THE ECOLOGICAL AND PHYSIOLOGICAL ROLES OF BACTERIAL CELL-CELL SIGNALLING

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Summary

Bacteria have developed a number of strategies to cope with environmental conditions in order to best exploit those conditions or to survive under unfavourable conditions. Equally important to the development of these adaptive phenotypes, are the mechanisms that bacteria use to control the expression of those phenotypes. This chapter describes a type of genetic regulation termed "quorum sensing" which is a form of cell to cell communication system that some bacteria utilise to evaluate their environment and to subsequently control gene expression at the population level as opposed to the level of individual cells. This process is mediated through the secretion and recognition of small, diffusible compounds. There are several different types of cell-cell signalling

systems, some of which are widely spread amongst bacteria, and some that are, to date, specific for a particular organism or family of related organisms. Interestingly, it is now appreciated that there also exist examples of naturally produced signal antagonists that are capable of disrupting quorum sensing processes. This latter phenomenon reflects the concept that bacteria and higher organisms have evolved together and this co-evolution is reflected in the diverse strategies for survival where some organisms may cooperate and others have a more antagonistic interaction. This chapter will discuss the genetics and diversity of quorum sensing systems as well as the ecology of quorum sensing and natural inhibitors of such systems.

1. Introduction

Until recently, the conventional view of bacterial cells has been that they represent independent units and do not require interaction with other cells. This view has been reinforced by the fact that each bacterial cell contains all of the required genetic material to exploit its local resources and to generate copies of itself; indeed, it is possible to generate litres of identical bacteria from a single cell. In contrast, higher organisms are typically made of highly differentiated cells, each with a specific taste they perform in concert with other cells to make up an organism. Such differentiated cells may contain identical genetic material, but they are incapable of regenerating a complete organism. In this sense, bacteria were thought to resemble biochemical factories engineered with the sole purpose of reproduction. This genetic competency makes bacteria attractive model systems for the study of the genetics, physiology and biochemistry of cellular activities because, with a few notable exceptions, each bacterial cell within a population can perform all of the required functions required for growth rather than divide the work up amongst specialised or differentiated cells. Some bacteria, such as the Myxobacteria, Rhizobia, and Cyanonbacteria, do form differentiated cells under specific conditions, but these were thought to be the exception to the norm. Thus if bacteria act independently, it also means that within a population of cells, portions of the population might be in slightly different phases of growth depending on differences in nutrient availability etc in their local micro-environment. In the laboratory, bacteria can be coordinated to grow synchronously for several generations, or can be grown in continuous culture systems, chemostats, to maintain the cells at a specific growth rate. However, in the environment, it was thought that coordination of bacterial behaviour did not occur. Two discoveries changed this perception of cells acting as individuals.

The observation that bacteria form complex community structures, known as biofilms, suggested that they could live and grow in close proximity and it was believed that the different members of the biofilm performed different physiological roles. Thus, the formation of the biofilm was driven by nutrient availability and physical-chemical properties such as surface sheer forces and diffusion (eg, oxygen penetration into the biofilm). The second discovery, which has begun to shift the paradigm from cells acting singularly towards potential co-operativity, was the discovery of cell-cell communication molecules that enabled the population to simultaneously express the same behaviour. Autoinduction and quorum sensing are two terms commonly used to describe bacterial communication systems. In essence, this phenomenon includes those systems where a chemical cue, or signal, is produced by a bacterium, the cue accumulates externally, recognition of the external cue leads to the induction of a gene

or genes in other members of the population and gene expression results in the coordinated expression of a specific phenotype, such as bioluminescence. Since its description in the 1970's, autoinduction, or quorum sensing as it has come to be known, has leapt from being a curious phenomenon of limited interest because of its presence in only a few bacteria, to a theme that currently permeates much of modern microbiology, from genetics to physiology to microbial ecology. It has now been demonstrated that bacterial communication is used to coordinate the production of virulence factors, biofilm formation, bioluminescence, secondary metabolite production and to mediate stress adaptation in a wide range of bacteria. Even now, it is emerging that bacterial signals do not only elicit a response from other bacteria, but may also interact with eukaryotes (higher organisms) to alter their behaviour. Moreover, this interaction is not a one-way event as it appears that eukaryotes have learned to exploit the fact that bacteria rely on chemical cues and thus, some eukaryotes produce mimic cues that can disrupt the bacterial signal cascade as a mechanism of protection from the effects of the bacteria. This chapter explores the genetics of different bacterial communication systems, the phenotypes controlled by those signals, the ecology of the signal systems and the chemical ecology of eukaryotes that produce mimic signals to effect the bacteria associated with them.

2. Bacterial Signalling systems

2.1 N-acylated homoserine lactones

The presence of an autoinducer molecule was first proposed based on the pattern of luminescence induction in some marine *Vibrio* spp. In essence, it was observed that at low cell density, the cells were dark or non-luminescent, but as the culture density increased, the luminescence response increased dramatically. This increase was faster than could be accounted for as a simple function of the number of cells present. Further studies postulated and subequently demonstrated that the cells secreted a compound or compounds into the surrounding medium that could induce the cells to luminesce. This was termed autoinduction as the cells were inducing their luminescence through a feedback mechanism. The ecological role of the autoinduced phenotype is discussed below in section 3. There have been several types of bacterial cell communication systems identified in a range of bacteria. *Vibrio fischeri* is presented here as a model for one type of bacterial cell communication system, the acylated homoserine lactone (AHL) mediated quorum sensing system, although the system generally reflects quorum sensing in other bacteria that utilise AHL signals.

The first recognised autoinducer was a non-polar compound isolated and purified using organic solvents to extract them from dense cultures of *V. fischeri* (Eberhard, 1972; Eberhard et al. 1981) and was identified as 3-oxo-*N*-(tetrahydro-2-oxo-3-furanyl) hexanamide or more commonly as *N*-(3-oxohexanoyl) homoserine lactone or OHHL (Fig. 1). Addition of the purified signal to cells of *V. fischeri* led to a rapid increase in the bioluminescence of the bacterial culture, indicating that this molecule was responsible for the control of bioluminescence and for induction of the genes involved. Subsequent to the identification of OHHL as the autoinducer in *V. fischeri*, other related compounds have been identified in a wide range of bacteria. These signals form a family or class of signals collectively known as the acylated homoserine lactones

(AHLs) in that the signals are chemically quite similar, consisting of the central lactone ring, but differing in the length or substitution of the side-chain. Moreover, comparison of the genes involved in the signal production and regulation of gene expression show significant homology, indicating they form a family of genes that have evolved from a common ancestor. These similarities then suggest that AHL quorum sensing will operate similarly in the various AHL positive organisms, allowing one to extrapolate information from one AHL system to another. The small differences that exist in this family of genes presumably reflects optimisation of each AHL system to recognise its specific AHL signal (eg. 3 oxo-hexanoyl homserine lactone vs 3 oxo-docecanoyl homoserine lactone-see Fig. 1) and to bind to regulatory regions of DNA that are particular to that bacterium. Thus, each bacterium that utilises AHL signalling may have some minor modifications that have been optimised for quorum sensing in that organism.

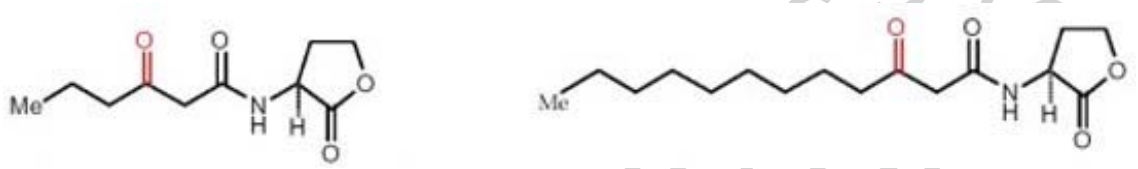

Figure 1. The structure of *N*-acyl homoserine lactones. Two AHL molecules are shown, 3-oxo-hexanoyl homoserine lactone (OHHL) from *Vibrio fischeri* on the left and 3-oxo-dodecanoyl homoserine lactone (OdDHL) from *Pseudomonas aeruginosa* on the right. These two compounds differ by the length of the side chain (6 carbons vs 12 carbons respectively). Other differences between various AHLs include the presence or absence of the oxygen at the C3 position (shown in red) of the chain and the presence or absence of other substitutions at the C3 position (eg. -OH).

The cloning and identification of the genes from *V. fischeri* that are involved in the autoinduction of bioluminescence was an important step in the understanding of AHL systems. As mentioned above, there are two genes that are common to AHL signal systems, the AHL synthase gene and the response regulator. The product of the synthase gene, *luxI* in *V. fischeri*, is an enzyme (LuxI) that catalyses the production of the signal from components of the fatty acid biosynthesis pathway (Schaefer *et al.* 1996). The protein product of the *luxR* gene, LuxR, is the response regulator in that it acts to bind the AHL signal, and when the ligand is bound, the LuxR protein is then able to bind to the promoter of specific genes and in most cases, induce the expression of that gene. Together, these proteins form the core of the genetics of AHL signalling, and as a family, are referred to as "I" and "R" proteins. As mentioned above, principle difference in AHL signal molecules, lies in the chain length of the *N*-acyl chain, and substitution at the 3rd carbon position of the chain. Such differences in the signal molecule are presumably matched by similar changes in the binding pocket of the receptor "R" protein, such that each "R" protein responds optimally to a specific AHL signal. Indeed, several studies into the structure-activity relationship of AHLs and their receptors have been performed. These studies have confirmed that the "R" protein will optimally respond to a narrow range of structural variants of the AHL and that some structural variants will in fact inhibit the activity of the R-protein. For example, LuxR from *V. fischeri* will optimally bind its native signal, OHHL. LuxR will not bind OdDHL as well is it does OHHL. In contrast, LasR, the "R" protein from *Pseudomonas*

aeruginosa, optimally binds OdDHL, but does not bind OHHL as efficiently. This has many implications for the ability of one bacterium to recognise and respond to the AHL produced and secreted by another bacterium in the local environment. Presumably, if *V. fischeri* and *P. aeruginosa* were to occur in the same environment, their signals would not be recognised by each other due to this loose specificity of the "R" protein for particular AHL signals.

Typically, genes that are quorum sensing controlled are phenotypic genes (as opposed to central cell functions such as metabolism), such as the bioluminescence machinery in *V. fischeri*. In many cases the phenotypes regulated by cell-cell signalling facilitate the association of bacteria with higher organisms or with surfaces (see section 3 below). For example, cell-cell signalling is involved in surface motility, swarming motility, in *Serratia liquefaciens* and biofilm formation in *S. liquefaciens* as well as in *P. aeruginosa* (see section 3.2 below). For some AHL induced genes, there is a specific recognition sequence in the promoter region that is bound by the R protein and this region has been called a *lux* box. In the case of *V. fischeri*, the *luxI* gene occurs in an operon along with the genes encoding the bioluminescence machinery; the order of these genes is *luxICDABEFG*. Therefore, in this bacterium, at low cell density, there is some basal expression of this gene cluster, which results in the expression of *luxI*, with the end result being the production of OHHL. The signal can diffuse out of the cell and into the external medium. When the concentration of OHHL reaches a threshold concentration, it binds to LuxR which then is capable of binding to the *lux* box in promoter region of *luxI*. This leads to a rapid increase in expression of LuxI and thus a rapid increase in OHHL production. Thus, the system represents a feed-back or autoinduction loop. It should be noted that the system is not a way of counting the number of cells present, but rather the amount or concentration of signal present. In this way, the cell density required to initiate the autoinduction circuit may differ from one environment to another, depending in part on diffusion limitations in those environments. It may be possible for 10 cells to accumulate sufficient signal if there is low diffusion of the signals away from them. However, if those same 10 cells are in an environment where the diffusion rate is high, then the signal concentration may never build up to a sufficient level to induce the autoinduction loop.

As with most genetic systems, the genes involved in quorum sensing are not only regulated by the "R" gene, but also by other regulatory pathways. The complexity of this regulation was investigated early in the history of quorum sensing, and there has been a recent interest in other regulators that control quorum sensing processes. For example, nutrient levels affect quorum sensing through one of the central energy metabolites, cAMP. Iron availability also effects quorum sensing phenotypes and more recently, it has been suggested that regulators such as two component systems (GacA/GacS) or alternative sigma factors (RpoS and RpoN) may also play important roles in the expression and timing of quorum sensing systems. Interestingly, it has been shown that one quorum sensing system can display control over another in the same organism. For example, *P. aeruginosa* has two quorum sensing circuits, one of which is dominant and is capable of repressing the second system. Thus, AHL mediated quorum sensing represents a mechanism for coordinating the expression of phenotypes within the population.

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Biographical Sketches

Scott Rice was born in Virginia (USA) and received his PhD in Microbiology (1996) at The University of Tennessee studying the distribution of retron elements in Myxobacteria. He then travelled to Sydney (Australia) where he took up a Post-doctoral position investigating the relationship between enteroviruses and motor neuron disease. He then took up his current position as senior research associate with the Centre for Marine Biofouling and Bio-Innovation at UNSW, working in the areas of quorum sensing, biofilm formation, and starvation/stress adaptation.

Michael Givskov, born in Denmark, received his PhD from the University of Copenhagen (Denmark) in 1988 in Microbiology. In 1996 he took up his current position as Associate professor of Microbiology,

Department of Microbiology, Technical University of Denmark. He has made major and significant contributions to the fields of molecular biology, the biology of *Serratia liquefaciens* and *Pseudomonas*, quorum sensing signalling in bacteria and most recently the effect of quorum sensing inhibitors on the pathogenesis of *P. aeruginosa* lung infections. This work has generated over 80 publications and a range of related patents.

Staffan Kjelleberg was born in Sweden where, in 1981, he received his PhD in Microbiology at the Univesity of Goteborg (Sweden). In 1993, he moved to Sydney (Australia) where he has continued his work on bacterial adaptation, focusing on starvation adaptation, quorum sensing, biofilms, and microbial ecology. He has published over 150 papers, reviews and book chapters and 7 patents on these topics. He serves as editor or on the editorial board for many international journals.