Quorum Sensing in Gram-negative and Gram-positive bacterial systems

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1 Introduction

Regulation of gene expression in response to cell density is the key phenomenon in quorum sensing and is employed by both Gram-negative and Gram-positive bacteria to regulate diverse physiological functions. In both the cases the production and detection of extracellular signalling molecules called auto inducers initiates the event. Newer experiments in the field have demonstrated that quorum sensing modulates intra and inter species cell-cell communication which enables bacteria to exhibit phenotypes of clinical significance.

1.1 Communication and quorum sensing in Gram-negative bacteria- the language of LuxI and LuxR genes

Gram-negative bacterial systems employ the autoinducer Homoserine Lactones synthesized by a LuxI homologue and a LuxR homologue. The LuxR homologue encodes a transcriptional activator protein which detects the cognate HSL inducing the appropriate phenotype. It has been established that such cell dependent phenomenon is exhibited by over 30 species of Gram-negative bacteria. Though LuxI/LuxR systems are used by a variety of bacteria for quorum sensing, there exists a layer of complexity in the LuxI/LuxR systems.

1.1.1 Quorum Sensing in Pseudomonas aeruginosa

Pseudomonas aeruginosa is a human pathogen responsible for opportunistic infection in cancer, AIDS and cystic fibrosis patients. Extracellular enzymes like elastase, protease, hemolysins, rhamno lipid, biosurfactants and phospholipases contribute to virulence in Pseudomonas aeruginosa. The micro organism Pseudomonas aeruginosa which has been the most investigated for Quorum sensing in recent involves two Quorum sensing systems unlike the simple system in Vibrio fischeri. LuxI/ LuxI/R pairs-LasI/R, RhlI/R, functions in tandem to control the expression of virulence factors in this opportunistic pathogen. In the Las system as in Fig 1, LasI, the homologue of LuxI is involved in the synthesis of the autoinducer N-(3-oxododecanoyl)-L-homoserine lactone.
(OdDHL). The homologue of LuxR-LasR and OdDHL are required to activate virulent genes encoding elastase (LasB), protease (LasA) and alkaline protease APR. The second system in *Pseudomonas aeruginosa* is the Rhl Quorum sensing system comprising transcriptional activator proteins RhlR and RhlI which primarily synthesize the autoinducers N-butonoyl-L-Homoserine Lactone (BHL) and smaller amounts of N-hexanoyl-L-homoserine lactone. The RhlI system regulates production of rhamno lipids, elastase, pyocyanin and lipases. Experiments have established the need for OdDHL production via LasI for the formation of biofilms.

![Fig 1. Quorum Sensing in *Pseudomonas aeruginosa*](image-url)
1.1.2 Bioluminescence in *Vibrio fischeri*

*Vibrio fischeri* is a marine bacterium which shows a rapid growth with the bacterium entering directly the exponential phase. The bioluminescence in such bacteria has been shown to increase only at the mid log phase of its growth, due to the transcriptional regulation of luciferase and due to the attainment of threshold cell density in the system. The bioluminescence gene cluster of *Vibrio fischeri* is comprised of eight Lux genes LuxA-E, LuxG, LuxI and LuxR arranged in two bidirectionally transcribed operons separated by 218bp as shown in the **Fig 2**.

![Fig 2. Quorum sensing in Vibrio fischeri at low and high cell density](image-url)
The operon on the right contains genes required for the synthesis of the autoinducers (LuxI) and those for bioluminescence (LuxCDABEG). This structure is called the Lux Regulon. The LuxI gene is responsible for the production of the autoinducer synthase required for the synthesis of N-(3-oxohexanoyl)-L-homoserine lactone (OHHL). The transcriptional activator LuxR is housed in the leftward operon.

At lower cell densities, LuxI undergoes basal transcription gradually accumulating OHHL in the medium until the threshold concentration is reached. As the population density increases achieving the critical concentration of OOHL, the OOHL diffuses back into the cell and binds to LuxR. Binding of the autoinducer to the N-terminal regulatory domain enhances multimer formation by LuxR inducing the C-terminal domain to activate transcription from both the Lux operons. A blue green light with a maximum intensity at 490nm then marks the bioluminescence phenotype in *Vibrio fischeri*. Here the LuxCDE encode products that form the multi enzyme complex which synthesizes the aldehyde substrate utilized by luciferase and Lux G gene encodes flavin reductase and is followed by a transcription termination site.

### 1.2 Peptide mediated Quorum sensing in Gram-positive bacteria

Gram-positive bacteria regulate variety of phenotypes in response to increasing cell population density. While gram-negative bacteria use HSLs as auto inducers Gram-positive bacteria use secreted peptides as autoinducers for Quorum sensing. Different bacterial species employ quorum sensing to regulate different phenotypes. For example, *Bacillus subtilis* and *Streptococcus pneumoniae* use quorum sensing to develop bacterial competence. *Staphylococcus aureus* regulates virulence while *Enterococcus faecalis* employs quorum sensing to regulate conjugation.

The peptide used as autoinducer in Gram-positive quorum sensing is secreted through an ATP Binding Cassette transporter (ABC). As stated earlier, the Gram-positive bacteria use two component adapter response proteins for the detection of the peptide autoinducer and a phosphorylation/ dephosphorylation cascade regulates the two component signalling. In a Gram-positive system, the peptide auto inducers increase in
concentration with increasing cell density. The secreted peptide signals are detected by two component sensor kinases in the genetic circuit. The interaction of the peptide ligand induces a phosphorylation cascade that results in the phosphorylation of a cognate response regulator protein. This activates the response regulator facilitating DNA binding and hence alters the transcription of target genes involved in quorum sensing as shown in Fig 3.

![Fig 3. Quorum sensing in Gram-positive bacteria](image)

**1.2.1 ComD/ComE competence system in *Streptococcus pneumoniae***

*Streptococcus pneumoniae* is the first system in which genetic transformation was described in 1931. This process involves inducing ‘competency’ in the recipient bacterium to acquire exogenous DNA molecules. The signalling events which induce the competence state in *Streptococcus pneumonia* are complex and are controlled by regulative quorum sensing. In *Streptococcus pneumoniae*, 5 genes have been implicated in the regulatory circuit for competence development, as in Fig 4. The auto inducing peptide signal to develop the competence state is called CSP (Competence Stimulating Peptide). The CSP is 17 amino acids long and is produced from a 41 amino acid precursor called ComC. The ATP Binding Cassette transporter of ComAB processes and secretes the competence stimulating peptide in the extracellular region.
The ComD is a sensor kinase protein which detects the accumulated CSP at higher cell density. ComD is a membrane bound receptor/kinase and functions through the response regulator ComE communicating CSP abundance to responder genes. Higher than the threshold levels of CSP induce auto phosphorylation of ComD transferring the phosphoryl group to the response regulator ComE. Phosphorylated ComE activates transcription of comX gene. comX is an alternative sigma factor required for the transcription of structural genes involved in the development of competence. The competence state in *Streptococcus pneumonia* is transient and occurs only during exponential growth phase. Therefore one can expect unidentified regulators responsible for transiting *Streptococcus pneumonia* out of the competence state. The remarkable competence property of *Streptococcus pneumonia* is its capability to take a foreign DNA irrespective of its sequence and the species of origin.

![Diagram of ComD/ComE competence exhibited by Streptococcus pneumoniae](image)

**1.2.2 ComP/ComA competence/sporulation system in Bacillus subtilis**

As against *Streptococcus pneumonia*, only 10% of *Bacillus subtilis* cells acquire competence and the competence state is achieved at the transition between logarithmic
and exponential phase. As the population enters stationary phase owing to cell lysis one can observe increased levels of exogenous DNA. Hypothesis exists in the field that the small sub population of competence cells use the acquired DNA as a repository of genetic material which is later exploited for repairing damaged or mutated chromosomes. Sporulation occurs in *Bacillus subtilis* when the nutrient supplied to the bacteria is depleted due to deteriorated environmental conditions. Under such conditions the bacteria undergo an asymmetric cell division forming dormant, environmentally resistant spores. Lower cell densities cause poor sporulation with starvation of *Bacillus subtilis* cells. At higher cell densities, sporulation requires extracellular and environmental signals and a part of this phenomenon is controlled through quorum sensing.

In *Bacillus subtilis*, peptides comX and CSF (Competence and Sporulation Factor) regulate competence and sporulation. The ComX is a 10 amino acid long peptide which undergoes a hydrophobic modification on a tryptophan residue required for initiating signalling. The processed comX is derived from a 55 amino acid precursor encoded by comX gene. ComQ facilitates production of the ComX peptide. A two component ComP/ComA signal kinase/Response regulator pair detects accumulated ComX signals as in earlier cases. Phosphorylated ComA activates the expression of comS gene, comS inhibits the proteolytic degradation of ComK which is a transcriptional activator that regulates the expression of structural genes involved in competence development in *Bacillus subtilis*. *Bacillus subtilis* also involves second quorum sensing pentapeptide called CSF. Extracellular CSF is imported into the bacterium by an ABC oligopeptide transporter called OPP. At lower intracellular concentrations of CSF it binds to and inhibits a phosphatase called RapC. CSF inhibits RapC elevating the levels of phospho ComA. Hence low levels of internal CSF promote competence in *Bacillus subtilis*.

Apart from these systems, bacteria like *Vibrio harveyi* exhibit an interesting cascade of events leading to hybrid quorum sensing involving two auto inducers in the circuit. This will be discussed in the next lecture.
2 References

2.1 Literature References


2. Charu Gera and S. Srivastava, Quorum- sensing: The phenomenon of microbial communication, Current science (2006), 90,666-676

2.2 Video Link

Bonnie Bassler (Princeton) Part 1: Bacterial Communication via Quorum Sensing

http://www.youtube.com/watch?v=saWSxLU0ME8