Protein family review

The peptidoglycan recognition proteins (PGRPs)

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Summary

Peptidoglycan recognition proteins (PGRPs) are innate immunity molecules present in insects, mollusks, echinoderms, and vertebrates, but not in nematodes or plants. PGRPs have at least one carboxy-terminal PGRP domain (approximately 165 amino acids long), which is homologous to bacteriophage and bacterial type 2 amidases. Insects have up to 19 PGRPs, classified into short (S) and long (L) forms. The short forms are present in the hemolymph, cuticle, and fat-body cells, and sometimes in epidermal cells in the gut and hemocytes, whereas the long forms are mainly expressed in hemocytes. The expression of insect PGRPs is often upregulated by exposure to bacteria. Insect PGRPs activate the Toll or immune deficiency (Imd) signal transduction pathways or induce proteolytic cascades that generate antimicrobial products, induce phagocytosis, hydrolyze peptidoglycan, and protect insects against infections. Mammals have four PGRPs, which are secreted; it is not clear whether any are directly orthologous to the insect PGRPs. One mammalian PGRP, PGLYRP-2, is an *N*-acetylmuramoyl-L-alanine amidase that hydrolyzes bacterial peptidoglycan and reduces its proinflammatory activity; PGLYRP-2 is secreted from the liver into the blood and is also induced by bacteria in epithelial cells. The three remaining mammalian PGRPs are bactericidal proteins that are secreted as disulfide-linked homo- and hetero-dimers. PGLYRP-1 is expressed primarily in polymorphonuclear leukocyte granules and PGLYRP-3 and PGLYRP-4 are expressed in the skin, eyes, salivary glands, throat, tongue, esophagus, stomach, and intestine. These three proteins kill bacteria by interacting with cell wall peptidoglycan, rather than permeabilizing bacterial membranes as other antibacterial peptides do. Direct bactericidal activity of these PGRPs either evolved in the vertebrate (or mammalian) lineage or is yet to be discovered in insects.

Gene organization and evolutionary history

Peptidoglycan recognition proteins (PGRPs) are innate immunity molecules that contain a conserved peptidoglycan-binding type 2 amidase domain that is homologous to bacteriophage and bacterial type 2 amidases [1-6]. PGRPs are ubiquitous in most animals. Insects have multiple PGRP genes that are classified into short (S) and long (L) transcripts and are often alternatively spliced into up to 19 different proteins (Table 1) [1-5]. PGRPs have also been identified in mollusks, echinoderms, and vertebrates (Table 1), but plants and lower metazoa, including nematodes such as *Caenorhabditis elegans*, do not have PGRPs. PGRP genes usually form clusters that suggest their origin by gene duplication.

Mammals have a family of four PGRPs, which were initially named PGRP-S, PGRP-L, and PGRP-Iα and PGRP-Iβ (for 'short', 'long', or 'intermediate' transcripts, respectively), by analogy to insect PGRPs [3]. Subsequently, the Human Genome Organization Gene Nomenclature Committee changed their symbols to PGLYRP-1, PGLYRP-2, PGLYRP-3, and PGLYRP-4, respectively. This terminology is also used for mouse PGRPs, and is beginning to be adopted for all

Table 1

Accession numbers, chromosomal locations, and functions of PGRPs

Organism (abbreviation)	Protein name*	Accession number†	Gene ID	Chromosome	PDB ID‡	Function§
Insects						
Anopheles gambiae, mosquito *(Ag)*	PGRP-LA	XM_314105	1274911	2L	-	-
	PGRP-LB	XM_321943	1281956	2R	-	Predicted amidase
	PGRP-LC1	XM_314103	1274909	2L	-	-
	PGRP-LC2	XM_558599	1274909	2L	-	-
	PGRP-LC3	XM_558600	1274909	2L	-	-
	PGRP-S1	XM_310547	1271702	X	-	-
	PGRP-S2	XM_557000	3290146	2L	-	-
	PGRP-S3	XM_316359	1276947	2L	-	Predicted amidase
	PGRP-SC2	XM_316360	1276948	2L	-	Predicted amidase
Apis mellifera, honey bee (*Am*)	PGRP-L	XM_392452	408924	LG7	-	-
	PGRP-S	XM_395941	412484	LG13	-	Predicted amidase
Bombyx mori, domestic silkworm (*Bm*)	BTL-LP1	AB017519	-	-	-	Predicted amidase
	BTL-LP2	AB017520	-	-	-	-
	PGRP	AF441723	-	-	-	-
	PGRP-S	AB016249	-	-	-	PPO activation [36]
Calpodes ethlius, Brazilian skipper butterfly (*Ce*)	PGRP-S	AF035445	-	-	-	-
Drosophila melanogaster, fruit fly (*Dm*)	PGRP-LA-C	NM_206306	39062	3L 67A7	-	-
	PGRP-LA-D(a)	NM_206305	39062	3L 67A7	-	-
	PGRP-LA-E	NM_206304	39062	3L 67A7	-	-
	PGRP-LA-F(b)	NM_206307	39062	3L 67A7	-	-
	PGRP-LB-A	NM_141822	41379	3R 86E8	1OHT	Amidase [7,40]
	PGRP-LB-B	NM_169393	41379	3R 86E8	-	Predicted amidase
	PGRP-LB-C	NM_169392	41379	3R 86E8	-	Predicted amidase
	PGRP-LC-A(x)	NM_168324	39063	3L 67A8	2F2L	Imd activation [19,25,29-34], phagocytosis [31]
	PGRP-LC-B(a)	NM_140041	39063	3L 67A8	1Z6I, 2F2L	Imd activation [19,29-34]
	PGRP-LC-C(y)	NM_206308	39063	3L 67A8	-	Imd activation [33]
	PGRP-LD-A	NM_001031942	3771920	3L 67A8	-	-
	PGRP-LE	NM_132850	32534	X 13F1	2CB3	Imd and PPO activation [35]
	PGRP-LF	NM_140042	39064	3L 67A8-67A9	-	-
	PGRP-SA	NM_132499	32099	X 10C6	1SXR, 1S2J	Toll activation [8], carboxypeptidase [12], phagocytosis [24]
	PGRP-SB1	NM_140660	39870	3L 73C1	-	Predicted amidase
	PGRP-SB2	NM_140659	39869	3L 73C1	-	Predicted amidase
	PGRP-SC1a¶	NM_136563	35859	2R 44E2	-	Amidase [14], Toll activation [24], phagocytosis [24]
	PGRP-SC1b¶	NM_136565	35861	2R 44E2	-	Amidase [14]
	PGRP-SC2	AJ55662	-	2R 44E2	-	Predicted amidase
	PGRP-SD	AJ556628	-	3L 66A8	-	Toll activation [23]
Glossina morsitans, tsetse fly (*Glm*)	PGRP-LB	DQ307160	-	-	-	Predicted amidase
	PGRP-LC	DQ307161	-	-	-	-
Galleria mellonella, greater wax moth (*Gm*)	PGRP-A	AF394583	-	-	-	-
	PGRP-B	AF394587	-	-	-	-
Holotrichia diomphalia, beetle (*Hd*)	PGRP-1	AB115774	-	-	-	PPO activation [38]
	PGRP-2	AB115775	-	-	-	-
	PGRP-3	AB115776	-	-	-	-
Manduca sexta, tobacco hornworm (*Ms*)	PGRP-1A	AF413068	-	-	-	-
	PGRP-1B	AF413061	-	-	-	-
Tenebrio molitor, yellow mealworm (*Tm*)	PGRP-SA	AB219970	-	-	-	PPO activation [37]
Trichoplusia ni, cabbage looper (*Tn*)	PGRP-S	AF076481	-	-	-	-
Mollusks						
Argopecten irradians, bay scallop (*Ai*)	PGRP	AY437875	-	-	-	Predicted amidase
Euprymna scolopes, Hawaiian bobtail squid (*Es*)	PGRP-1	AY956811	-	-	-	Predicted amidase
	PGRP-2	AY956812	-	-	-	Predicted amidase

Table 1 *(continued)*

Organism (abbreviation)	Protein name*	Accession number†	Gene ID	Chromosome	PDB ID‡	Function§
	PGRP-3	AY956813	-	-	-	Predicted amidase
	PGRP-4	AY956814	-	-	-	-
Echinoderms						
Asterias rubens, European starfish (*Ar*)	PGRP-S1a	DQ222477	-	-	-	Predicted amidase
	PGRP-S2a	DQ222478	-	-	-	Predicted amidase
Strongylocentrotus purpuratus, purple sea urchin (*Sp*)	PGRP-S	XM_781925	581948	-	-	Predicted amidase
Fish						
Danio rerio, zebrafish (*Dr*)	PGLYRP-2	DQ447202	568634	8	-	Predicted amidase
	PGLYRP-5	DQ447203	553387	18	-	Predicted amidase
	PGLYRP-6	DQ447204	571817	-	-	Predicted amidase
Tetraodon nigroviridis, spotted green pufferfish (*Ten*)	PGLYRP-2	CAG06114	-	-	-	Predicted amidase
Amphibians						
Xenopus laevis, African clawed frog (*Xl*)	PGLYRP-5	BC087429	496035	-	-	Predicted amidase
Xenopus tropicalis, Western clawed frog (*Xt*)	PGLYRP-1	NM_001030455	595014	-	-	Predicted amidase
	PGLYRP-5	NM_001015775	548492	-	-	Predicted amidase
Birds						
Gallus gallus, chicken (*Gg*)	PGLYRP-2	AY740510	-	-	-	Predicted amidase
Mammals						
Bos taurus, cow (*Bt*)	PGLYRP-1	NM_174573	282305	18	-	Bactericidal [46,47]
	PGLYRP-2	XM_588006	510803	7	-	Predicted amidase
	PGLYRP-3	XM_611696	532575	3	-	Predicted bactericidal¥
Camelus dromedaries, camel (*Cd*)	PGLYRP-1	AJ409286	-	-	-	Predicted bactericidal
Canis familiaris, dog (*Cf*)	PGLYRP-1	XM_849945	612209	1	-	Predicted bactericidal
	PGLYRP-2	XM_847906	610405	20	-	Predicted amidase
Homo sapiens, human (*Hs*)	PGLYRP-1	NM_005091	8993	19q13.2-q13.3	1YCK	Bactericidal [17]
	PGLYRP-2	NM_052890	114770	19p13.12	-	Amidase [9,16]
	PGLYRP-3	NM_052891	114771	1q21	1SK3, 1SK4, 1TWQ, 2APH	Bactericidal [17]
	PGLYRP-4	NM_020393	57115	1q21	-	Bactericidal [17]
Mus musculus, mouse (*Mm*)	PGLYRP-1	NM_009402	21946	7 A3	-	Antibacterial [45,48]
	PGLYRP-2	AY282722	57757	17	-	Amidase [15]
	PGLYRP-3	NM_207247	242100	3 F1	-	Predicted bactericidal
	PGLYRP-4	NM_207263	384997	3 F1	-	Predicted bactericidal
Pan troglodytes, chimpanzee (*Pt*)	PGLYRP-2	XM_512455	455797	19	-	Predicted amidase
Rattus norvegicus, rat (*Rn*)	PGLYRP-1	NM_053373	84387	1q21	-	Predicted bactericidal
	PGLYRP-2	BC088306	299567	7q11	-	Predicted amidase
	PGLYRP-3	XM_57498	499658	2q34	-	Predicted bactericidal
	PGLYRP-4	XM_227383	310611	2q34	-	Predicted bactericidal
Sus scrofa, pig (*Ss*)	PGLYRP-1	NM_001001260	397213	-	-	Predicted bactericidal
	PGLYRP-2A	AF541955	-	-	-	Amidase [44]
	PGLYRP-2B	AF541956	-	-	-	Amidase [44]

*Vertebrate PGRPs were initially named PGRP-S, PGRP-L, and PGRP-Iα and PGRP-Iβ (for short, long, and intermediate transcripts). The human and mouse PGRPs have been renamed PGLYRP-1, PGLYRP-2, PGLYRP-3, and PGLYRP-4, respectively, and this new nomenclature is followed here for all vertebrate PGRP orthologs. Current nomenclature of *D. melanogaster* PGRP-LA, -LB, and -LC isoforms (-A, -B, and so on) is indicated. Previous names are also included, indicated by lower case letters in parentheses. For *D. melanogaster* PGRP-LD, isoforms -A, -B, and -C have the same amino-acid sequence, and only isoform A is shown. †Accession numbers starting with XM are predicted proteins. ‡A dash in the PBD ID column indicates that a structure or function has not been determined. §Amidase activities were predicted on the basis of the presence of all four Zn^{2+}-binding amino acids and other amino acids required for the amidase activity, as described [9,14,15]. PPO, prophenol-oxidase. ¶*D. melanogaster* PGRP-SC1a and PGRP-SC1b are encoded by two adjacent genes translated into proteins with identical amino acid sequences. ¥Bactericidal activities were predicted on the basis of homology to human PGLYRPs.

vertebrate PGRPs. In this article, the abbreviation PGRP will be used for all invertebrate members and PGLYRP for all vertebrate members of the PGRP family.

Phylogenetic analysis of insect PGRPs reveals an early separation of PGRPs into enzyme-active amidases and the remaining PGRPs, which activate signal transduction pathways and proteolytic cascades (Figure 1). PGRPs from other animals cannot easily be grouped with any individual insect PGRPs, so they are considered separately here. The non-insect PGRPs also evolved into two groups. The first group are all amidases, which in echinoderms, mollusks, fish, and amphibians are evolutionarily older and which more recently evolved into the mammalian amidases (PGLYRP-2; Figure 2). The second group are mammalian bactericidal proteins, which separated into two well defined branches: PGLYRP-1 (present in phagocytic granules) and PGLYRP-3 and PGLYRP-4 (present on skin and mucous membranes; Figure 2). The only probable orthologs between non-insect and insect PGRPs are the amidase-active PGRPs (Figures 1,2 and Table 1).

Characteristic structural features

Most PGRPs have one carboxy-terminal type 2 amidase domain (approximately 165 amino acids-long; Figure 3), which is homologous to bacteriophage and bacterial type 2 amidases [1-4]. It is also called a PGRP domain, because it is longer at its amino terminus than a type 2 amidase domain and contains a PGRP-specific segment not present in type 2 amidases [7]. Across all animals, the PGRP domains are approximately 42% identical and about 55% similar. The short PGRPs (invertebrate PGRP-S and vertebrate PGLYRP-1) are about 200 amino acids long, have a signal peptide and one PGRP domain, and have a molecular weight

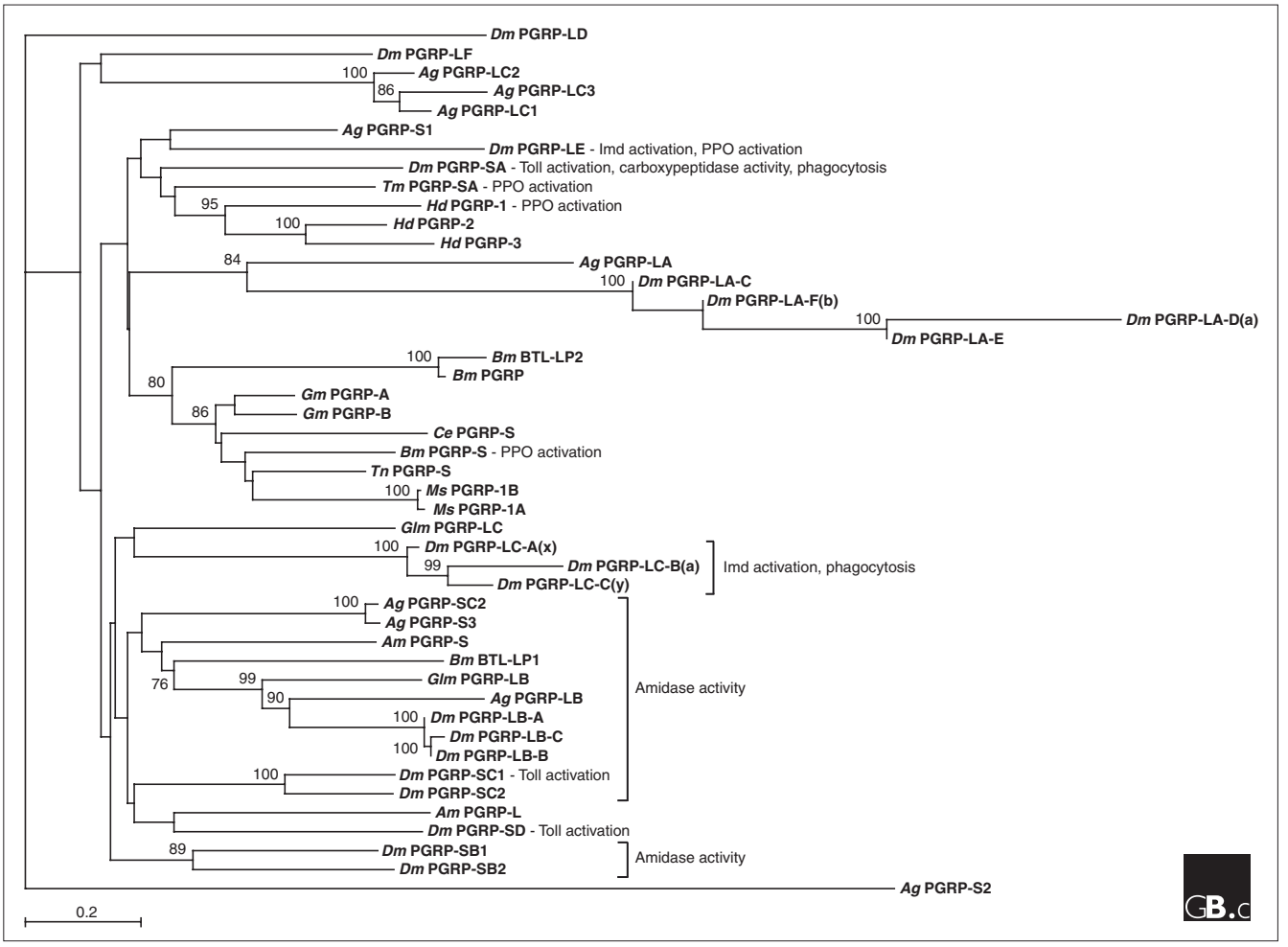

Figure 1
A phylogenetic tree of insect PGRPs, indicating their known and deduced functions. For branches supported by bootstrap analysis with the proportion of 1,000 replications higher than 70%, the percentage is indicated. The bar indicates the p-distance. Abbreviations: *Ag, Anopheles gambiae; Am, Apis mellifera; Bm, Bombyx mori; Ce, Calpodes ethlius; Dm, Drosophila melanogaster; Glm, Glossina morsitans; Gm, Galleria mellonella; Hd, Holotrichia diomphalia; Ms, Manduca sexta; Tm, Tenebrio molitor; Tn, Trichoplusia ni.* Accession numbers and references are listed in Table 1. PPO, prophenol-oxidase.

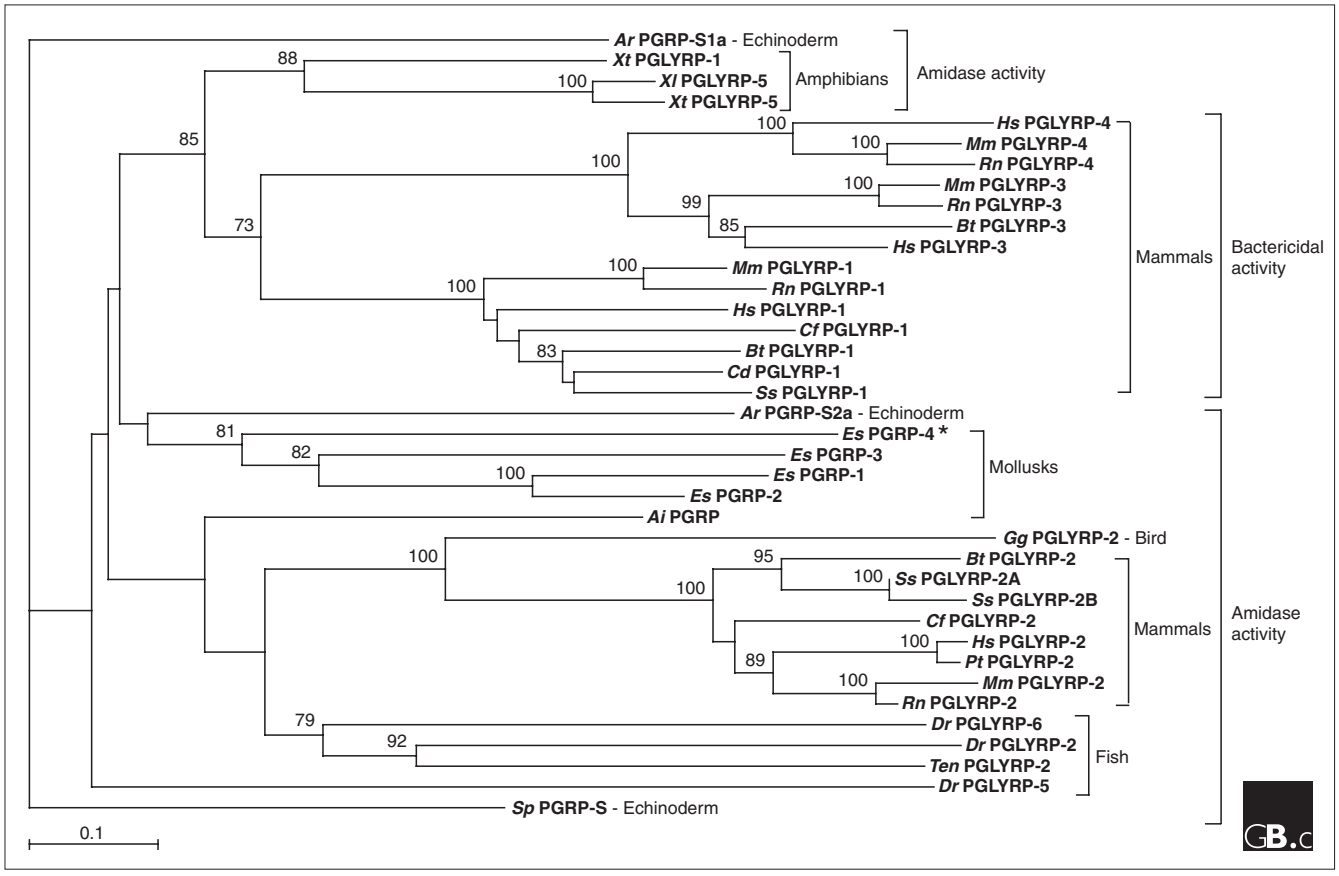

Figure 2
A phylogenetic tree of mollusk, echinoderm, and vertebrate PGRPs, indicating their known and deduced functions . Bootstrap analysis and p-distance are indicated as in Figure 1. Abbreviations: *Ai, Argopecten irradians; Ar, Asterias rubens; Bt, Bos taurus; Cd, Camelus dromedaries; Cf, Canis familiaris; Dr, Danio rerio; Es, Euprymna scolopes; Gg, Gallus gallus; Hs, Homo sapiens; Mm, Mus musculus; Pt, Pan troglodytes; Rn, Rattus norvegicus; Sp, Strongylocentrotus purpuratus; Ss, Sus scrofa; Ten, Tetraodon nigroviridis; Xl, Xenopus laevis; Xt, Xenopus tropicalis.* Accession numbers and references are listed in Table 1. The asterisk indicates that *Es* PGRP-4 is not a predicted amidase.

of about 18-20 kDa. Most long or intermediate-sized PGRPs (invertebrate PGRP-L and vertebrate PGLYRP-2) are at least twice as large and have one carboxy-terminal PGRP domain and an amino-terminal sequence of variable length that is not conserved and is unique for a given PGRP. These amino-terminal sequences have no homology to other PGRPs or any other proteins, and they lack easily identifiable functional motifs. Some PGRPs, such as *Drosophila* PGRP-LC, are transmembrane molecules, whereas most other PGRPs have a signal peptide and are secreted, or do not have a signal peptide and therefore are either intracellular or are secreted by another mechanism. Some PGRPs, most notably all mammalian PGLYRP-3 and PGLYRP-4 and some insect PGRPs (such as *Drosophila* PGRP-LF), have two PGRP domains, but these are not identical (for example, in human PGLYRP-3 and PGLYRP-4 they have only 37-43% identity).

Almost all PGRPs have two closely spaced conserved cysteines in the middle of the PGRP domain that form a disulfide bond, which is needed for the activity of PGRPs. A mutation in one of these cysteines in *Drosophila* PGRP-SA (Cys80Tyr) abolishes the ability of PGRP-SA to activate the Toll pathway and to induce a protective response against Gram-positive bacteria [8], whereas a mutation in one of these cysteines in human PGLYRP-2 (Cys419Ala) abolishes its amidase activity [9]. Most vertebrate PGLYRPs and some invertebrate PGRPs have two additional conserved cysteines that form a second disulfide bond, and many mammalian PGLYRPs (PGLYRP-1 and the carboxy-terminal PGRP domain of PGLYRP-3 and PGLYRP-4) have another conserved pair of cysteines that form a third disulfide (Figure 3).

The crystal structures of PGRPs reveal a general design similar to type 2 bacteriophage amidases: they all have three peripheral α helices and several central β-sheet strands (Figure 3) [7,10-13]. The front face of the molecule has a cleft that forms a peptidoglycan-binding groove (Figure 3), and the back of the molecule has a PGRP-specific segment (not present in bacteriophage amidases), which is often hydrophobic and is also

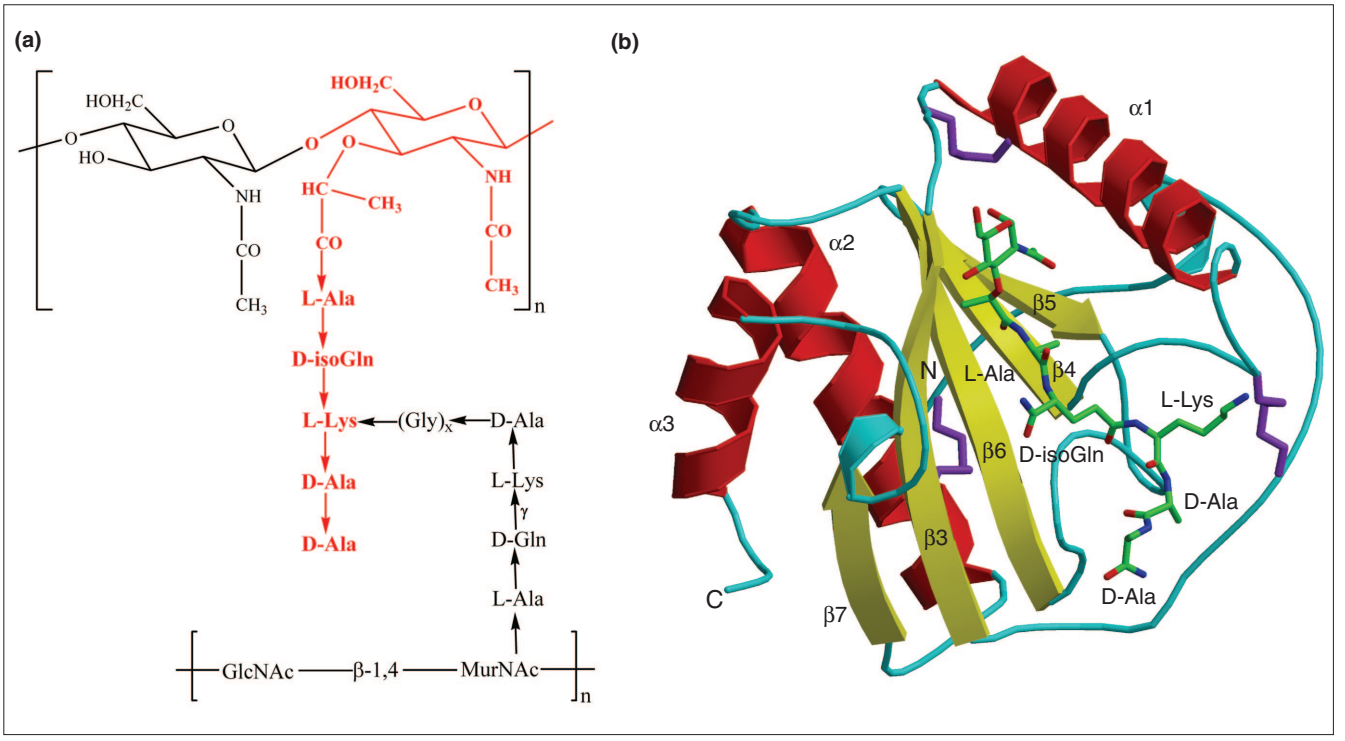

Figure 3
The structures of **(a)** Lys-type peptidoglycan and **(b)** the carboxy-terminal PGRP domain of human PGLYRP-3 complexed with MurNAc-pentapeptide. (a) Lys-type peptidoglycan; two repeating disaccharide units crosslinked by a peptide are shown; the MurNAc-pentapeptide is in red; the arrows represent the direction of the peptide bond; D-isoGln, D-isoglutamine. (b) The PGRP domain has three α helices (red), five β strands (yellow) and coils (cyan); the three disulfide bonds are in purple; MurNAc-pentapeptide is drawn in stick representation, with carbon, nitrogen, and oxygen atoms in green, blue, and red, respectively. N, amino terminus; C, carboxyl terminus. Reproduced with permission from [58].

more diverse among various PGRPs. All amidase-active PGRPs (invertebrate and vertebrate) have a conserved Zn^{2+}-binding site in the peptidoglycan-binding groove, which is also present in bacteriophage type 2 amidases and consists of two histidines, one tyrosine, and one cysteine (Cys168 in *Drosophila* PGRP-SC1 and Cys530 in human PGLYRP-2). In non-amidase PGRPs, this cysteine is substituted with serine; the presence of this cysteine can therefore be used to predict the amidase activity of PGRPs (Figures 1,2 and Table 1) [9,14,15].

All mammalian PGLYRPs are secreted, and PGLYRP-1, PGLYRP-3, and PGLYRP-4 form disulfide-linked homodimers [16,17]. Moreover, if PGLYRP-3 and PGLYRP-4 are expressed in the same cells, they almost exclusively form disulfide-linked heterodimers [17]. Insect PGRPs have not been shown to form disulfide-linked dimers, but binding to their ligands may induce dimerization [18,19].

Localization and function

Insect PGRPs

Both invertebrate and vertebrate PGRPs function as pattern-recognition and effector molecules in innate immunity. Consistent with their role in insect immunity, most insect PGRPs are expressed in immune-competent organs [1,2,20-22]. Insect PGRP-S and other short PGRPs are present in the hemolymph and cuticle and are constitutively synthesized or induced, mainly in the fat-body cells, and some also in the epidermal cells, in the gut, and to a lesser extent in hemocytes. Long insect PGRPs are expressed mainly in hemocytes, although some are also present in the hemolymph (for example *Drosophila* PGRP-LE). The expression of several short and long insect PGRPs is upregulated by exposure to bacteria or purified bacterial peptidoglycan, which is an essential cell wall component of virtually all bacteria. Differential induction of expression of different PGRPs by different stimuli suggests specificity of induction and effector function of different PGRPs [21,22].

Insect PGRPs have recognition, signaling, and effector functions, all of which are important for antimicrobial innate immunity (Figure 4). Three *Drosophila* PGRPs - PGRP-SA, PGRP-SD, and PGRP-SC1 - recognize bacterial peptidoglycan and activate proteases that cleave Spaetzle, an extracellular cytokine-like protein present in insect hemolymph, which in turn serves as an endogenous activator of Toll [8,23,24] (Figure 4a). Activation of Toll initiates a signal

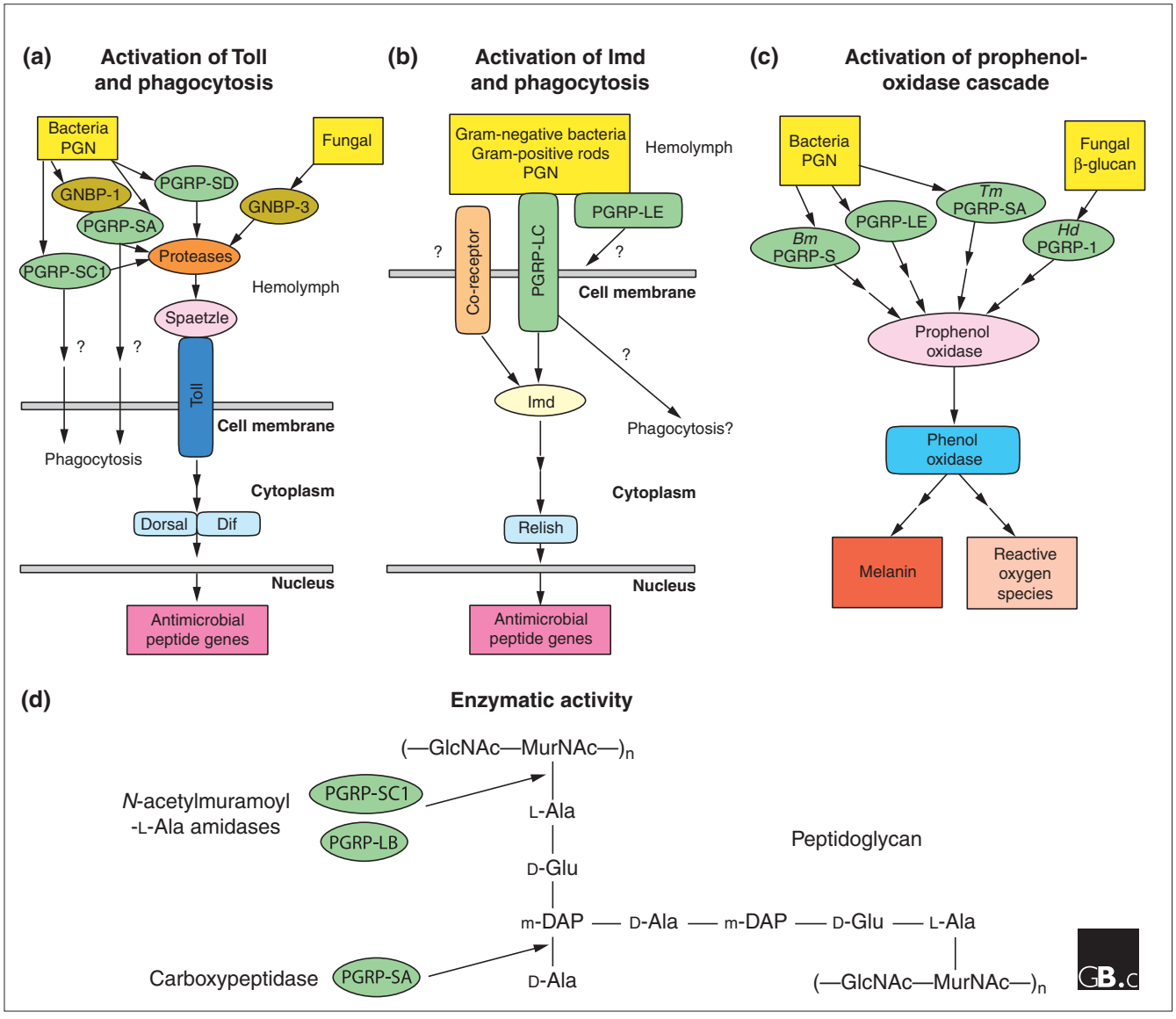

Figure 4
Functions of insect PGRP proteins. In response to peptidoglycan (PGN) from bacteria or other stimulants (yellow), insect PGRPs activate the **(a)** Toll and **(b)** Imd pathways and **(c)** the prophenol-oxidase cascade, which results in the production of antimicrobial products. **(d)** The structure of DAP-type peptidoglycan, indicating the positions at which proinflammatory peptidoglycan can be hydrolyzed by some PGRPs, reducing inflammation. *Drosophila* PGRPs are shown (green) unless otherwise indicated (*Bm*, *Bombyx mori*; *Hd*, *Holotrichia diomphalia*; *Tm*, *Tenebrio molitor*). Multiple arrows signify multiple steps; question marks signify unconfirmed or controversial functions. PGN, peptidoglycan; m-DAP, *meso*-DAP. See text for more details of the pathways shown.

transduction pathway that results in the activation of the Dorsal and Dif transcription factors (which are similar to mammalian nuclear factor NF-κB), which translocate into the nucleus, bind to the NFκB sites in the genome, and initiate transcription of drosomycin and other antimicrobial peptides, which are mainly active against Gram-positive bacteria and fungi (Figure 4a). This pathway is essential for *Drosophila* immunity to Gram-positive bacteria: 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 [8,23,24].

Peptidoglycan is a polymer of β(1-4)-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc), crosslinked by short peptides containing alternating L- and D-amino acids (Figures 3a, 4d and 5c). In position 3, the peptide has either diaminopimelic acid (DAP-type peptidoglycan, found in all Gram-negative bacteria and in Gram-positive bacilli; Figure 4d) or L-lysine (Lys-type peptidoglycan, found in most other Gram-positive bacteria, Figures 3a and 5c).

The Toll pathway is preferentially triggered by the Lys-type peptidoglycan and only weakly by the DAP-type peptidoglycan

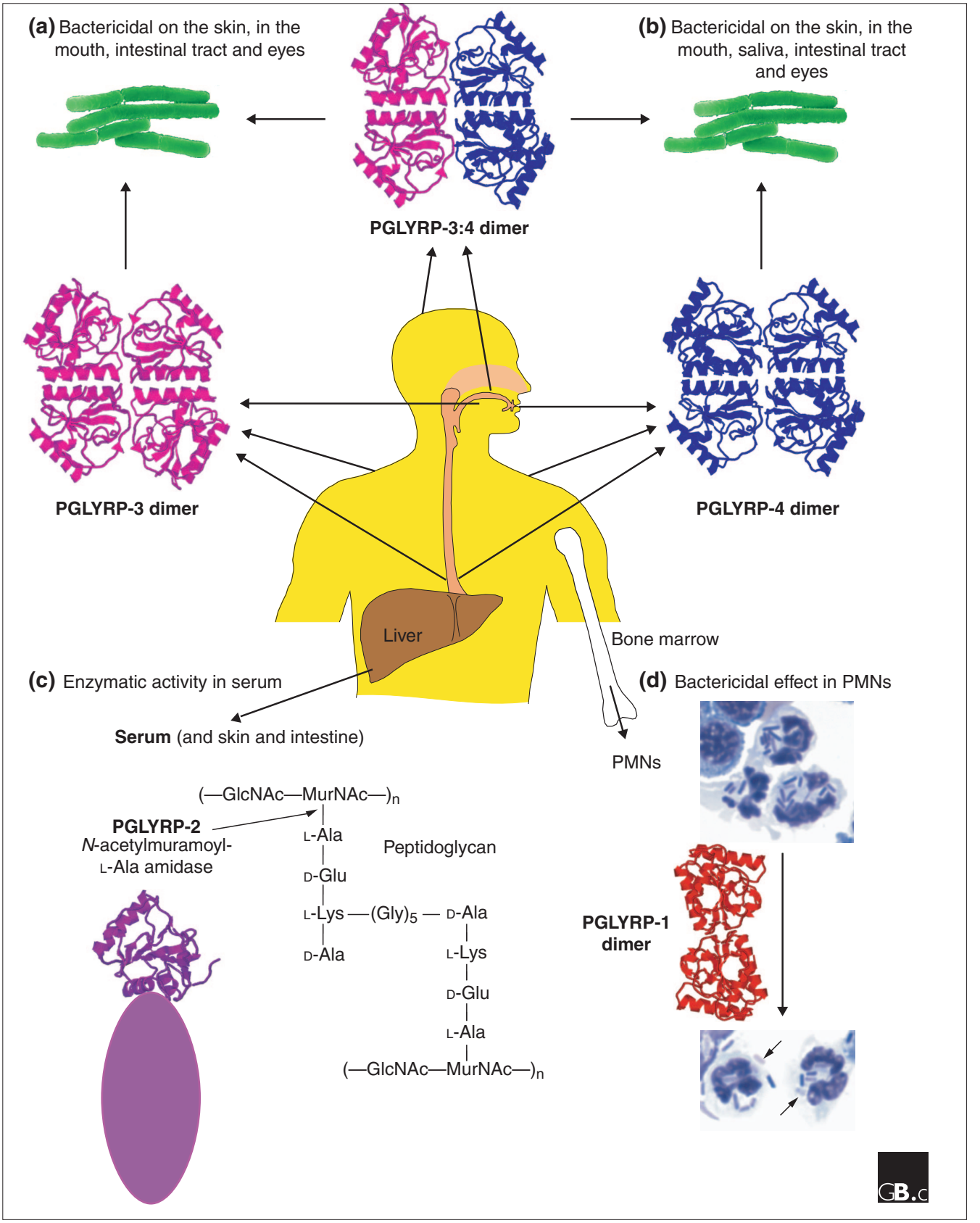

Figure 5 *(see legend on following page)*

[25], although both types of peptidoglycan bind to PGRP-SA [12]. The probable 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 [12] (Figure 4d). Efficient triggering of the Toll pathway by PGRP-SA requires cooperation (and probably formation of a complex) with another pattern-recognition molecule, Gram-negative binding protein (GNBP)-1 [26,27] (Figure 4a). GNBP-1 digests peptidoglycan and generates free reducing ends of MurNAc, which are then recognized by PGRP-SA [28]. *Drosophila* PGRP-SC1 and PGRP-SD [23,24], as well as other pattern-recognition molecules such as GNBP-3, also activate the Toll pathway (Figure 4a). 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 may also trigger phagocytosis by an as yet unidentified mechanism [24].

Activation of *Drosophila* PGRP-LC by Gram-negative bacteria and Gram-positive bacilli (also called rods) triggers another signal transduction pathway, the Imd pathway [19,25,29-34] (Figure 4b). Binding of peptidoglycan to *Drosophila* PGRP-LC induces its oligomerization and recruitment and activation of the death-domain-containing Imd protein [19]. The Imd pathway is Toll-independent and 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 active primarily against Gram-negative bacteria [29-31]. PGRP-LC responds primarily to DAP-type peptidoglycan. It is a transmembrane protein and has three alternative splice forms (LC-A, LC-B, and LC-C), which differ in the extracellular PGRP domains; they probably cooperate with each other and have somewhat different recognition specificities [25,29,32-34]. PGRP-LC activates the Imd pathway in cooperation with PGRP-LE [35] and also probably with another, as yet unidentified co-receptor (Figure 4b). *Drosophila* PGRP-LC may also have 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 of *Staphylococcus aureus* [31]; the mechanism of this phenomenon is still unclear, however.

Silkworm (*Bombyx mori*) and mealworm (*Tenebrio molitor*) PGRP-S are present in the hemolymph and cuticle, bind bacteria and Lys- and DAP-peptidoglycan, and activate the prophenol-oxidase cascade (Figure 4c) [36,37]. This generates antimicrobial products, such as melanin and reactive oxygen species, surrounds the infection site with melanin, and contains the infection. *Drosophila* PGRP-LE [35] and beetle (*Holotrichia diomphalia*) PGRP-1 [38] (and probably 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.

Drosophila PGRP-SC1 and PGRP-LB are *N*-acetylmuramoyl-L-alanine amidases [7,14], which hydrolyze the amide bond between MurNAc and L-alanine and thus remove stem peptides from peptidoglycan (Figure 4d). Stem peptides are the four to five amino acids directly bound to MurNAc. Digestion of peptidoglycan with amidase reduces or eliminates the ability of polymeric peptidoglycan to stimulate insect cells [14], and thus the function of amidase PGRPs *in vivo* may be to prevent excessive activation of the immune system by bacteria [39,40]. On the basis of the conserved structure of the active site of the amidase, several other insect PGRPs are predicted to have amidase activity, whereas several others are not [9,14,15] (Figure 1 and Table 1). One PGRP that is not an amidase, *Drosophila* PGRP-SA, has an L,D-carboxypeptidase activity with specificity for the bond between DAP and D-Ala of the stem peptide present in peptidoglycan of Gram-negative bacteria and Gram-positive rod bacteria [12] (Figure 4). The biological significance of this carboxypeptidase activity is not certain.

Mammalian PGLYRPs

Mammalian PGLYRPs are differentially expressed in various organs and tissues and have two major functions: amidase

Figure 5 *(see figure on previous page)*
Functions and expression of mammalian PGLYRP proteins. The diagram in the center shows the regions of the human body where each PGLYRP is expressed; note that the information shown applies to other mammals as well as humans. **(a)** Mammalian PGLYRP-3 has direct bactericidal activity and is expressed in the skin, eyes, tongue, esophagus, stomach, and intestines. **(b)** PGLYRP-4 and the PGLYRP-3:4 dimer also have direct bactericidal activity in the same tissues; PGLYRP-4 is also expressed in the salivary gland, mucus-secreting glands in the throat and also in saliva. **(c)** PGLYRP-2, which is constitutively produced in the liver and secreted into the blood, is also induced in the skin and intestine. It is an *N*-acetylmuramoyl-L-alanine amidase that hydrolyzes proinflammatory peptidoglycan. The structure of Lys-type peptidoglycan is shown, to indicate where in the molecule PGLYRP-2 hydrolyzes it. **(d)** PGLYRP-1 is present in the granules of the polymorphonuclear leukocytes (PMNs) which are produced in the bone marrow. PGLYRP-1 is bactericidal for phagocytosed bacteria; the images show killing of bacillus by PMNs. The images of scanning electron micrographs of *Bacillus* in (a) and (b) are copyright Dennis Kunkel Microscopy, Inc and are reproduced with permission. PGLYRP structures were rendered by RasMol and arranged as homodimers or heterodimers. The structure of PGLYRP-1 is based on PDB entry 1yckA; the structure of the carboxy-terminal PGRP domain of PGLYRP-2 was predicted by Swiss-Model on the basis of the crystal structure of *D. melanogaster* PGRP-SA (PDB entry 1s2jB); the amino-terminal portion of PGLYRP-2 cannot be predicted and hence is shown as an oval; the structures of PGLYRP-3 and PGLYRP-4 were predicted by Swiss-Model based on the crystal structure of carboxy-terminal half of PGLYRP-3 (PDB entry 1SK3A).

activity and antibacterial activity. Mammalian PGLYRP-2 (and probably other vertebrate PGLYRP-2s) is an *N*-acetylmuramoyl-L-alanine amidase that hydrolyzes the lactyl bond between the MurNAc and L-alanine in bacterial peptidoglycan (Figure 5c) [9,15]. PGLYRP-2 is constitutively produced in the liver and is secreted from the liver into the blood [16]. This liver PGLYRP-2 and serum *N*-acetylmuramoyl-L-alanine amidase (which was identified earlier but not cloned) are the same protein, encoded by the *PGLYRP2* gene [16]. The function of this amidase is probably to eliminate the proinflammatory peptidoglycan and thus to prevent overactivation of the immune system and excessive inflammation.

Mammalian PGLYRP-2 is also expressed in the intestinal follicle-associated epithelial cells [41]. PGLYRP-2 is not expressed in healthy human skin, but its expression is induced in keratinocytes and other epithelial cells by exposure to bacteria and cytokines [42,43]. 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*-acetylmuramoyl-L-alanine amidase activity, and the long form has 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 [44].

Mammalian PGLYRP-1 is highly expressed in the bone marrow [1,3], and the protein is almost exclusively present in the granules of polymorphonuclear leukocytes [45-49] (Figure 5d). Mammalian PGLYRP-3 and PGLYRP-4 proteins are selectively expressed in the skin epidermis, hair follicles, sebaceous glands and sweat glands; in the eye's ciliary body (which produces aqueous humor that fills the 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 PGLYRP-4, but not PGLYRP-3); in the tongue and esophagus in squamous epithelial cells; in the stomach in acid-secreting parietal cells (PGLYRP-3) and glycoprotein-secreting neck mucous cells (PGLYRP-4); and in the small and large intestine in the columnar absorptive cells, but not in mucus-secreting goblet cells and not in Paneth cells in the crypts, which produce antimicrobial peptides [17,50] (Figure 5a,b). Bacteria and their products increase the expression of PGLYRP-3 and PGLYRP-4 in keratinocytes [17] and oral epithelial cells [51], probably through activation of the Toll-like receptors TLR2, TLR4, Nod1, and Nod2.

Human PGLYRP-1, PGLYRP-3, PGLYRP-4, the heterodimer formed by PGLYRP-3 and PGLYRP-4, (PGLYRP-3:4), and bovine PGLYRP-1 are bactericidal for many pathogenic and nonpathogenic Gram-positive and Gram-negative bacteria [17,46,47] (Figure 5a,b,d). PGLYRP-1, PGLYRP-3, and PGLYRP-4 from other mammalian species are also likely to have similar bactericidal activity. Bovine PGLYRP-1 also has some microbicidal activity against a fungus, *Cryptococcus neoformans* [46,47]. This broader spectrum of microbicidal activity of bovine PGLYRP-1 could reflect a true difference between the human and bovine orthologs, or it might simply reflect a difference in the protein purification methods and assay conditions.

Mechanism

Crystallographic analysis of human PGLYRP-1 and the carboxy-terminal PGRP domain of PGLYRP-3, as well as insect PGRP-LB, -SA, -LC and -LE, show that all these PGRPs have a ligand-binding groove that binds peptidoglycan and is specific for MurNAc bound to three peptide-bonded amino acids (muramyl-tripeptide), which is the minimum peptidoglycan fragment hydrolyzed by PGLYRP-2 [7,9,10-13,52-55]. It can accommodate a larger structure, such as GlcNAc-MurNAc-tetrapeptide or MurNAc-pentapeptide (Figure 3), but it does not bind muramyl-dipeptide or a peptide without MurNAc [56-58]. These results are consistent with the specificity of human PGLYRP-2 for muramyl-tripeptide and with the specificity and high affinity (K_d = 13 nM) of murine PGLYRP-1 for uncrosslinked polymeric peptidoglycan but not muramyl-dipeptide or pentapeptide [45]. The high-affinity binding of peptidoglycan to PGLYRP is achieved by burying both the peptide and MurNAc portions of peptidoglycan in a deep cleft that completely excludes solvent [52].

Human PGLYRP-1 and a carboxy-terminal fragment of PGLYRP-3 bind muramyl-tetrapeptide and muramyl-pentapeptide with higher affinity than muramyl-tripeptide [56,58]. Moreover, binding of muramyl-pentapeptide (but not muramyl-tripeptide) to the carboxy-terminal fragment of PGLYRP-3 induces a conformational change in the PGLYRP-3 molecule that locks the ligand in the binding groove (Figure 3) [58]. Some PGRPs (such as a carboxy-terminal fragment of human PGLYRP-3) have a preference for binding the Lys-type over the DAP-type peptidoglycan, whereas others (such as human PGLYRP-1 or *Drosophila* PGRP-LCx and PGRP-LE) bind DAP-type peptidoglycan with higher affinity than Lys-type peptidoglycan [54-57]. The only difference between Lys and DAP is the presence of an additional carboxylate at carbon 1 of DAP. Discrimination between Lys- and DAP-type peptidoglycan is based on three amino acids in the peptidoglycan-binding groove, corresponding to Asn236, Phe237, and Val256 in human PGLYRP-3 for binding Lys, or Gly68, Trp69, and Arg88 in human PGLYRP-1 in the same position for binding DAP, or Gly234, Trp235 and Arg254 in *Drosophila* PGRP-LE for binding DAP [54-57]. The importance of these Asn and Phe or Gly and Trp for binding Lys and DAP is verified by mutations in these positions that can change the specificity of the binding from Lys to DAP or DAP to Lys [57]. This allows prediction of binding specificity of various PGRP domains for Lys- or DAP-type peptidoglycan. Moreover, both human and

insect PGRPs have a dual strategy for discrimination among different types of peptidoglycan, using detection of Lys or DAP in the stem peptide together with the type of peptide crossbridge [57]. Detection of peptide-crosslinked peptidoglycan would require engagement of two peptidoglycan-binding sites in two PGRP domains, which could be accomplished by PGRPs with two PGRP domains and/or by dimeric PGRPs, which is consistent with recent demonstration of dimeric PGRPs in mammals [17] and insects [18,19].

There is likely, however, to be considerable variation in the fine specificity of different PGRPs, because the residues in and around the peptidoglycan-binding groove are relatively variable; they are less than 50% conserved among PGRPs [7,11,52]. This structural variation may correspond to different ligand specificities of different PGRPs. Mammalian PGLYRPs bind to both Gram-positive and Gram-negative bacteria and also some fungi [17,47], and some insect PGRPs (such as *H. diomphalia* PGRP-1) bind fungal β-glucan [38]. Therefore, binding to peptidoglycan is not always responsible for PGRP binding, and even with bacteria there are indications that some PGRPs may also bind to other polymers, such as lipoteichoic acid and lipopolysaccharide [17,45,47]. Human and mouse PGLYRPs have the highest affinity for peptidoglycan, however, and much lower affinities for lipoteichoic acid and lipopolysaccharide [17,45], whereas bovine PGLYRP-1 seems to have high affinity for lipoteichoic acid and lipopolysaccharide [47]. It is not clear, however, whether these other ligands bind to the peptidoglycan-binding groove or to another portion of the PGLYRP molecule, such as the hydrophobic region on the opposite side of the molecule. Binding of peptidoglycan outside the peptidoglycan-binding groove was recently shown, which contributes to the formation of PGRP-LE oligomers [54] or PGRP-LCx:PGRP-LCa dimers [55].

The diversity of PGRP specificities is also increased by duplication of PGRP domains and dimerization. PGLYRP-3 and PGLYRP-4 both have two PGRP domains, and each PGRP domain has one ligand-binding site [52]. Thus, whereas PGLYRP-1 monomers and dimers have one and two identical ligand-binding sites, respectively, PGLYRP-3 and PGLYRP-4 monomers and dimers have two and four ligand-binding sites, respectively (Figure 5). Because these PGRP domains in PGLYRP-3 and PGLYRP-4 are not identical (they have 37-43% identity), however, the fine binding specificity or affinity of each PGRP domain in these PGLYRP molecules is probably different. For example, the carboxy-terminal and amino-terminal PGRP domains in human PGLYRP-3 are specific for DAP-type and Lys-type peptidoglycan, respectively [57]. The diversification of PGLYRP specificities is then further increased by formation of PGLYRP-3:4 heterodimers, which have four different binding sites. In this way, the host can fine-tune the specificities of PGLYRPs by expressing PGLYRP-3 and PGLYRP-4 either in the same or in separate cells, to form hetero- or homodimers, respectively. In addition, PGRPs 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 [7]. In mammalian PGLYRPs, however, these hydrophobic domains may either have a role in the interaction of PGLYRPs with bacteria, or in the formation of dimers.

Mammalian PGLYRP-1, PGLYRP-3, and PGLYRP-4 form a new class of bactericidal proteins that have a different structure, mechanism of action, and expression from those of currently known mammalian antimicrobial peptides [6,17]. PGLYRPs are much larger than all currently known vertebrate antibacterial peptides: PGLYRP-1, PGLYRP-3, PGLYRP-3:4, and PGLYRP-4 proteins are disulfide-linked glycosylated 44 kDa, 89 kDa, 98 kDa, and 115 kDa dimers [17], and vertebrate antimicrobial peptides are typically 3 kDa to 15 kDa. PGLYRPs require divalent cations and *N*-glycosylation for bactericidal activity, which are not usually required by membrane-permeabilizing antibacterial peptides, such as defensins or magainin [17]. Mammalian PGLYRPs also differ from antimicrobial peptides in their mechanism of bactericidal activity: they kill bacteria by interacting with cell-wall peptidoglycan, whereas antimicrobial peptides do so by permeabilizing bacterial membranes [17]. Furthermore, the expression patterns of mammalian PGLYRPs and antimicrobial peptides are different, and some cells that produce large amounts of these peptides, such as Paneth cells (which produce defensins, phospholipase A_2, and lysozyme), do not express PGLYRPs [17].

Frontiers

Despite enormous progress since the discovery of PGRPs in 1996 [36], much remains to be done. The structures and specificities of many insect and mammalian PGRPs still need to be determined. For example, the PGRP/amidase domain of mammalian PGLYRP-2 or many insect long PGRPs is located in the carboxy-terminal one third of the molecule, but the role and the structure of the remaining amino-terminal two thirds of PGLYRP-2 or several insect long PGRPs is unknown, as this portion has no homology to any other PGRPs or to any other known proteins [3,9]. These amino-terminal portions of PGLYRP-2 and several insect long PGRPs may therefore have unique and so far unidentified functions.

The functions of many insect PGRPs and their mechanisms of action also still need to be determined (Figure 1 and Table 1). It should be especially interesting to look for direct antimicrobial activity of insect PGRPs, which will establish whether this function developed in mammalian or vertebrate PGLYRPs or whether it was already present in their common ancestor with insects. PGRPs in other invertebrates and in nonmammalian vertebrates (fish, amphibians, reptiles, and birds) are beginning to be discovered and nothing is known about their functions, although most of them are predicted to have amidase activity (Figure 2 and Table 1).

The exact mechanism of antibacterial activity of mammalian PGLYRPs needs to be determined. Moreover, although the main functions of mammalian PGLYRPs have been identified, it remains possible that they 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 (Figure 4), and pig PGLYRP-2 has two splice forms, both of which have amidase activity but also seem to have a role in the induction of β-defensin synthesis [44].

The role and significance of mammalian PGLYRPs *in vivo* also need to be established, as well as their clinical significance, including any possible associations with diseases. For example, human *PGLYRP3* and *PGLYRP4* genes are located in the epidermal differentiation gene cluster in the psoriasis sensitivity *PSORS4* locus, and, thus mutations in *PGLYRP3* and *PGLYRP4* genes may contribute to the pathogenesis of psoriasis [59]. It is likely that associations of other PGLYRPs with disease will be found in the future.

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