Bacterial Intercellular Signalling and Infectious Disease

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For many years bacteria were generally regarded as single-celled organisms, responding to altered amounts of nutrients and other changes in the environment but not directly responding to the existence of other neighbouring microorganisms. However, it has now become clear that far from being solitary organisms, bacterial cells make extensive use of intercellular signalling molecules to communicate with their neighbours. In addition, rather than being motile single cells, bacteria spend much of their time in complex communities, often as biofilms (see below), that may involve many different species and extensive intercellular signalling. This has important implications for our understanding of infectious diseases because biofilms are a major factor in chronic diseases and the colonisation of surgical implants. This review will focus on signalling between bacterial cells and its importance in microbial infection. Due to space limitations here, only a few highlights of bacterial cell-cell signalling are dealt with here; the interested reader is referred to recent reviews for broader coverage of this field (1-3).

Quorum sensing in Gram negative bacteria

One bacterial signalling system that is currently the subject of intensive study involves the secretion of acylated homoserine lactones (AHSLs) by Gram negative bacteria. Control of gene expression through production of AHSLs was first reported for a marine bacterium, *Vibrio fischeri*, in 1981. However this was generally regarded as a scientific curiosity until the discovery that the human pathogen *Pseudomonas aeruginosa* also synthesises an AHSL, 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL) (**Fig. 1A**) that controls production of virulence factors by this bacterium (4). **Fig. 1B** shows how the 3-oxo-C12-HSL regulatory system works. It does not involve cell-surface receptor proteins and signal-transducing proteins of the kind associated with signalling molecules in higher organisms. Instead, the secreted 3-oxo-C12-HSL diffuses back into the *P. aeruginosa* cells through the cell envelope to interact directly with a transcriptional activator LasR to control its activity. So when the bacteria are at low cell density, the level of secreted 3-oxo-C12-HSL is low, and consequently, the intracellular concentration of 3-oxo-C12-HSL and the activity of LasR are also low. However as the bacterial cell density increases, so does the secreted and intracellular 3-oxo-C12-HSL concentration and this causes the upregulation of LasR-dependent genes.

This form of cell density-dependent regulation of gene expression has been termed quorum sensing. The upregulated genes encode enzymes such as elastases that are associated with the ability of *P. aeruginosa* to cause chronic infections in the lungs of patients with cystic fibrosis. Activated LasR also causes increased expression of an operon comprising the *lasR* gene and the *lasI* gene that encodes an enzyme required for synthesis of 3-oxo-C12-HSL. Consequently, auto-induction occurs, with the presence of 3-oxo-C12-HSL triggering increased 3-oxo-C12-HSL production. Significant concentrations of 3-oxo-C12-HSL can be detected in the sputa of cystic fibrosis patients infected with *P. aeruginosa* (5) and mutants lacking

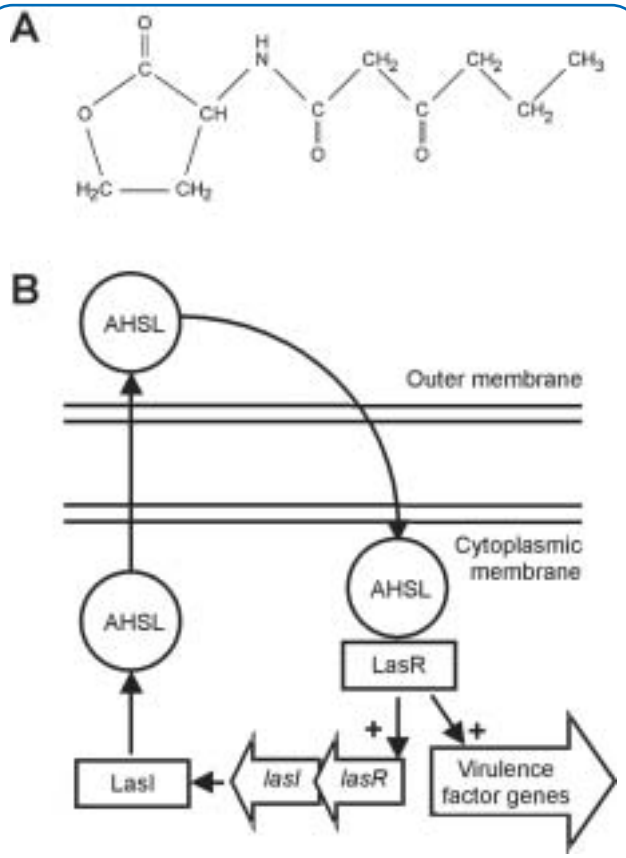

Fig. 1. Acyl homoserine lactone from *P. aeruginosa*.
A. Structure of 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL), an acyl homoserine lactone from *P. aeruginosa*.
B. The Las system from *P. aeruginosa*. Synthesis of 3-oxo-C12-HSL (AHSL) is catalysed by the LasI gene product. 3-oxo-C12-HSL diffuses freely across bacterial membranes and at high concentration (a result of high cell density) it interacts with the transcriptional activator LasR. LasR then directs increased expression of target genes including genes encoding virulence factors and *lasI* and *lasR*.

this cell-signalling system have reduced virulence in animal models of disease (see ref. 6) emphasising the involvement of this system in the infectious process.

How widespread are quorum-sensing systems? Homologues of the *P. aeruginosa* regulatory proteins (LasI and LasR) are present in many Gram negative bacteria and over 25 species of Gram negative bacteria have now been identified as having AHSL-based quorum-sensing systems (2). The AHSLs can differ in the size and nature of the acyl component. Some species secrete more than one AHSL, but the control circuitry invariably mirrors the archetypal Las system shown in **Fig. 1B**, with a transcriptional activator responding to the AHSL in a concentration-dependent (and cell density-dependent) manner. AHSL synthesis genes are amongst those that are upregulated so that a regulatory cascade takes place. The other genes that are upregulated reflect the biology of the particular bacterial species and encode functions as diverse as virulence factor production, bioluminescence, antibiotic

synthesis, exopolysaccharide production and formation of biofilms. *P. aeruginosa* itself has at least two quorum-sensing systems, emphasising the importance of these systems in cell-cell communication in this organism, and these control expression of different, though overlapping, sets of genes (3).

Peptide-mediated cell-cell signalling in Gram positive bacteria

Synthesis of AHSLs has not been demonstrated for Gram positive bacteria. However, a large number of Gram positive species communicate though small secreted peptides. One relatively well characterised example is control of virulence production in the opportunistic pathogen *Staphylococcus aureus* (7). These bacteria secrete a short peptide termed auto-inducing peptide (**Fig. 2A**). This is secreted into the environment and subsequently detected by the cell-surface AgrC receptor protein (**Fig. 2B**). AgrC is the histidine kinase component of a so-called two-component bacterial regulatory system; binding of auto-inducing peptide triggers phosphorylation of the transcriptional regulator AgrA. Phosphorylated AgrA causes increased expression of genes encoding the proteins required for synthesis, secretion and detection of auto-inducing peptide and also of proteins that are required for pathogenicity of *S. aureus* including toxins, haemolysins and proteases. Other Gram positive bacteria, including pathogenic species of *Streptococcus* and *Enterococcus*, also secrete signalling peptides (2). These can be quite different from the auto-inducing peptide shown in **Fig. 2A** although all of the peptides are synthesised on ribosomes and secreted following enzymatic cleavage of a larger precursor polypeptide. Peptide-mediated signalling controls functions as diverse as production of antimicrobial compounds and competence for uptake of DNA, as well as production of virulence factors.

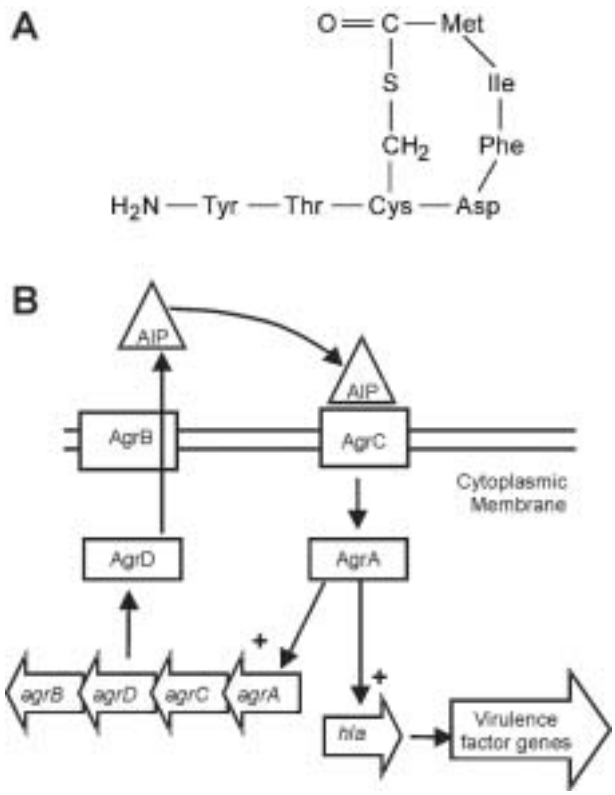

Fig. 2. A cyclic thiolactone-peptide (auto-inducing peptide) from *S. aureus*.

A. Structure of thiolactone, formed by condensation of the cysteine side chain and the terminal carboxyl group of the methionine residue. Amino acids are otherwise represented by the standard three-letter code.

B. The Agr system from *S. aureus*. The auto-inducing peptide (AIP) is synthesised as part of a larger precursor AgrD. Processing of AgrD to give AIP occurs concomitantly with secretion of the peptide by the AgrB protein. At inducing concentrations, AIP binds to the membrane-bound histidine-sensor-kinase protein AgrC. This catalyses phosphorylation of the transcriptional activator AgrA and results in increased expression of the *agr* genes and also of *hla*, which encodes a regulatory RNA that causes increased expression of genes associated with virulence.

Siderophores as signalling molecules

Many bacteria secrete small (300-1500 Da) iron-scavenging molecules termed siderophores. These chelate Fe^{3+} ions in the environment and the ferri-siderophore complexes are then taken up by the bacteria via cell surface receptor proteins, with the iron being released inside the bacteria for incorporation into iron-containing proteins. We have recently shown that the siderophore pyoverdine (**Fig. 3A**) secreted by *P. aeruginosa* also acts as a signalling molecule, controlling the production of at least two virulence factors, exotoxin A and a protease, as well as its own production (8). The signalling pathway is activated by binding of pyoverdine to a receptor, FpvA, that is located in the outer membrane of the bacteria. This signal is transmitted to a transducing protein, FpvR, that spans the cytoplasmic membrane and then to an alternative transcription factor, a sigma factor, PvdS, that induces expression of target genes (**Fig. 3B**).

In contrast to the AHSL-based signalling systems that are also found in Gram negative bacteria, this system involves transmission of a signal through two membranes rather than internalisation of the signalling molecule into the cytoplasm. Other siderophores also induce gene expression inside cells (9), although thus far there are no other described examples of siderophores controlling production of virulence factors. However, given the large number of bacterial species that secrete siderophores, and the large number of siderophores (>500) that have been described, it would be surprising if there were not other examples of siderophores being involved in regulation of virulence factor production.

Cell-cell signalling and biofilms

Biofilms are microbial colonies encased in an adhesive, usually polysaccharide material, that are attached to a surface. The complex structure of biofilms suggests that cell-cell signalling is likely to play an important role in their development and ongoing existence. Fortuitously, *P. aeruginosa* is a model organism for biofilm development as well as for the study of cell-cell signalling. Linking these processes, it has been shown that mutants that are affected in AHSL-mediated signalling form altered biofilms (10). In particular, an AHSL controls production of rhamnolipid that is an important factor in maintaining biofilm structure (11). The presence of AHSLs in the sputa of patients with cystic fibrosis has been taken as evidence that *P. aeruginosa* exists as biofilms in the cystic fibrosis lung (5). Gram positive bacteria also form biofilms and there is evidence that intercellular signalling molecules, including auto-inducing peptides, contribute to biofilm formation (12,13). A full appreciation of the roles of signalling molecules in biofilms will require a more complete understanding of biofilm physiology; it is an area that merits considerable attention.

Conclusions and perspectives

Research on cell-cell signalling is in its infancy, having only become part of mainstream microbial biochemistry about 10 years ago. However the molecular circuitry is already well understood for the three systems described above and for other related systems, and it is clear that they play important roles in at least some infectious diseases. Some common themes are also evident. All of the systems that have been described involve auto-induced upregulation of production of signalling molecules as well as upregulation of genes encoding virulence factors or other functions.

What advances can we expect in the next 10 years? First, it would be surprising if there were not other bacterial signalling molecules that have yet to be discovered. Second, research has focussed on recognition of signalling molecules by the producer organism. However, in nature, including episodes of infection, bacteria do not live in monoculture but rather in the company of other strains and species of microbes. Many of these may be competitors but some may have collaborative properties, for example producing complementary metabolites. It would seem likely that intercellular signalling contributes to interactions between different bacteria. Indeed, an unusual signalling molecule that contains boron and is produced by a large number of bacterial species has been characterised by Bassler and colleagues (2,14), may well enable interspecies communication. Third, can signalling molecules secreted by bacteria also affect the host organism during infection? This question is being actively researched (15) but a clear picture has yet to emerge. Finally, intercellular signalling represents an attractive target for interfering with the infection process. Analogues of homoserine lactones that interfere with signalling have already been developed (16) – this and similar approaches may form the basis for new strategies to combat infectious disease. It is clear that bacterial cell-cell signalling is an active field of research and we should expect to see significant advances in our understanding of its mechanisms and our appreciation of its importance in the next few years.

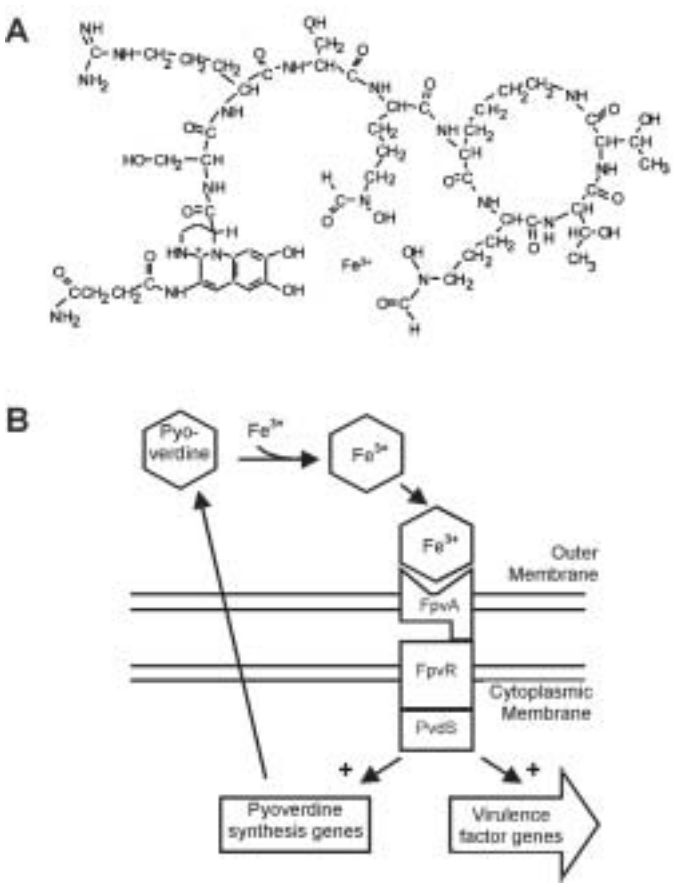

Fig. 3. Ferripyoverdine from *P. aeruginosa*.
A. Structure of ferripyoverdine.
B. The ferripyoverdine system from *P. aeruginosa*. Approximately 15 genes are required for synthesis of pyoverdine. The molecule is secreted from *P. aeruginosa* by an unknown mechanism and chelates Fe^{3+} ions. At inducing concentrations, ferripyoverdine is bound by the receptor protein FpvA. This results in signal transmission through the FpvR protein to the alternative sigma factor PvdS and results in increased expression of pyoverdine synthesis genes and of genes encoding virulence factors.

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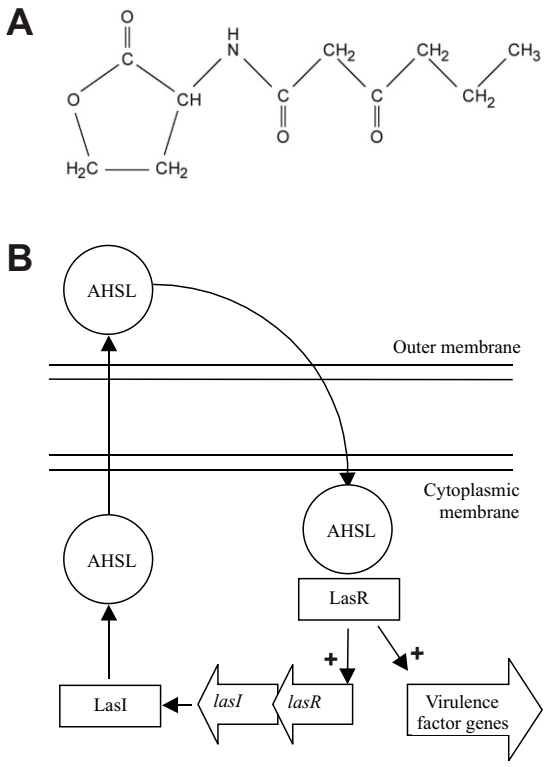

Fig. 1

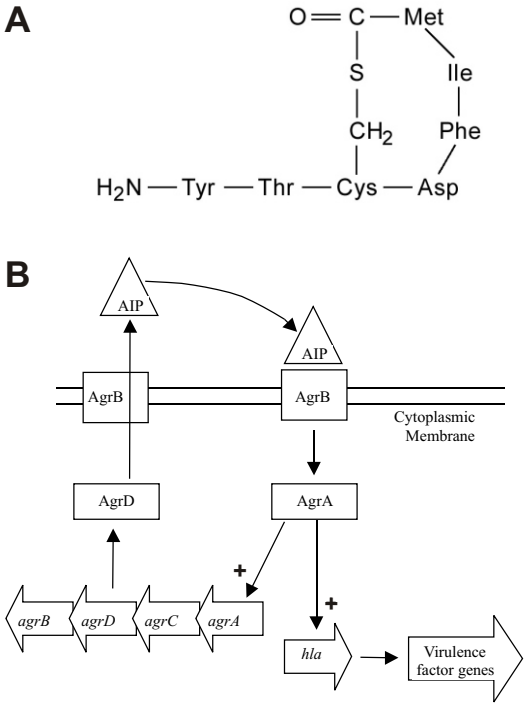

Fig. 2

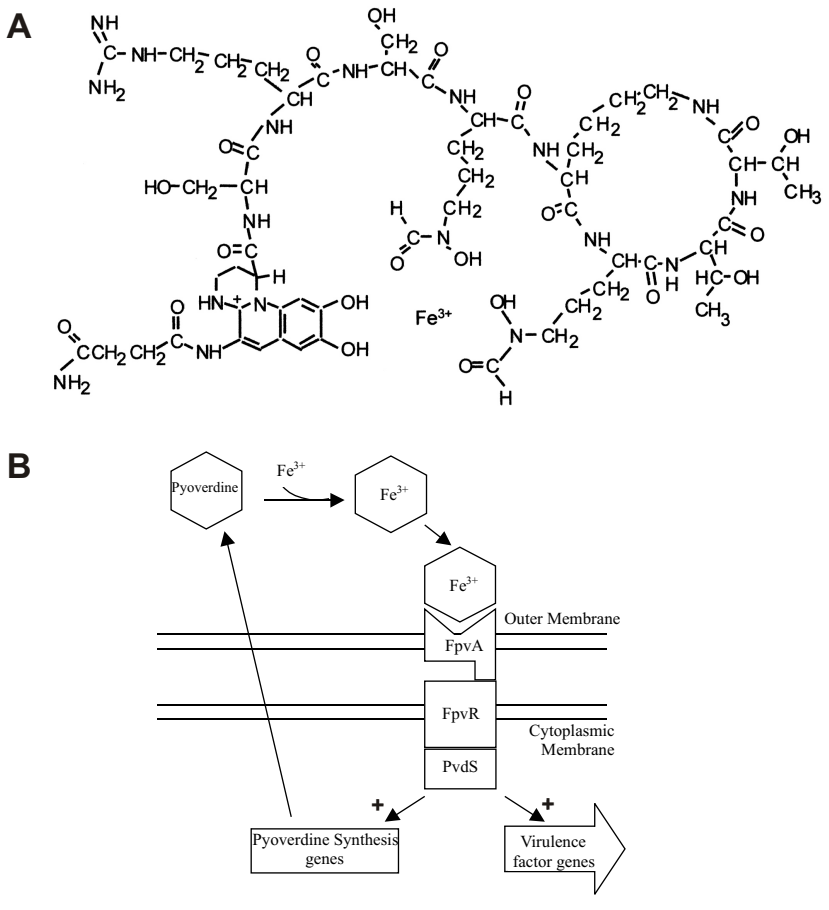

Fig. 3