Microreview

Mammalian PGRPs: novel antibacterial proteins

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Summary

Peptidoglycan recognition proteins (PGRPs) are innate immunity molecules conserved from insects to mammals. Insects have up to 19 PGRPs, which activate Toll or Imd signal transduction pathways or induce proteolytic cascades that generate antimicrobial products, induce phagocytosis, hydrolyse peptidoglycan, and protect insects against infections. Mammals have four PGRPs, which were hypothesized to function as signal-transducing pattern recognition receptors. However, all mammalian PGRPs are secreted, usually as disulphide-linked homo- and heterodimers. One mammalian PGRP, PGLYRP-2, is an N-acetylmuramoyl-L-alanine amidase that hydrolyses bacterial peptidoglycan and reduces its proinflammatory activity. PGLYRP-2 is secreted from liver into blood, and is also induced by bacteria in epithelial cells. The three remaining mammalian PGRPs are bactericidal or bacteriostatic proteins. PGLYRP-1 is expressed primarily in the granules of polymorphonuclear leucocytes (PMNs), and PGLYRP-3 and PGLYRP-4 are expressed in the skin, eyes, salivary glands, throat, tongue, esophagus, stomach and intestine, and protect the host against infections. They kill bacteria by interacting with their cell wall peptidoglycan, rather than permeabilizing their membranes. These PGRPs therefore are a new class of bactericidal and bacteriostatic proteins that have different structure, mechanism of action, and expression pattern from currently known vertebrate antimicrobial peptides. Direct bactericidal activity of these PGRPs either evolved in vertebrates or mammals, or it is yet to be discovered in insects.

Introduction: innate immunity, peptidoglycan and peptidoglycan recognition proteins

Innate immune system recognizes microorganisms through germline-encoded receptors and soluble molecules specific for microbial components. Peptidoglycan is an excellent target for recognition by the eukaryotic innate immune system, because it is an essential and unique component of the cell walls of virtually all bacteria, and because it is not present in eukaryotic cells. Peptidoglycan is a polymer of β(1–4)-linked N-acetylglosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), with all lactyl groups of MurNAc substituted with stem peptides, typically containing four alternating L- and D-amino acids. The stem peptides from adjacent strands are often cross-linked, either directly or through short peptides. While the structure of the polysaccharide backbone of peptidoglycan is conserved in all bacteria, the composition of the peptide varies: Gram-negative bacteria and Gram-positive bacilli have m-diaminopimelic acid (m-DAP) as the third amino acid, and most other Gram-positive bacteria (including Gram-positive cocci) have L-lysine as the third amino acid (see peptidoglycan structures in Figs 1 and 2).

Peptidoglycan Recognition Proteins (PGRPs) are innate immunity molecules that recognize bacteria and function in antibacterial immunity (Kang et al., 1998; Werner et al., 2000; Dziarski, 2004). The first PGRP was discovered in silkworm as a protein that recognizes bacterial peptidoglycan and initiates activation of the prophe- nol-oxidase cascade (Yoshida et al., 1996). Subsequently, its moth orthologue was identified and cloned (Kang et al., 1998). Since then, families of PGRPs have been identified in insects and mammals, showing that PGRPs are highly conserved in evolution. All PGRPs have at least one, or sometimes two PGRP domains, that are homologous to bacteriophage and bacterial type 2 amidases, which raises the possibility that type 2 amidase gene has been conserved in evolution from prokaryotes to eukaryotes. PGRPs, however, are not ubiquitous: plants and lower metazoa, such as nematodes (Caenorhabditis elegans), do not have PGRPs.
Functions of insect PGRPs

Insects have many PGRPs with recognition, signalling and effector functions, all of which are important for the antimicrobial innate immunity. Consistent with immune function, insect PGRPs are expressed in immune competent organs, such as the fat body, gut, and haemocytes, and their expression is upregulated by injections of peptidoglycan and bacteria (Kang et al., 1998; Werner et al., 2000; Christophides et al., 2002; Dimopoulos et al., 2002; Dziarski, 2004). A fruit fly (*Drosophila melanogaster*) has 13 PGRP genes that are transcribed into at least 19 proteins (Werner et al., 2000) and a mosquito (*Anopheles gambiae*) has seven PGRP genes that are transcribed into at least nine proteins (Christophides et al., 2002; Dimopoulos et al., 2002). Other insects are also likely to have large families of PGRPs. Insect PGRPs are grouped into two classes: short PGRPs (PGRP-S), which are small extracellular proteins, and long PGRPs (PGRP-L), which have long transcripts and are either intracellular, extracellular, or membrane-spanning proteins (Werner et al., 2000; Dziarski, 2004). Insect PGRPs have several known functions (Fig. 1).

**Activation of Toll and phagocytosis**

Three *Drosophila* PGRPs, PGRP-SA, PGRP-SD and PGRP-SC1, recognize bacterial peptidoglycan and activate proteases that cleave Spaetzle, an extracellular...

**Enzymatic activity**

| Reaction | Product |
|----------|---------|
| GlcNAc   | MurNAc  |
| L-Ala    | D-Glu   |
| D-Ala    | m-DAP   |
| D-Ala    | m-DAP   |
| D-Glu    | L-Ala   |
| GlcNAc   | MurNAc  |

**Peptidoglycan**

Fig. 1. Functions of insect PGRP proteins. In the lower-right panel, the structure of DAP-type peptidoglycan is also shown.
cytokine-like protein present in insect haemolymph, which in turn serves as an endogenous activator of Drosophila Toll (Michel et al., 2001; Bischoff et al., 2004; Garver et al., 2006). Activation of Toll initiates a signal transduction pathway that results in the activation of Dorsal and Dif transcription factors (which are similar to mammalian NF-κB), that translocate into the nucleus, bind to the κB sites, and initiate transcription of drosomycin and other antimicrobial peptides, which are mainly active against Gram-positive bacteria and fungi (Fig. 1) (Lemaitre et al., 1996). This pathway is essential for Drosophila immunity to Gram-positive bacteria, because mutations in recognition or signal transduction molecules for this pathway make the flies highly susceptible to infections with Gram-positive, but not Gram-negative, bacteria (Michel et al., 2001; Bischoff et al., 2004; Garver et al., 2006).

The Toll pathway is preferentially triggered by the Lys-type peptidoglycan and only weakly by the DAP-type peptidoglycan (Leulier et al., 2003), although both types of peptidoglycan bind to PGRP-SA (Chang et al., 2004). The likely reason for the weak Toll-activating capacity of DAP-type peptidoglycan is that this peptidoglycan, but not Lys-type peptidoglycan, is the substrate for the carboxypeptidase activity of PGRP-SA (Chang et al., 2004) (Fig. 1). Efficient triggering of the Toll pathway by PGRP-SA requires cooperation (and likely formation of a complex) with another pattern recognition molecule, Gram-negative binding protein (GNBP)-1 (Gobert et al., 2003; Pili-Floury et al., 2004). GNBP-1 digests peptidoglycan and generates free reducing ends of MurNAc, which are then recognized by PGRP-SA (Filipe et al., 2005). Drosophila PGRP-SC1 and PGRP-SD (Bischoff et al., 2004; Garver et al., 2006), as well as other pattern recognition molecules, such as GNBP-3, also activate the Toll pathway (Fig. 1). Both PGRP-SA and PGRP-SC1 are required for the activation of Toll pathway, whereas PGRP-SD is not essential, but enhances Toll activation. Recognition of bacteria by PGRP-SC1 and PGRP-SA also triggers phagocytosis by an as yet unidentified mechanism (Garver et al., 2006).
Activation of Imd

*Drosophila* also has the *Imd* pathway that is Toll-independent and is activated by Gram-negative bacteria and some Gram-positive bacilli (Lemaître et al., 1995; Leulier et al., 2003; Kaneko and Silverman, 2005). This pathway results in the activation of Relish transcription factor (which is also similar to mammalian NF-κB) and induction of transcription of diptericin and other antimicrobial peptides that are primarily active against Gram-negative bacteria.

Activation of the *Imd* pathway by Gram-negative bacteria and Gram-positive bacilli in *Drosophila* is mediated through PGRP-LC (Choe et al., 2002; Gottar et al., 2002; Ramet et al., 2002) (Fig. 1). PGRP-LC responds primarily to DAP-type peptidoglycan. It is a transmembrane protein and has three alternative splice forms (LCa, LCx and LCy), which differ in the extracellular PGRP domains and likely cooperate with each other and have somewhat different recognition specificities (Choe et al., 2002; Leulier et al., 2003; Werner et al., 2003; Stenbak et al., 2004). PGRP-LC activates the *Imd* pathway in cooperation with PGRP-LE (Takehana et al., 2004) and also likely with another, as yet unidentified coreceptor (Fig. 1). *Drosophila* PGRP-LC may also play a role in phagocytosis of Gram-negative bacteria, because inhibition of PGRP-LC expression in *Drosophila* S-2 cells diminishes phagocytosis of *Escherichia coli*, but not *Staphylococcus aureus* (Ramet et al., 2002). However, direct evidence for the function of PGRP-LC as a phagocytic receptor is still lacking.

**Activation of prophenol-oxidase cascade**

Silkworm (*Bombyx mori*) and mealworm (*Tenebrio molitor*) PGRP-S are present in the haemolymph and cuticle, bind bacteria and Lys- and DAP-peptidoglycan, and activate the prophenol-oxidase cascade (Fig. 1) (Yoshida et al., 1996; Park et al., 2006), which generates antimicrobial products, such as melanin and reactive oxygen species, surrounds the infection site with melanin, and contains the infection. *Drosophila* PGRP-LE (Takehana et al., 2004) and beetle (*Holotrichia diomphalia*) PGRP-1 (Lee et al., 2004) (and likely other PGRPs) also activate the prophenol-oxidase cascade, but *H. diomphalia* PGRP-1 responds to 1,3-β-D-glucan, a common constituent of fungal cell walls.

**Amidase and carboxypeptidase activity**

*Drosophila* PGRP-SC1 and PGRP-LB are N-acetylmuramoyl-L-alanine amidases (Kim et al., 2003; Mellroth et al., 2003), which hydrolyse the amide bond between MurNAc and L-Ala and thus remove stem peptides from peptidoglycan (Fig. 1). Digestion of peptidoglycan with amidase reduces or eliminates the ability of polymeric peptidoglycan to stimulate insect cells (Mellroth et al., 2003). Based on the conserved structure of the active site of the amidase, several other insect PGRPs are predicted to have an amidase activity, whereas several others are not (Gelius et al., 2003; Mellroth et al., 2003; Wang et al., 2003). However, one PGRP that is not an amidase, *Drosophila* PGRP-SA, has an L,D-carboxypeptidase activity with specificity for the bond between m-DAP and D-Ala of the stem peptide present in peptidoglycan of Gram-negative bacteria and Gram-positive rods (Chang et al., 2004) (Fig. 1). The biologic significance of this carboxypeptidase activity is not certain.

**Functions of mammalian PGRPs**

Mammals have a family of four PGRPs, which were initially named PGRP-S, PGRP-L, and PGRP-αC and PGRP-β (for 'short', 'long' or 'intermediate' transcripts respectively), by analogy to insect PGRPs (Liu et al., 2001). Subsequently, the Human Genome Organization Gene Nomenclature Committee changed their names to Peptidoglycan Recognition Protein 1, 2, 3 and 4 (PGLYRP-1, PGLYRP-2, PGLYRP-3 and PGLYRP-4) respectively (Table 1). This terminology is also beginning to be used for mouse and all vertebrate PGRPs.

Mammalian PGLYRPs were initially hypothesized to function as pattern recognition receptors based on analogy to mammalian Toll-like receptors (TLRs) or signal-transducing insect PGRPs, and also based on initial computer predictions of transmembrane domains in mammalian PGLYRP-2, PGLYRP-3 and PGLYRP-4 (Liu et al., 2001). However, recent crystallographic (Guan et al., 2004a,b; 2005) and biochemical (Lu et al., 2006) data show that all four mammalian PGLYRPs are secreted, and are not transmembrane molecules. Functional studies also failed to reveal any role for mammalian PGLYRPs in TLR-induced cell activation, and instead, showed direct bacterial recognition and effector functions for mammalian PGLYRPs.

**Amidase**

Mammalian PGLYRP-2 is an N-acetylmuramoyl-L-alanine amidase that hydrolyses the lactyl bond between the

| Table 1. Nomenclature of human PGRPs. |
|---------------------------------------|
| Original name | Current human genome name* |
|--------------|-----------------------------|
| PGRP-S       | PGLYRP-1                    |
| PGRP-L       | PGLYRP-2                    |
| PGRP-αC      | PGLYRP-3                    |
| PGRP-β       | PGLYRP-4                    |

* Current gene and protein abbreviation approved by the Human Genome Organization Gene Nomenclature Committee.
MurNAc and L-Ala in bacterial peptidoglycan (Fig. 2) (Gelius et al., 2003; Wang et al., 2003). PGLYRP-2 is constitutively produced in the liver and is secreted from the liver into blood (Zhang et al., 2005). This liver PGLYRP-2 and serum N-acetylmuramyl-L-alanine amidase (that was earlier identified but not cloned) are the same protein encoded by the pglyrp2 gene (Zhang et al., 2005).

PGLYRP-2 is also expressed in the intestinal follicle-associated epithelial cells (Lo et al., 2003). PGLYRP-2 is not expressed in healthy human skin, but its expression in keratinocytes is induced by bacteria and cytokines (Wang et al., 2005). This induction is limited to epithelial cells, does not involve TLR2 or TLR4, and correlates with keratinocyte differentiation and stress responses that proceed through the activation of the p38 mitogen-activated protein kinase (Wang et al., 2005).

Some mammals express multiple splice forms of PGLYRP-2 that may have different expression and possibly multiple functions. For example, pigs have two PGLYRP-2 splice forms, short and long. They both have N-acetylmuramyl-L-alanine amidase activity, and the long form has a similar expression to human PGLYRP-2, whereas the short form is constitutively expressed in several tissues, including bone marrow, intestine, liver, spleen, kidney and skin (Sang et al., 2005).

The in vivo role of PGLYRP-2 amidase activity could be threefold. First, it could function as a scavenger of proinflammatory peptidoglycan. In mammals, recognition of extracellular polymeric peptidoglycan occurs through TLR2 (Schwandner et al., 1999; Takeuchi et al., 1999; Yoshimura et al., 1999; Dziarski and Gupta, 2005), which likely requires glycan chains and stem peptides. Digestion of peptidoglycan with amidase reduces or eliminates cell-activating proinflammatory activity of polymeric peptidoglycan (Højjer et al., 1997; Meleroth et al., 2003). Recognition of intracellular peptidoglycan fragments occurs through Nod1 and Nod2. Digestion of peptidoglycan with amidase would abolish its Nod2-activating capacity, which requires, at minimum, a muramyl dipeptide peptidoglycan fragment (Chamaillard et al., 2003; Girardin et al., 2003a). Second, PGLYRP-2, could have direct antibacterial activity or, similarly to lysozyme, could enhance the killing activity by antibacterial peptides. And third, PGLYRP-2 digestion of polymeric peptidoglycan could generate a Nod1-activating peptide, because the minimum structure that activates Nod1 is a peptide derived from the stem peptide of DAP-type peptidoglycan (without the glycan) (Chamaillard et al., 2003; Girardin et al., 2003b). Therefore, digestion of peptidoglycan with amidase could have a scavenger function to reduce proinflammatory activity of peptidoglycan, direct antibacterial function, or could generate Nod1-activating peptides, which would enhance antimicrobial responses.

Bactericidal and bacteriostatic activity

What is the function of the three remaining mammalian PGLYRPs? PGLYRP-1 was the first cloned mammalian PGLYRP (Kang et al., 1998). Initially, PGLYRP-1 was shown to be highly expressed in the bone marrow (Kang et al., 1998; Liu et al., 2001), and later it was shown to be almost exclusively present as a soluble protein in the granules of polymorphonuclear leucocytes (PMNs) (Liu et al., 2000; Tydell et al., 2002; 2006; Dziarski et al., 2003; Cho et al., 2005). This suggested its role in antibacterial activity, and indeed PGLYRP-1 knockout mice are more susceptible to infection with some Gram-positive bacteria, and their PMNs are defective in killing and digesting Gram-positive bacteria (Dziarski et al., 2003). The rate of bacterial uptake is not impaired in PGLYRP-1 knockout mice, and exogenous PGLYRP-1 added to bacteria and leucocytes has no effect on the rate of bacterial uptake by PMNs, which indicates that PGLYRP-1 is not an opsonin. However, PGLYRP-1 restores the normal killing and digestion of PMNs from PGLYRP-1 knockout mice (Dziarski et al., 2003). Although in initial studies purified mouse or human PGLYRP-1 were only bacteriostatic (Liu et al., 2000; Dziarski et al., 2003; Cho et al., 2005), the latest results demonstrate that human PGLYRP-1 is bactericidal and that the cidal activity requires Ca^{2+} (Lu et al., 2006); the earlier preparations were not bactericidal because they did not contain Ca^{2+}. Bovine PGLYRP-1, purified from leucocyte granules, is also directly bactericidal for both Gram-positive and Gram-negative bacteria (Tydell et al., 2002; 2006). Therefore, PGLYRP-1 is a part of PMNs’ antibacterial arsenal, but it is also present in milk (Kappeler et al., 2004) and in intestinal M cells (Lo et al., 2003).

The functions of the remaining two mammalian PGLYRPs, PGLYRP-3 and PGLYRP-4, until very recently, were still unknown. The initial results showed very selective expression of PGLYRP-3 and PGLYRP-4 in esophagus (out of 76 tissues and cell types tested) (Liu et al., 2001), and computer analysis initially identified them as transmembrane molecules (Liu et al., 2001), leading to the hypothesis that they could function as cell surface receptors. However, recent crystallization of the C-terminal half of PGLYRP-3 demonstrated the lack of transmembrane domain, suggesting that PGLYRP-3 and PGLYRP-4 may be secreted (Guan et al., 2004a,b). Indeed, the most recent biochemical results revealed that both PGLYRP-3 and PGLYRP-4, as well as PGLYRP-1, are secreted primarily as disulphide-linked homodimers (Lu et al., 2006). Moreover, when PGLYRP-3 and PGLYRP-4 are coexpressed in the same cells, they are exclusively secreted as PGLYRP-3:4 heterodimers (Lu et al., 2006).

Because pglyrp3 and pglyrp4 genes are located in the epidermal differentiation complex gene cluster on...
with bacteria. Enzymes and peptides are often found in the same secretions, because PGL YRPs and these antimicrobial products increase the expression of PGL YRP-3 and PGL YRP-4 proteins are selectively expressed in tissues that come in contact with the environment (Lu et al., 2006): in the skin epidermis, hair follicles, sebaceous glands and eccrine sweat glands; in the eye’s ciliary body (which produces aqueous humour that fills anterior and posterior chambers of the eye); in the eye’s corneal epithelium; in the mucus-secreting cells of the main salivary (submandibular) gland and in mucus-secreting glands in the throat (both mucus-secreting glands selectively express PGL YRP-4, but not PGL YRP-3); in the tongue and esophagus in squamous epithelial cells; in the stomach in acid-secreting Parietal cells (PGL YRP-3) and glycoprotein-secreting neck mucous cells (PGL YRP-4); and in the small and large intestine in the columnar absorptive cells, but not in mucus-secreting goblet cells and not in the crypts in Paneth cells, which produce antimicrobial peptides (Lu et al., 2006). Mouse PGL YRP-3 and PGL YRP-4 are also expressed in the intestinal tract and salivary glands (Mathur et al., 2004). Bacteria and their products increase the expression of PGL YRP-3 and PGL YRP-4 in keratinocytes (Lu et al., 2006) and oral epithelial cells (Uehara et al., 2005), likely through activation of TLR2, TLR4, Nod1 and Nod2.

The latest results demonstrate that human PGL YRP-3, PGL YRP-4, PGL YRP-3:4, as well as PGL YRP-1, are bactericidal for pathogenic (Listeria monocytogenes and S. aureus) and non-pathogenic (Bacillus and Lactobacillus) Gram-positive bacteria (Lu et al., 2006). They are markedly less bactericidal or bacteriostatic for all other bacteria tested, including normal flora Gram-positive bacteria (Micrococcus luteus, Staphylococcus epidermidis and several Streptococci) and Gram-negative bacteria. PGL YRP-3 and PGL YRP-4 are active in vivo on mucous membranes (which is the site of their production) in an intranasal S. aureus lung infection in mice (Lu et al., 2006). PGL YRPs are bactericidal (kill 99% of bacteria) at 0.1–1 μM, and thus are more active than most antibacterial peptides, such as defensins or magainin, but less active than phospholipase A2, which is the most active human bactericidal peptide (Lu et al., 2006). PGL YRPs act synergistically with bacteriolytic enzymes (lysozyme and lysostaphin) and antimicrobial peptides (defensins and phospholipase A2) (Cho et al., 2005; Lu et al., 2006). This synergism should greatly enhance their effectiveness in vivo, because PGL YRPs and these antimicrobial enzymes and peptides are often found in the same secretions or on the same cell surfaces that come in contact with bacteria.

**Spectrum of activity and specificity of mammalian PGL YRPs**

Human PGL YRP-1, PGL YRP-3, PGL YRP-4 and PGL YRP-3:4 are bactericidal for Bacillus, Lactobacillus, L. monocytogenes and S. aureus, which are both non-pathogenic and pathogenic Gram-positive bacteria. They are markedly less bactericidal or bacteriostatic for all the remaining Gram-positive bacteria tested (S. epidermidis, M. luteus, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus faecalis) and for Gram-negative bacteria (E. coli, Enterobacter cloacae, Proteus vulgaris), but they have no effect on fungi (Candida albicans, Saccharomyces cerevisiae). Each PGL YRP, however, has a different spectrum of bactericidal activity (Lu et al., 2006). These results suggest that normal flora bacteria have developed resistance to bactericidal mechanisms constitutively present in the skin, eyes and mucous membranes (such as PGL YRP-3 and PGL YRP-4), and can colonize these areas. Bacteriostatic effect of PGL YRPs on normal flora bacteria makes perfect sense for host physiology – normal flora bacteria are not killed, but their overgrowth is limited. Non-pathogenic *not* normal flora bacteria (transient flora, such as *Bacillus* and *Lactobacillus*) are uniformly sensitive to killing by PGL YRPs. L. monocytogenes, a pathogen that does not infect skin and mucous membranes, is also highly sensitive. S. aureus, a pathogen that often infects skin, has intermediate sensitivity to killing by PGL YRPs, with some strains highly sensitive and some less sensitive, whereas S. pyogenes, a pathogen that frequently infects throat and skin and has high carrier rate in the throat, is more resistant to killing by PGL YRPs. These results demonstrate how normal flora adapt to their environment and how successful pathogens evade the immune system at the site of infection.

Mouse PGL YRP-1 seems to have similar spectrum of activity to human PGL YRP-1, whereas bovine PGL YRP-1 is bactericidal for both Gram-positive (*L. monocytogenes,* S. aureus) and Gram-negative (*Salmonella typhimurium*) bacteria, and also has some microbicidal activity against a fungus, *Cryptococcus neoformans* (Tydell et al., 2002; 2006). The broader spectrum of cidal activity of bovine PGL YRP-1 may reflect a true difference between the human and bovine orthologues, or may simply reflect a difference in the protein purification methods and assay conditions.

All mammalian PGL YRPs tested so far bind peptidoglycan and bacteria (Kang et al., 1998; Werner et al., 2000; Liu et al., 2000; 2001; Mellroth et al., 2003; Lu et al., 2006; Tydell et al., 2006). Mammalian PGL YRPs bind to both Gram-positive and Gram-negative bacteria (Liu et al., 2000; 2001; Cho et al., 2005; Lu et al., 2006; Tydell et al., 2006). Binding to peptidoglycan may not be always
responsible for binding to intact bacteria, because in Gram-negative bacteria peptidoglycan is located underneath the outer membrane and is not easily accessible on the bacterial surface (although penetration of PGL YRPs through the outer membrane cannot be excluded). PGL YRPs also bind to fungi, which do not have peptidoglycan. Therefore, PGL YRPs may also bind to other polymers, such as lipoteichoic acid and lipopolysaccharide (Imoto et al., 2000; Lu et al., 2006; Tydell et al., 2006). However, human and mouse PGL YRPs have the highest affinity for peptidoglycan and much lower for lipoteichoic acid and lipopolysaccharide (Liu et al., 2000; Lu et al., 2006), whereas bovine PGL YRP-1 seems to have high affinity for lipoteichoic acid and lipopolysaccharide (Tydell et al., 2006). It is not clear, however, whether these other ligands bind to the peptidoglycan binding groove (see next section) or to another portion of the PGL YRP molecule, such as the hydrophobic region on the opposite side of the molecule.

Also, the binding specificities of the amidase-active PGRPs may be broader than their enzymatic specificity, which is reminiscent of the vertebrate lysozyme. This lysozyme has highly specific lytic activity for peptidoglycan’s polysaccharide backbone, but it nevertheless also binds to other compounds, such as Gram-negative bacteria and their lipopolysaccharide component (Imoto et al., 1972; Ohno and Morrison, 1989).

**Molecular basis of recognition and function of mammalian PGL YRPs**

All PGRPs have a ~165-amino-acid-long PGRP domain. In PGL YRP-1, one PGRP domain comprises most of the entire ~190-amino-acid sequence, and in PGL YRP-2, one C-terminal PGRP domain comprises ~1/3 of the sequence. PGL YRP-3 and PGL YRP-4 both have two PGRP domains. Each PGRP domain has one ligand binding site (Guan et al., 2004b). Thus, PGL YRP-3 and PGL YRP-4 monomers and dimers have two and four ligand binding sites respectively, whereas PGL YRP-1 and PGL YRP-2 monomers and dimers have one and two identical ligand binding sites respectively (Fig. 2). However, because these PGRP domains are not identical (have 37–43% identity), the fine binding specificity or affinity of each PGRP domain in each PGL YRP molecule is likely different. The diversification of PGL YRPs’ specificities is then further increased by formation of PGL YRP-3:4 heterodimers, which have four different binding sites. This way the host can fine-tune the specificities of PGL YRPs by expressing PGL YRP-3 and PGL YRP-4 either in the same or in separate cells, to form hetero- or homodimers respectively. In addition, PGL YRPs have hydrophobic domains on the opposite side of the molecule from the ligand binding groove, which were previously hypothesized to interact with signal transduction molecules (Kim et al., 2003). In mammalian PGL YRPs, however, these hydrophobic domains may either play a role in interaction of PGL YRPs with bacteria, or in formation of dimers.

Crystallographic analysis of human PGL YRP-1 and C-terminal PGRP domain of PGL YRP-3, as well as insect PGRP-LB, -SA and -LCa, show that all these PGRPs have a ligand binding groove that binds peptidoglycan and is specific for muramyl-tripeptide (Kim et al., 2003; Chang et al., 2004; 2005; Guan et al., 2004a, b; 2005; Reiser et al., 2004). It can accommodate a larger structure, such as GlcNAc-MurNAc-tetrapeptide, but it does not bind muramyl-dipeptide or a peptide without MurNAc (Kumar et al., 2005; Swaminathan et al., 2006). These results are consistent with the specificity of human PGL YRP-2 for muramyl-tripeptide, which is the minimum peptidoglycan fragment hydrolysed by PGL YRP-2 (Wang et al., 2003), and with the specificity and high affinity (Kd = 13 nM) of murine PGL YRP-1 for uncleaved polymeric peptidoglycan, but not muramyl-dipeptide or pentapeptide (Liu et al., 2000). The high-affinity binding of peptidoglycan to PGL YRP is achieved by burying both the peptide and MurNAc portions of peptidoglycan in a deep cleft that completely excludes solvent (Guan et al., 2004b).

Human PGL YRP-1 and C-terminal fragment of PGL YRP-3 bind muramyl-tetrapeptide and muramyl-pentapeptide with higher affinity than muramyl-tripeptide (Kumar et al., 2005). C-terminal fragment of PGL YRP-3 has a preference for binding the Lys-type over the DAP-type peptidoglycan, whereas PGL YRP-1 binds DAP-type peptidoglycan with higher affinity than Lys-type peptidoglycan (Kumar et al., 2005; Swaminathan et al., 2006). Moreover, both human and insect PGRPs employ a dual strategy for discrimination among different types of peptidoglycan, based on detection of Lys or DAP in the stem peptide and the type of peptide cross-bridge (Swaminathan et al., 2006). Discrimination between Lys- and DAP-type peptidoglycan is based on two amino acids in the peptidoglycan binding groove in position 236 and 237 in PGL YRP-3. The validity of this model is verified by mutations in these positions that can change the specificity of the binding from Lys to DAP or DAP to Lys (Swaminathan et al., 2006). This allows prediction of binding specificity of various PGRP domains for Lys- or DAP-type peptidoglycan. Detection of peptide-cross-linked peptidoglycan would require engagement of two peptidoglycan binding sites in two PGRP domains, which could be accomplished by PGL YRPs with two PGRP domains and/or by dimeric PGL YRPs, which is consistent with recent demonstration of dimeric PGRPs in mammals (Lu et al., 2006) and insects (Meliroth et al., 2005).

Although the amino acids that contact peptidoglycan in the C-terminal portion of human PGL YRP-3 have been established, there is likely to be considerable variation in
the fine specificity of different PGRPs, because both contact and non-contact residues in and around peptidoglycan binding groove are quite variable and less than 50% conserved among PGRPs (Kim et al., 2003; Guan et al., 2004a,b). Therefore, this structural variation may correspond to different ligand specificities of different PGRPs.

Amidase-active PGLYRP-2 have a conserved Zn\(^{2+}\) binding site in the peptidoglycan binding groove, which consists of two His, one Tyr and one Cys (C530 in human PGLYRP-2), and which is also present in all amidase-active insect PGRPs and in homologous bacteriophage type 2 amidases. In non-amidase PGLYRPs, this Cys is substituted with Ser. PGLYRP-2 have at least four other amino acids that are required for their activity, because mutations in these amino acids greatly reduce or abolish their amidase activity (Gelius et al., 2003; Wang et al., 2006).

Mechanism of antibacterial activity

To determine the mechanism of bactericidal activity of PGLYRPs, their effects on bacteria were compared with the effects of membrane-permeabilizing antibacterial peptides, peptidoglycan-lytic enzymes, and inhibitors of peptidoglycan biosynthesis. Vertebrate antibacterial peptides (e.g. defensins) kill bacteria by traversing bacterial cell wall and permeabilizing bacterial cytoplasmic membrane. Therefore, kinetics of membrane permeabilization by anti-bacterial peptides correlates with the kinetics of bacterial killing. Peptidoglycan-lytic enzymes kill bacteria by destroying the physical integrity of peptidoglycan, which causes osmotic lysis, because the function of peptidoglycan in bacterial cell wall is to counteract the high osmotic pressure of bacterial protoplast. This osmotic lysis results in rapid membrane permeabilization, and the kinetics of membrane permeabilization correlates with the kinetics of bacterial killing. Antibiotics (such as penicillin) that target cell wall kill bacteria by inhibiting peptidoglycan synthesis. However, in contrast to peptidoglycan-lytic enzymes, penicillin does not cause early permeabilization of bacterial membranes, because penicillin-treated bacteria only die when they start to grow and are unable to synthesize peptidoglycan. Therefore, in penicillin-killed bacteria membrane permeabilization is substantially delayed until the bacteria have grown without synthesizing peptidoglycan. The kinetics of bacterial killing and membrane permeabilization by PGLYRPs resembles those by penicillin, thus suggesting that PGLYRPs target cell wall peptidoglycan rather than cell membranes (Lu et al., 2006).

When bacteria lose their cell wall peptidoglycan due to digestion with an enzyme or due to inhibition of peptidoglycan synthesis by antibiotics, such as penicillin, they can be kept alive in the protoplast medium containing 0.75 M sucrose, until they rebuild their cell walls. Consistent with this model, killing of bacteria and membrane permeabilization by peptidoglycan-lytic enzymes, penicillin, and also by PGLYRPs are prevented in the protoplast medium containing 0.75 M sucrose, because sucrose protects the cells from osmotic lysis (Lu et al., 2006), which further supports that PGLYRPs target cell wall peptidoglycan rather than cell membranes. By contrast, neither membrane permeabilization nor killing by membrane-damaging antibacterial peptides is prevented in the protoplast medium.

Therefore, the mechanism of bacterial killing by PGLYRPs is different from the mechanism of killing by peptidoglycan-lytic enzymes and membrane-permeabilizing antibacterial peptides, and it most resembles the effect of antibiotics that inhibit peptidoglycan synthesis. Antibiotics inhibit peptidoglycan synthesis by two basic mechanisms: (i) they either block the active site of peptidoglycan-synthesizing enzymes (e.g. β-lactams); or (ii) they bind to metabolic precursors of peptidoglycan and prevent their use in peptidoglycan synthesis (e.g. vancomycin, mersacidin and actagardine) (Lu et al., 2006). Because PGLYRPs avidly bind to peptidoglycan or its fragments (e.g. GlcNAc-MurNAc-pentapeptide), they may exert their antibacterial effect by inhibiting peptidoglycan synthesis through binding to peptidoglycan biosynthetic precursors or by limiting access to peptidoglycan or its precursors by enzymes that participate in cell wall synthesis. This mechanism of action of PGLYRPs is consistent with the ability of exogenous peptidoglycan to inhibit bactericidal activity of PGLYRPs (Lu et al., 2006).

Another possible mechanism of bacterial killing by PGLYRPs is enzymatic digestion of peptidoglycan by PGLYRPs, which would also be consistent with inhibition of killing by exogenous peptidoglycan. This is a less likely mechanism for several reasons (Lu et al., 2006). First, as already mentioned, peptidoglycan-lytic enzymes kill bacteria by destroying physical integrity of peptidoglycan, which causes immediate osmotic lysis of bacteria, manifested as permeabilization of cytoplasmic membrane that correlates with bacterial killing. PGLYRPs, however, do not cause early permeabilization of cytoplasmic membranes. Second, PGLYRP-1, PGLYRP-3 and PGLYRP-4, in contrast to PGLYRP-2, do not have any detectable amidase activity (Wang et al., 2003; Lu et al., 2006), likely because they do not have a conserved Cys, which corresponds to C530 in PGLYRP-2 and which is required for Zn\(^{2+}\) binding and amidase activity. Third, no significant bacteriolytic activity of PGLYRP-1, PGLYRP-3 and PGLYRP-4 was detected under the conditions that killed 99% of bacteria. And fourth, although bovine PGLYRP-1 does have bacteriolytic activity, this activity does not correlate with bactericidal activity, because the former is heat-labile and the latter is heat-stable (Tydell et al., 2006). However, it is still possible that peptidoglycan...
hydrolytic activity could be responsible for the bactericidal activity of PGLYRPs, if the binding of PGLYRPs to peptidoglycan was of high affinity (essentially irreversible), but the rate of hydrolysis was very slow. Such a slow rate of peptidoglycan hydrolysis is exhibited by insect PGRP-SA, which is a D-Ala-carboxypeptidase, and which, similarly to mammalian PGLYRP-1, PGLYRP-3 and PGLYRP-4, does not have the conserved Zn$^{2+}$ binding Cys (Chang et al., 2004). Such a carboxypeptidase activity of PGLYRPs would not have to be bacteriocytic, as it could convert the peptidoglycan biosynthetic precursor, GlcNAc-MurNAc-pentapeptide, to GlcNAc-MurNAc-tetrapeptide, and make it unsuitable for further steps in the biosynthetic pathway, such as transpeptidation. These possibilities will have to be explored experimentally.

Finally, it is also possible that bactericidal activity of PGLYRPs is unrelated to their peptidoglycan binding ability. This mechanism is the least likely, although it is consistent with the inhibition of PGLYRPs binding to bacteria (Lu et al., 2006) and killing of bacteria (Tydell et al., 2006) by lipoteichoic acid and lipopolysaccharide.

Conclusions

In conclusion, mammalian PGLYRPs function as both recognition and effector molecules: three PGLYRPs (PGLYRP-1, PGLYRP-3 and PGLYRP-4) are bactericidal, and are produced in PMNs or in epithelial cells and in body secretions. They protect the host against infections (Lu et al., 2006) and mutations in their genes may be linked to skin diseases, such as psoriasis (Sun et al., 2006). They form a new class of bactericidal proteins that have a different structure, mechanism of action, and expression from currently known mammalian antimicrobial peptides. PGLYRPs are much larger than all currently known vertebrate antibacterial peptides: PGLYRP-1, PGLYRP-3, PGLYRP-3:4 and PGLYRP-4 proteins are disulphide-linked glycosilated 44 kDa, 89 kDa, 98 kDa and 115 kDa dimers (Lu et al., 2006), whereas vertebrate antimicrobial peptides are typically 5–15 kDa. PGLYRPs require divalent cations (especially Ca$^{2+}$) and N-glycosilation for bactericidal activity, which are not usually required by membrane-permeabilizing antibacterial peptides, such as defensins or magainin (Lu et al., 2006). Mammalian PGLYRPs also differ from antimicrobial peptides in their mechanism of bactericidal activity: the former kill bacteria by interacting with cell wall peptidoglycan and the latter by permeabilizing bacterial membranes (Lu et al., 2006).

Furthermore, the expression patterns of mammalian PGLYRPs and antimicrobial peptides are different, and some cells that produce large amounts of these peptides, e.g. Paneth cells (which produce defensins, phospholipase A2 and lysozyme) do not express PGLYRPs (Lu et al., 2006). One mammalian PGLYRP, PGLYRP-2, is a peptidoglycan-lytic enzyme, N-acetylmuramoyl-L-alanine amidase that likely functions to reduce proinflammatory activity of peptidoglycan.

By contrast to mammalian PGLYRPs, several insect PGRPs function as bacterial recognition molecules that trigger proteolytic or signal transduction pathways, which result in production of antimicrobial peptides, melanin, or reactive oxygen species. Some insect PGRPs trigger phagocytosis or are peptidoglycan-lytic enzymes, but there are no insect PGRPs with known direct microbicidal activity. Thus, direct bactericidal activity of PGRPs either evolved in vertebrates or mammals, or it is yet to be discovered in insects.

Although the main functions of mammalian PGLYRPs have been identified, it is still possible that mammalian PGLYRPs have other unidentified functions, because many mammalian proteins have evolved to have multiple functions. Indeed, even some insect PGRPs, such as Drosophila PGRP-SA, have multiple functions (Fig. 1), and pig PGLYRP-2 has two splice forms, both of which have amidase activity, and also seem to play a role in induction of β-defensin synthesis (Sang et al., 2005). The amidase domain of PGLYRP-2 is located in the C-terminal one-third of the molecule, and the role of the remaining N-terminal two-thirds of PGLYRP-2 molecule is unknown, as it has no homology to any other PGRPs or to any other known proteins (Liu et al., 2001; Wang et al., 2003). This N-terminal portion of PGLYRP-2 therefore may have a unique and so far unidentified function.

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References

Bischoff, V., Vignal, C., Boneca, I.G., Michel, T., Hoffmann, J.A., and Royet, J. (2004) Function of the Drosophila pattern-recognition receptor PGRP-SD in the detection of Gram-positive bacteria. Nat Immunol 5: 1175–1180.

Chamaillard, M.M., Hashimoto, Y., Horie, J., Masumoto, S., Qiui, L., Saab, Y., et al. (2003) An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol 7: 702–707.

Chang, C.I., Pili-Floury, S., Herve, M., Parquet, C., Chelliah, Y., Lemaitre, B., et al. (2004) A Drosophila pattern recognition receptor contains a peptidoglycan docking groove and unusual L,D-carboxypeptidase activity. Plos Biol 2: 1293–1302.

Chang, C.I., Ihara, K., Chelliah, Y., Mengin-Lecreulx, D., Wakatsuki, S., and Deisenhofer, J. (2005) Structure of the ectodomain of Drosophila peptidoglycan-recognition protein LCA suggests a molecular mechanism for pattern recognition. Proc Natl Acad Sci USA 102: 10279–10284.

Cho, J.H., Fraser, I.P., Fukase, K., Kusumoto, S., Fujimoto, Y., Stahl, G.L., and Ezekowitz, R.A. (2005) Human
peptidoglycan recognition protein S is an effector of neutrophil-mediated innate immunity. Blood 106: 2552–2558.

Choe, K.-M., Werner, T., Stoven, S., Hultmark, D., and Anderson, K.V. (2002) Requirement for a peptidoglycan recognition protein (PGRP) in Relish activation and antibacterial immune responses in Drosophila. Science 296: 359–362.

Christophides, G.K., Zdobnov, E., Barillas-Mury, C., Birney, E., Blandin, S., Blass, C., et al. (2002) Immunity-related genes and gene families in Anopheles gambiae. Science 298: 159–165.

Dimopoulos, G., Christophides, G.K., Meister, S., Schultz, J., White, K.P., Barillas-Mury, C., and Kaftos, F.C. (2002) Genome expression analysis of Anopheles gambiae: responses to injury, bacterial challenge, and malaria infection. Proc Natl Acad Sci USA 99: 8814–8819.

Dziarski, R. (2004) Peptidoglycan recognition proteins (PGRPs). Mol Immunol 40: 877–886.

Dziarski, R., and Gupta, D. (2005) Staphylococcus aureus peptidoglycan is a Toll-like receptor 2 activator: a reevaluation. Infect Immun 73: 5212–5216.

Dziarski, R., Platt, K.A., Gelius, E., Steiner, H., and Gupta, D. (2003) Defect in neutrophil killing and increased susceptibility to infection with non-pathogenic Gram-positive bacteria in peptidoglycan recognition protein-S (PGRP-S)-deficient mice. Blood 102: 689–697.

Filipe, S.R., Tomasz, A., and Ligoxygakis, P. (2005) Requirements of peptidoglycan structure that allow detection by the Drosophila Toll pathway. EMBO Rep 6: 327–333.

Garver, L.S., Wu, J., and Wu, L.P. (2006) The peptidoglycan recognition protein PGRP-SC1a is essential for Toll signaling and phagocytosis of Staphylococcus aureus in Drosophila. Proc Natl Acad Sci USA 103: 660–665.

Gelius, E., Persson, C., Karlsson, J., and Steiner, H. (2003) A mammalian peptidoglycan recognition protein with N-acetylmuramoyl-L-alanine amidase activity. Biochem Biophys Res Commun 306: 988–994.

Girardin, S.E., Travassos, L.H., Herve, M., Blanot, D., Bonnec, I.G., Philpott, D.J., et al. (2003a) Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. J Biol Chem 278: 41702–41708.

Girardin, S.E., Boneca, I.G., Carneiro, L.A.M., Antignac, A., Jehanno, M., Viala, J., et al. (2003b) Nod1 detects a unique muropeptide from Gram-negative bacterial peptidoglycan. Science 300: 1584–1587.

Gobert, V., Gottar, M., Matskevich, A.A., Rutschmann, S., Royet, J., Belvin, M., et al. (2003) Dual activation of the Drosophila Toll pathway by two pattern recognition receptors. Science 302: 2126–2130.

Gottar, M., Gobert, V., Michel, T., Belvin, M., Duyk, G., and Hoffmann, J.A. (2002) The Drosophila immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature 416: 640–644.

Guan, R., Malchiodi, M.L., Wang, Q., Schuck, P., and Mariuzza, R.A. (2004a) Crystal structure of the C-terminal peptidoglycan-binding domain of human peptidoglycan recognition protein Ix. J Biol Chem 279: 31873–31882.

Guan, R., Roychowdhury, A., Ember, B., Kumar, S., Boons, G.-J., and Mariuzza, R.A. (2004b) Structural basis for peptidoglycan binding by peptidoglycan recognition proteins. Proc Natl Acad Sci USA 101: 17168–17173.

Guan, R., Wang, Q., Sundberg, E.J., and Mariuzza, R.A. (2005) Crystal structure of human peptidoglycan recognition protein S (PGRP-S) at 1.7 A resolution. J Mol Biol 347: 683–691.

Hojier, M.A., Melief, M.-J., Debets, R., and Hazenberg, M.P. (1997) Inflammatory properties of peptidoglycan are decreased after degradation with human N-acetylmuramyl-L-alanine amidase. Eur Cytokine Netw 8: 375–381.

Imoto, T., Johnson, L.N., North, A.C.T., Phillips, D.C., and Rupley, J.A. (1972) Vertebrate lysozyme. In The Enzymes, Vol. VII. Boyer, P.D. (ed.). New York: Academic Press, pp. 566–568.

Kane, T., and Silverman, N. (2005) Bacterial recognition and signaling by the Drosophila IMD pathway. Cell Microbiol 7: 461–469.

Kang, D., Liu, G., Lundstrom, A., Gelius, E., and Steiner, H. (1998) A peptidoglycan recognition protein in innate immunity conserved from insects to mammals. Proc Natl Acad Sci USA 95: 10078–10082.

Kappeler, S.R., Heuberger, C., Farah, Z., and Puhan, Z. (2004) Expression of the peptidoglycan recognition protein, PGRP, in the lactating mammary gland. J Dairy Sci 87: 2660–2668.

Kim, M.-S., Byun, M., and Oh, B.-H. (2003) Crystal structure of peptidoglycan recognition protein LB from Drosophila melanogaster. Nat Immunol 4: 787–793.

Kumar, S., Roychowdhury, A., Ember, B., Wang, Q., Guan, R., Mariuzza, R.A., and Boons, G.-J. (2005) Selective recognition of synthetic lysine and meso-diaminopimelic acid-type peptidoglycan fragments by human peptidoglycan recognition proteins Ix and S. J Biol Chem 280: 37005–37012.

Lee, M.H., Osaki, T., Lee, J.Y., Baek, M.J., Zhang, R., Park, J.W., et al. (2004) Peptidoglycan recognition proteins involved in 1,3-β-D-glucan-dependent prophenoloxidase activation system of insect. J Biol Chem 279: 3218–3227.

Lemaitre, B., Kromer-Metzger, E., Michaut, L., Nicolas, E., Meister, M., Georgel, P., et al. (1995) A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the Drosophila host defense. Proc Natl Acad Sci USA 92: 9465–9469.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., and Hoffmann, J.A. (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86: 973–983.

Leuiller, F., Parquet, C., Pili-Floury, S., Ryu, J.-H., Caroff, M., Lee, W.-J., et al. (2003) The Drosophila immune system detects bacteria through specific peptidoglycan recognition. Nat Immunol 4: 478–484.

Liu, C., Gelius, E., Liu, G., Steiner, H., and Dziarski, R. (2000) Mammalian peptidoglycan recognition protein binds peptidoglycan with high affinity, is expressed in neutrophils, and inhibits bacterial growth. J Biol Chem 275: 24490–24499.

Liu, C., Xu, Z., Gupta, D., and Dziarski, R. (2001) Peptidoglycan recognition proteins: a novel family of four human innate immunity pattern recognition molecules. J Biol Chem 276: 34686–34694.

Lo, D., Tynan, W., Dickerson, J., Mendy, J., Chang, H.-W., Scharf, M., et al. (2003) Peptidoglycan recognition protein expression in mouse Peyer’s Patch follicle associated epithelium suggests functional specialization. Cell Immunol 224: 8–16.

Lu, X., Wang, M., Qi, J., Wang, H., Li, X., Gupta, D., and Dziarski, R. (2006) Peptidoglycan recognition proteins are...
a new class of human bactericidal proteins. J Biol Chem 281: 5895–5907.

Mathur, M., Murray, B., Crowell, T., Gardner, H., Allaie, N., Hu, Y.-M., et al. (2004) Murine peptidoglycan recognition proteins Pglyrp1α and Pglyrp1α are encoded in the epidermal differentiatation complex and are expressed in epidermal and hematopoietic tissues. Genomics 83: 1151–1163.

Mellroth, P., Karlsson, J., and Steiner, H. (2003) A scavenger function for a Drosophila peptidoglycan recognition protein. J Biol Chem 278: 7059–7064.

Mellroth, P., Karlsson, J., Hakansson, J., Schultz, N., Gold- man, W.E., and Steiner, H. (2005) Ligand-induced dimerization of Drosophila peptidoglycan recognition proteins in vitro. Proc Natl Acad Sci USA 102: 6455–6460.

Michel, T., Reichhart, J.-M., Hoffmann, J.A., and Royet, J. (2001) Drosophila Toll is activated by Gram-positive bacte ria through a circulating peptidoglycan recognition protein. Nature 414: 756–759.

Ohno, N., and Morrison, D.C. (1989) Lipopolysaccharide interaction with lysozyme. Binding of lipopolysaccharide to lysozyme and inhibition of lysozyme enzymatic activity. J Biol Chem 264: 4434–4441.

Park, J.W., Je, B.-R., Piao, S., Inamura, S., Fujimoto, Y., Fukase, K., et al. (2006) A synthetic peptidoglycan fragment as a competitive inhibitor of the melanization cascade. J Biol Chem 281: 7747–7755.

Pili-Floury, S., Leulier, F., Takahashi, K., Saigo, K., Samain, E., Ueda, R., and Lemaitre, B. (2004) Peptidoglycan recognition protein-L is an N-acetylmuramoyl-L-alanine amidase. Proc Natl Acad Sci USA 101: 7059–7064.

Reiser, J.-B., Teyton, L., and Wilson, I.A. (2004) Crystal structure of the Drosophila peptidoglycan recognition protein (PGRP)-SA at 1.56 A resolution. J Mol Biol 340: 909–917.

Swaminathan, C.P., Brown, P.H., Roychowdhury, A., Wang, Q., Guan, R., Silverman, N., et al. (2006) Dual strategies for peptidoglycan discrimination by peptidoglycan recognition proteins (PGRPs). Proc Natl Acad Sci USA 103: 684–689.

Takehana, A., Yano, T., Mita, S., Kotani, A., Oshima, Y., and Kurata, S. (2004) Peptidoglycan recognition protein (PGRP)-LE and PGRP-LC act synergistically in Drosophila immunity. EMBO J 23: 4690–4700.

Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T., et al. (1999) Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. Immunity 11: 443–451.

Tydell, C.C., Yount, N., Tran, D., Yuan, J., and Selsted, M. (2002) Isolation, characterization, and antimicrobial properties of bovine oligosaccharide-binding protein. J Biol Chem 277: 19658–19664.

Tydell, C.C., Yuan, J., Tran, P., and Selsted, M.E. (2006) Bovine peptidoglycan recognition protein-S: antimicrobial activity, localization, secretion, and binding properties. J Immunol 176: 1154–1162.

Uehara, A., Sugawara, Y., Kurata, S., Fujimoto, Y., Fukase, K., Kusumoto, S., et al. (2005) Chemically synthesized pathogen-associated molecular patterns increase the expression of peptidoglycan recognition proteins via toll-like receptors, NOD1 and NOD2 in human oral epithelial cells. Cell Microbiol 7: 675–686.

Wang, H., Gupta, D., Li, X., and Dziarski, R. (2005) Peptidoglycan recognition protein 2 (N-acetylmuramoyl-L-alanine amidase) is induced in keratinocytes by bacteria through the p38 kinase pathway. Infect Immun 73: 7216–7225.

Wang, Z.-M., Li, X., Cocklin, R.R., Wang, M., Wang, M., Fukase, K., et al. (2003) Human peptidoglycan recognition protein-L is an N-acetylmuramoyl-L-alanine amidase. J Biol Chem 278: 49044–49052.

Werner, T., Liu, G., Kang, D., Ekenbren, S., Steiner, H., and Hultmark, D. (2000) A family of peptidoglycan recognition proteins in the fruit fly Drosophila melanogaster. Proc Natl Acad Sci USA 97: 13772–13777.

Werner, T., Borge-Renberg, K., Mellroth, P., Steiner, H., and Hultmark, D. (2003) Functional diversity of the Drosophila PGRP-LC gene cluster in the response to lipopolysaccharide and peptidoglycan. J Biol Chem 278: 26319–26322.

Yoshida, H., Kinoshita, K., and Ashida, M. (1996) Purification of peptidoglycan recognition protein from hemolymph of the silkworm, Bombyx mori. J Biol Chem 271: 13854–13860.

Yoshimura, A., Lien, E., Ingalls, R.R., Tuomanen, E., Dziarski, R., and Golenbock, D. (1999) Recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. J Immunol 163: 1–5.

Zhang, Y., van der Fits, L., Voerman, J.S., Mellef, M.-J., Laman, J.D., Wang, M., et al. (2005) Identification of serum N-acetylmuramoyl-L-alanine amidase as liver peptidoglycan recognition protein 2. Biochim Biophys Acta 1752: 34–46.