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GUEST COMMENTARY

Peptidoglycan Monomer Release and *Vibrio fischeri*▽

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Peptidoglycan (PG) is the major component of the bacterial cell wall and is recognized by animals as a signaling molecule indicating the presence of bacteria. PG recycling during cell growth and division is well regulated, but a few gram-negative bacteria also release monomeric forms of PG (2). Until recently, the interaction between these PG monomers and animal host cells was thought to be restricted to pathogenic associations. In this issue, Adin et al. describe a mechanism by which the beneficial bacterium *Vibrio fischeri* releases PG monomers, lending insight into the role that these microbe-associated molecular pattern (MAMP) molecules play in host morphogenesis of the Hawaiian bobtail squid *Euprymna scolopes* (1).

PG monomers. In gram-negative bacteria, PG consists of subunits of *N*-acetylglucosamine and *N*-acetylmuramic acid connected to a short pentapeptide side chain of L-alanyl-D-γ-glutamyl-*meso*-diaminopimelyl-D-alanyl-D-alanine (2). During normal recycling of PG, lytic transglycosylases cleave the *N*-acetylmuramic acid-β-1,4-*N*-acetylglucosamine linkage, generating PG monomers (2, 7). In *Escherichia coli*, a permease, AmpG, then aids these PG monomers in entering the cytoplasm, where they are recycled and incorporated into the remodeled cell wall (2, 9). Some gram-negative bacteria, however, release PG monomers that can have a dramatic effect on eukaryotic host epithelial cells. This phenomenon was first described for the pathogens *Bordetella pertussis* and *Neisseria gonorrhoeae*, where release of PG monomers (identical in structure but named tracheal cytotoxin in *B. pertussis* and PG cytotoxin in *N. gonorrhoeae*) causes sloughing of ciliated epithelial cells and contributes to the etiology of whooping cough and gonorrhea, respectively (2, 10, 12).

The squid-vibrio association and PG. The symbiosis between the benthic Hawaiian bobtail squid, *Euprymna scolopes*, and the bioluminescent bacterium *Vibrio fischeri* is used as a model system to study the effects of beneficial bacteria on the development of animal host tissues (6, 8, 13). Each generation, the host is colonized by *V. fischeri* cells from the environment. Upon entry, these symbionts are housed in a structure called a light organ, the light of which is used by the host to camouflage itself during its nocturnal feeding behavior. Because the potential symbionts represent only a small fraction of the ambient microbial assemblage found in the surrounding seawater, *E. scolopes* and *V. fischeri* have evolved mechanisms that increase the probability of successful colonization, while discouraging infection of host tissues by nonspecific microorganisms. One such mechanism is a process by which the host harvests *V. fischeri* from seawater by using mucus secretions originating from superficial ciliated epithelia that aggregate environmental bacteria (reviewed in reference 8). The induction of these mucus secretions is initiated by bacterial PG. *V. fischeri* is able to outcompete other environmental bacteria in this mucus and migrate to and colonize epithelium-lined crypt spaces located in the center of the light organ, which is positioned below the superficial ciliated epithelium that is responsible for the mucus secretions (Fig. 1) (8). Following successful colonization, *V. fischeri* induces apoptosis and regression of these superficial ciliated fields via the synergistic action of PG monomers (identical to those released by *B. pertussis* and *N. gonorrhoeae*) and lipopolysaccharide (4). *V. fischeri* PG monomers have also been shown to cause the trafficking of host phagocytic hemocytes into the ciliated fields, presumably a process that aids in the symbiont-induced host cell morphogenesis (5).

Significance of this work. The discovery that a beneficial symbiont employs PG monomers to induce eukaryotic cell morphogenesis as part of the normal developmental program of an animal host broadens the role of MAMPs in microbe-animal interactions beyond that of recognition and removal of pathogens by the innate immune system (4). Adin et al. continue this story by describing a potential mechanism by which *V. fischeri* accumulates extracellular PG monomers. The authors identified and targeted *V. fischeri* homologues to *ampG*, which transports PG into the cytoplasm, and lytic transglycosylase genes (*ltgA*, *ltgD*, and *ltgY*) for mutagenesis. These genes were chosen because *B. pertussis* and *N. gonorrhoeae* employ different mechanisms to make their PG monomers. Whereas in *B. pertussis* the disruption of *ampG* leads to the release of PG monomer into the extracellular environment, *N. gonorrhoeae* uses lytic transglycosylase activity to generate and release PG monomer (reviewed in reference 2). Based on a number of elegant experiments, the major findings of this study include the following: (i) *ampG* mutants have a 100-fold increase in PG monomer release; (ii) mutations of transglycosylase genes in *V. fischeri* led to a decrease in PG monomer release; and (iii) a triple mutant lacking *ltgA*, *ltgD*, and *ltgY* colonized the host but left these squid open to secondary infection.

Because the *V. fischeri ampG* mutant led to a significant increase in PG monomer release while the inactivation of three transglycosylase genes led to the opposite result, the authors conclude that PG monomer release in *V. fischeri* more resembles that of *N. gonorrhoeae*. Interestingly, knocking out all three lytic transglycosylase genes led to susceptibility to a su-

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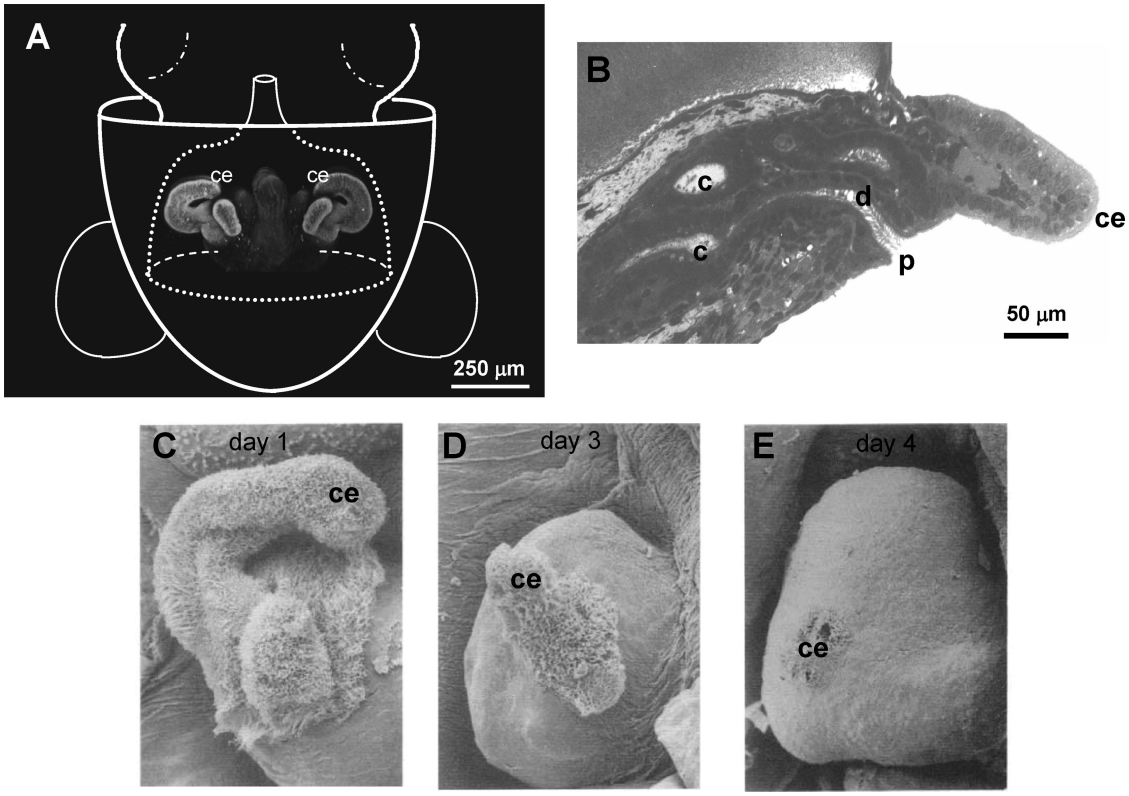

FIG. 1. Effect of *V. fischeri* monomers on host light-organ morphogenesis. (A) At hatching, the juvenile host's light organ is positioned in the center of the mantle cavity, where it is exposed to seawater and a background of environmental bacteria. A ciliated epithelium (ce) located on the surface of the light organ aids *V. fischeri* cells in colonizing the host (7a). (B) During this process, the symbionts migrate through pores (p) on the surface, down a ciliated duct (d), and into epithelium-lined crypt spaces (c), where colonization takes place. (C to E) *V. fischeri* PG monomers along with lipopolysaccharide lead to cell death and regression of the superficial ciliated epithelium (ce) over a 4-day period. (Adapted from reference 2a with the permission of the publisher.)

perinfection of host light organs by another strain of *V. fischeri*, suggesting that the inability to produce PG monomers leads to less regression of the superficial ciliated epithelium and presumably continued colonization of the host light organ. One of the amazing aspects of the squid-vibrio association is the high level of specificity (i.e., only *V. fischeri* has been demonstrated to colonize the light organ, even though the host crypt spaces are physically open to the surrounding seawater, containing on average 10^6 cells/ml of nonsymbiotic bacteria) (8). As the authors so aptly state, PG monomers may help establish this specificity by "closing the door" and preventing future colonization by potential pathogens or interlopers.

Future questions. Besides the results outlined above, this study also generated a number of very useful future tools, *V. fischeri* strains that result in significant increased and decreased PG monomer activity. *V. fischeri* PG monomer and PG derivatives have been implicated in a number of developmental events in the squid host (8, 13). These mutants will no doubt prove to be crucial resources for elucidating the role(s) that PG monomers play in establishing, and perhaps also in maintaining, the squid-vibrio association. Besides the induction of host mucus secretions preceding bacterial aggregation, hemocyte trafficking, and regression of the superficial ciliated epithelium, a number of other developmental events in this association may be mediated by *V. fischeri* PG monomer and/or PG derivatives. For example, by 48 h after colonization, host mucus accumulates in the crypt spaces while mucus secretion from the cells of the superficial epithelium ceases (8). Furthermore, the presence of *V. fischeri* in the crypt spaces is required to prevent continued mucus secretion, as removal of the symbionts from the light organ with the use of antibiotics restores the ability of the host to secrete mucus (8). Does PG or its derivatives play a role in regulating these changes in host mucosecretory behavior?

The host light organ continues to develop after colonization as the hatchling animal grows into an adult. The crypt spaces must accommodate a 1,000-fold increase in *V. fischeri* cells during this period, and a number of changes occur in the overall architecture of the tissues that house the symbionts. Are PG monomers involved with initiating any of these later developmental events, as they are during establishment of the association? The host also has a unique behavior in that every day at dawn, greater than 95% of the symbionts are expelled from the light organ along with a component of host cells comprised of sloughed epithelial cells, epithelial cell fragments, and phagocytic blood cells or hemocytes, the main cellular component of the host's innate immune system (7). This behavior serves two purposes, to seed the environment with symbionts for future host generations and to presumably regulate *V. fischeri* growth in the crypt spaces. The role of the

sloughed epithelial cells in the association is unknown, but this cellular debris may serve as a nutritional source for the symbionts. PG monomers from *B. pertussis* and *N. gonorrhoeae* have been implicated in ciliated cell sloughing in humans (2). The mutants generated in this study may help researchers to understand if *V. fischeri* PG monomers are involved with this daily restructuring of the host epithelium as well as mediation of the host's immune response, leading to the establishment and maintenance of homeostasis in the crypt spaces.

How might *V. fischeri* MAMPs such as PG monomers interact with the host at the molecular level? A number of pattern recognition receptors such as Toll-like receptor and PG recognition proteins along with downstream signaling pathways such as the NF-κB signaling pathway have been implicated in recognizing MAMPs and mediating host responses to microorganisms (2, 11). In *E. scolopes*, homologues to all of these have been found, and now host gene expression of these various receptors and pathways may be studied in response to varying *V. fischeri* PG monomer release in vivo (3).

Research on host-microbe interactions continues to reveal an intricate and conserved repertoire of signals used to mediate molecular cross talk in both pathogenic and beneficial associations. The use of MAMPs such as PG monomers in the initiation and establishment of both types of relationships demonstrates that cell-cell communication between bacteria and eukaryotes is ubiquitous in nature, often serving critical functions in these associations.

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