Cell-cell communication and its role in biofilm formation
This lecture will present a fundamental description of bacterial communication through quorum sensing mechanisms and explain how this communication system contributes to the formation of biofilms. A brief introduction, to be expanded upon in later chapters, will be presented. For information on the course can be found at the following website:
http://www2.hawaii.edu/~mcooney/oest740/.
1. **Cell - Cell communication**

**Definition**
One type of bacterial cell-to-cell communication is referred to as quorum sensing which is defined as a cell-density dependent regulation of gene expression [1, 2]. This mechanism allows bacterial populations to act in a coordinated fashion [3], to undertake behaviors that are unproductive for single, isolated cells, but effective when executed as a group [2]. Cell to cell communication is now thought to be the mechanism by which populations of bacteria coordinate complex behavior such as bioluminescence, biofilm formation, swarming, and virulence.

**Basic principle**
The basic principle of quorum sensing is the same for all bacteria, though the signaling pathways and the molecules that regulate them, known as autoinducers, vary between species [2]. In gram-positive bacteria such as *pneumococci*, autoinducing peptides move into the environment until they reach a certain concentration at which time sensors on the cell surfaces can recognize them (Figure 1, [4]). The sensors then initiate a phosphorylation cascade, which activates a DNA binding protein that modifies gene expression. In gram-negative bacteria, such as *Pseudomonas aeruginose*, where peptides could potentially get stuck in the cell membrane, acyl-homoserine lactones (AHLs) are utilized (Figure 1, [4]). The AHLs are synthesized by LuxI-type proteins in the cytoplasm, diffuse across the cell membrane until the AHL concentration is the same both inside and outside the cell. When the external cell concentration achieves a critical concentration (and thus the internal concentration as well), the internal AHLs form complexes with LuxR-type proteins that bind promoters of target genes, thus activating transcription of density dependent phenotypes. The number of AHLs per bacteria can vary, depending upon the number of genes controlled by quorum sensing. Some bacterial control
hundreds of genes (with quorum sensing) while others control only a few genes [5]. Products produced from quorum sensing controlled genes can include: toxins, enzymes, exopolysaccharides, lipopeptides, and even antibiotics [3, 6-10].

**Autoinduction and autoinducers**

Autoinduction defines an environmental sensing system that allows bacteria to monitor their own population [3]. The bacteria produce a compound, termed an autoinducer, which accumulates in the surrounding environment during growth. Autoinducers are the compounds used by bacteria to monitor their population, to assess whether (or not) a sufficient number “quorum” is present. In both gram-negative and gram-positive bacteria, the autoinducers are produced intracellularly but accumulate outside the cell, reaching a stimulatory concentration only when sufficient number of cells, a quorum, is present [2]. In gram-negative bacteria, the autoinducers diffuse through the membrane while in gram-positive bacteria the autoinducers are actively transported outside the cell [3].
Autoinducers are different between gram-negative and gram-positive bacteria. In gram-negative the autoinducers are acylated homoserine lactone (AHL) synthesized by a LuxI-type enzyme. In general, these signal molecules are composed of a fatty acyl chain ligated to a lactonized homoserine through an amide bond [11]. There is considerable structural variety between acyl-HSLs from different bacteria and even between different acyl-HSLs synthesized by the same bacterium (Figure 3, [4]). Over 50 - 70 species of gram-negative bacteria produce AHLs that differ only in the acyl side chain moiety, and each luxR-type protein is highly selective for its cognate AHL signal molecule [2, 12]. The acyl chains can vary between 4 and 16 carbons, usually by increments of two carbon units (C6, C8). The third carbon in the acyl chain might be a fully oxidized carbonyl, carry a hydroxyl, or be fully reduced [11]. The nomenclature used usually indicates the oxidation state of the third carbon (3-oxo, 3-OH, or no prefix), followed by the chain length (C4, C6, C8 etc.) and the abbreviation HSL.

In gram-positive bacteria the autoinducers are short, usually modified peptides processed from precursors [2]. Oligopeptide autoinducers are synthesized by cleavage from longer precursor peptides. They typically consist of 5-17 amino acids, sometimes containing unusual side chain moieties[12]. Some peptide signals contain side chain modifications that include lactones or thio-lactone rings and other as yet undefined hydrophobic moieties [2]. Gram-positive bacteria have never been shown to use AHL autoinducers. In further contrast to AHL signaling, the bacterial cell membrane is not permeable to autoinducing peptides, necessitating the dedicated cell-surface oligopeptide transporters to facilitate AIP secretion into the extracellular environment [12]. Detection of AIPs is mediated by a two-component sensory system (Figure 1b).

Two component systems exist in a wide variety of gram-negative and gram-positive bacteria. These circuits are responsible for detecting fluctuations in a vast assortment of extracellular cues and transducing that sensory information into the cell to appropriately modulate gene expression in response to a changing environment [13].
Species specific cell – cell communication

In a typical gram-negative bacterial quorum sensing circuit the LuxI-type enzyme synthesizes the AHL. This cytoplasmically synthesized autoinducer diffuses passively through the bacterial membrane and accumulates both intra and extracellularly in proportion to cell density. When the stimulatory concentration of AHL is achieved, the LuxR-type protein binds it and forms a complex that can bind to promoters of quorum sensing-regulated target genes and activate transcription (Figure 4, [12]). Additional complexity exists in the many of these circuits, such as the use of multiple AHL autoinducers and LuxR proteins that can act in parallel or in series [2, 4].

The quorum sensing circuit of a gram-positive bacterium utilizes short peptides that are actively transported out of the cell and interact with external domains of membrane bound sensors (Figure 4, [12]). Signal transduction occurs by a phosphorylation cascade that culminates in the activation of a DNA bind protein that controls transcription of target genes. Specificity exists because each sensor protein is highly selective for a given peptide signal[2, 4]. Gram-positive bacteria can use multiple autoinducers and sensors. Some of the peptide transducers operate exclusively outside, some elicit a specific set of
gene expression changes from the outside and are transported back into the cell where they trigger a different set of behavioral changes[2, 4].

**Interspecies cell-cell communication**

While species-specific quorum sensing apparently allows the recognition of self in a mixed population, bacteria can also use signal molecules to detect the presence of another species (Figure 4c, [12]). The classic system is that of the gram-negative bacterium *Vibrio harveyi*, the same one that produces the bioluminescence. *V. harveyi* produces two autoinducers (HAI-1 and AI-2), the first of which is a typical gram-negative-like AHL, although its synthesis is not dependent upon the LuxI-like enzyme. The second autoinducer AI-2, is a furanosyl borate diester. Both autoinducers act via a gram-positive phosphorylation cascade. The AI-2 autoinducer binds to the soluble periplasmic AI-2 binding protein LuxP [2]. In this regulatory circuit, it appears that one signal is used for intraspecies communication and the second signal is used for interspecies communication [12].

Similar to other gram-negative bacteria, *V. harveyi* produces and responds to an AHL-type autoinducer termed AI-1 (Figure 3A). By contrast to gram-negative bacteria, two-component signal transduction proteins carry out the autoinducer-mediated signal relay in *V. harveyi*. Additionally, *V. harveyi* possesses a novel signal molecule, denoted AI-2 [14]. Genetic and biochemical evidence suggests that AI-1 mediates intraspecies communication and AI-2 mediates interspecies cell-cell communication [12].

In *V. harveyi*, the AHL signal is AI-1 and synthesized by the protein LuxLM (Figure 2 and 4 [12]). LuxLM shares no homology with the LuxI family of AHL autoinducer synthases; however it performs identical biochemical tasks to the LuxI-type enzymes [15]. AI-1 is detected by the two-component hybrid sensor kinase-response regulator protein LuxN [15]. The second signal, AI-2, is synthesized by an enzyme called LuxS and is a furanosyl borate diester (Figure 3). Detection of AI-2 requires two proteins: LuxP and LuxQ [16]. LuxP is the AI-2-binding protein which is a soluble periplasmic protein resembling sugar-binding proteins. The LuxP-AI-2 complex interacts with the
second protein required for detection, LuxQ. LuxQ is similar to LuxN and is a hybrid two-component protein containing both sensor kinase and response regulator modules. Sensory information from both LuxN and LuxPQ is transduced to the phosphotransferase protein LuxU, and LuxU transmits the signal to the downstream response regulator LuxO (Figure 4 [12]).

Under conditions of low cell density (i.e. in the absence of autoinducers), LuxN and LuxQ act as kinases. Analogous to all other two-component sensors, they autophosphorylate on conserved histidine residues. Phosphate is sequentially transferred to the conserved region aspartate residue on the response regulators of the hybrid proteins, and then to a conserved histidine on LuxU, and finally to a conserved aspartate on LuxO. Phospho-LuxO is indirectly responsible for repression of the LuxCDABE operon, which encodes the luciferase enzymes necessary for light production. At high cell density, interactions of the signals with LuxN and LuxPQ cause the sensors to switch from kinase mode to phosphatase mode. The net results are the dephosphorylation and inactivation of LuxO, leading to the derepression of LuxCDABE [12].

**Conclusions**

The discovery of species-specific and universal intercellular signaling molecules reveals that bacteria interact with one another using surprisingly sophisticated mechanisms of communication. In nature, bacteria are rarely found in isolation and evolution has allowed them to detect when they are in heterogeneous communities, to assess the proportions of self and others in mixed-species environments, and to respond to this information by appropriately modulating gene expression.
References


