Cell–cell communication in Gram-negative bacteria

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Over the last decade or so, a wealth of research has established that bacteria communicate with one another using small molecules. These signals enable the individuals in a population to coordinate their behaviour. In the case of pathogens, this behaviour may include decisions such as when to attack a host organism or form a biofilm. Consequently, such signalling systems are excellent targets for the development of new antibacterial therapies. In this review, we assess how Gram-negative bacteria use small molecules for cell–cell communication, and discuss the main approaches that have been developed to interfere with it.

Bacteria as social organisms: Love thy neighbor

Ever since the pioneering studies of Koch and Pasteur in the latter half of the 19th century, bacteria have been tagged as archetypal single-celled organisms. However, over the last few decades, this classical view of the microbial world has become challenged increasingly. It is now clear that many (if not most) bacteria have the potential to form highly structured communities, and that the individuals within these assemblies exhibit some form of social hierarchy.[1] These communities are often ''multicultural'', and composed of more than one species.[2] Indeed, recent work suggests that most bacteria absolutely *require* the presence of other microbial partners for growth. This may explain why around 99% of bacterial species are uncultivatable in pure form.[3] Quite where this discovery leaves Koch's postulate† is open to debate.

Probably, most bacteria spend *most* of their time in social communes, which manifest themselves as thin (*ca.* a few hundred microns) sheets known as ''biofilms''.[4] The slippery layer on the pebble from the bottom of a fast-flowing stream, the growth around the surface of a long-forgotten cup of tea, or even the plaque-layer on unbrushed teeth, are all examples of bacterial biofilms (Fig. 1). This sessile biofilm state may be far more representative of the normal state-of-affairs in most ecological niches than

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† Koch proposed that in order to prove that a microorganism causes a disease, it must be cultivatable in pure form, and the cultivated organism must be able to reproduce the disease when introduced into a healthy host.

Martin Welch

Martin Welch grew up near Newmarket and went on to study Biochemistry at Oxford University. In 1989 he started a PhD with Professor Michael Eisenbach at the Weizmann Institute of Science, where he worked on the mechanism of switching in the bacterial flagellar motor. On completing his PhD in 1994, he joined the laboratory of Professor Jean-Pierre Samama in Toulouse as a long term EMBO fellow, where he solved the X-ray crystal structure of one of the protein complexes involved in bacterial chemotaxis. He moved back to the UK in 1997, where he joined the laboratory of Professor George Salmond, establishing an interest in quorum sensing. After a brief stint as a Royal Society University Research Fellow, in 2002 he took up a lectureship in the Department of Biochemistry in Cambridge. Martin is a Fellow of New Hall, where he teaches molecular medicine. His research interests lie in understanding how antibiotics affect cell function.

David Spring

David Spring was born in West Bromwich and attended Oxford University for his undergraduate chemistry degree, graduating in 1995. He stayed at Oxford under the supervision of Sir Jack Baldwin and received his DPhil in 1998 for work on the proposed biosynthesis of the manzamine alkaloids. David then spent two and a half years as a Wellcome Trust postdoctoral fellow and Fulbright scholar at Harvard University with Stuart Schreiber. In 2001 he returned to the UK as a BBSRC David Phillips Fellow and Fellow of Queens' College at Cambridge, where he is researching diversity-oriented synthesis and chemical genetics.

Fig. 1 Photograph of a bacterial biofilm (predominantly *Erwinia carotovora*) grown in just 36 hours. The biofilm was poured off into a bioassay dish to facilitate photography.

the dispersed "planktonic" cultures studied in many labs.

Although bacterial biofilms have been known about for decades, until recently they received scant attention from the mainstream microbiological community. However, this situation is now changing. This resurgence in interest in biofilms has been driven by three things; (*i*) the realization that biofilms are intimately associated with many disease states in humans,[5] (*ii*) the fact that biofilm formation follows a defined developmental profile – a process that requires social interaction(s) between the cells involved, and (*iii*) observations showing that, in many cases, biofilm genesis involves cell–cell signalling *via* small molecules.[6]

From the clinical perspective, biofilms are a major problem, since these structures display greatly increased resistance to physical and chemical insults,[7] which is probably a major reason why they form in the first place! Crucially, biofilms are much more resistant to antibiotic treatment than their planktonic counterparts, making them particularly difficult to eliminate from patients and contaminated surgical equipment. Consequently, biofilms are an excellent potential target for new anti-microbial therapies; an issue that has not escaped the attention of several groups.[8,9] Current efforts are focused on understanding how and why biofilms form in the first place, and targeting the key components for inhibition through chemical intervention.

Biofilms – especially those formed in a flowing environment – are complex structures, with different cells expressing

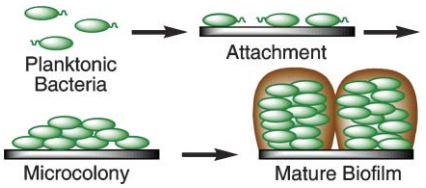


Fig. 2 Many bacteria can develop into sessile biofilms, consisting of numerous bacterial cells attached to a surface and embedded within a self-produced matrix material. Quorum sensing is involved during the maturation of *Pseudomonas aeruginosa* biofilms, which causes chronic infections in cystic fibrosis patients' lungs.

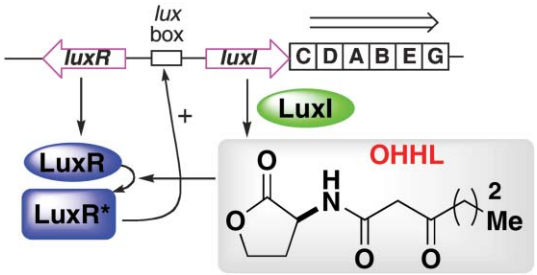
different sets of genes in a temporally and spatially resolved manner throughout the colony (Fig. 2). Typically, maturing biofilms growing in free-flowing systems consist of mushroom-like microcolonies separated by channels that apparently carry nutrients and dissolved oxygen to the interior of the structure.[10] In some cases, the biofilm may be further sculpted through responses to cell-derived chemical cues, and even apoptotic events[11] – changes of the sort that until now, have been associated with the morphogenesis of structured body plans in the higher eucaryotes. The remarkable thing about all of these developmental processes is that they seem to be controlled by cell–cell communication. That is, the individuals within a bacterial colony "talk" to one another, conveying information through the colony about its physiological, developmental, and morphological state. But what is the *lingua franca* of the microbial world? And, by blocking this inter-cellular communication, can we alleviate biofilm formation?

Seeing the light: Self-perception and discovery of quorum sensings

The recent history of bacterial cell–cell communication has now (albeit somewhat erroneously) become synonymous with that of "quorum sensing". The phenomenon of quorum sensing was first recognized in the early 1970's, although at that time – and for several years afterwards – the subject remained an intriguing but relatively obscure backwater of microbiological study. The story begins with a series of observations made by Hastings and colleagues, who were investigating light production by the marine bacterium, *Vibrio fischeri* (reviewed in ref. 12). Although this species can be found in open seawater, it also occupies an unusual ecological niche; *V. fischeri* is a symbiont, which colonizes the light-producing organ of certain marine fish and squid. Here, due to a ready supply of host-supplied branched-chain amino acids, *V. fischeri* often exceeds a population density of 10^{10} cells mL^{-1}. Unlike their free-living counterparts (which rarely achieve a cell density greater than 10^2 cells mL^{-1}) *V. fischeri* growing within a light organ can luminesce, generating visible light.

Although the potential benefits to the host of having a mobile biological "light bulb" are superficially obvious (attracting prey, repelling predators *etc.*), the question of precisely how *V. fischeri* produces light, and why this only occurs at high cell densities, occupied researchers for several years. The problem was all-the-more intriguing since the bacteria within the light organ apparently coordinate their efforts to produce light; the transition to light production is sharp, and involves a concerted effort on behalf of the whole population. A key breakthrough came when Hastings and colleagues discovered that cell–cell signalling lies at the heart of this remarkable biological switch.[12,13]

We now know that *V. fischeri* conducts a more-or-less continuous population census. When the population cell density exceeds a certain "threshold" value, transcription of the gene cluster encoding the light-production machinery (the *luxCDABE* operon) is activated in a highly concerted way. The term "quorum sensing" was coined to describe this sort of democratic behavior, since the population must reach a "quorum" before any light is produced.[14] But how is the quorum measured? How does *V. fischeri* "count"? The answer proved to be deceptively simple. Each cell within the population produces a continuous (albeit low) level of a freely diffusible signalling molecule – an *N*-acylated homoserine lactone (HSL) derivative known as *N*-(3-oxohexanoyl)-HSL, or "OHHL", made by the enzyme LuxI[14] (Scheme 1). OHHL is sufficiently amphipathic to be able to cross cell membranes passively and accumulate throughout the culture. Consequently, although the amount of OHHL produced by individual cells is



Scheme 1 LuxIR quorum sensing system. The regulation of bioluminescence in *Vibrio fischeri*. At low cell density, transcription of the genes for bioluminescence (*luxICDABEG*) is weak and insufficient for light emission due to low levels of OHHL. At high cell density, a critical concentration of OHHL is reached. OHHL binds to LuxR (indicated as LuxR*) and stimulates transcription of *luxICDABEG*, leading to rapid amplification of the OHHL signal and emission of light.

low, *en masse* the population can accumulate the compound to high concentrations. Eventually, the concentration of OHHL rises high enough to enable the ligand to bind to an intracellular (cytoplasmic) receptor – a transcriptional regulator known as LuxR. This causes LuxR to dimerize and adopt the activated conformation, leading to transcription of the *luxCDABE* cluster. The LuxR·OHHL complex also activates further the expression of *luxI* itself, generating a positive feedback loop. This behavior is known as autoinduction,[14] and *N*-acyl HSLs are sometimes known as ''autoinducers''. It is interesting to note that in chemical terms, the cell density at which a ''quorum'' of cells is reached is determined by the dissociation constant (K_d) of the ligand for LuxR, and by the affinity of the activated LuxR·OHHL complex for the *luxCDABE*/*luxI* promoters.

Due to space limitations, the remainder of this review will remain focused on *N*-acyl-HSL-mediated cell–cell signalling in the Gram-negative bacteria. However, other signalling systems have also been described, and the main ones deserve acknowledgement. These include the peptide-based signalling molecules (often linear or cyclic oligopeptides) employed by Gram-positive organisms for quorum sensing. These signal molecules are often detected by membrane-associated sensor kinases, which belong to the two-component family of signal transducing proteins. These, in turn, activate cognate ''response regulators'', which subsequently modulate the expression of quorum sensing-regulated genes (reviewed in ref. 15). Another signalling system that has attracted a lot of attention over the last few years involves ''autoinducer-2'' or AI-2. This molecule, probably a furanosyl borate diester,[16] is so named because it was the second autoinducer molecule identified in *Vibrio harveyi*. AI-2 synthesis is directed by the LuxS protein, and has been implicated in interspecies communication.[17] AI-2 also seems to play an important role in biofilm formation by certain Gram-positive organisms, although its role as a universally recognized molecular ''esperanto'' has been questioned.[18]

N-Acyl-HSL-dependent quorum sensing as a strategy for pathogenicity

Over the decade or so following the work of Hastings and colleagues, several teams showed that *N*-acyl HSL-mediated cell signalling is common among many species of *Proteobacteria*; an effort which has pushed cell–cell signalling to the forefront of microbiological research.[19] Interestingly, this list includes many animal and plant pathogens, such as *Yersinia* sp., *Pseudomonas* sp., the *Burkholderia cepcia* complex, *Serratia marsescens*, *Aeromonas hydrophila*, *Agrobacterium tumefaciens*, and *Erwinia* sp., as well as non-pathogenic organisms like *Rhodobacter sphaeroides*. Through thorough molecular analyses of each system, we know that although the *N*-acyl HSL-mediated quorum sensing systems in these organisms are often subtly different compared to that of *V. fischeri*, they all share a common feature: the small molecule. In each case, the *N*-acyl HSL is synthesized by a protein orthologous to LuxI, and the cognate receptor(s) are orthologous to LuxR (reviewed in ref. 14). Mostly, the *luxI* and *luxR* orthologs within a given organism are tightly linked‡ (although there are some exceptions to this).

Unlike *V. fischeri*, relatively few of the species mentioned in the previous paragraph inhabit highly specialized niches, which raises the intriguing question of why do they need quorum sensing in the first place? And why is quorum sensing frequently associated with pathogens? It turns out that quorum sensing is a powerful *global* regulatory mechanism; it allows the organism to modulate concomitantly the expression of tens or even hundreds of unlinked genes in a cell-density-dependent manner. This type of control circuit is known as a ''regulon''. The simultaneous activation or repression (sometimes both) of multiple genes acts as a biological switch that allows the bacterium to undergo major phenotypic alterations in a highly concerted way. In the case of pathogens, such changes include the elaboration of secreted virulence factors, changes in cell-surface components, the production of secondary metabolites, and the switch to a sessile biofilm lifestyle, as well as the alterations in basal metabolism that necessarily accompany these modifications.[20,21]

The strategy of using quorum sensing to control virulence and biofilm formation may have arisen to minimize the host-response to an invading organism by ensuring that the pathogen does not produce virulence factors (*i.e.*, become ''aggressive'') until its numbers are sufficiently high to ensure that it can overwhelm the host-defences. However, this rather anthropocentric view of the pathogen as ''a wolf in sheep's clothing'' is unlikely to be generally true, since most immune systems will not tolerate *any* bacterial presence, irrespective of whether or not the pathogen is producing virulence factors. Also, in at least one well-studied case (that of *P. aeruginosa*), expression of the genes in the quorum sensing regulon is not linked to a predefined ''threshold'' concentration of

‡ ''Linkage'' is genetic parlance for two or more loci being closely associated on the genome. In this case, *luxI* and *luxR* homologs are usually adjacent to one another.

N-acyl HSL. Instead, many of these genes are transcribed over a continuum of signal molecule concentrations during the late log- and early stationary-phases of the growth curve.[20]

A case study: *Pseudomonas aeruginosa*

One of the best-characterized quorum sensing systems is seen in the opportunistic human pathogen, *Pseudomonas aeruginosa* (Fig. 3). This organism is a major cause of nosocomial (hospital-acquired) infections in the industrialized world, and is the main contributor to progressive lung degeneration in cystic fibrosis (CF) patients (reviewed in ref. 22). Indeed, the quorum sensing signalling molecules can be isolated readily from the sputum of CF patients. One of the main problems with *P. aeruginosa* infections is that the organism is resistant intrinsically to the action of many antibiotics, a situation that is exacerbated when it forms biofilms. *P. aeruginosa* is slightly unusual in that it operates a two-tiered quorum sensing system, with the *rhl* component subordinate to the *las* component (Scheme 2). The *Pseudomonas* quorum sensing system is complicated further by the presence of an additional signalling component, known as the *Pseudomonas* quinolone signal

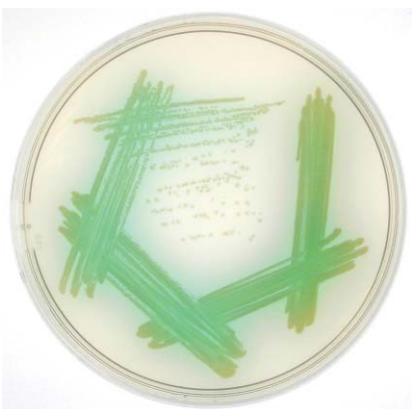
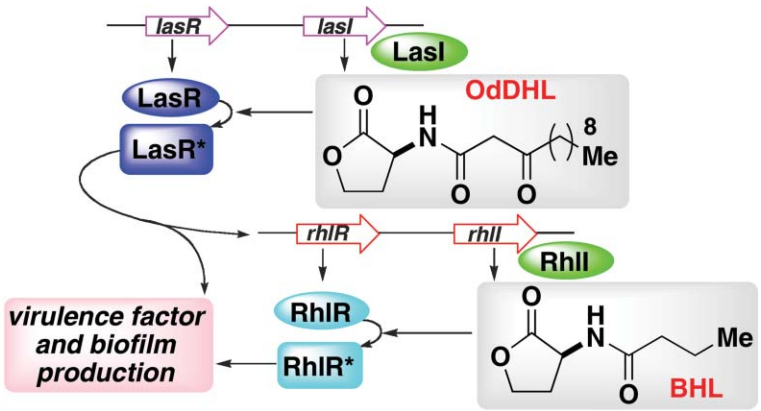


Fig. 3 The photograph shows colonies of an antibiotic-resistant strain of *Pseudomonas aeruginosa* growing on an agar plate. Note the hyper-production of diffusible green/blue pigments (mainly pyocyanin). Like many other secondary metabolites from *P. aeruginosa*, the synthesis of pyocyanin is under quorum sensing control. Wounds infected by *P. aeruginosa* ooze blue pus as a result of pycocyanin production.



Scheme 2 The *las* and *rhl* quorum sensing signalling pathways in *Pseudomonas aeruginosa*. In *P. aeruginosa*, the quorum sensing system is hierarchical, involving two signalling pathways acting sequentially. The enzyme LasI produces OdDHL, which binds to and activates a LuxR homolog called LasR. LuxR homologs are ligand-activated transcriptional regulators. Activated LasR (labelled as LasR*) turns on transcription of a subset of virulence genes (*e.g.* leading to production of rhamnolipids, alkaline protease, LasA, LasB, pyocyanin, cyanide, the Xcp secretory pathway, RpoS) and of a gene encoding another LuxR homolog, *rhlR*. RhlR binds the second signalling molecule BHL, (produced by RhlI) and turns on the expression of another set of virulence genes.

(PQS), which apparently functions to link the *las* and *rhl* systems. Superimposed on this arrangement are various other layers of control, mediated by the proteins QscR, Vfr, MvaT, GacA, RsmA and RpoS, to name but a few. Together, the LasIR signalling system (which generates and senses *N*-3-oxododecanoyl-HSL, known as OdDHL) and the RhlIR signalling system (which generates and senses *N*-butanoyl-HSL, known as BHL) control production of a diverse variety of tissue-degrading enzymes and other exo-products. In line with this, quorum sensing mutants are less lethal in burned-mouse models than their wild-type progenitors;[23] a finding that reinforces the potential of quorum sensing blockers as promising therapeutic agents.

In addition to regulating virulence factor production, the quorum sensing system also seems to control biofilm formation by *P. aeruginosa* (at least in certain circumstances *in vitro*). The seminal studies on the quorum sensing-dependence of biofilm formation suggested that the *las* branch of the signalling system plays a key role in biofilm maturation, since *lasI* mutants formed thin, easily dispersed biofilms.[24] However, other workers have challenged this conclusion and proposed that the *rhl* branch of the quorum sensing system is more important.[25] Yet others have found little difference between the biofilms formed by quorum sensing mutants and the wild-type (reviewed recently in ref. 1). Biofilm formation, therefore, appears to be typically multi-factorial, and there is likely to be a complex interplay between the input from the quorum sensing system and from environmental factors. For example, a simple change in carbon source can be sufficient to elicit biofilm disaggregation *in vitro*. This notwithstanding, there is mounting evidence that quorum sensing blockers have therapeutic potential as inhibitors of both virulence and biofilm formation *in vitro*.

Cell–cell communication as an Achilles heel?

Several different strategies are being pursued to look for quorum sensing inhibitors. One of the most successful avenues of investigation performed to date has exploited the fact that the red marine alga, *Delisea pulchra*, produces compounds that apparently have anti-fouling properties. Hentzer and colleagues showed that the synthetic derivative **1** (Fig. 4) of halogenated furanone compounds from *D. pulchra* was able to (*i*) inhibit quorum sensing-dependent gene expression in *P. aeruginosa in vitro*, (*ii*) increase the susceptibility of *P. aeruginosa* biofilms to tobramycin and SDS, and (*iii*) promote immune-clearance of *P. aeruginosa* in a mouse

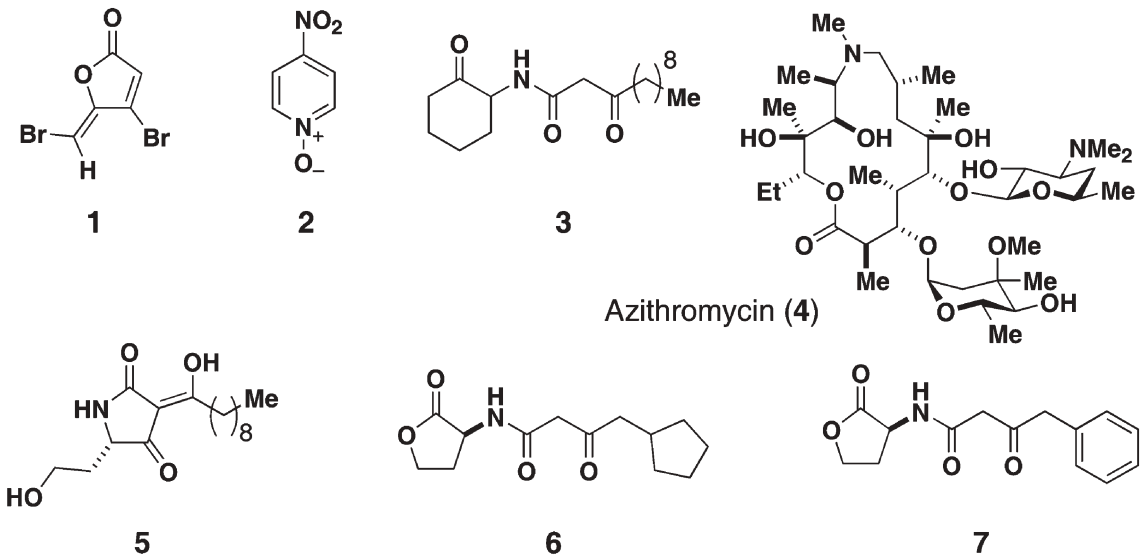


Fig. 4 Structures of small molecules **1**–**7**.

pulmonary infection model.[26,27] The same workers have now extended these findings by developing a genetic selection system to enable facile screening of quorum sensing inhibitors generally. Using this, they found that selected synthetic compounds and extracts from a variety of natural sources have quorum sensing- and biofilm-inhibitory activity.[28] In particular, 4-nitropyridine *N*-oxide (**2**) reduced the virulence of wild-type *P. aeruginosa* in a *Caenorhabditis elegans* pathogenesis model to levels comparable to that of a *lasI rhlI* mutant. More recently, these workers showed that penicillic acid and patulin (extracted from cultures of *Penicillium* moulds) also exhibited quorum sensing inhibitory activity, although subsequent transcriptomic analyses showed that their effect(s) were not specific to quorum sensing.[44]

Many groups have carried out detailed structure–activity relationship analyses on *N*-acyl-HSLs and we cannot possibly do justice to these efforts in this review. Instead, we will concentrate on those studies that have been aimed specifically at identifying quorum sensing blockers. Recently, Suga and colleagues[29] used a gfp§-based reporter system to screen analogs of OdDHL and BHL for antagonist activity. They found that replacement of the HSL headgroup in OdDHL with a 2-aminocyclohexanol moiety yielded a quorum sensing antagonist (**3**). This compound was able to reduce biofilm formation and virulence factor production, although only when applied at high concentrations (*ca.* 0.1 mM). However, subsequent work in our laboratories[30] has cast some doubt over the stability of this antagonist in aqueous solutions, so its precise mechanism of action may not be *via* straightforward competitive inhibition. The Suga team extended their preliminary findings by screening an *N*-acyl-HSL "headgroup library" for antagonist activity.[31] Several novel antagonists were identified; in most of these the HSL ring had been replaced by an aromatic derivative. Interestingly, the mechanism of action of some of these compounds appears to differ from that of the aminocyclohexanol derivative described in their earlier report. This highlights the need to refine the initial screening assay(s) during more detailed SAR studies *e.g.*, through direct measurements of protein–ligand interactions. A simple fluorescence-based assay that allows the binding of *N*-acyl-HSLs to purified LuxR homologs to be put on a quantitative footing has been described.[32]

The synthetic azalide antibiotic, azithromycin (**4**), has also shown promise as a potential quorum sensing blocker, both *in vitro* and *in vivo*. Azithromycin is often taken by CF sufferers, some of whom report that it alleviates their bacterial infections. Like other macrolides, azithromycin inhibits ribosomal function, although it does so only at concentrations that are not safely achievable in the clinic. However, at sub-growth inhibitory concentrations (as low as 2 mg L^{-1}), azithromycin has been shown to block quorum sensing and attenuate biofilm formation *in vitro*.[33] The mechanism of action of this drug is not yet clear, although this is an active area of study by several laboratories, including ours. As an aside, one speculative (and, as yet, unproven) hypothesis is that many of the antibiotics produced by soil microorganisms may have their origins as cell–cell communication molecules,[34] and that their anti-microbial action at higher concentrations is a secondary effect.

Research into cell–cell signalling has also thrown up one of the finest examples of how studies into fundamental biological processes can reveal valuable, and entirely unexpected, insights into clinical problems. It transpires that OdDHL also has effects on the host. In particular, it has been shown (*i*) to activate COX-2, a membrane-associated prostaglandin E synthase, resulting in inflammation of the lung tissue, and (*ii*) to influence the host immune response through modulation of interleukin-8 and interleukin-12 production. OdDHL also inhibits lymphocyte proliferation and tumour necrosis factor α production by lipopolysaccharide-stimulated macrophages.[35–37] OdDHL and its analogs therefore have the potential to make excellent immune-suppressants, which may find

§ "gfp" is a standard notation for **g**reen **f**luorescent **p**rotein, a naturally occurring fluorescent protein, originally derived from jellyfish and now widely used as a reporter gene in many studies.

use, *e.g.*, in preventing rejection of organ transplants. There has been some suggestion that azithromycin also has immune-modulatory activity, which may contribute towards the efficacy of this compound in combating *P. aeruginosa* infections.

OdDHL may have other physiological functions. Kaufmann *et al.*[38] showed recently that this *N*-acyl-HSL undergoes non-enzymatic conversion to the tetramic acid derivative **5**. This compound turns out to have antibacterial properties, especially against Gram-positive strains. Plausibly, *P. aeruginosa* might utilize this toxicity as an interference strategy to prevent encroachment by competing bacteria – a hypothesis that has been mooted on previous occasions to account for the quorum sensing-dependence of antibiotic production by other bacteria.[39] Unexpectedly, the OdDHL-derived tetramate compound also turned out to be an excellent siderophore (K_d for $Fe^{3+} = 1.6 \times 10^{-29}$ M^3; greater than the affinity of pyochelin or EDTA for Fe^{3+}, but lower than that of the main *P. aeruginosa* siderophore, pyoverdin, for Fe^{3+}). Since iron uptake is known to be a key requirement for *P. aeruginosa* pathogenicity, compounds that reduce OdDHL production (*e.g.*, azithromycin) are likely to impinge on this aspect of virulence too.

Reverchon *et al.*[40] recently returned to the *V. fischeri* system to screen for quorum sensing modulators. Their approach was to use the quorum sensing-dependency of bioluminescence to screen synthetic *N*-acyl-HSL analogs for antagonist activity. They focused their study on OHHL derivatives carrying modified acyl-chains, and found that while analogs carrying acyclic or cyclic alkyl substituents on the C4 position of the chain showed inducing activity (*e.g.* **6**), the presence of an aromatic moiety at this position yielded antagonist activity (*e.g.* **7**). These workers speculated that this might reflect an interaction between aromatic amino acids in the receptor protein, LuxR, and the arylated ligands, although this has yet to be tested experimentally. It will be interesting to see whether compounds based on these *V. fischeri* antagonists are active in other organisms.

Recently, Bauer and colleagues showed that exudates from pea seedlings (*Pisum sativum*) and other plant sources (including the unicellular soil-freshwater alga, *Chlamydomonas reinhardtii*) were found to contain a range of compounds that mimicked *N*-acyl-HSL signals in several bacterial reporter strains (reviewed in ref. 41). In some cases, these extracts inhibited quorum sensing-dependent phenotypes, suggesting that the active compounds may have potential as quorum sensing-blockers. Although the chemical nature of the active mimic compounds is not (yet) known, they are apparently not *N*-acyl-HSL. Bauer *et al.* have speculated that these compounds may play a role in determining the outcome of interactions between higher plants and a diversity of pathogenic, symbiotic, and saprophytic bacteria. Interestingly, the secretion of *N*-acyl-HSL mimics by germinating seeds and seedlings was found to change substantially with developmental age, and the secretion of some of these activities is probably dependent upon prior exposure of the plants to bacteria.

The discussion above gives some indication of the field as it currently stands. However, it is crucial to appreciate the shortcomings of the approaches described. For example, although many compounds have been shown to affect quorum sensing-dependent phenotypes, in only a very few cases have any attempts been made to investigate their specificity for these signalling systems (*e.g.*, ref. 27). Also, very few studies address explicitly issues relating to differential compound uptake across the cell envelope or compound efflux *via* membrane-associated pumps, and how these might affect the apparent quorum sensing inhibitory "activity" of the agent. These problems could be largely overcome if some way could be found to assay conveniently the binding of putative drugs to purified LuxR orthologs. Progress in this regard seems to have been hampered primarily by the intransigent nature of many LuxR-type proteins to purification in soluble form. However, a few years ago, the authors showed that the active, ligand-binding domain of at least one LuxR ortholog (CarR, from the phytopathogen *Erwinia carotovora*) can be purified readily in soluble form.[32] We extended this work recently by purifying, in soluble form, all three of the known LuxR homologs from *Erwinia* and assaying these for ligand binding.[47] Also, the field now has a high-resolution crystal structure available for one of the LuxR homologs (TraR from *Agrobacterium tumefaciens*), which should facilitate *in silico* structure-based drug design.[45]

What is on the horizon?

In the last five years, Biology has entered a new epoch; the "post-genomic" era. Advances in technology, and the funding of collaborative inter-disciplinary "mega" projects, means that now we can accomplish more in a single experiment than would have previously been possible in a lifetime of work. For example, in the case of *P. aeruginosa*, we not only have a complete genome sequence, but also a commercially-available micro-array for transcriptome analysis, a library of defined "off-the-shelf" mutants, and a library of cloned genes. Several groups have been quick to exploit these developments by defining the quorum sensing-controlled regulon in *P. aeruginosa*[20,21,27] and the set of genes whose transcription is modulated during biofilm formation.[42] Although such studies are doubtless helpful, it must be recalled that there is very often little correlation between the measured transcriptome and the more physiologically-relevant proteomic and metabolomic data. Also, it is becoming increasingly clear that the results obtained in such microarray studies are often crucially dependent on the growth conditions employed. Indeed, in three separate analyses of the quorum sensing-regulon in *P. aeruginosa*, some 600 or so genes have been identified collectively as being modulated, yet fewer than 100 of these genes are common to all three studies![43] Clearly, the field is crying out for the introduction of "standard" protocols to allow meaningful comparisons to be made. However, proper analysis of the output data *e.g.*, through the application of multivariate statistics (Principal Components analysis, Partial Least Squares Discriminant analysis, Hierarchical Cluster analysis, *etc.*), will be required for effective interrogation of such data-sets, and for prioritization of the key players in downstream target selection. The ultimate hope is that these kinds of data will form the basis for *in silico*

models of quorum sensing and biofilm formation; such models should prove invaluable as resources for pinpointing nodes for selective chemical intervention. This notwithstanding, in the few cases where detailed biochemical information is available, the data suggest that the quorum sensing signalling circuitry is highly dynamic, and that subtle changes (*e.g.*, in the amount of the signalling proteins in the cell, or in their signalling state) can result in drastic alterations to the signal output of the system.[46] Consequently, it is becoming clear that in order to construct truly *useful* quorum sensing models, we will need to combine the functional genomics approach with additional, detailed biochemical information.

In summary, the quorum sensing field is a very exciting place to be; it is ripe for small molecule modulation and new approaches and technologies are available to look at gene regulation at the global level. The study of quorum sensing at the chemistry–biology interface will no doubt reveal more unexpected insights in basic biology, and has the potential to deliver new antibacterial chemotherapeutics with novel modes of action compared with the antibiotics currently in clinical use. With multidrug resistance becoming an increasing problem, antibiotics with new modes of action are highly sought-after. Chemical, biological and medicinal collaborations are required to bring this dream into a reality.

Acknowledgements

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