Signaling between Bacteria and Their Hosts

Chemical crosstalk between bacteria and hosts may enhance virulence or, in some cases, tames pathogens

Y Nguyen and Vanessa Sperandio

One early challenge in life is to learn how to communicate, a skill that is not limited to the animal kingdom. Microorganisms communicate through cell-to-cell chemical signaling, called quorum sensing (QS), that was recognized as early as 1970 by J. Woodland Hastings and his collaborators at the Marine Biological Laboratory in Woods Hole, Mass. They reported that *Vibrio fischeri,* a bioluminescent bacterium, produces an extracellular molecule, later identified as acyl-homoserine lactone (AHL), that activates light production when these cells reach a particular population density. The bioluminescent *Vibrio fischeri* emits light in response to AHLs within their own species when colonizing the light organs of squid or fishes, where these bacteria colonize as a homogeneous community.

Many other gram-negative bacteria also depend on AHLs to communicate within and across species. The possibility that this communication extends beyond kingdoms, meaning that bacteria might communicate with their animal hosts, has interested scientists since the discovery of the QS phenomenon.

Coevolving Microbe-Host Pairs

Microorganisms and their eukaryotic hosts have coevolved for millions of years, both subject to pressures to adapt to changing surroundings. Such organism pairings lend themselves to intercommunication between microbes and hosts. Further, experimental evidence indicates that microbes communicate with their hosts via hormones and hormone-like compounds through a process called interkingdom signaling.

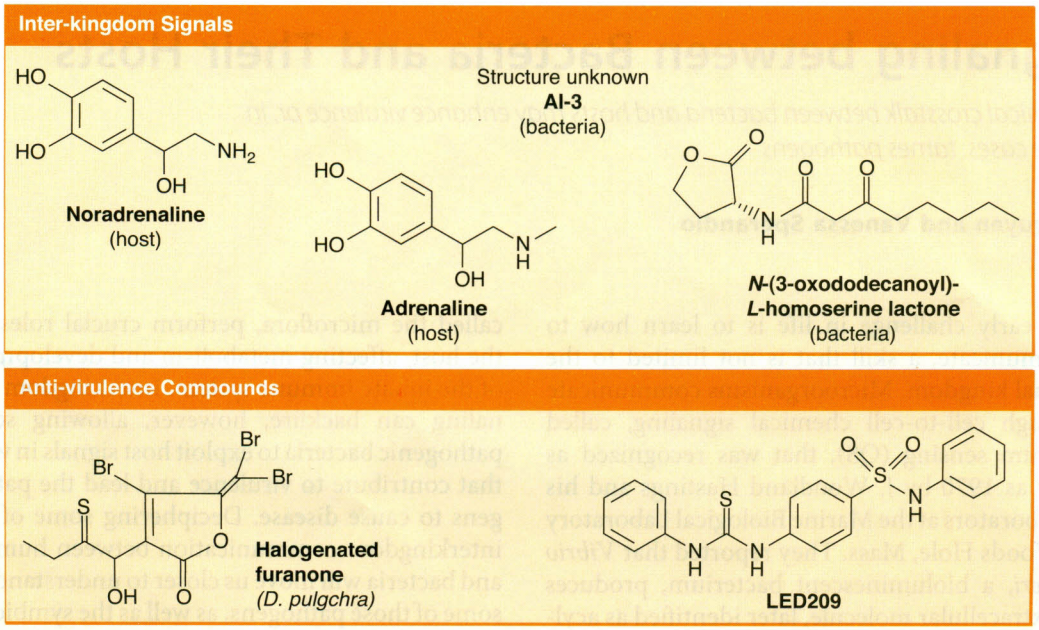
Consider, for example, the human gastrointestinal (GI) tract, which contains approximately 10^{14} bacteria from more than 1,000 different species. The host provides nutrients needed for the bacteria to survive. In return, those bacteria, called the microflora, perform crucial roles for the host, affecting metabolism and development of the innate immune system. Interkingdom signaling can backfire, however, allowing some pathogenic bacteria to exploit host signals in ways that contribute to virulence and lead the pathogens to cause disease. Deciphering some of the interkingdom communication between humans and bacteria will move us closer to understanding some of those pathogens, as well as the symbionts that affect host functions without causing disease.

Of great interest is the interrelationship between pathogenic bacteria and their human hosts. Expressing virulence traits can be metabolically expensive for these microorganisms. However, by living within the GI tract of their human or other mammalian hosts, resident bacteria maximize the nutrients available to them. To avoid wasting metabolic energy, some pathogenic bacteria have mechanisms with which they sense host signals that alert them to their surroundings. These host signals may activate one or several virulence factors or, instead, may indicate that the environment is hospitable and thus suitable for them to colonize.

SUMMARY

- Many gram-negative bacteria release specific variants of acyl-homoserine lactones (AHLs) as part of quorum sensing-based, cell-to-cell signaling.
- In some cases, AHLs become part of interkingdom signaling mechanisms between bacteria, including pathogenic species, and their plant or animal hosts.
- Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) can sense and respond to human adrenergic hormones, adrenaline and noradrenaline, as well as autoinducer-3 from microflora, in ways that affect the virulence of this bacterial pathogen.
- Some bacterial AHLs that resemble eukaryotic lipid-based signaling molecules can regulate specific host genes.
- Some hosts have developed mechanisms with which they counteract pathogens by degrading their AHL-based signals.

FIGURE 1



Structures of interkingdom signaling molecules and antivirulence compounds.

Signaling between EHEC and Its Human Host

Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) is one such pathogen that unleashes potentially deadly virulence factors in response to very specific human signals—in this case, two human adrenergic hormones, adrenaline and noradrenaline (Fig. 1). EHEC causes outbreaks of bloody diarrhea and hemolytic uremic syndrome worldwide. These bacteria attach to host intestinal epithelial cells, where they form attaching and effacing (A/E) lesions while colonizing the large intestine (Fig. 2). From this perch, EHEC can sense adrenaline and noradrenaline through the histidine sensor kinase QseC.

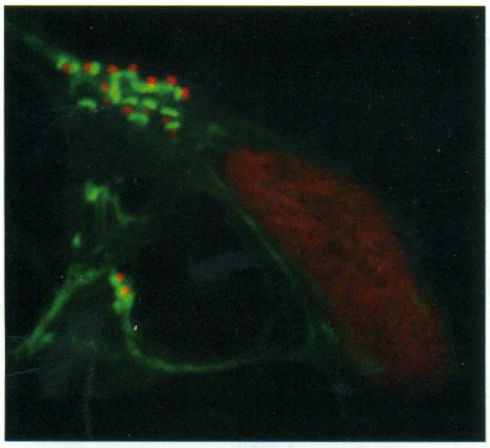
QseC in EHEC also senses a bacterial signal, autoinducer 3 (AI-3), synthesized by gastrointestinal microbial flora. Moreover, QseC homologues are present in at least 25 human and plant pathogens. Further, enterotoxigenic *E. coli* (ETEC), *Salmonella enterica* serovar Typhi, *Salmonella enterica* serovar Typhimurium, *Vibrio parahaemolyticus*, and *Francisella tularensis* also sense adrenaline and noradrenaline through QseC. QseC-defective mutants of EHEC, uropathogenic *E. coli* (UPEC), *Salmonella* spp., and *F. tularensis* express reduced virulence and colonize their hosts less effectively, demonstrating the crucial role of QseC in bacterial pathogenesis.

QseC is the first example of a receptor capable of sensing both human and bacterial signals. Although QseC is being carefully studied in EHEC, several important questions remain unanswered. For example, noradrenaline, adrenaline, and AI-3 are all found within the GI tract. Does QseC distinguish among these signals and, if so, how is each one regulated? Are there other bacterial adrenergic receptors?

In our laboratory, we found that another sensor kinase, QseE, also detects adrenaline to regulate EHEC virulence traits such as the production of Shiga toxin. Several commensal bacteria in the GI also express QseC. What function does QseC have in these populations?

Do human cells have receptors for recognizing signals from bacterial cells? AI-3 likely acts as a QS signal among the gut microflora, yet pathogenic microorganisms have evolved a means to exploit the system to infect and cause disease in the host. Elucidating the structures of AI-3 and QseC will provide further insights into ligand specificity and assist in the better design of compounds to inhibit QseC interkingdom signaling. However, small-molecule inhibitors must

FIGURE 2



EHEC forms AE lesions on host cells. Cell nuclei and bacterial cells are stained in red (propidium iodide) and actin is stained in green (fluorescein isothiocyanate-phalloidin).

be designed and tested carefully to treat infections without disrupting the gut microbiota.

Some Bacteria Signal Their Hosts

Noradrenaline and adrenaline are host hormones that modulate gene expression in some bacteria, particularly pathogens. Some of these bacteria turn the tables on their mammalian or plant hosts—for instance, with bacterial QS molecules regulating host gene expression to further the infection (Fig. 3).

Several gram-negative bacteria produce AHL signals, each of which contain a conserved lactone ring attached by an amide bond to a variable side acyl chain. The acyl chain variants provide specificity by binding to different receptors. Similar to members of the eicosanoid family of lipid-based signaling molecules or steroid hormones that are found in eukaryotes, most AHLs diffuse freely across cell membranes. AHLs function as ligands, each binding to its specific receptor, which is also a transcription factor. When bound to an AHL, the transcription factor alters its conformation in such a way that it binds specific target gene promoters, or in some cases the AHL can act as a folding switch to its receptor protein, aiding its proper folding and preventing its degradation (Fig. 3).

Because bacterial AHLs and eukaryotic lipid-based signaling molecules are structurally similar, some researchers suspected that AHLs could bind to mammalian receptors to regulate gene expression. Indeed, AHLs can enter host cells and regulate mammalian biological functions.

For instance, one such AHL, namely N-(3-oxododecanoyl)-L-homoserine lactone, promotes apoptosis in various types of mammalian cells, including neutrophils, macrophages, fibroblasts, and human vascular endothelial cells. Additionally, this AHL can modulate host immune responses in ways that benefit the pathogen during different stages of an infection. More specifically, relatively low concentrations of this AHL inhibit host immune responses and can prevent clearance of the pathogen during early stages of an infection. Meanwhile, higher concentrations of this AHL elicit a host inflammatory response, favoring bacterial dissemination from the site of infection.

AHLs from plant bacterial pathogens also alter host responses. AHLs at nanomolar concentrations can modify an array of phenotypes in plants, including changing host defense responses, primary metabolism, stress and hormone responses, gene regulation, and protein and cytoskeleton dynamics.

Of the variety of host responses that AHLs elicit, the peroxisome proliferator activated receptors (PPAR$\beta/\alpha/\delta$) were the first such eukaryotic receptors to be identified as also being responsive to AHLs. Doubtless other eukaryotic receptors for AHLs are yet to be found in nature. Additionally, AHLs are only one class of QS molecules; other chemical types of QS signals are likely to be also capable of altering host responses.

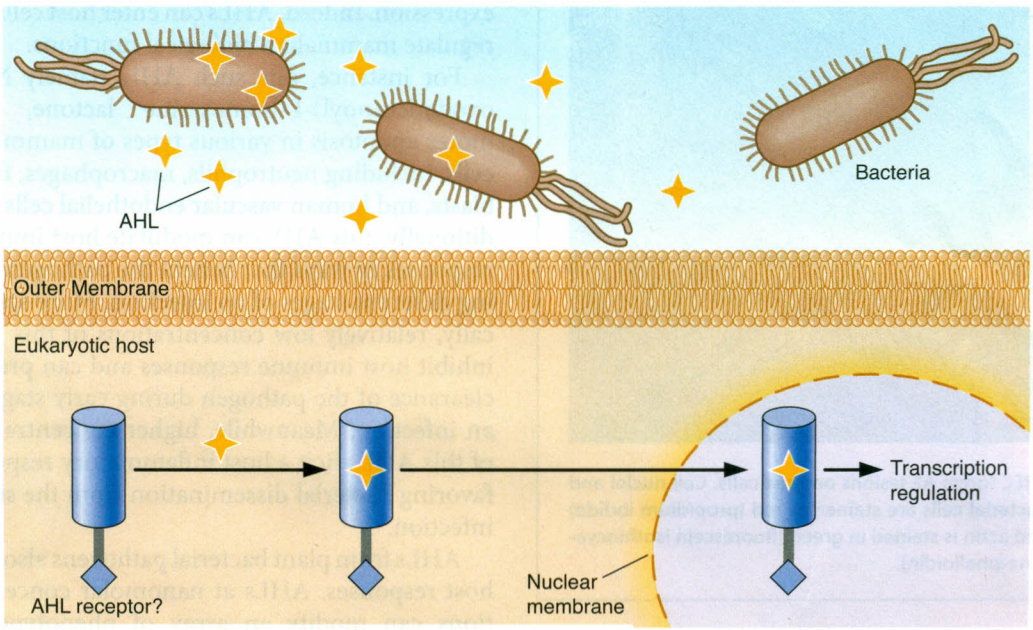
Some Hosts Resist or Disrupt Bacterial Signals

Bacteria exploit their own QS molecules to manipulate their hosts. Although pathogenic bacteria sometimes use their own chemical signaling to disrupt host defense mechanisms, some hosts have developed mechanisms with which they fight back against such pathogens, including inactivation of AHL signals.

Among the best examples are the mammalian produced paraoxonases, which act like bacterial lactonases by degrading AHLs. These factors may have evolved to prevent AHL from accumulating in host cells and interfering with host functions.

Unlike mammals, plants do not carry genes encoding AHL-degrading enzymes. However,

FIGURE 3



Gram-negative bacteria use quorum sensing molecule AHL to modulate host response.

plants and algae secrete compounds that mimic bacterial AHLs that apparently disrupt bacterial cell-to-cell signaling. For example, the red alga *Delisea pulchra* synthesizes halogenated furanones that mimic specific bacterial AHLs (Fig. 1). These halogenated furanones compete for binding with bacterial AHL receptors and inhibit AHL function by promoting degradation of those receptors. Other plant species, including rice, soybean, tomato, and crown vetch, as well as the unicellular green alga *Chlamydomonas reinhardtii,* produce other AHL mimics that interfere with bacterial QS. The chemical identities of these compounds are currently under investigation.

Value in Learning More about Bacteria-Host Signaling

Improving our understanding of signaling between bacteria and their eukaryotic hosts will provide further insights and might lead to novel ways of treating patients who are infected with potentially deadly pathogens. Antimicrobial therapeutic options are narrowing in the face of expanding antibiotic resistance among many pathogenic strains. Traditional antimicrobial agents target bacterial processes such as cell wall synthesis, DNA replication, and protein synthesis. Although these agents can be highly effective, they place high selective pressure on bacterial populations, encouraging drug-resistant mutants to emerge.

Hence, new strategies are needed to combat these antibiotic-resistant microbial pathogens. One potential alternative strategy is to disrupt interkingdom signaling. By interfering with the synthesis of virulence factors instead of blocking bacterial growth, this approach could relieve some of the evolutionary pressure that traditional antibiotics exert. Such signaling strategies in some cases might interfere specifically with the ability of the bacterial pathogen to sense environmental cues that promote colonization.

Some scientists are actively screening for new antagonists of AHLs. Identifying inhibitors that block AHL signaling is proving to be a challenge. One reason is that several gram-negative bacterial pathogens, including *Pseudomonas* spp., produce and sense different types of AHLs.

One promising approach to overcome this challenge involves inhibiting QseC and preventing interkingdom communication. Another advantage to targeting QseC is that this sensor ki-

AUTHOR PROFILE

Sperandio: from Solving Complex Biological Puzzles to Training for Marathons

Vanessa Sperandio once thought her passion for ancient history and archeology would lead her to become an Egyptologist or classicist. But her love for biology proved stronger. "I just really wanted to know how things worked," she says. "Biology was always a big mystery to me, a complex puzzle to be solved.... It requires creativity and curiosity, [and] you are constantly learning something. Science is a humbling profession because you are always realizing that you should do more, and that you are far from knowing everything."

Sperandio, 42, professor of microbiology and biochemistry at the University of Texas Southwestern Medical Center in Dallas, continues in solving puzzles and learning new things. Her focus is on trying to understand bacterial-host chemical signaling, whether beneficial or harmful.

Sperandio grew up in the Brazilian city of Londrina, in the southern part of the country. Both her parents were professors, her mother in sociology, and her father in business. They divorced when she was ten, but remained good friends. She has a younger brother, who works in marketing. "In my household there was always a huge emphasis in academia, and I always enjoyed reading a lot," she says. Her mother and father, and her mother's partner, a philosopher, became her role models. "All three of them taught me to work really hard—there was no complaining about things being difficult," she says.

Sperandio earned her B.S. in biological sciences in 1991, her M.S. in molecular genetics, and her Ph.D. in molecular genetics in 1995, all at the State University of Campinas in the state of São Paulo, Brazil. However, she trained in the United States from May 1993 until October 1994 on what she calls a "sandwich" fellowship at the University of Maryland School of Medicine in Baltimore with James Kaper, before returning to Brazil to defend her dissertation. She did postdoctoral research at the University of São Paulo from 1995 to 1997 before returning to work again with Kaper between 1997 and 2001. She joined the University of Texas Southwestern Medical Center as an assistant professor in 2001.

Now a U.S. citizen, Sperandio is married to a software engineer, and they have three children—a daughter, 10, and twin boys, 7. In her spare time, she enjoys reading novels and likes to run. "I always have a book with me," she says, adding: "I ran a couple of marathons, and several half-marathons. I am trying to get into the mode of training for a full marathon again."

When asked what it is like to be a woman in science, "it always puzzles me," she says. "I always had wonderful male and female colleagues and friends, and usually gender became invisible. Men and woman are different, thank God, otherwise it would be boring. But I don't think this changes anything professionally. A good professional is a good professional."

Marlene Cimons

Marlene Cimons lives and writes in Bethesda, Md.

nase is not found in mammalian cells, suggesting that inhibitors will not induce side effects in infected human hosts. Additionally, the QseC sensor kinase is conserved among several human and plant pathogens. Thus, effective inhibitors of QseC might be used in treating an array of different pathogens.

After screening 150,000 low-molecular-weight organic compounds, we identified one of them, designated LED209, to be capable of blocking the binding of AI-3, adrenaline, and noradrenaline to QseC. LED209 also prevents QseC autophosphorylation and subsequent downstream activation of virulence genes in three different bacterial pathogens. We are continuing to study this and other molecules as we search for other cross-kingdom signals that are part of the intricate and delicate relationships that develop between bacteria and their eukaryotic hosts.

Y Nguyen is a graduate student in the Molecular Microbiology program and Vanessa Sperandio is a Professor in the Departments of Microbiology and Biochemistry, UT Southwestern Medical Center, Dallas, Tex.

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