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MINIREVIEW

Acyl-Homoserine Lactone Quorum Sensing in Bacteria

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Recent advances in studies of bacterial gene expression and light microscopy show that cell-to-cell communication and community behavior are the rule rather than the exception. One type of cell-cell communication, quorum sensing in Gram-negative bacteria involves acyl-homoserine lactone signals. This type of quorum sensing represents a dedicated communication system that enables a given species to sense when it has reached a critical population density, and to respond by activating expression of specific genes. The LuxR and LuxI proteins of *Vibrio fisheri* are the founding members of the acyl-homoserine lactone quorum sensing signal receptor and signal generator families of proteins. Acyl-homoserine lactone signaling in *Pseudomonas aeruginosa* is one model for the relationship between quorum sensing, community behavior, and virulence. In the *P. aeruginosa* model, quorum sensing is required for normal biofilm maturation and virulence. There are multiple quorum-sensing circuits that control the expression of dozens of specific genes in *P. aeruginosa*.

Key words: Acyl-homoserine lactone, quorum sensing, biofilm, virulence, *P. aeruginosa*

Recent advances have shown that bacteria often function as groups that are capable of more than the sum of the individuals. Like other organisms, bacteria organize into functional groups or communities (4, 8, 36). Bacteria use chemicals to signal each other, and to coordinate their activities. Many Gram-positive bacteria use small peptide signals (6, 22), Gram-negative bacteria appear to use small molecule signals of various sorts (1, 8-10, 30). Perhaps the best-studied signaling system is the Gram-negative acyl-homoserine lactone (acyl-HSL) system. This type of bacterial cell-to-cell communication was first discovered in the context of microbial ecology but it is now evident that acyl-HSL signaling is important in plant and animal (including human) diseases. Acyl-HSL signaling is a dedicated communication system that is used by bacteria to control specific genes in response to population density. Acyl-HSLs (Fig. 1) are small molecule signals with no known function other than signaling. These chemical signals are produced by specific enzymes, and they are detected by specific receptors. Because acyl-HSL signaling provides a mechanism by which a bacterial species can monitor its own population density, this type of signaling and other signaling systems that achieve the same purpose have been termed quorum sensing systems (11). This sort of dedicated signaling system should be distinguished from metabolite cross-feeding or sensing of a build-up of a metabolic intermediate during culture growth.

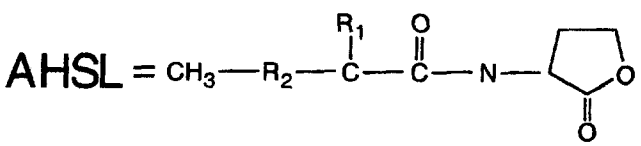

Fig. 1. A schematic diagram of an acyl-HSL (R_1, H, OH, or O; R_2, $(CH_2)_{2\text{-}14}$, or CH_2-CH_2-CH=CH-CH_2-CH_2). The substrates for the acyl-HSL synthase are an acylated acyl carrier protein (Acyl-ACP), and *S*-adenosylmethionine (SAM). AHSL, acyl-homoserine lactone.

Acyl-HSL signals are generated by the activity of a single enzyme that uses as substrates, *S*-adenosylmethionine and an intermediate of fatty acid biosynthesis, acyl-acyl carrier protein (14, 15, 20, 25, 37). The enzyme is generally a member of the LuxI family of acyl-HSL synthases. Different LuxI homologs generate different acyl-HSLs. Thus the *Pseudomonas aeruginosa* RhlI primarily catalyzes the synthesis of *N*-butyryl-HSL (C4-HSL) and the *P. aeruginosa* LasI directs the synthesis of *N*-(3-oxododecanoyl)-HSL (3OC12-HSL). The acyl side-chain length and the substitutions on the side chain provide signal specificity. Acyl side chains of these signals can be

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fully saturated, they can have hydroxyls or carbonyls on the third carbon, and acyl-HSLs with side chain lengths of 4 to 16 carbons have been identified (10), Short-chain signals like C4-HSL diffuse freely through the cell membrane (16, 28), and 3OC12-HSL partitions into cells, presumably in the membrane. This signal can diffuse into the surrounding environment but export is enhanced by the *mexAB-oprM*, and perhaps other efflux pumps (7, 28). Regardless, the cellular concentration of an acyl-HSL is defined by the environmental concentration, and environmental concentrations can rise only when there is a sufficient population of the signal producing bacterium.

The specific receptors for acyl-HSL signals are members of the LuxR family of transcriptional regulators. LuxR family members have been proposed to consist of 2 domains, a C-terminal DNA-binding domain, and an N-terminal acyl-HSL-binding domain (39). Within the C-terminal domain there is a helix-turn-helix motif. For LuxR this motif has been shown to make contact with the regulatory DNA in the promoter of the luminescence genes. Recently, alanine-scanning mutagenesis has been used to identify amino acids in the helix-turn-helix that interact with the DNA, and other residues that seem to form an activating patch that interacts with RNA polymerase (K. Egland and E. P. Greenberg, In Press).

Quite often the two regulatory genes (the R and I genes) are linked but not always. The orientation of the two genes with respect to each other is variable. In some cases the R protein serves as an activator, and in other cases the R protein functions as a repressor.

Acyl-HSL quorum sensing was first discovered to control the luminescence of *Vibrio fischeri*, a bacterium that forms a mutualistic light organ symbiosis with certain marine animals (21, 34). Here quorum sensing is critical to the symbiosis. Acyl-HSL signaling is essential for virulence of the plant pathogen *Erwinia carotovora* (32), and for virulence of *P. aeruginosa* in mouse models of lung (42) and burn infections (35), in invertebrates (19, 40, 41), and in plants (33). Here *P. aeruginosa* serves as a model for the role of bacterial communication in community behaviors important in pathogenesis.

P. aeruginosa can be isolated from soil and water. It is also an opportunistic pathogen of humans, other animals, and plants. One of the reasons *P. aeruginosa* is a successful opportunistic pathogen is that it produces a battery of secreted virulence factors. These virulence factors include exoproteases, siderophores, exotoxins, and lipases. Many of these virulence factors are regulated by quorum sensing (8, 29, 43). Of what advantage to *P. aeruginosa* is quorum sensing control of virulence factors? First it is economical to produce extracellular factors only after a critical population has been achieved. A mass of cells is required to produce sufficient quantities of these factors to influence the surrounding environment. Second, in the host, timing of the deployment of virulence factors may be critical. The pathogen can amass without displaying its virulence factors, and then the pathogen can mount a surprise attack in which the arsenal of virulence factors is deployed in a coordinated and overwhelming fashion.

Genetic studies have revealed two quorum-sensing systems in *P. aeruginosa*. Both of these systems have linked R and I genes. They are the LasR-I and RhlR-I quorum sensing systems (2, 13, 18, 23, 24, 26). In addition, the recently completed *P. aeruginosa* genome sequencing project has revealed a third LuxR homolog that is adjacent to a cluster of quorum sensing controlled (qsc) genes (44). However, a third LuxI homolog is not evident from the sequence, and the function of the third LuxR homolog is as yet unknown. LasR is a transciptional regulator that responds primarily to the LasI-generated signal, 3-OC12-HSL, and RhlR is a transcriptional regulator that responds best to the RasI-generated, C4-HSL. The current model for the quorum sensing in *P. aeruginosa* is that at low population densities LasI produces a basal level of 3-OC12-HSL. As density increases, 3-OC12-HSL builds to a critical concentration at which it interacts with LasR. This LasR-3-OC12-HSL complex then activates transcription of a number of genes including *rhlR* (12, 13, 26, 29, 38). The activation of *rhlR* by LasR results in a quorum sensing regulatory cascade, in which activation of the *rhl* system requires an active *las* system. RhlR responds best to the RhlI-generated C4-HSL. RhlR then activates expression of genes required for production of a variety of secondary metabolites such as hydrogen cyanide and pyocyanin (29). A DNA sequence with dyad symmetry called a *lux*-box-like sequence can easily be identified in the promoter regions of many quorum-sensing controlled (qsc) genes (11, 27, 31, 44). By analogy to other acyl-HSL quorum sensing systems we deduce that the *lux*-box like sequences function as binding sites for LasR and RhlR. It is not yet clear how RhlR and LasR discriminate between their respective binding sites. In fact many genes show partial activation with either LasR or RhlR and the appropriate acyl-HSL (for example see *30, 37*). One explanation for this is that binding site discrimination is less than perfect and either LasR or RhlR can bind with varying efficiency to any *lux* box-like element. In fact evidence is beginning to accumulate that the gene designations lasI and lasR may in fact be misnomers and that in P. aeruginosa the lasB gene is controlled primarily by RhlR and RhlI (44).

A recent study used a random mutagenesis approach to identify 39 gene that were highly regulated (minimum 5-fold induction, maximum 740-fold induction) by quorum sensing (44). The genes were divided into 4 different classes, two of which respond to 3-OC12-HSL, and two of which required both C4-HSL and 3-OC12-HSL for maximum induction. The qsc genes map throughout the *P. aeruginosa* chromosome, confirming the view that quorum sensing in this bacterium represents a global reg-

ulatory system (29). The 39 genes revealed by the random mutagenesis study represent only a subset of the qsc genes in *P. aeruginosa*. It was estimated that as many as 4% of the roughly 6,000 *P. aeruginosa* genes are controlled by quorum sensing (44).

One report indicates that transcription of *rpoS*, a gene encoding an RNA polymerase subunit involved in expression of stationary phase factors is activated by RhlR and C4-HSL (17). This raises the possibility that many genes may be controlled indirectly rather than directly by quorum sensing. It is also an enticing hypothesis because it lends itself to the idea that one specific cue that enables a cell to anticipate stationary phase is crowding. Unfortunately, quorum-sensing control of *rpoS* transcription is an example for which there is limited evidence. It is also an example for which there are low levels of induction at best (3-fold). In fact recent investigations suggest that quorum sensing may have no significant influence on *rpoS* transcription in *P. aeruginosa* (45).

Bacteria often tend to attach to surfaces and form communities enmeshed in a self-produced polymeric matrix. These communities are called a biofilm (3, 4). *P. aeruginosa* is often found in naturally occurring biofilms. Under the appropriate laboratory conditions, *P. aeruginosa* forms characteristic biofilms that can be several hundred micrometers thick. Development of a mature biofilm proceeds through a programmed series of events (4). After attachment, cells multiply to form a layer on a solid surface. Individuals in the layer then exhibit a surface motility called twitching. Twitching is dependent on Type IV pili. As a result of twitching motility small groups of *P. aeruginosa* called microcolonies form. Microcolonies then differentiate to form a mature biofilm. Microcolonies in a mature biofilm have tower and mushroom-shaped architectures. The cells in these structures are encased in an extracellular polysaccharide matrix. Water channels that allow the flow of nutrients into and waste products out of the biofilm innervate these structures. There is a significant physiological heterogeneity within biofilms. This heterogeneity in physiological activity makes studying biofilms with traditional molecular microbiological techniques difficult. Bacteria in these mature biofilms are phenotypically resistant to microbiocidal agents including antibiotics. Thus biofilms cause many different types of chronic or persistent bacterial infections (4).

Recent studies have linked quorum sensing and biofilm maturation (5). This is a particularly gratifying finding because quorum sensing functions to control gene expression in groups of bacteria, and biofilms are just that, organized groups of bacteria. A mutation in *lasI* has a dramatic affect on biofilm maturation. LasI mutants are incapable of 3O-C12-HSL synthesis, and the development of LasI mutant biofilms is arrested after microcolony formation but prior to maturation of the microcolonies into thick structured assemblages. Thus LasI mutant biofilms appear flat and undifferentiated. The normal biofilms architecture can be restored to the mutant by addition of the LasI-generated quorum sensing signal 3O-C12-HSL. A RhlI mutant exhibits normal biofilm development and architecture. The 3O-C12-HSL-responsive qsc genes involved in biofilm maturation remain unknown.

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