iBiology.org Teaching Tools
Bonnie Bassler's Lecture Part 1:
Intra- and Inter-Species Communication

Teaching Tools were prepared by Julia Van Kessel and Bonnie Bassler.

Contents

---

1. Keywords and Terms

quorum sensing; *Vibrio fischeri*; bioluminescence; group behavior; virulence; high-cell-density; low-cell-density; autoinducer; receptor; phosphorylation; acyl homoserine lactone; peptide; chemical signaling

2. Lecture Notes

Introduction

Quorum sensing is a process of chemical communication that bacteria use to assess the species composition and cell number in the vicinity. Quorum sensing involves the production, release, and detection of signal molecules called autoinducers. Extracellular

autoinducer levels increase in proportion to increasing cell-population density. When autoinducer levels increase above a particular threshold level, it “informs” the bacteria that they have reached a certain cell density. The bacteria detect the autoinducers and respond as a group to collectively change their gene expression patterns, and, in turn, their behavior. Because quorum sensing allows bacteria to behave in a coordinated fashion, it allows bacteria to take on many of the characteristics of multicellular organisms.

Some bacterial processes are not effective when single cells carry them out alone but, rather, these processes only become successful when undertaken in synchrony by a group of cells. Bacteria can use quorum sensing to determine if there are a sufficient number of cells present to successfully initiate particular tasks. One classic example of this is the production of bioluminescence by the marine bacterium V*ibrio fischeri*, a symbiont of the Hawaiian bobtail squid *Euprymna scolopes*. V*. fischeri* only exists at high cell density in the squid light organ, not free-living in the seawater. Thus, it is only beneficial to the bacteria to synthesize the light producing (luciferase) enzymes in the squid light organ. The bacteria detect when they are in the light organ by measuring the accumulation of autoinducers inside the squid light organ, and in response to the molecules, the bacteria make light. By contrast, autoinducers do not accumulate to any significant level the free ocean, so under this condition, *V. fischeri* does not make light. Light production by the bacteria enables the squid to eliminate its shadow on the shallow ocean floor and thus light is used by the squid in a strategy to evade predators.

Originally assumed to be a phenomenon unique to Vibrios, it is becoming clear that many or all bacteria use quorum sensing to count their numbers, to recognize when they are alone versus when they are in a community, to distinguish self from non-self, and to control important collective activities. While the quorum sensing strategies and outcomes differ between species, the common trait is that bacteria control behaviors at the level of the group.

Quorum sensing: chemical communication between bacterial cells

Bacteria use production and detection of small molecules called autoinducers to communicate and to ‘vote’ on when a minimum threshold number of cells are present to initiate group activities instead of acting as individual cells. Bacteria produce and respond to autoinducers in a species-specific manner, and many different quorum sensing autoinducer molecules have been identified from numerous bacteria.

Gram-negative bacteria typically use acyl homoserine lactone (AHL) molecules as autoinducers, and each species uses a distinct AHL to communicate with members of its own species. AHLs are produced by LuxI-type proteins and AHLs diffuse freely across the Gram-negative cell membrane. At high concentrations, the autoinducer is bound by a transcription factor of the LuxR type in the cytoplasm. The LuxR-AHL complexes bind DNA and activate gene expression for group-specific processes (e.g. bioluminescence).

Gram-positive bacteria typically use small peptides as autoinducers, and these are bound by receptors present in the cell membrane. Upon binding of the autoinducing peptide, the receptor undergoes a conformational change that results in phosphorylation of proteins in the cytoplasm. Ultimately, a transcription factor is phosphorylated, which changes its activity, and in turn, promotes changes in gene expression patterns.

Processes controlled by quorum sensing

The list of processes that bacteria coordinate via quorum sensing is extensive, and typically these activities are unproductive when carried out by small numbers of cells. Often quorum sensing controls pathogenesis. Specifically, pathogenic bacteria do not express canonical virulence traits initially after infection. Rather, the bacteria “wait” until their numbers have increased, which they detect by sensing the buildup of released autoinducers, and then, only at high cell density, do they “attack” the host, For instance, genes encoding virulence factors, and proteins required for biofilm production are controlled by quorum sensing in many pathogens (for example, *Pseudomonas aeruginosa*). In the plant pathogen *Agrobacterium tumefaciens*, mating and transfer of DNA are coordinated, making the bacterial cell community more infective and better at causing disease. In addition, some quorum sensing plant pathogens such as *Erwinia carotorova* produce virulence factors to successfully infect the host, and they simultaneously produce antibiotics to which they are resistant but that kill competitor bacteria that might also try to infect the host. All these strategies allow bacteria to take effective actions as a group.

Quorum sensing in *Vibrio harveyi*: a variation on a theme

Vibrio harveyi, another marine bacterium that produces bioluminescence at high-cell-density, uses a different quorum sensing mechanism than most Gram-negative bacteria, and notably, this mechanism differs significantly from that of *V. fischeri*. In *V.*

harveyi, autoinducer receptors are transmembrane proteins that detect autoinducer externally, and then transmit that information internally through a phosphorylation cascade similar to the mechanism used by Gram-positive bacteria. *V. harveyi* uses two autoinducers, named autoinducer-1 (AI-1) and autoinducer-2 (AI-2) for quorum sensing. AI-1 is used for intra-species communication, while AI-2 enables inter-species communication. These two signals have different structures, bind to different receptors, and enable the bacteria to differentiate between the presence of *V. harveyi* cells and the presence of other species of bacteria.

The *V. harveyi* AI-1 is an AHL, analogous to autoinducers in other Gram-negative species. However, AI-2 has a unique structure, and is produced by a protein called LuxS. The luxS gene is present in more than half of the bacterial genomes for which sequences are available, supporting the hypothesis that AI-2 is used by bacteria for inter-species communication. LuxS functions in the pathway for S-adenosylmethionine (SAM) utilization, an important molecule in central metabolism. One of the products of the LuxS reaction is 4,5-dihydroxy-2,3-pentanedione (DPD), a reactive molecule that interconverts into a variety of cyclic molecules at equilibrium. In order to determine which of the rearranged molecules is the active AI-2 autoinducer, the *V. harveyi* LuxP protein, (which binds AI-2 and acts in conjunction with the transmembrane receptor LuxQ to detect AI-2), was used to bind and isolate the active AI-2 moiety. The active AI-2 molecule is formed by DPD that cyclizes and reacts with borate. Incorporation of boron in the AI-2 molecule is another unique and interesting aspect of the *V. harveyi* quorum sensing mechanism because boron is plentiful in the ocean, the primary habitat of *V. harveyi*, whereas boron is uncommon in other environments. Consistent with this, structural studies show that *Salmonella typhimurium* uses a different rearranged DPD moiety as its active AI-2 signal molecule, and this molecule lacks boron. Nonetheless, the *V. harvyei* and *S. typhimurium* AI-2 molecules spontaneously inter-convert which allows the bacteria to communicate across species.

Summary

In a process called quorum sensing, bacteria use chemical molecules called autoinducers to communicate. Using quorum sensing, bacteria can distinguish self from non-self, determine population numbers, and coordinate group behaviors. Groups of bacteria can thus behave analogously to multicellular organisms by displaying synchronized gene expression and carrying out processes as a collective. Although the *V. harveyi* and *V. fischeri* quorum sensing circuits provide clues about bacterial strategies that have evolved for communication, there are many molecules left to be

discovered and the information processing mechanisms remain to be understood. One practical aspect of these studies is to develop strategies to interfere with bacterial quorum sensing as novel treatments against bacterial pathogens.

3. Review Questions

1. Bacteria use quorum sensing to assess
    A. the bacterial species present
    B. the number of cells present
    C. the bacterial growth rate
    D. all of the above
    E. all of the above except c

2. How do bacteria assess population density?

    A. Chemicals
    B. Touch
    C. Sound
    D. Pressure
    E. Nutrient availability

3. Why is bacterial quorum sensing often compared to multicellularity?

    A. Because there are more than one bacterium present in a particular environment.
    B. Because it enables the bacteria to behave as a group.
    C. Because it enables the group to move.
    D. Because bacteria that use quorum sensing are present in multicellular organisms.
    E. None of the above.

2. Most Gram-negative bacteria produce and detect autoinducer molecules with which type of structure:

    1. a protein

2. a peptide
3. an acyl homoserine lactone
4. an S-adenosylmethionine moiety

3. Which molecule(s) is(are) used for quorum sensing communication by *V. harveyi*?

1. AI-1
2. AI-2
3. SAM
4. Both (a) and (b)
5. All of the above

4. Describe two activities that bacteria control using quorum sensing.

5. Describe one benefit you can imagine that could come from research to discover the structures of autoinducer molecules.

6. How do bacteria benefit from using multiple autoinducer signals for quorum sensing?

7. Discuss at least one way in which the *V. harveyi* quorum sensing system is unique from those of other Gram-negative bacteria.

8. How was the final structure of the *V. harveyi* AI-2 molecule determined?

4. Answers to Review Questions

1. E. Quorum sensing via release and detection of autoinducers provides information about the bacterial species composition and cell number present. Quorum sensing does not provide information about growth rate.

2. A. Autoinducer molecules are chemicals that are sensed by bacterial cell receptor proteins.

3. B. Cells of multicellular organisms do not act individually but instead coordinate gene expression as a group. Likewise, quorum sensing bacteria count their numbers and when there are enough cells present, they express genes as a group.

4. C. Gram-negative bacteria use autoinducers that are acyl homoserine lactones (Gram-positive bacteria use peptides)

5. D. *V. harveyi* uses two autoinducers, AI-1, and AI-2. AI-2 is produced as a bi-product of the SAM biosynthetic pathway, but the cells do not use SAM to communicate.

6. Bacteria control expression of numerous genes using quorum sensing. These can include genes that encode proteins involved in production of biofilms, virulence factors, and antibiotics, as well as proteins required for mating and DNA uptake.

7.
    a. Autoinducer structural information provides clues for how collective behaviors evolved and/or for how organisms can use chemicals to distinguish self from other.
    b. Understanding autoinducers allows researchers to experimentally manipulate bacteria to assess the role of quorum sensing in bacterial physiology.
    c. By determining the structures of molecules that bacteria use to control virulence, these molecules or derivatives of them, can be synthesized and potentially used as antibacterial therapeutics. In addition, some bacteria are beneficial to humans, and manipulating their autoinducer molecules could be used to enhance advantageous bacterial activities.

8.
    a. Multiple signals provide bacteria with multiple ‘languages’ to communicate. One signal can be used to determine self (species-specific), whereas an additional signal can be used to determine if other bacterial species are present
    b. It is also possible that bacteria can detect signals from their eukaryotic hosts in order to establish symbiosis
    c. Using multiple signals can facilitate diverse responses depending on the environmental conditions.

9.

a. The *V. harveyi* receptors that bind autoinducers are transmembrane proteins, and usually cytoplasmic proteins (e.g. LuxR) bind autoinducers in the cytoplasm of Gram-negative bacteria.
b. The membrane-bound receptors are similar to Gram-positive bacteria, while the *V. harveyi* AI-1 autoinducer signal is an acyl homoserine lactone analogous to other Gram-negative bacteria
c. Information transmitted by the membrane-bound autoinducer receptors occurs by a phosphorylation cascade to activate downstream genes. This mechanism is similar to Gram-positive bacteria that also use phosphorylation to activate transcription factors, whereas the transcription factors in Gram-negative bacteria (such as LuxR) typically bind autoinducers directly in the cytoplasm.
d. *V. harveyi* uses boron in its AI-2 molecule, which to date has only been found in Vibrios.

10. One of the bi-products of the SAM utilization pathway is 4,5-dihydroxy-2,3-pentanedione (DPD), which suggested that this, or a rearranged form of DPD, might be the AI2 molecule. Furthermore, LuxP (the *V. harveyi* protein that binds the AI-2 molecule) resembles the *E. coli* ribose binding protein, and since DPD is derived from ribose, this provided support for the hypothesis that DPD is a precursor to the AI-2 molecule. However, DPD is a reactive species that rapidly cyclizes to form multiple structures at equilibrium, so it was necessary to develop a method to isolate the active AI-2 structure from the equilibrium mixture. To solve this problem, purified LuxP protein was used to isolate the active AI-2 moiety. The crystal structure of LuxP bound to AI-2 was solved, showing the structure of bound AI-2. The AI-2 mass was determined by mass spectrometry. The inclusion of boron in the molecule was determined by boron-NMR.

5. Discussion Questions

1. How do Gram-negative bacteria that use AHLs communicate within their own species?

2. Explain the mechanism of signal transduction in Gram-positive bacterial quorum sensing cascades.

3. How did the organization of the *Borrelia luxS* operon provide clues about the function of *luxS* and the structure of AI-2?

4. Why was the discovery of the *Salmonella* AI-2 structure important?

5. How might you develop an anti-quorum sensing strategy?

6. Answers to Discussion Questions

1. Differences in the acyl side chains can provide species-specificity in quorum sensing mediated communication. Even minor changes in AHL structure can make a large difference in how the molecule interacts with its cognate receptor, and this provides signaling specificity.

2. Gram-positive bacteria use small peptides as autoinducers that are bound by receptors located in the cell membrane. Unbound receptors (at low-cell-density) do not act as kinases. Therefore, downstream transcription factors are not phosphorylated, and thus, are not active. Peptide-bound receptors (at high-cell-density) undergo conformational changes that result in phosphorylation of downstream proteins in the cytoplasm. Most often, the phospho-relay culminates in phosphorylation of a DNA binding transcription factor which is responsible for controlling changes in gene expression.

3. luxS was identified in a *V. harveyi* screen for mutants that could not produce AI-2. Among the many bacteria that possess the luxS gene, Borrelia sp. contained the luxS gene in an operon with two other known genes: pfs and metK. These two genes encode proteins known to function in SAM utilization. However, in the known SAM pathway, one enzyme had not yet been identified, and in addition, one of the products of SAM utilization did not have a described function. This suggested that LuxS and AI-2 might be the missing enzyme and functional molecule, respectively.

4. The AI-2 of Salmonella is synthesized via the SAM pathway identically to V. harveyi. In addition, the Salmonella AI-2 is also formed from rearranged DPD. However, a different moiety, with the opposite stereochemistry at the chiral carbon and lacking

boron is the S. typhimurium AI-2. The *V. harveyi* and *S. typhimurium* AI-2s interconvert. These findings provided support for the hypothesis that different AI-2s exist but nonetheless, because of the interconversion process, bacteria can communicate across species boundaries.

5. Anti-quorum sensing technology could be developed by screening for natural molecules or by making synthetic molecules that inhibit bioluminescence in *V. harveyi*. A molecule that, when added to *V. harveyi*, causes a decrease in bioluminescence at high-cell-density could indicate that quorum sensing had been blocked. Such molecules could subsequently be tested for blocking quorum sensing mediated virulence in pathogenic bacteria.

7. Explain or Teach These Concepts to a Friend

1. Explain why *V. cholerae* is a unique pathogen with regard to the mechanism of infection.
2. Explain how *V. cholerae* cells behave when present alone versus in a group.
3. Explain how autoinducers could be used as a treatment for *V. cholerae.*
4. Explain one method for assaying quorum sensing activity in *V. cholerae.*

8. Research the Literature on Your Own

How does *V. cholerae* quorum sensing control biofilm production? How might this affect *V. cholerae* pathogenesis? (HINT: look up the role of cyclic di-GMP and VpsT in *V. cholerae* biofilm production)

What are some differences between quorum sensing in classical *V. cholerae* strains compared to El Tor strains? (HINT: look up biofilm production and sRNA regulation)

9. Papers for Journal Club

Higgins DA, Pomianek ME, Kraml CM, Taylor RK, Semmelhack MF, Bassler BL. The major Vibrio cholerae autoinducer and its role in virulence factor production. (2007). Nature, 6;450(7171): 883-6.

In this paper, the structure of the *V. cholerae* autoinducer CAI-1 was determined, as described in the lecture. In addition, synthetic CAI-1 was produced, as well as similar molecules, all of which were tested for their effectiveness as quorum sensing molecules in *V. cholerae*. Exogenous addition of CAI-1 to cultures of *V. cholerae* resulted in decreased expression of one of the toxin co-regulated pilis genes (tcpA), demonstrating a critical role for quorum sensing in pathogenesis.

Waters CM, Lu W, Rabinowitz JD, Bassler BL. Quorum sensing controls biofilm formation in Vibrio cholerae through modulation of cyclic di-GMP levels and repression of vpsT. (2008). J Bacteriol., 190(7): 2527-36.

This paper examines the role of cyclic di-GMP, a cytoplasmic small molecule second messenger, in quorum sensing and in biofilm production in *V. cholerae*. The data show that quorum sensing activates expression of a set of genes that alter cyclic di-GMP levels, which in turn, decreases biofilm production. This finding connects two chemical-sensing systems resulting in the control of expression of genes required for *V. cholerae* pathogenesis.