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Bonnie Bassler's Lecture Part 2:
Quorum Sensing and Developing Novel Antibiotics

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Contents

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1. Keywords and Terms

quorum sensing; *Vibrio cholerae*; group behavior; pathogen; virulence; biofilms; high-cell-density; low-cell-density; autoinducer; chemical signaling; antibiotics

2. Lecture Notes

Vibrio cholerae, like many other bacteria, uses quorum sensing to synchronize gene expression on a population-wide level. Upon infection of its human host, *V. cholerae* immediately initiates expression of virulence genes and causes an acute infection.

This immediate use of quorum sensing stands in stark contrast to bacterial pathogens that cause persistent infections and only initiate virulence factor expression after

reaching high-cell-density in the host. The unique *V. cholerae* quorum sensing mechanism provides an opportunity to explore strategies in which quorum sensing autoinducer molecules are supplied to treat bacterial infections.

Toward this end, the structure of the *V. cholerae*-specific autoinducer, CAI-1, was determined and synthetic CAI-1 was produced. Indeed, addition of CAI-1 to cultures of *V. cholerae* decreased production of virulence factors, and protected a mouse from infection with *V. cholerae.* The results of these experiments underscore the importance of identifying quorum sensing molecules and characterizing their potential use in novel antibacterial treatments.

3. Review Questions

1. What genes are expressed at high-cell-density in *V. cholerae*?

A. Toxin genes
B. Biofilm genes
C. Protease genes
D. Attachment genes
E. All of the above

2. What is the order of events in *V. cholerae* infection?

A. Ingestion, attachment (TcpA), bacterial cell accumulation, toxin release, detachment (proteases), escape from host.
B. Ingestion, attachment (TcpA), toxin release, bacterial cell accumulation, detachment (proteases), escape from host.
C. Ingestion, bacterial cell accumulation, attachment (TcpA), toxin release, detachment (proteases), escape from host.
D. None of the above.

3. Adding CAI-1 to a culture of *V. cholerae* results in:

A. a decrease in TcpA production
B. an increase in TcpA production

C. no change in TcpA production

4. What is unique about the timing of virulence gene expression in *V. cholerae* compared to many other pathogens?

5. What method was used to measure activity of the CAI-1 autoinducer?

4. Answers to Review Questions

1. C. V. cholerae turns on genes encoding proteases at high-cell-density to facilitate release of the bacterial cells from the intestinal wall. By contrast, virulence genes (required for attachment and secretion of toxins) and biofilm genes are turned on at low-cell-density and turned off at high-cell-density.

2. B. First the bacterial cells are ingested by the host. At low cell density, they express attachment genes and toxin genes, and then they grow in number. Once the cells have reached high cell density, detachment genes encoding proteases are expressed, and the cells are released from the intestinal wall and pass out of the host.

3. A. TcpA (toxin co-regulated pilus) is expressed at low-cell-density and low autoinducer concentration. Addition of autoinducers results in the V. cholerae cells behaving as if they are in high-cell-density mode, and they turn off expression of tcpA, as well as other virulence genes.

4. *V. cholerae* turns on expression of genes encoding proteins required for virulence and biofilm production at low-cell-density and turns off these genes at high-cell-density. Most pathogens only turn on virulence and biofilm gene expression at high-cell-density.

5. Bioluminescence expression. The genes encoding luciferase were introduced into V. cholerae so that the production of light would be controlled by quorum sensing. When the *V. cholerae* cells reached high-cell-density, and CAI-1 had accumulated

(or CAI-1 was added), light production turned on, analogous to what happens in V. harveyi.

5. Discussion Questions

1. What do you predict would occur if the CAI-1 molecule was altered? For example, if the chain length was one carbon shorter? What would happen if this molecule was added to cells simultaneously with CAI-1?
2. If you mutated the *V. cholerae* CAI-1 receptor CqsS so that it does not respond to CAI-1, and compared the gene expression pattern of a strain carrying the mutated CqsS to that of a wild-type *V. cholerae* strain at low-cell-density what would you expect? What would happen at high-cell-density?
3. Describe the differences between developing anti-quorum sensing molecules for pathogens that turn on virulence genes at low-cell-density (eg. *V. cholerae*) and pathogens that turn on virulence genes at high-cell-density (eg. *P. aeruginosa*).

6. Answers to Discussion Questions

1. One possible outcome is that the new molecule might not bind the CqsS receptor as effectively, causing a decrease in the quorum sensing response. Alternatively, the new molecule might be able to inhibit quorum sensing, even in the presence of CAI-1 by blocking CAI-1 access to the receptor

2. If the mutant cells cannot sense the autoinducer CAI-1, they will behave as if they are in low-cell-density-mode at all times. At low-cell-density, both the wild type and the mutant strain will behave identically. However, at high-cell-density, there will be large differences in gene expression patterns between the wild type and the mutant. The mutant will express the low-cell-density gene expression program which includes virulence, attachment, and biofilm genes. The wild-type will not express these genes, but instead will express high-cell-density-specific genes including genes for detachment.

3. For pathogens such as *V. cholerae*, discovering small molecules that promote quorum sensing will make good strategies for therapies. Addition of such molecules could result in a decrease in virulence gene expression. This could be true for other pathogens that cause acute infections. Conversely, for pathogens such as *P. aeruginosa* that cause persistent infections, an ideal treatment would be to inhibit quorum sensing. This could be accomplished by screening for molecules that block or antagonize the native autoinducers.

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.