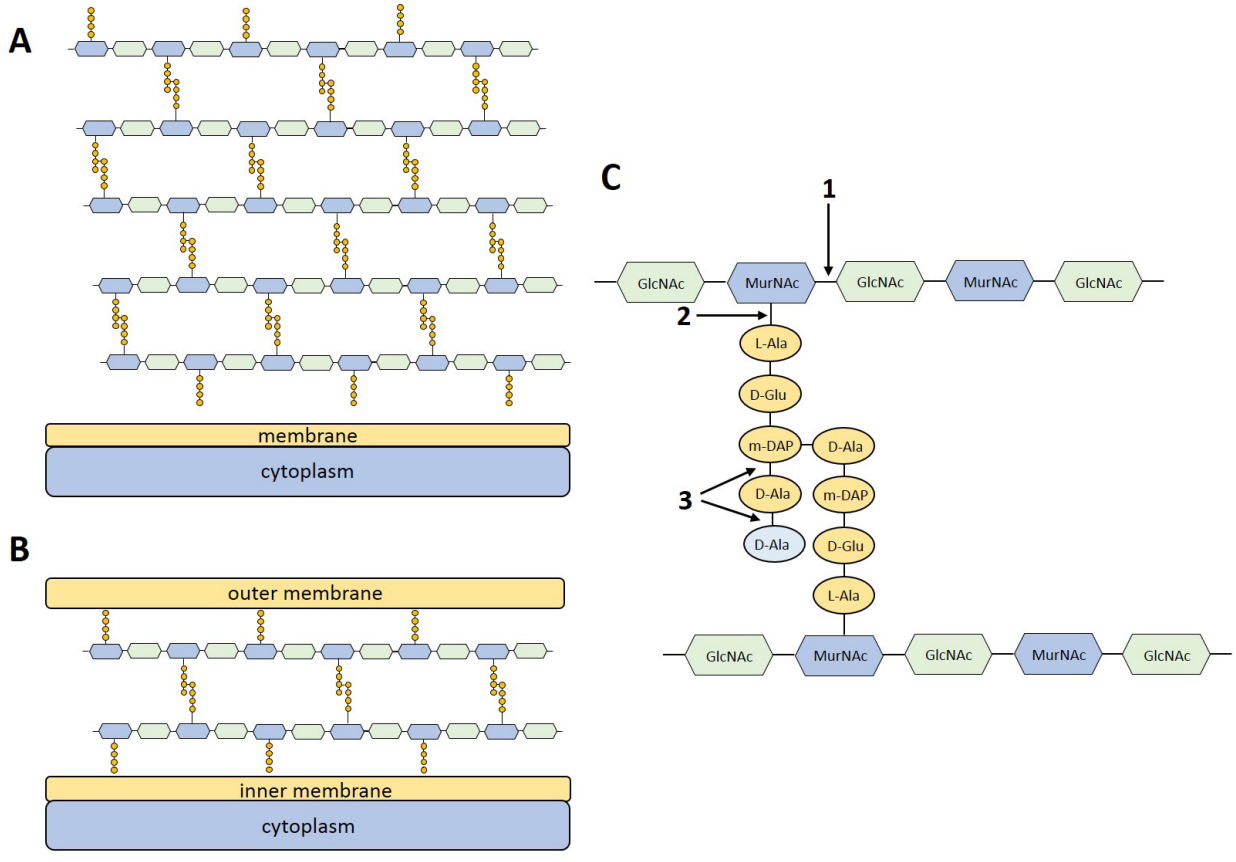

Figure 1. Scheme of the cell wall of Gram (+) and Gram (–) bacteria and the peptidoglycan structure.
A–B. Gram-positive bacteria are characterized by a thick outer layer of peptidoglycan, which is easily accessible for lysozyme. In contrast, Gram-negative bacteria usually possess a single layer of peptidoglycan between membranes. This structure provides protection against exogenous hydrolases such as lysozyme. C. Basic structure of Gram-negative peptidoglycan. In Gram-positive bacteria, the variation in peptide bonds occurs at the 2nd (D-Glu→D-iGln) and 3rd (m-DAP→L-Lys) positions. Arrows indicate the possible enzymatic activities of PGRPs and lysozyme. (1) Muramidase activity cleaving the β-1-4 bond between MurNAc and GlcNAc. (2) Amidase activity breaking the amide bond between MurNAc and the peptide bridge. (3) Carboxypeptidase activity of PGRP-SA, which specifically recognize and cleave only diaminopimelic acid-type tetrapeptide PGN (Chang *et al.*, 2004). The blue-marked D-Ala is lost during the maturation of PGN.

doglycan can be efficiently cleaved by various enzymatic PGRPs (see the text below) and lysozyme. Lysozyme is a muramidase, which cleaves the β-1-4 bond between MurNAc and GlcNAc (Paredes *et al*., 2011). However, due to the architecture of the Gram-negative cell wall, the muramidase activity is efficient only against Gram-positive bacteria. The sites of PGRPs and lysozyme enzymatic activities are presented in Fig. 1. Peptidoglycan and other PAMPs are recognised by pattern-recognition receptors (PRRs), which are molecules present in the host. PRR molecules can be found both inside and on the surface of cells. They can also be secreted to the hemocoel. PRRs are represented by peptidoglycan recognition proteins (PGRPs) (Hultmark, 2003; Kurata, 2004; Steiner, 2004; Wang *et al*., 2019).

PEPTIDOGLYCAN RECOGNITION PROTEINS – PGRPS

Peptidoglycan recognition proteins (PGRPs) were discovered by Yoshida and co-authors, who purified a 19-kDa protein from the hemolymph and cuticle of *Bombyx mori* in 1996 (Yoshida *et al.,* 1996). It has been proven that this protein has a high affinity toward peptidoglycan derived from Gram-negative bacteria and is involved in activation of prophenoloxidase responsible for the production of melanin (Steiner, 2004). Further research focused on cloning of orthologues of its gene. Shortly after the discovery of PGRPs in *Bombyx mori*, these proteins were identified in *Trichoplusia ni* and were shown to increase their level significantly in response to bacterial infection (Kang *et al.,* 1998). Further studies have shown that PGRPs are widespread and occur in other invertebrates (except lower metazoan like *Coenorhabditis elegans*) and in vertebrates, including humans, where 4 PGRP groups were identified (Liu *et al.,* 2000; Royet, 2004). *Drosophila* genome sequencing revealed 13 genes encoding over 20 PGRP proteins. The genes are located at eight *loci* on three chromosomes (Werner *et al.,* 2000; Neyen *et al.,* 2016). In terms of the size of their transcripts, PGRPs can be divided into two main classes. The first group includes so-called short PGRPs, which have a signal sequence at the N-terminal end (PGRP-SA, PGRP-SB1, PGRP-SB2, PGRP-SC1A, PGRP-SC1B, PGRPSC2, PGRP-SD) (Steiner, 2004). The second group has long transcripts resulting in long transmembrane PGRPs (PGRP-LA a, PGRP-LA b, PGRP-LC x, PGRP-LC y, PGRP-LD, PGRP-LF) and secretory PGRPs (PGRP-LA c, PGRP- LB, PGRP- LE) (Werner *et al.,* 2000; Steiner, 2004).

The main sites of expression of PGRP genes are tissues involved in the insect immune response. It is worth noting that the induction and expression of a number of PGRP genes occur in specific locations of the body, which is associated with specific functions assigned to specific PGRPs (El Chamy *et al.,* 2008). PGRP-SB1 and

Table 1. Types of PGRPs and summary of their expression and function

	Type of PGRP	Expression (type/ place)	Function	Literature
PGRP-SA	short, secretory	inducible/fat body	Toll activation	Michel *et al*., 2001; Royet & Dziarski 2007
PGRP-SB1	short, secretory	inducible/ fat body	Antibacterial amidase function	Ligoxygakis, 2002; Kurata, 2010
PGRP-SB2	short, secretory	inducible/ fat body	Amidase function	Kurata, 2010; Mellroth & Steiner, 2006
PGRP-SC1A	short, secretory	constitutive/ fat body, gut	Amidase function, phagocytosis	Dziarski, 2004; Kurata, 2010
PGRP-SC1B	short, secretory	constitutive/ fat body, gut	Amidase function	Mellroth *et al*., 2003; Kurata, 2010
PGRP-SC2	short, secretory	constitutive/fat body, gut	Amidase function	Ligoxygakis, 2002; Dziarski, 2004; Kurata, 2010
PGRP-SD	short, secretory	inducible/ fat body	Toll activation	Ligoxygakis, 2002; Dziarski, 2004; Kurata, 2010
PGRP-LA a	long, transmembrane	constitutive/ hemocytes	Imd activation	Dziarski, 2004; Gendrin *et al*. 2013
PGRP-LA b	long, transmembrane	constitutive/ hemocytes	Imd activation	Dziarski, 2004; Gendrin *et al*. 2013
PGRP-LC	long, transmembrane	constitutive/ hemocytes	Imd activation, phagocystosis	Choe *et al*., 2002; Ramet *et al*., 2002
PGRP-LD	long, transmembrane	constitutive/ hemocytes	Amidase function	Dziarski, 2004
PGRP-LF	long, transmembrane	constitutive/ hemocytes	Negative regulation of Imd pathway	Dziarski, 2004; Kurata 2010
PGRP-LA c	long, secretory	constitutive/ hemocytes, epithelial cells	Imd activation	Dziarski, 2004; Dziarski & Gupta, 2006
PGRP- LB	long, secretory	constitutive/ gut	Amidase function	Kim *et al*., 2003; Mellroth & Steiner, 2006; Zaidman- Remy *et al*., 2006
PGRP- LE	long, secretory	constitutive/ fat body, hemolymph	Imd, autophagy and PPO activation	Takehana *et al*., 2002; Kurata 2010

PGRP-SD2 genes are expressed in the fat body, i.e. one of the main organs involved in immune response. In the intestine, where contact with many entomopathogens occurs, PGRP-SC is expressed constitutively (Liu *et al.,* 2000; Ligoxygakis, 2002). The expression of long forms of PGRPs takes place in hemocytes, which are morphotic constituents of hemolymph engaged in cellular immunity (Dziarski & Gupta, 2006). Different types of PGRPs are presented in Table 1.

PGRP STRUCTURE

Due to the common ancestry, regardless of the group membership, in each PGRP one can distinguish at least one C-terminal PGRP domain, which structurally resembles bacterial N-acetylmuramyl-alanine muramidase and bacteriophage T7 lysozyme due to the presence of three peripheral α-helices and several central β-strands (Reiser *et al.,* 2004; Guan *et al.,* 2006). There is a groove in front of the molecule with a strong affinity toward peptidoglycan, which is the PGN binding site. About half of the residues in this hydrophilic groove are highly conserved, with tyrosine (Y^{76} in PGRP-SA) and threonine (T^{156} in PGRP-SA) residues, together with a water molecule that is supposed to be essential for PGN binding (Reiser *et al.,* 2004). The binding of the peptidoglycan molecule leads to structural changes in the PGRP domain or interaction with subsequent PGRP.

There are two main groups of PGRPs: catalytic and recognition proteins. The latter bind peptidoglycan but do not cleave it. The differences in these types of PGRPs lie in the bottom of the PGRP binding grove, which does not affect the capability to bind PGRP but determines whether a given PGRP has amidase activity. The enzymatic PGRPs contain conserved residues for Zn ion binding, which are absent in the recognition PGRPs, thus they are not able to bind Zn ions necessary for catalysis (Reiser *et al.,* 2004). The PGRP domain length is about 165 amino acids, which corresponds to most PGRP short sequences. Long PGRPs have an additional highly variable sequence at the N-terminus (Royet *et al.,* 2004; Stainer, 2004; Dziarski & Gupta, 2006).

The backside molecule creates a hydrophobic region with high variability, which has the ability to bind non-peptidoglycan molecules and ligands like lipopolysaccharide and teichoic acid (Mellroth *et al.,* 2003; Stainer, 2004; Dziarski & Gupta, 2006).

PGRP SPECIFICITY

Research on PGRPs proves that these structures bind to bacteria and peptidoglycan, regardless of their origin (invertebrates and vertebrates) (Yoshida *et al.,* 1996; Kim *et al.,* 2003). Individual PGRPs show specific preferences for selected types of peptidoglycan. For example, PGRP-SA has a greater affinity for lysine-type peptidoglycan, which builds the cell wall of most Gram-positive bacteria. Interactions between PGRP-SA and Lys-type peptidoglycan lead to activation of the Toll/ Dif pathway (Werner *et al.,* 2000; Kurata, 2004; Neyen *et al.,* 2016). DAP-type peptidoglycan, which occurs in many Gram-negative and some Gram-positive bacteria e.g. *Bacillus* species, is recognized by PGRP-LC and PGRP-LE (Liu *et al.,* 2000; Dziarski, 2004). Recognition of DAP-type peptidoglycan by PGRP-LC and PGRP-LE leads to activation of the Imd pathway (Takehana *et al.*, 2002). PGRP-LC is a specific receptor for the DAP-type peptidoglycan pattern, which is a component of the cell wall of most Gram-negative bacteria (Kurata, 2004). As mentioned above, the residues in the PGN binding groove are highly conserved but the residues at the edges of the groove are more variable and tailor PGRPs for different types of PGNs (Kim *et al.,* 2003).

An important aspect of specificity is also the variability occurring within some PGRP families. Compared to other PGRPs, over 50% less conservative residues and high structural variability was observed in the ligand-binding groove in PGRP-LB. Although PGRPs bind mainly bacterial molecules and peptidoglycan, at least some of them have the ability to bind also other structures (Tydell *et al.,* 2002). Each characteristic change in the PGN-binding groove can result in a higher affinity for such bacterial components as LPS (Ohno & Morrison, 1989; Dziarski, 2004). Selective differentiation of pathogens by PRRs helps in more effective fight against the intruder and facilitates activation of appropriate immune reactions, activation of proteolytic cascades, and epithelial or systemic activation of the production of immune peptides (Kurata, 2004).

SENSORY ROLE OF PGRPS

The main task of PGRP molecules in the insect body is recognition of PAMPs and transmission of a signal to induce effector response. It has been shown that three PGRPs identified in *Drosophila*: PGRP-SA, PGRP-SD, and PGRP-SC1 activate proteases that cleave proSpatzle into Spatzle, which binds to the Toll receptor activating the signalling pathway (Dziarski & Gupta, 2006). As a result of the Toll pathway activation, the Dif transcription factor is released from its inhibitor Cactus (homologue of the mammalian NF-κB inhibitor), which is phosphorylated and proteolytically degraded (Sun *et al.,* 2004; Dziarski & Gupta, 2006). The released transcription factor induces the expression of genes encoding antimicrobial peptides (Michel, 2001; Dziarski & Gupta, 2006; Charroux & Royet, 2010). Gram-negative bacteria only weakly activate the Toll pathway, since DAP-PGN is a substrate for PGRP-SA carboxypeptidase activity (Chang *et al.,* 2004). Mutations in genes encoding PGRPs engaged in activation of the Toll pathway make the fly susceptible to infections with Gram-positive bacteria and fungi (Leulier *et al.,* 2003; Garver *et al.,* 2006). In addition to PGRP-SA, so-called Gram-negative binding proteins (GNBPs) are also involved in the activation of the Toll pathway. GNBP-1 digests peptidoglycan, thereby generating free MurNAc ends recognized by PGRP-SA (Gobert *et al.,* 2003; Filipe SR *et al.,* 2005). Thus, PGRP molecules co-operate with other molecules to induce more effective action. Another example is PGRP-SD, which, although is not mandatory for the activation of the Toll/Dif pathway, it however increases the effectiveness of its activation mechanism guided by PGRP-SC1 and PGRP-SD (Buchon, 2009).

PGRP-LC molecules found in *Drosophila* possess an ability to recognise DAP-type peptidoglycan and activate the Imd pathway, resulting in the activation of the Relish transcription factor, a member of the NF-κB family (Gottar *et al.,* 2002; Choe *et al.*, 2005). Activation of the Relish factor leads to the induction of expression of genes that encode antimicrobial peptides. Besides PGRP-LC molecules, PGRP-LE also participates in the activation of the Imd pathway and acts in response to intracellular pathogens (Takehana *et al.,* 2002; Takakeana *et al.,* 2004). PGRP-LE occurs on the surface of immune cells together with PGRP-LC and acts as their co-receptor,

as well as within immunocompetent cells. Several studies have proven that the participation of the PGRP-LE protein increases the efficiency of expression of genes encoding antimicrobial peptides (Steiner, 2004; Kurata, 2010).

Studies of PGRP-LCa or PGRP-LCx have shown that these molecules can be overexpressed to trigger the Imd pathway and have the ability to oligomerize in the absence of peptidoglycan (Basbous *et al.*, 2011). The process of spontaneous dimerization and activation of the Imd pathway is prevented by transmembrane PGRP-LF, which blocks PGRP-LC isoforms, forming heterodimers with them and leading to negative regulation of immune response (Basbous *et al.*, 2011; Kurata, 2014). The high PGRP specificity, modulating properties, and a number of processes regulating PGRP activity may suggest that the immune response against pathogenic bacteria is highly selective and tailor-made (Buckley & Rast, 2015).

ENZYMATIC PROPERTIES OF PGRPS

In *Drosophila*, there are six PGRPs: -LB, -SB1, -SB2, -SC1a, -SC1b, and -SC2 exhibiting amidase activity. This property allows degradation of the amide bonds in the peptidoglycan structure. These bactericidal properties place PGRPs among effector molecules, which can directly fight pathogens and among modulators of immune response (Mellroth & Steiner, 2006). For example, PGRP-LB antagonises the function of PGRP-SD in the regulation of the IMD pathway, which contributes to the maintenance of a certain number of bacteria in the digestive tract. Degradation of peptidoglycan by PGRP-LB prevents induction of an immune response despite the presence of a certain number of bacteria in the gut (Steiner, 2004; Filipe *et al.,* 2005; Kurata, 2014). Only the number of bacteria exceeding the catalytic capabilities of PGRPs facilitates the activation of the Imd pathway (Zaidman-Remy *et al.,* 2006). The regulation of gut homeostasis is schematically presented in Fig. 2. Paredes and others (Paredes *et al.*, 2011) reported that flies lacking all six catalytic PGRPs exhibited deleterious immune responses to innocuous gut infections.

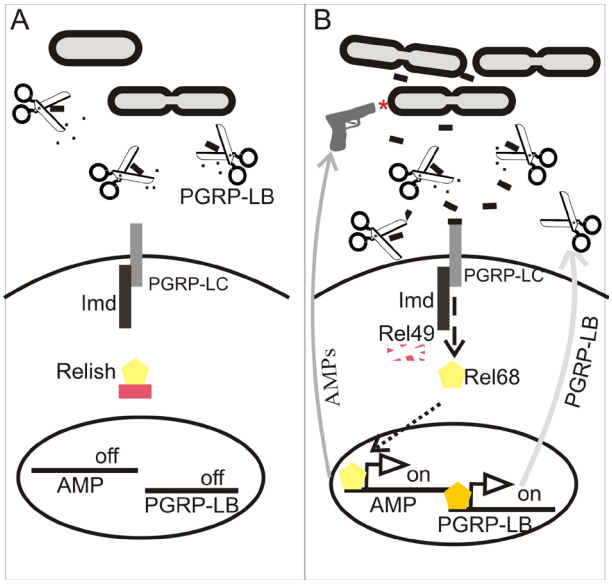

Figure 2. The role of catalytic PGRP in modulation of the Imd pathway.
Bacteria are often ingested by insects. There are also bacteria present permanently in the gut that do not activate the immune response, however their number is controlled. Dividing bacteria release some PGN, which is recognised by PGRP-LB and, due to its amidase activity, digested to small fragments (dots) which are not recognised by PGRPs. In this situation, despite the presence of bacteria, the Imd pathway is turned off (A). When the bacteria are proliferating intensively, many PGNs are released, which exceed the number of PGRPs. Next, the undigested PGN binds to PGRP-LC, activating the Imd pathway. As a result, expression of genes encoding AMPs is triggered by Rel-68. Appearing AMPs kill the excess bacteria. Furthermore, the activation of the Imd pathway results in an increased level of PGRP-LB gene expression, which results in an increased level of this PRR. Additionally, lysozyme present on the gut digests B-glycosidic bonds creating smaller fragments, which are still able to induce immune response. These short pieces of digested PGRPs are able to pass the intestinal wall and bind to PGRP-LC on the surface of fat body cells, thereby inducing the systemic immune response. Based on Zaidman-Remy (2006).

PGRP AS MOLECULES ACTIVATING OTHER IMMUNE PROCESSES

Some PGRP molecules are actively involved in the cellular branch of immunity. PGRP-SC1 and PGRP-SD have the ability to induce the phagocytosis process (Chang *et al.,* 2004; Dziarski & Gupta, 2006; Garver *et al.,* 2006). Extracellular PGRP-SC1a has the ability to bind bacterial molecules, as it acts as opsonin. While bound to bacteria, PGRP-SC1a can be recognised by the receptor Eater present on the surface of hemocytes (Garver *et al.,* 2006; Mellroth & Steiner, 2006). On the other hand, PGRP-LC is involved in phagocytosis of Gram-negative bacteria cells (Ramet *et al.,* 2002). In contrast to the sensory role of PGRP-LC and its interaction with PGRP-LA and PGRP-LD, the presence of these molecules is not necessary to carry out an effective process of phagocytosis (Ramet *et al.,* 2002; Kurata, 2004).

Studies conducted with the use of the silkworm *Bombyx mori* and the mealworm *Tenebrio molitor* revealed that insect hemolymph and epidermis are rich in PGRP-S. PGRP-S as well as *Drosophila* PGRP-LE molecules can activate the prophenoloxidase cascade (Park *et al.,* 2006; Yoshida *et al.,* 1996). The recognition and binding of PGN by these PGRPs lead to activation of a cascade of serine proteases, which are synthesized as zymogens. The activation of the final protease, called prophenoloxidase activating protease (PAP), results in proteolytic cleavage of the prophenoloxidase (pro-PO) zymogen to active phenoloxidase (PO) (Stączek *et al.,* 2017; Wang *et. al.,* 2019). The number of enzymes involved in the activation of proPO and those directly involved in its activation varies and depends on the species. In *D. melanogaster*, the initial protease is ModSP (modular serine protease), which is activated after joining the complex formed by PGRP-SA, and GNBP1 on the surface of the intruding pathogen leads to the activation of PAP (Park *et al.*, 2007). In *M. sexta*, two proteases are involved in the activation of PAP; active HP14 protease (HP – hemolymph protease) converts proHP21 to active HP21 protease, which in turn activates proPAP into the active PAP form, which exists in three isoforms: PAP1-3 (Lu *et al.*, 2014). Active phenoloxidase catalyses the synthesis of melanin, i.e. the dark pigment important in insect immunity. It can be deposited on the surface of pathogens, thus isolating the intruder from the rest of the host's body. Moreover, melanin can enhance the immune properties of other molecules. Reactive oxygen species produced during melanin synthesis have a detrimental effect on pathogens, thereby participating in infection control (Dziarski & Gupta, 2006; Park *et al.,* 2006). In *D. mela-*

nogaster, in addition to the activation of the Imd/Relish pathway, PGRP-LE molecules are involved in the activation of the prophenoloxidase cascade (Dziarski & Gupta, 2006).

PARTICIPATION OF PGRPS IN THE XENOPHAGY PROCESS

Autophagy is an evolutionarily conservative and very old process common to all cells of eukaryotic organisms. Autophagy is a catabolic process whose main task is intracellular degradation of macromolecular components of the cytoplasm and whole organelles. Xenophagy is one of the varieties of autophagy that makes the host organism degrade bacteria and the viruses (Travassos *et al.,* 2010).

In addition to the important role in the regulation of the Toll and Imd pathways, it has been proved that PGRPs are involved in the activation of xenophagy. In the insect organism, autophagy is induced in hemocytes. As previously mentioned, PGRPs can be located inside and on the surface of cells (Kuo *et al.,* 2018). Research conducted by Yano *et al.* (2008) has shown that PGRP-LE recognizes DAP-type peptidoglycan from *Listeria monocytogenes* and induces processes leading to xenophagy (Yano *et al.*, 2008). LC3/Atg8 is targeted at the bacteria, which are then absorbed *via* xenophagy. In addition, it has been shown that the xenophagy process induced by the presence of *L. monocytogenes* protected *Drosophila* against *Escherichia coli* infection (Travassos *et al.,* 2010; Kuo *et al.,* 2018). *Mycobacterium marinum, Salmonella enterica, Escherichia coli*, and *Wolbachia* are also eliminated *via* autophagy. Induction of the autophagy (and xenophagy) process in the insect's body is carried out independently of the activation of the Imd and Toll pathways, but it is not yet known exactly how the regulation of xenophagy by PGRP-LE works (Fig. 3). It has been proved that autophagic defects in the Atg5 component involved in the formation of autophagous follicles, as well as PGRP-LE, have a superior role in the induction and regulation of autophagy. Further studies have shown that PGRP-LE and the Toll receptor may also be involved in the induction of xenophagy processes in response to some viral infections (Shelly *et al.,* 2009; Kuo *et al.,* 2018). Shelly and others (Shelly *et al.*, 2009) has discovered that mammalian vesicular stomatitis virus (VSV) can lead to the development of an infection in *D. melanogaster*. In studies on the role of individual elements of the immune system in antiviral defence, it has been proven that silencing the Atg1/Ulk1, Atg5, Atg8a/Lc3, and Atg18/Wipi2 genes responsible for the induction of autophagy leads to a decrease in the survival of infected insects (Shelly *et al.* 2009). Autophagy is also commonly found in other invertebrates. This key process is involved in defending the organism of the nematode *Caenorhabditis elegans* against pathogens. Infection with the pathogen *Nematocida parisii*, results in ubiquitination and recruitment of Atg/LGG-1, which leads to activation of the xenophagy process. Infection of the nematode with *Bacillus thuringiensis* bacterium, which produces the Cry5b toxin, leads to induction of expression of genes indirectly associated with the activation of autophagy through TFEB/HLH30 transcription factors. Autophagy serves as a defensive function, participates in many physiological processes of the organism, and maintains its homeostasis (Kuo *et al.,* 2018).

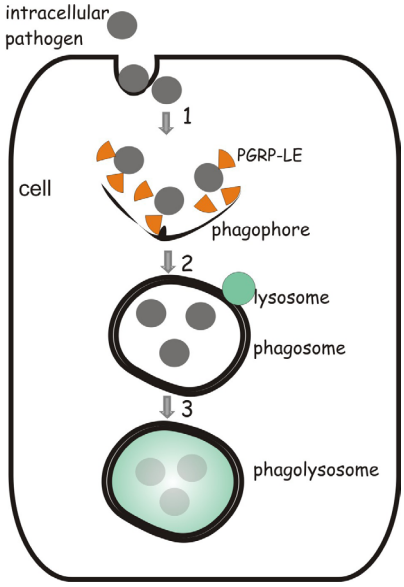

Figure 3. Scheme illustrating the role of PGRP-LE in the defence against intracellular pathogens.
After entering the cell, pathogenic bacteria are recognised by PGRP-LE, i.e. a PRR that is able to enter the cell. The binding of PGRPs to the pathogen induces the process of xenophagy (occurring in the same way as macroautophagy but it is directed against pathogens). A membrane called the phagophore is formed around bacteria-PGRP complexes (1). The phagosome is completed when the membrane forms a bubble around bacteria (2). The phagosome is fused with lysosome (3), whose enzymes cause destruction of pathogenic bacteria. Based on Polewska (2012) and Kurata (2010).

SUMMARY

The discovery of PGRPs significantly contributed to scientific progress in the field of immunobiology (Yoshida *et al.,* 1996). In insects, PGRPs play very important defence roles (Dziarski, 2004). They are responsible for the recognition of PAMPs and activation of the Toll and Imd signalling pathways. Some PGRPs are involved in activating the prophenoloxidase cascade and inducing phagocytic processes. PGRPs are also involved in xenophagy processes after infection with intracellular pathogens (Takehana *et al.,* 2004). The function of PGRPs in insect immunity is summarized in Fig. 4. These molecules take part in the maintenance of homeostasis in

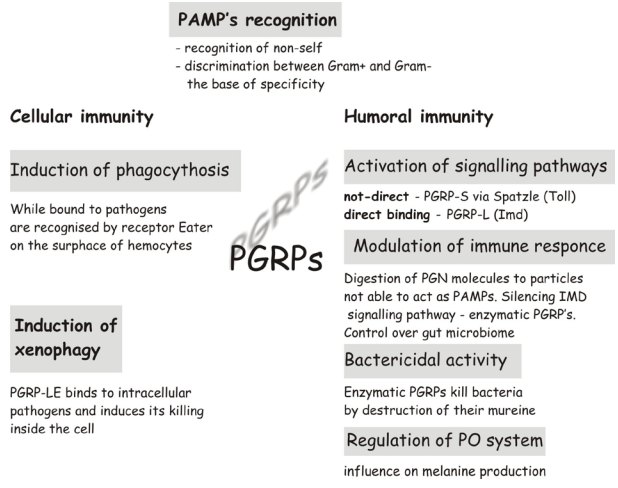

Figure 4. Scheme summarizing the function of PGRPs in insect immunity.

the host organism. The presence of PGRP receptors maintaining a wide spectrum of activity on the surface and inside of cells as well as in differentiated tissues allows a conclusion that they are an effective and common weapon against intruders (Kurata, 2010; Könner & Bruning, 2011). Although they were discovered a long time ago, PGRPs are still an interesting subject of scientific research. PGRPs are present in both invertebrates and vertebrates. The PGRP phylogenetic tree of both groups has common branches (Dziarski, 2004; Royet, 2004). Some of the functions found in insects have been evolutionarily conserved and transferred to higher organisms (Dziarski & Gupta, 2006; Kurata, 2010). However, it should be remembered that, as a result of evolutionary changes within species, many PGRPs have acquired new functions (Sang *et al.,* 2005). The structural abundance of PGRPs and the various properties exhibited by single receptors suggest that they may have unique and yet unidentified functions whose discovery requires further research.

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