Mammalian Peptidoglycan Recognition Proteins Kill Bacteria by Activating Two-Component Systems and Modulate Microbiome and Inflammation

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Peptidoglycan recognition proteins (PGRPs) are conserved from insects to mammals and function in antibacterial immunity. We have revealed a novel mechanism of bacterial killing by innate immune system, in which mammalian PGRPs bind to bacterial cell wall or outer membrane and exploit bacterial stress defense response to kill bacteria. PGRPs enter Gram-positive cell wall at the site of daughter cell separation during cell division. In *Bacillus subtilis* PGRPs activate the CssR-CssS two-component system that detects and disposes of misfolded proteins exported out of bacterial cells. This activation results in membrane depolarization, production of hydroxyl radicals, and cessation of intracellular peptidoglycan, protein, RNA, and DNA synthesis, which are responsible for bacterial death. PGRPs also bind to the outer membrane in *Escherichia coli* and activate functionally homologous CpxA-CpxR two-component system, which also results in bacterial death. We excluded other potential bactericidal mechanisms, such as inhibition of extracellular peptidoglycan synthesis, hydrolysis of peptidoglycan, and membrane permeabilization. *In vivo*, mammalian PGRPs are expressed in polymorphonuclear leukocytes, skin, salivary glands, oral cavity, intestinal tract, eyes, and liver. They control acquisition and maintenance of beneficial normal gut microflora, which protects the host from enhanced inflammation, tissue damage, and colitis.

Discovery and Functions of PGRPs

PEPTIDOGLYCAN RECOGNITION PROTEINS (PGRPs or PGLYRPs) were discovered in 1996 in the silkworm, *Bombyx mori*, as the sensors of bacterial peptidoglycan that activate prophenoloxidase cascade.⁴⁴ This cascade is a part of innate immune response in invertebrates and generates quinones, which are toxic to microorganisms, and melanin pigments, which restrict spreading of the microorganisms within the host. The first PGRP was cloned in a moth *Trichoplusia ni*, which led to the discovery of highly conserved human and mouse orthologs.¹⁹ Sequencing of the fruit fly, mosquito, and human genomes led to the discovery of diversified families of PGRPs, conserved from insects to mammals.^{3,29,41}

PGRPs function in antibacterial defenses and innate immunity. Insects have many PGRPs with diverse functions. Insect PGRPs sense bacteria and trigger host defense pathways, which generate antibacterial products, defend against infections, and regulate insects' microbiomes. Some insect PGRPs hydrolyze or nonenzymatically neutralize proinflammatory bacterial peptidoglycan and thus limit inflammation.^{9,33,34}

Mammals have four PGRP genes, *Pglyrp1*, *Pglyrp2*, *Pglyrp3*, and *Pglyrp4*, which were initially named PGRP-S, PGRP-L, PGRP- α , and PGRP- β (for "short," "long," or "intermediate" transcripts, respectively), by analogy to insect PGRPs.²⁹ PGLYRP1, PGLYRP3, and PGLYRP4 are directly bactericidal for both Gram-positive and Gram-negative bacteria,^{11,28,30,37,39} and PGLYRP2 is a peptidoglycan-lytic amidase^{14,40,45} and also has bactericidal activity. PGLYRP1 is mainly present in PMN's granules, PGLYRP2 is made in the liver and secreted into blood and is also induced in epithelial cells, and PGLYRP3 and PGLYRP4 are produced on the skin and mucous membranes, and in sweat, sebum, and saliva.^{11,26,28–30,35–40,45}

This review will first focus on the question how PGRPs kill bacteria, and then will briefly discuss the *in vivo* consequences of antibacterial activity of PGRPs—their effect on microbiome and inflammation.

How do PGRPs Kill Bacteria?—The Initial Hypotheses

Our first hypothesis was that PGRPs kill bacteria by inhibiting the transglycosylation or transpeptidation steps in peptidoglycan synthesis^{10,30,39} because (a) PGRPs bind to the

MurNAc-pentapeptide fragments present both in mature peptidoglycan and in peptidoglycan precursors used in these biosynthetic steps, and (b) crystallographic analysis showed that this binding locks peptidoglycan in a conformation that should prevent transpeptidation.² Our alternative hypotheses were that PGRPs kill bacteria by either hydrolyzing peptidoglycan and causing osmotic cell lysis, or by directly permeabilizing bacterial cytoplasmic membranes.

PGRPs Inhibit an Intracellular Step in Peptidoglycan Synthesis

Indeed, PGRPs completely inhibit total peptidoglycan biosynthesis in both *Staphylococcus aureus* and *Bacillus subtilis*, but they do not inhibit transglycosylation or transpeptidation, the two extracellular steps of peptidoglycan synthesis.²⁰ These results indicate that PGRPs inhibit an earlier intracellular step in peptidoglycan synthesis.

PGRPs Kill Gram-Positive Bacteria by Localizing to Cell Separation Sites

To selectively and directly inhibit an intracellular step in peptidoglycan synthesis, PGRPs would have to enter the cytoplasm. However, PGRPs do not enter the cytoplasm. Rather, PGRPs exclusively localize at the separation sites of the newly formed daughter cells in *B. subtilis*, *S. aureus*, and *Listeria monocytogenes*.²⁰ PGRPs do not co-localize with vancomycin; the latter localizes at the sites of new peptidoglycan synthesis, which is primarily at the synthesis of new septa.²⁰

Cell separation after cell division is carried out by dedicated peptidoglycan-lytic endopeptidases, which in *B. subtilis* are LytE, LytF, and CwlS^{13,43} and whose expression is limited to the cell separation sites. PGRPs co-localize with LytE and LytF in the cell separation sites.²⁰ This localization is necessary for bacterial killing, because mutants that lack LytE and LytF and do not separate after cell division are less efficiently killed by PGRPs than the wild-type (WT) strain.²⁰ These mutants also do not show specific binding of PGRPs,²⁰ suggesting that the cell-separating LytE and LytF enzymes are required for efficient PGRP binding to bacteria and bacterial killing. This effect is selective for LytE and LytF, because deficiencies in peptidoglycan-lytic amidase (LytC) and glucosaminidase (LytD), which function as autolytic but not cell-separating enzymes, have no effect on bacterial sensitivity to PGRP-induced killing.²⁰ Thus, in Gram-positive bacteria, PGRPs trigger their lethal effect from this extracellular site without entering the cytoplasm.

PGRPs Inhibit Protein, RNA, and DNA Synthesis

PGRPs also rapidly and completely inhibit protein, RNA, and DNA synthesis in *S. aureus* and *B. subtilis*.²⁰ Similar inhibition is caused by magainin (due to the loss of membrane integrity) and lysostaphin (due to the loss of peptidoglycan and immediate osmotic lysis). By contrast, antibiotics selectively inhibit either peptidoglycan, or protein, or RNA, or DNA synthesis.²⁰

PGRPs do not Kill by Osmotic Lysis or by Hydrolyzing Peptidoglycan

To determine whether PGRPs kill by hydrolyzing peptidoglycan, we used 0.5 M sucrose to prevent osmotic lysis.

About 0.5 M sucrose prevents lysostaphin-induced, but not PGRP-induced, inhibition of protein, RNA, and DNA synthesis, indicating that PGRPs do not kill bacteria by hydrolyzing peptidoglycan and inducing osmotic lysis.²⁰ Consistent with these results, bactericidal PGRPs do not hydrolyze insoluble *S. aureus* or *B. subtilis* peptidoglycan, uncross-linked soluble polymeric peptidoglycan, synthetic peptidoglycan fragments, or heat-killed *S. aureus* and *B. subtilis* bacteria.²⁰ Thus, bactericidal PGRPs do not have amidase, carboxypeptidase, or any other peptidoglycan-hydrolytic activity. PGRP-induced killing is also not due to the activation of autolytic enzymes.²⁰

PGRPs do not Directly Permeabilize Cell Membranes

Direct permeabilization of bacterial cell membranes by PGRPs would explain their rapid and simultaneous inhibition of all biosynthetic reactions that is not prevented by hyperosmotic medium (and thus resemble the effect of membrane-permeabilizing peptides, such as magainin). However, PGRPs do not permeabilize bacterial cell membranes over a period of 6 hr, despite rapid killing that exceeds 99% in 2–4 hr and is not prevented by 0.5 M sucrose.²⁰

Thus, the mechanism of bactericidal activity of PGRPs is distinct from the bactericidal activity of antibiotics that inhibit peptidoglycan, protein, RNA, or DNA synthesis and is also distinct from membrane-permeabilizing peptides and from enzymes that hydrolyze the bacterial cell wall.

PGRPs induce Membrane Depolarization and •OH Production

We next considered whether the loss of membrane potential is responsible for inhibition of intracellular biosynthetic reactions and killing of bacteria by PGRPs, because all these reactions require energy from ATP, whose production is largely dependent on the ATP synthase driven by the proton gradient maintained by the membrane potential.^{8,16}

Indeed, PGRPs at bactericidal concentrations induce rapid and sustained membrane depolarization in *B. subtilis*. Membrane depolarization was accompanied by intracellular production of toxic hydroxyl radicals (•OH), which are part of the bacterial stress response.²⁰ Similar effect is also seen in *S. aureus*.²⁰

To determine whether •OH production is responsible for the PGRP-induced bacterial killing and how •OH is generated, we tested the effect of dipyridyl and thiourea on PGRP-induced •OH production and bacterial killing. Dipyridyl is a membrane-permeable selective Fe²⁺ chelator that inhibits Fenton reaction-mediated •OH production, whereas thiourea is an •OH scavenger.^{22–24} Both dipyridyl and thiourea significantly inhibit PGRP-induced bacterial killing. These results indicate that •OH is generated through the Fenton reaction from FeS clusters and is required for PGRP-induced bacterial killing.²⁰

Membrane depolarization is sufficient to kill bacteria, because chemical membrane potential decoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP) induces membrane depolarization and kills bacteria, and the killing is not inhibited by dipyridyl. However, membrane depolarization and •OH have synergistic bactericidal effect.²⁰

PGRPs Kill *B. Subtilis* by Activating the CsrR-CssS System

We then tested whether the CsrR-CssS two-component system in *B. subtilis* is involved in PGRP-induced membrane depolarization, $\bullet$ OH production, and bacterial killing, because a functionally homologous CpxA-CpxR two-component system in *Escherichia coli* detects misfolded proteins in antibiotic-treated bacteria and is responsible for antibiotic-induced membrane depolarization, $\bullet$ OH production, and killing.^{23,24}

PGRP-induced membrane depolarization and $\bullet$ OH production is significantly reduced in both Δ cssS and Δ cssR mutants compared with isogenic WT *B. subtilis*.²⁰ Membrane depolarization by CCCP is similar in WT and Δ cssS and Δ cssR mutants, indicating that these mutants do not have an inherent defect in maintaining membrane potential and that CsrS and CsrR selectively mediate the effect of PGRPs. These results indicate that both membrane depolarization and $\bullet$ OH production induced by PGRPs are mediated through the CsrR-CssS two-component system.

PGRPs also cause rapid high-level induction of *htrA* mRNA in WT *B. subtilis*, but not in Δ cssS and Δ cssR mutants (HtrA is membrane-bound protease directly regulated by CsrR). These results indicate that PGRPs induce CsrS- and CsrR-dependent activation of the CsrR-CssS two-component system and activate the regulator (the transcription factor CsrR) through the sensor (CsrS).²⁰

To test whether the CsrR-CssS two-component system is responsible for the bactericidal activity of PGRPs, we compared the sensitivity of Δ cssS and Δ cssR mutants and isogenic WT *B. subtilis* to PGRPs. Whereas WT *B. subtilis* is readily killed by all PGRPs, Δ cssS and Δ cssR mutants are ~100 times more resistant to killing by all four PGRP proteins.²⁰ We confirmed these results by complementing Δ cssS and Δ cssR mutants with *cssS*- or *cssR*-expressing plasmids. Thus, the CsrR-CssS two-component system in *B. subtilis* is required for bacterial killing by all four PGRP proteins.²⁰

PGRPs Kill *E. Coli* by Activating the CpxA-CpxR System

To determine whether PGRPs also kill Gram-negative bacteria through protein-sensing two-component systems, we compared the sensitivity to PGRPs of WT *E. coli* and its

isogenic Δ cpxA and Δ cpxR mutants. *E. coli* CpxA-CpxR is a functional homologue of *B. subtilis* CsrR-CssS^{4,15,17,18,24}; both systems sense extracytoplasmic misfolded proteins and induce membrane depolarization, $\bullet$ OH production, and stress response.²⁰ WT *E. coli* bacteria are readily killed by PGRPs, whereas Δ cpxA and Δ cpxR mutants are 100–1,000 times more resistant to PGRP-mediated killing.²⁰ We confirmed these results by complementing Δ cpxA and Δ cpxR mutants with *cpxA*- and *cpxR*-expressing plasmids.²⁰

PGRPs activate the CpxA-CpxR two-component system, as shown by PGRP-induced triggering of rapid high-level induction of *cpxP* mRNA (a stress-response gene directly regulated by CpxR^{7,24}) in WT *E. coli*, but not in Δ cpxA and Δ cpxR mutants.²⁰ These results indicate that PGRPs activate the regulator (the transcription factor CpxR) through the sensor (CpxA).

PGRPs Bind to the Entire *E. Coli* Outer Membrane

How do PGRPs access the CpxA-CpxR two-component system in *E. coli*? The entire *E. coli* outer membrane uniformly binds PGRPs at all stages of growth,²⁰ which contrasts selective localization of PGRPs to the cell separation sites in Gram-positive bacteria. Similarly to Gram-positive bacteria, PGRPs do not enter the cytoplasm in *E. coli*.²⁰ Thus, in Gram-negative bacteria, PGRPs bind to the outer membrane and are sensed by the CpxA-CpxR two-component system, consistent with the ability of *E. coli* CpxA-CpxR to sense bacterial proteins in the outer membrane.⁴

The Model: PGRPs Kill Bacteria by Activating Two-Component Systems

PGRPs kill bacteria by activating bacterial two-component stress response defense systems²⁰ (Fig. 1), which detect extracytoplasmic misfolded and aggregated proteins. Such proteins are generated in bacteria under stress, exported from the cell, and degraded by proteases.^{4,5,17,23,24,42}

In Gram-positive bacteria, PGRPs bind to peptidoglycan in bacterial cell walls at the sites of hydrolysis by the enzymes that separate daughter cells after cell division (LytE and LytF in *B. subtilis*). In Gram-negative bacteria, PGRPs bind to the entire outer membrane, which is composed of lipopolysaccharide (LPS). It should be noted that the name PGRPs is somewhat of

FIG. 1. The model of PGRP-induced killing of bacteria by activation of two-component systems, induction of membrane depolarization, production of $\bullet$ OH, and inhibition of biosynthesis of macromolecules. PGRP, peptidoglycan recognition protein.

FIG. 2. PGRPs protect mice from *Staphylococcus aureus* and *Escherichia coli* lung infection. Intranasal application of PGLYRP3 (4.5 μ g) or PGLYRP4 (4.5 μ g) followed after 15 min by 3×10^8 *S. aureus* (left), or of PGLYRP3 (6 μ g) or PGLYRP3 (6 μ g)+phospholipase A₂ (PLA₂, 4 μ g) followed after 30 min by 4×10^4 invasive *E. coli* (strain Bort, right) significantly reduces the numbers of bacteria recovered from the lungs at 4 hr postinfection (*S. aureus*, $n=6$; *E. coli*, $n=7$).

a misnomer, because PGRPs, in addition to binding peptidoglycan, also bind LPS and other molecules^{1,21,27–29,37} using binding sites outside of the peptidoglycan-binding groove. Binding of PGRPs to peptidoglycan or LPS probably induces their oligomerization into ribbon-like structures,²⁷ which are

then detected by *B. subtilis* CssR-CssS or *E. coli* CpxA-CpxR two-component systems.²⁰ Inhibition of binding of PGRPs to bacteria by addition of isolated peptidoglycan or LPS inhibits PGRP-mediated killing of Gram-positive and Gram-negative bacteria, respectively.^{30,37,39}

Activation of the protein-sensing two-component systems by PGRPs induces a sustained high-level stress response in bacteria, which induces membrane depolarization, the production of toxic $\bullet$ OH, and inhibition of all major intracellular biosynthetic reactions.²⁰ The latter are probably caused by depleting of energy stores, such as ATP, whose generation depends on the membrane potential.^{6,8,12,16}

Some PGRPs also bind to and kill unicellular fungi, such as *Cryptococcus neoformans*.³⁷ Because fungi also have envelope stress response systems (but animals do not), activation of these systems by PGRPs could explain fungicidal activity of PGRPs.

PGRPs are Antibacterial *In Vivo*

Do PGRPs have antibacterial activity *in vivo*? In zebrafish, PGRPs protect the embryos from infection.²⁵ In mice, PGRPs protect mice from infection. Sequential intranasal application of PGRPs, followed by a challenge with *S. aureus* or *E. coli*, protects mice from lung infection; it results in reduced numbers of bacteria recovered from the lungs³⁰ (Fig. 2). This effect is synergistic with antibacterial peptides, such as phospholipase A₂, which is consistent with synergistic bactericidal activity of PGRPs and antimicrobial peptides.³⁹

In addition to PGRPs, mammals have many bactericidal defense mechanisms, including oxidative killing by phagocytic cells, the complement system, antimicrobial peptides, and antibacterial lectins present in phagocytic cells, on body surfaces, in secretions, and in tissue fluids. The loss of one antibacterial system is often compensated by other systems. Thus, in general, *Pglyrp*-deficient mice do not suffer from serious infections, although *Pglyrp1*^{-/-} mice are more sensitive than WT mice to infections with some Gram-positive bacteria.¹¹

FIG. 3. Mammalian PGRPs promote beneficial intestinal microflora and protect from colitis. PGRPs promote beneficial gut microflora, which does not induce cytokines and chemokines and does not cause recruitment of inflammatory cells, and thus protect against colitis. Lack of PGRPs results in the overgrowth of harmful intestinal microflora, which upon intestinal injury with DSS results in increased production of natural killer (NK) cell-attracting and activating chemokines (CXCL9, CXCL10, and CXCL11), recruitment of NK cells to the colonic epithelium, production of interferon (IFN)- γ by NK cells, activation of IFN-inducible genes (including NK cell-attracting chemokines), and eventually colitis, manifested by hyperplasia of the lamina propria, loss of colonic epithelium, ulceration, and bleeding. DSS, dextran sulfate sodium.

PGRPs Maintain Healthy Gut Microbiome and Protect the Host from Colitis

PGRPs are expressed in the salivary glands and on mucous membranes in the mouth, throat, esophagus, and intestines and they play a role in maintaining healthy gut microbiome.³⁴ *Pglyrp1*^{-/-}, *Pglyrp2*^{-/-}, *Pglyrp3*^{-/-}, and *Pglyrp4*^{-/-} mice all have significant and distinct changes in the abundance of various bacterial groups in the stools, and especially they have decreased numbers of *Lactobacillus/Lactococcus* group and *Clostridium perfringens* group,³⁵ which are generally considered beneficial for the host. *Pglyrp1*^{-/-}, *Pglyrp2*^{-/-}, *Pglyrp3*^{-/-}, and *Pglyrp4*^{-/-} mice are also more sensitive than WT mice to ulcerative colitis induced by epithelial-cell-damaging agent, dextran sulfate sodium.³⁵ This increased sensitivity is due to the loss of protective microflora in the gut and the dominance of predisposing microflora in *Pglyrp*-deficient mice, because germ-free WT mice gavaged with stools from WT mice show resistance to colitis, whereas germ-free WT mice gavaged with stools from *Pglyrp*-deficient mice are sensitive to colitis.³⁵ Thus, in WT mice, PGRPs promote maintenance of the beneficial gut microbiota that protects the colon from developing a severe colitis following intestinal injury (Fig. 3).

PGRPs Protect the Host from Excessive Skin Inflammation

PGRPs are also expressed in the skin and play a role in protecting the skin from excessive inflammatory response to chemicals and allergens. However, various PGRPs have differential effects in various models of inflammation.^{31,32} *Pglyrp2*^{-/-} mice (but not *Pglyrp1*^{-/-}, *Pglyrp3*^{-/-}, or *Pglyrp4*^{-/-} mice) are more sensitive to 12-O-tetradecanoylphorbol 13-acetate (TPA) and develop more severe TPA-induced psoriasis-like inflammation than WT mice.³¹ By contrast, *Pglyrp3*^{-/-} and *Pglyrp4*^{-/-} mice (but not *Pglyrp1*^{-/-} or *Pglyrp2*^{-/-} mice) develop more severe oxazolone-induced atopic dermatitis than WT mice.³² In both models of inflammation this increased sensitivity to TPA or oxazolone is due to reduced recruitment of regulatory T cells and enhanced production and activation of Th17 cells in the affected skin. Thus, in WT mice PGRPs prevent an aberrant activation of Th17 cells by promoting accumulation of regulatory T cells at the site of inflammation, and this protects the skin from an exaggerated inflammatory response. It is not known, however, whether these effects of PGRPs are due to their effects on the microbiome (similar to colitis), or due to their direct effect on the inflammatory response in the skin.

Acknowledgment

This work was supported by USPHS Grants AI028797 and AI073290 from NIH.

Disclosure Statement

The authors have no conflicts of interest and no competing financial interests.

References

- Chang, C.I., Y. Chelliah, D. Borek, D. Mengin-Lecreux, and J. Deisenhofer. 2006. Structure of tracheal cytotoxin in complex with a heterodimeric pattern-recognition receptor. *Science* **311**:1761–1764.
- Cho, S., Q. Wang, C.P. Swaminathan, D. Heseck, M. Lee, G.J. Boons, S. Mobashery, and R.A. Mariuzza. 2007. Structural insights into the bactericidal mechanism of human peptidoglycan recognition proteins. *Proc. Natl. Acad. Sci. U. S. A.* **104**:8761–8766.
- Christophides, G.K., E. Zdobnov, C. Barillas-Mury, E. Birney, S. Blandin, C. Blass, et al. 2002. Immunity-related genes and gene families in *Anopheles gambiae*. *Science* **298**: 159–165.
- Danese, P.N., W.B. Snyder, C.L. Cosma, L.J. Davis, and T.J. Silhavy. 1995. The Cpx two-component signal transduction pathway of *Escherichia coli* regulates transcription of the gene specifying the stress-inducible periplasmic protease, DegP. *Genes Dev.* **9**:387–398.
- Darmon, E., D. Noone, A. Masson, S. Bron, O.P. Kuipers, K.M. Devine, and J.M. van Dijk. 2002. A novel class of heat and secretion stress-responsive genes is controlled by the autoregulated CsxRS two-component system of *Bacillus subtilis*. *J. Bacteriol.* **184**:5661–5671.
- Deckers-Hebestreit, G., and K. Altendorf. 1996. The F0F1-type ATP synthases of bacteria: structure and function of the F0 complex. *Annu. Rev. Microbiol.* **50**:791–824.
- DiGiuseppe, P.A., and T.J. Silhavy. 2003. Signal detection and target gene induction by the CpxRA two-component system. *J. Bacteriol.* **185**:2432–2440.
- Dimroth, P., G. Kaim, and U. Matthey. 2000. Crucial role of the membrane potential for ATP synthesis by F1Fo ATP synthases. *J. Exp. Biol.* **203**(Pt 1):51–59.
- Dziarski, R., and D. Gupta. 2006. The peptidoglycan recognition proteins (PGRPs). *Genome Biol.* **7**:232.
- Dziarski, R., and D. Gupta. 2006. Mammalian PGRPs: novel antibacterial proteins. *Cell Microbiol.* **8**:1056–1069.
- Dziarski, R., K.A. Platt, E. Gelius, H. Steiner, and D. Gupta. 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.
- Friedrich, T., and D. Scheide. 2000. The respiratory complex I of bacteria, archaea and eukarya and its module common with membrane-bound multisubunit hydrogenases. *FEBS Lett.* **479**:1–5.
- Fukushima, T., A. Afkham, S. Kurosawa, T. Tanabe, H. Yamamoto, and J. Sekiguchi. 2006. A new D,L-endopeptidase gene product, YojL (renamed CwlS), plays a role in cell separation with LytE and LytF in *Bacillus subtilis*. *J. Bacteriol.* **188**:5541–5550.
- Gelius, E., C. Persson, J. Karlsson, and H. Steiner. 2003. A mammalian peptidoglycan recognition protein with N-acetylmuramoyl-L-alanine amidase activity. *Biochem. Biophys. Res. Commun.* **306**:988–994.
- Gerken, H., O.P. Leiser, D. Bennion, and R. Misra. 2010. Involvement and necessity of the Cpx regulon in the event of aberrant beta-barrel outer membrane protein assembly. *Mol. Microbiol.* **75**:1033–1046.
- Hurdle, J.G., A.J. O'Neill, I. Chopra, and R.E. Lee. 2011. Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. *Nat. Rev. Microbiol.* **9**:62–75.
- Hyryläinen, H.L., A. Bolhuis, E. Darmon, L. Muukkonen, P. Koski, M. Vitikainen, M. Sarvas, Z. Prágai, S. Bron, J.M. van Dijk, and V.P. Kontinen. 2001. A novel two-component regulatory system in *Bacillus subtilis* for the survival of severe secretion stress. *Mol. Microbiol.* **41**:1159–1172.

18. Jones, C.H., P.N. Danese, J.S. Pinkner, T.J. Silhavy, and S.J. Hultgren. 1997. The chaperone-assisted membrane release and folding pathway is sensed by two signal transduction systems. *EMBO J.* 16:6394–6406.
19. Kang, D., G. Liu, A. Lundström, E. Gelius, and H. Steiner. 1998. A peptidoglycan recognition protein in innate immunity conserved from insects to humans. *Proc. Natl. Acad. Sci. U. S. A.* 95:10078–10082.
20. Kashyap, D.R., M. Wang, L.H. Liu, G.J. Boons, D. Gupta, and R. Dziarski. 2011. Peptidoglycan recognition proteins kill bacteria by activating protein-sensing two-component systems. *Nat. Med.* 17:676–683.
21. Kim, M.-S., M. Byun, and B.-H. Oh. 2003. Crystal structure of peptidoglycan recognition protein LB from *Drosophila melanogaster*. *Nat. Immunol.* 4:787–793.
22. Kohanski, M.A., D.J. Dwyer, and J.J. Collins. 2010. How antibiotics kill bacteria: from targets to networks. *Nat. Rev. Microbiol.* 8:423–435.
23. Kohanski, M.A., D.J. Dwyer, B. Hayete, C.A. Lawrence, and J.J. Collins. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 130:797–810.
24. Kohanski, M.A., D.J. Dwyer, J. Wierzbowski, G. Cottarel, and J.J. Collins. 2008. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell* 135:679–690.
25. Li, X., S. Wang, J. Qi, S.F. Echtenkamp, R. Chatterjee, M. Wang, G.-B. Boons, R. Dziarski, and D. Gupta. 2007. Zebrafish peptidoglycan recognition proteins are bactericidal amidases essential for defense against bacterial infections. *Immunity* 27:518–529.
26. Li, X., S. Wang, H. Wang, and D. Gupta. 2006. Differential expression of peptidoglycan recognition protein 2 in the skin and liver requires different transcription factors. *J. Biol. Chem.* 281:20738–20748.
27. Lim, J.H., M.S. Kim, H.E. Kim, T. Yano, Y. Oshima, K. Aggarwal, W.E. Goldman, N. Silverman, S. Kurata, and B.H. Oh. 2006. Structural basis for preferential recognition of diaminopimelic acid-type peptidoglycan by a subset of peptidoglycan recognition proteins. *J. Biol. Chem.* 281:8286–8295.
28. Liu, C., E. Gelius, G. Liu, H. Steiner, and R. Dziarski. 2000. Mammalian peptidoglycan recognition protein binds peptidoglycan with high affinity, is expressed in neutrophils and inhibits bacterial growth. *J. Biol. Chem.* 275:24490–24499.
29. Liu, C., Z. Xu, D. Gupta, and R. Dziarski. 2001. Peptidoglycan recognition proteins: a novel family of four human innate immunity pattern recognition molecules. *J. Biol. Chem.* 276:34686–34694.
30. Lu, X., M. Wang, J. Qi, H. Wang, X. Li, D. Gupta, and R. Dziarski. 2006. Peptidoglycan recognition proteins are a new class of human bactericidal proteins. *J. Biol. Chem.* 281:5895–5907.
31. Park, S.Y., D. Gupta, R. Hurwich, C.H. Kim, and R. Dziarski. 2011. Peptidoglycan recognition protein Pglyrp2 protects mice from psoriasis-like skin inflammation by promoting regulatory T cells and limiting Th17 responses. *J. Immunol.* 187:5813–5823.
32. Park, S.Y., D. Gupta, C.H. Kim, and R. Dziarski. 2011. Differential effects of peptidoglycan recognition proteins on experimental atopic and contact dermatitis mediated by Treg and Th17 cells. *PLoS One* 6:e24961.1–16.
33. Royet, J., and R. Dziarski. 2007. Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defenses. *Nat. Rev. Microbiol.* 5:264–277.
34. Royet, J., D. Gupta, and R. Dziarski. 2011. Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. *Nat. Rev. Immunol.* 11:837–851.
35. Saha, S., X. Jing, S.Y. Park, S. Wang, X. Li, D. Gupta, and R. Dziarski. 2010. Peptidoglycan recognition proteins protect mice from experimental colitis by promoting normal gut flora and preventing induction of interferon- γ . *Cell Host Microbe* 8:147–162.
36. Saha, S., J. Qi, S. Wang, M. Wang, X. Li, Y.-G. Kim, G. Núñez, D. Gupta, and R. Dziarski. 2009. PGLYRP-2 and Nod2 are both required for peptidoglycan-induced arthritis and local inflammation. *Cell Host Microbe* 5: 137–150.
37. Tydell, C.C., J. Yuan, P. Tran, and M.E. Selsted. 2006. Bovine peptidoglycan recognition protein-5: antimicrobial activity, localization, secretion and binding properties. *J. Immunol.* 176:1154–1162.
38. Wang, H., D. Gupta, X. Li, and R. Dziarski. 2005. Peptidoglycan recognition protein 2 (N-acetylmuramoyl-L-alanine amidase) is induced in keratinocytes by bacteria through p38 kinase pathway. *Infect. Immun.* 73:7216–7225.
39. Wang, M., L.H. Liu, S. Wang, X. Li, X. Lu, D. Gupta, and R. Dziarski. 2007. Human peptidoglycan recognition proteins require zinc to kill both Gram-positive and Gram-negative bacteria and are synergistic with antibacterial peptides. *J. Immunol.* 178:3116–3125.
40. Wang, Z.-M., X. Li, R.R. Cocklin, M. Wang, M. Wang, K. Fukase, S. Inamura, K. Kusumoto, D. Gupta, and R. Dziarski. 2003. Human peptidoglycan recognition protein-L is an N-acetylmuramoyl-L-alanine amidase. *J. Biol. Chem.* 278:49044–49052.
41. Werner, T., G. Liu, D. Kang, S. Ekengren, H. Steiner, and D. Hultmark. 2000. A family of peptidoglycan recognition proteins in the fruit fly *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* 97:13772–13777.
42. Westers, H., L. Westers, E. Darmon, J.M. van Dijk, W.J. Quax, and G. Zanen. 2006. The CsrRS two-component regulatory system controls a general secretion stress response in *Bacillus subtilis*. *FEBS J.* 273:3816–3827.
43. Yamamoto, H., S. Kurosawa, and J. Sekiguchi. 2003. Localization of the vegetative cell wall hydrolases LytC, LytE and LytF on the *Bacillus subtilis* cell surface and stability of these enzymes to cell wall-bound or extracellular proteases. *J. Bacteriol.* 185:6666–6677.
44. Yoshida, H., K. Kinoshita, and M. Ashida. 1996. Purification of a peptidoglycan recognition protein from hemolymph of the silkworm, *Bombyx mori*. *J. Biol. Chem.* 271: 13854–13860.
45. Zhang, Y., L. van der Fits, J.S. Voerman, M.-J. Melief, J.D. Laman, M. Wang, H. Wang, M. Wang, X. Li, C.D. Walls, D. Gupta, and R. Dziarski. 2005. Identification of serum N-acetylmuramoyl-L-alanine amidase as liver peptidoglycan recognition protein 2. *Biochim. Biophys. Acta* 1752:34–46.

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