Quorum Sensing

How Bacteria Talk to Each Other

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Traditionally, social cooperation has been considered the preserve of higher organisms. Only in relatively recent times have biologists begun to appreciate that bacteria are also highly interactive creatures. They exhibit complex cooperative behaviours, such as conjugal plasmid transfer, biofilm maturation and virulence. Many of these behaviours are regulated by a process known as quorum sensing.

Introduction

Let us consider a population of bacteria. Each individual bacterium is capable of producing a signaling molecule (inducer) and each bacterium also has a receptor for the inducer. When the inducer binds to the receptor, it activates the transcription of certain genes, including those responsible for the synthesis of the inducer itself.

Imagine that only a few bacteria of the same kind are nearby. Diffusion reduces the concentration of the inducer in the surrounding medium to a negligible amount, so each bacterium produces a very small amount of the inducer.

However, as the bacterial population grows, the concentration of the inducer in the surroundings increases, causing more inducer molecules to be synthesized. This forms a positive feedback loop and the concentration of the molecule keeps increasing. Once a threshold concentration is attained, activation of the receptor leads to a signal transduction cascade to switch on specific genes in the bacterial cells, leading to a coordinated population response. As a group, bacteria behave in one way when there are few bacteria around them and in a different way when there are many bacteria present.

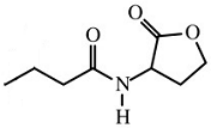
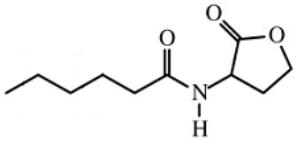
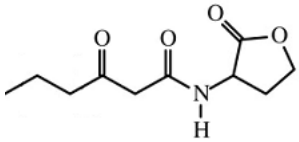
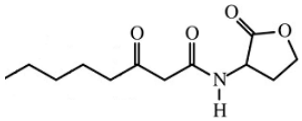
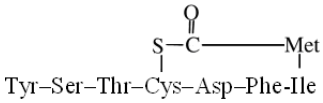
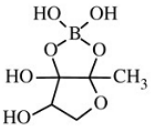
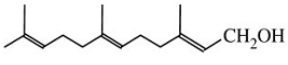
Keywords
Quorum sensing, bioluminescence, cell-cell communication.

Quorum sensing thus enables bacteria to co-ordinate and respond quickly to environmental changes, such as the availability of nutrients, other microbes or toxins in their environment.

Signal Molecules Involved in Quorum Sensing

Most quorum sensing signals are small organic molecules or peptides (*Box* 1). For example, gram-negative bacteria employ N-acyl homoserine lactones (AHLs), alkyl quinolones (AQs)

Box 1. Structures of Some Quorum Sensing Signal Molecules

Signal	Structure	Organisms
C4-HSL (an AHL)		*Aeromonas hydrophila, Pseudomonas aeruginosa*
C6-HSL		*Erwinia carotovora, Pseudomonas aureofaciens, Yersinia enterocolitica*
3-Oxo-C6-HSL		*E. carotovora, Vibrio fischeri, Y. enterocolitica*
3-Oxo-C8-HSL		*Agrobacterium tumefaciens*
Autoinducing Peptide (AIP)-I		*Straphylococcus aureus* Group I strains
AI-2 (*S*-THMF-borate)		*Vibrio harveyi*
Competence and Sporulation Stimulating Factor (CSF)	Glu – Arg – Gly – Met – Thr	*Bacillus subtilis*
Farnesol		*Candida albicans*

and fatty acid methyl esters. Gram-positive bacteria use peptides like the autoinducing peptides (AIPs). The streptomycetes synthesize butyrolactones such as A-factor. AHL-mediated quorum sensing is one of the best characterized cell-to-cell communication mechanisms. More than 70 bacterial species are known to produce AHL-type quorum-sensing signals, with many producing multiple AHLs.

Examples of Quorum Sensing

Quorum sensing was originally discovered in the luminescent bacterium *Vibrio fischeri*. These bacteria exist as free-living cells or as symbionts in the light-producing organ of an animal host, such as the Hawaiian bobtail squid. The host provides a nutrient-rich environment for the bacterium and the bacterium provides light for the host.

It was observed that liquid cultures of *V. fischeri* produced light only when large numbers of bacteria were present. The initial explanation for this was that the culture medium contained an inhibitor of luminescence, which was removed when large numbers of bacteria were present. However, it was later shown that this was not the case.

When a *V. fischeri* cell is alone, the autoinducer (3-oxo-C6-HSL, an AHL) is at a low concentration. At high cell concentrations, the level of the autoinducer becomes sufficient to induce transcription of the genes that produce the enzyme luciferase, leading to bioluminescence (*Figure* 1). On reflection, this system is clearly a sensible one. A single cell is not capable of producing enough luciferase to cause visible luminescence. Trying to do so would be a waste of valuable resources. Using quorum sensing, the cell can save its effort for the time when sufficient similar cells are around, so that their combined action produces a visible glow. The bacteria thus behave differently in the free-living and symbiotic states.

It is important for pathogens to co-ordinate their virulence to escape the immune response of the host and establish a successful infection. Quorum sensing is required for full virulence of pathogens like *S. aureus* and *Vibrio cholerae*. Also, bacteria sometimes group together to form an organized 'biofilm' covered by a polymer. Biofilms are resistant to UV radiation, desiccation and antibiotics. In several bacteria, disrupting quorum sensing adversely affects biofilm formation.

The pathogen *Pseudomonas aeruginosa* uses quorum sensing to coordinate behaviours such as biofilm formation, swarming motility, and aggregation. These bacteria grow inside a host organism without harming it, until they reach a threshold concentration. Then, having detected that their number is sufficient to overcome the host's immune system, they become aggressive and form a biofilm, causing disease. This pathogen uses AHL-mediated quorum sensing to regulate the production of many factors needed for virulence.

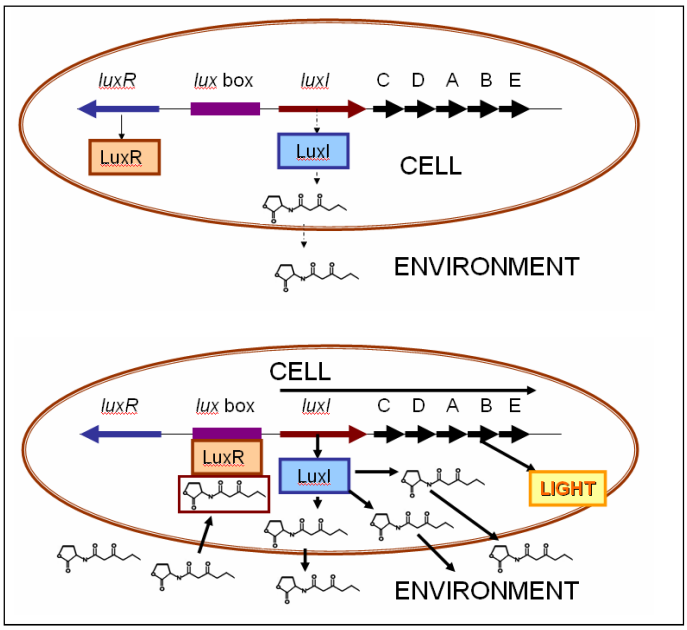


Figure 1. In V. fischeri, the signal molecule (3-oxo-C6-HSL) is synthesized by the protein LuxI and sensed by the protein LuxR. (a) When there are few bacteria nearby, the cell produces very little AHL. (b) As the concentration of cells in the surroundings increases, the signal accumulates. The AHL binds to LuxR and the LuxR/AHL complex binds to a region of DNA called the lux box. This activates the transcription of genes whose products give rise to luminescence. LuxI production also increases, leading to increased synthesis of the AHL. Homologues of the V. fischeri LuxR and LuxI proteins have been identified in over 25 species of gram-negative bacteria.

Quorum Sensing in Bacterial Crosstalk

The discovery that several bacteria make identical signal molecules prompted the idea that these signals may be exploited as a cross-talk mechanism between distinct species.

Another luminescent bacterium, *Vibrio harveyi*, produces two autoinducers. The first (AI-1) is an AHL used for communication only among *V. harveyi* bacteria. The other, AI-2 (see *Box* 1), is synthesized from S-adenosyl methionine. The enzyme which catalyzes the final step in this synthesis is called LuxS. The gene for LuxS is found in many different bacteria, all of which make and respond to AI-2. This suggests that perhaps AI-2 allows bacteria to sense and react to not only members of their own species, but also to all other species that produce AI-2.

In complex environments like the rhizosphere, with many different quorum sensing bacteria, one might imagine an intricate quorum sensing – driven signaling network to maintain ecological balance.

Quorum Sensing in Prokaryote–Eukaryote Interactions

Although quorum sensing signal molecules have largely been considered effectors of prokaryotic gene expression, they can also affect the behaviour of eukaryotic cells.

AHLs are known to have immunomodulatory effects. They also induce relaxation of blood vessels. Apparently some bacteria have the power to influence the host's immune responses to

their benefit, and stimulate the delivery of nutrients for their survival by increasing the blood supply.

But signal molecules may also benefit the host. 'Probiotic' bacteria are thought to be beneficial to the host organism and are added as dietary supplements in health-promoting food. Cultures of *Bacillus subtilis*, for example, have been used to treat dysentery and other intestinal problems. Recently, it was revealed that *B. subtilis* produces a quorum sensing signal molecule, the competence-and-sporulation-stimulating factor (CSF, *Box* 1), which induces the synthesis of the heat shock protein Hsp27 in the intestine. This protects intestinal cells against oxidative damage and loss of barrier function.

The marine alga *Ulva* releases zoospores into the water. These attach to a suitable surface and differentiate into new algae. The zoospores are known to settle preferentially on to sites of concentrated AHL biosynthesis.

Quorum Sensing in Insects

Social insect colonies are decentralized systems, because no individual is in charge of making decisions. It is interesting to see that several social insect species use quorum sensing to make collective decisions in matters that affect the entire colony.

A very interesting example is that of the small ants of the species *Temnothorax albipennis*, which typically nest in rock crevices. Suppose that an ant colony is in need of a new home. Scouts are sent out to explore the surroundings and hunt for potential nesting sites. When a scout finds a suitable site, she assesses its quality. She then returns to the old nest, where she waits for a certain period of time before recruiting other ants to follow her to the place she found. A worker that found a poor site will wait longer than one that encountered a good site.

New ants visiting the site make their own assessment and return. They will similarly wait for some time depending on their assessment of nest quality, and then recruit others. Because of the difference in waiting time, the number of ants in the best nest increases much faster than in others. Eventually, the ants at a particular site sense that the rate at which they encounter their nest mates has exceeded a threshold, i.e., the quorum has been reached. They then rapidly transport the entire colony to the new nest. The important feature of this process is that *no single worker may have compared all the options*. Quorum sensing enables the colony as a whole to make the best decisions in a short time.

Quorum Quenching

The most important implication of all this for human beings is that once we understand how

Box 2. Quorum-Quenching Mechanisms

Conventional antibiotics kill bacteria by interfering with essential functions like DNA, RNA and protein synthesis. However, the emergence of antibiotic-resistant 'superbugs' has led to an urgent need to develop novel antibacterial drugs. Quorum quenching is a promising approach.Many quorum-quenching chemicals and enzymes have been identified. These include halogenated furanones from the seaweed *Delisea pulchra*, which are structural mimics of quorum-sensing signals. Enzymes such as AHL-lactonase, AHL-acylase and paraoxonases degrade AHLs. Synthetic AHL and AIP analogues have been developed to compete with quorum-sensing signals. The antibacterial Triclosan inhibits enoyl-ACP reductase which produces an essential intermediate in AHL biosynthesis. Many of these compounds have been tested for antimicrobial activity with promising results. Expression of an AHL lactonase in potato plants conferred strong resistance to the pathogen E. carotovora, which uses AHL signals to activate the expression of virulence genes. Treatment of mice with furanones decreased *P. aeruginosa* cells in infected lung tissues. Research on quorum sensing and quorum quenching has progressed rapidly in recent years. It is very likely that more quorum-quenching mechanisms will be discovered in the near future.

bacteria talk, we can find ways to block their communication. It is hardly surprising that there has been a widespread surge of interest in 'quorum quenching', i.e., blocking quorum sensing in bacteria.

Already, many higher organisms including plants and animals have been found to produce AHL- inactivating enzymes. Their purpose is presumably to inhibit quorum sensing and defend the organism against bacterial infections. In mammals, enzymes that inactivate AHLs have been found in serum and airway epithelia. Such natural quorum-quenching mechanisms may be used to develop a new generation of antimicrobials. Already, the crystal structures of several quorum-quenching enzymes have been found and their catalytic mechanisms elucidated.Thus, quorum sensing is emerging as an area that has immense research potential to understand how microorganisms communicate among themselves as well as with other organisms.

Suggested Reading

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