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MicroMeeting

Socializing, networking and development: a report from the second 'Young Microbiologists Symposium on Microbe Signalling, Organization and Pathogenesis'

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Summary

In mid-June, the second Young Microbiologists Symposium took place under the broad title of 'Microbe signalling, organization and pathogenesis' on the picturesque campus of University College Cork, Ireland. The symposium attracted 150 microbiologists from 15 different countries. The key feature of this meeting was that it was specifically aimed at providing a platform for junior scientists to present their work to a broad audience. The meeting was principally supported by Science Foundation Ireland with further backing from the Society for General Microbiology, the American Society for Microbiology and the European Molecular Biology Organization. Sessions focused on microbial gene expression, biogenesis, pathogenicity and host interaction. In this MicroMeeting report, we highlight some of the most significant advances and exciting developments reported during various talks and poster presentations given by the young and talented microbiologists.

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Introduction

Microorganisms exhibit a stunning array of features that allow them to adapt and thrive in various environments. With the introduction of molecular microbiology, our understanding of microbial life has changed rapidly in a very short period of time. Much has been learned about the mechanistic basis of microbial gene expression, regulation, physiology and importantly, pathogenicity and host interaction.

On 21 and 22 June 2012, 150 microbiologists descended on University College Cork, Ireland, for the second 'Young Microbiologists Symposium' containing 30 talks and almost 80 posters. Like the previous meeting held in 2009, this one was held under the wide-ranging title of 'Microbe signalling, organization and pathogenesis' (Ryan *et al.*, 2009). The meeting was co-chaired by **Delphine Caly** (University College Cork, Ireland) and **Robert Ryan** (University College Cork, Ireland). The key and relatively unusual aspect of this meeting was that it was specifically designed to provide a platform for junior scientists to present their research. Each of the four main sessions was chaired by a distinguished academic who has made considerable contribution to an area covered by the session, but the majority of the talks were given by young researchers from leading laboratories around the world.

During broad theme-based sessions, many different aspects of microbial life were discussed including intracellular signalling, intercellular signalling between microbes, microbe–host interactions, and structure and transport across bacterial membranes. The meeting highlighted the remarkable breadth of the microbial field, and the range of approaches being utilized to address some important questions about microbial life. In this MicroMeeting report, we draw attention to new findings, highlight unanswered questions and reveal anticipated future directions in a variety of areas, as described in both oral and poster presentations. Because of space limitations, we can only summarize some of the highlights of the meeting and we

apologize to participants whose excellent work could not be mentioned here.

Rotary motors, protein dynamics and bacterial behaviour

Judy Armitage (University of Oxford, UK) delivered the European Molecular Biology Organization lecture focusing on two important protein complexes in bacteria, which have been the subject of intense research scrutiny for a number of years.

Judy started by explaining that until recently, the bacterial flagellar motor had been considered a stable multimeric complex. This assumption has been forced to change, with a growing body of evidence showing that this protein complex is far more dynamic than previously thought with some key proteins in a constant state of flux. In the case of *Escherichia coli*, the bacterial flagellar motor contains at least 13 different proteins, mostly multicopy, and rotation is driven by a transmembrane ion flux through a ring of stator complexes that push on a central rotor (the complete system was recently reviewed by Brown *et al*., 2011). The direction of rotation randomly changes in response to binding of the phosphorylated response regulator CheY to the rotor switch component FliM. Judy continued by describing how this system provides an excellent model to study the protein dynamics that underpin the functionality of this motor complex. Her laboratory recently demonstrated this by showing that the rotor contains approximately 30 FliM molecules separated into two populations. The first population is tightly associated with the motor and the other undergoes turnover, which is dependent on the regulator CheY (Delalez *et al*., 2010). To summarize, Judy postulated that upon further investigation these observations of dynamic protein turnover in the bacterial flagellar motor will perhaps be seen in other large molecular complexes in microbial life.

In the second segment of the lecture, Judy discussed her group's recent approach aimed at teasing out the mechanism by which plasmid partitioning Par-like systems control localization of protein complexes. Judy described how their original experiments in *Rhodobacter sphaeroides* examined various orphan ParA proteins and showed these proteins had a key role in controlling the segregation of cytoplasmic protein clusters involved in chemotactic signalling (Thompson *et al*., 2006). Judy further explained how her laboratory has recently demonstrated that one of these homologues, PpfA, uses non-specific chromosome binding to separate cytoplasmic clusters of chemotaxis proteins upon cell division. With the use of fluorescent microscopy and site-directed mutagenesis, she explained how they have been able to show that dynamic chromosome binding and ATPase activity are essential for cluster segregation (Roberts *et al*., 2012). Furthermore, it appears that the N-terminal domain of a cytoplasmic chemoreceptor encoded next to *ppfA* is also required for segregation, most likely acting as a ParB analogue to control PpfA ATPase activity. In wrapping up she speculated that as many bacterial genomes encode such orphan ParAs, this may be a common mechanism regulating segregation of proteins and protein complexes.

To end, and to inspire everyone to get involved in the spirit of the upcoming Olympic Games, Judy treated the audience to the 100 μm freestyle swimming event from the inaugural Microbial Olympics held at the Oxford microscopic pool. Judy explained that like the early human Olympic Games, the competitors were chosen on the basis of their ability to travel to the event; in other words they were easily recovered from Oxford freezers. The race pitted monotrichous contestants like *R. sphaeroides* and *Pseudomonas aeruginosa* against multi-flagellated bacteria including *E. coli* and *Yersinia enterocolitica*, and looked at the ability of sodium-driven motors to drive bacteria faster than proton-driven motors, in particular pitting a wild-type proton-driven *E. coli* against a mutant strain driven by sodium. As bacteria generally swim to optimize their environment, they typically change swimming direction randomly (reviewed recently by Porter *et al*., 2011). For this reason, half of the competitors were genetically modified to lack sensory responses to partake in this 100 μm straight sprint. *R. sphaeroides* (one proton-driven flagellum) won by a body length in a time of 2.02 s; the sodium-driven *E. coli* finished second in 2.08 s but held off a late challenge from proton-driven *P. aeruginosa*, who finished with a respectable time of 2.12 s. For other wacky events at the inaugural Microbial Olympics and the designed rules for each competition, please see the wonderfully inventive article by Youle *et al*. (2012).

Nucleotide second messengers and other regulators in environmental adaptation

The Gene Regulation and Intracellular Signalling session brought together speakers from a diverse range of fields, who described research ranging from detailed structure-function analyses of specific signalling pathways through to genome-wide studies of transcriptional regulation. An interesting example of the former was presented by **Max Dow** (University College Cork, Ireland) in the opening Science Foundation Ireland lecture. Max discussed his group's recent research on RpfG and cyclic di-GMP (c-di-GMP) signalling in the plant pathogen *Xanthomonas campestris*. RpfG is a response regulator with a HD-GYP phosphodiesterase (PDE) domain and functions together with its cognate sensor kinase RpfC as part of the cell-to-cell diffusible signalling factor (DSF) quorum sensing pathway (Ryan *et al*., 2006). RpfG regulates various phenotypic outputs including the synthesis of virulence factors and exopolysaccharides, the onset of biofilm for-

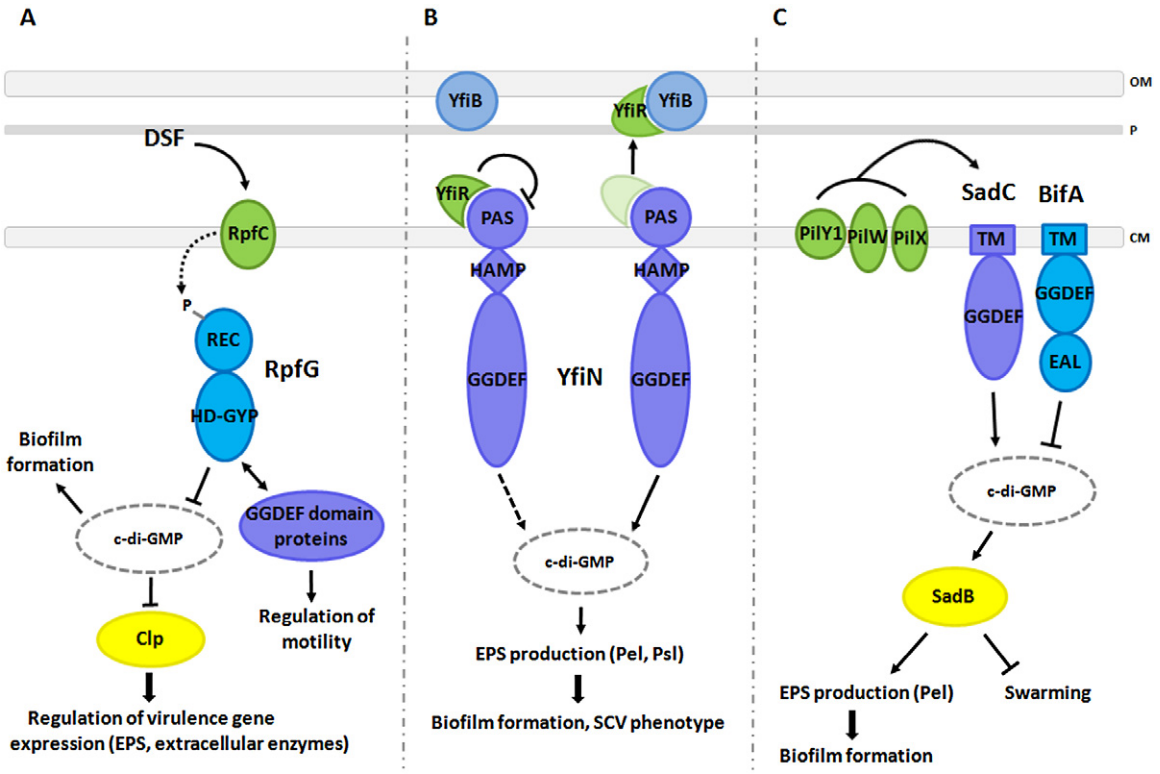

Fig. 1. Models of cyclic di-GMP-mediated regulation of virulence, motility and biofilm formation in *Xanthomonas campestris* and *Pseudomonas aeruginosa*.
A. In *X. campestris*, DSF and the Rpf proteins regulate virulence gene expression, biofilm formation and motility, in a c-di-GMP-dependent manner. When RpfG is inactive, c-di-GMP is bound to the transcriptional activator Clp, which inhibits the expression of virulence genes by preventing Clp binding to their promoters. Upon perception of the DSF signal, the hybrid sensor kinase RpfC activates the PDE RpfG. Activation of RpfG leads to a reduction in c-di-GMP levels, which inhibits biofilm formation and activates the expression of virulence-related extracellular enzymes and exopolysaccharides (EPS). RpfG is also known to regulate motility through direct interaction with GGDEF domain proteins (Ryan *et al.*, 2006; 2010).
B. The YfiBNR signalling system regulates biofilm formation and the appearance of small colony variants (SCV) by controlling c-di-GMP levels in *P. aeruginosa*. In response to unknown signals, the DGC YfiN is indirectly activated by the membrane protein YfiB, through sequestration of the periplasmic repressor protein YfiR. The resulting elevated c-di-GMP levels trigger the production of Pel and Psl EPS, which contribute to biofilm formation and to the appearance of SCV. Figure adapted from Malone *et al.* (2012).
C. In *P. aeruginosa*, the DGC SadC and the PDE BifA control swarming and biofilm formation, by modulating c-di-GMP levels. Activation of SadC and the consequent elevated levels of c-di-GMP promote EPS synthesis and biofilm formation, whereas low levels of c-di-GMP, maintained by BifA, promote swarming motility. The minor pilins PilW and PilX, and the pilus tip-associated protein PilY1 were shown to be involved in SadC activation, although the specifics of the interaction with SadC remain unknown. Figure adapted from Merritt *et al.* (2007) and Kuchma *et al.* (2012). OM, outer membrane; P, peptidoglycan layer; CM, cytoplasmic membrane.

mation, and pilus-mediated motility. Max explained how RpfG regulates these various phenotypes by different mechanisms. For example, extracellular enzyme production is a c-di-GMP-dependent process and involves the PDE activity of RpfG (Ryan *et al.*, 2010). Conversely, motility control relies on direct physical interaction between the GYP motif of RpfG and two GGDEF domain-containing diguanylate cyclases (DGCs), and is independent of the enzymatic activity of the two proteins (Fig. 1A). A combination of peptide array screening and FRET analysis has allowed the Dow group to identify the 'MLDLDD' motif that mediates GGDEF domain binding to RpfG. These HD-GYP binding residues are conserved among GGDEF domains, suggesting that GGDEF–HD-GYP interactions may be similarly widespread (Ryan *et al.*, 2012).

Matthew Bush (John Innes Centre, UK) presented another detailed structure-function study with his research on the bacterial enhancer binding protein NorR from *E. coli*. NorR functions as part of the *E. coli* σ^{54}-dependent response to nitric oxide (NO)-mediated stress, and is a transcriptional regulator with ATPase (AAA+), N-terminal (GAF) and C-terminal (DNA-binding) domains. NorR binds as a hexamer to conserved sites upstream of the *norVW* NO reductase genes. NO binding to the GAF domain releases its repression of AAA+ ATP hydrolysis, promoting higher-order nucleoprotein complex formation with σ^{54} and consequent *norVW* transcription (D'Autreaux *et al.*, 2005). To investigate the regulatory mechanism of NorR, Matthew and co-workers isolated and characterized mutations in the AAA+ domain that bypassed GAF-

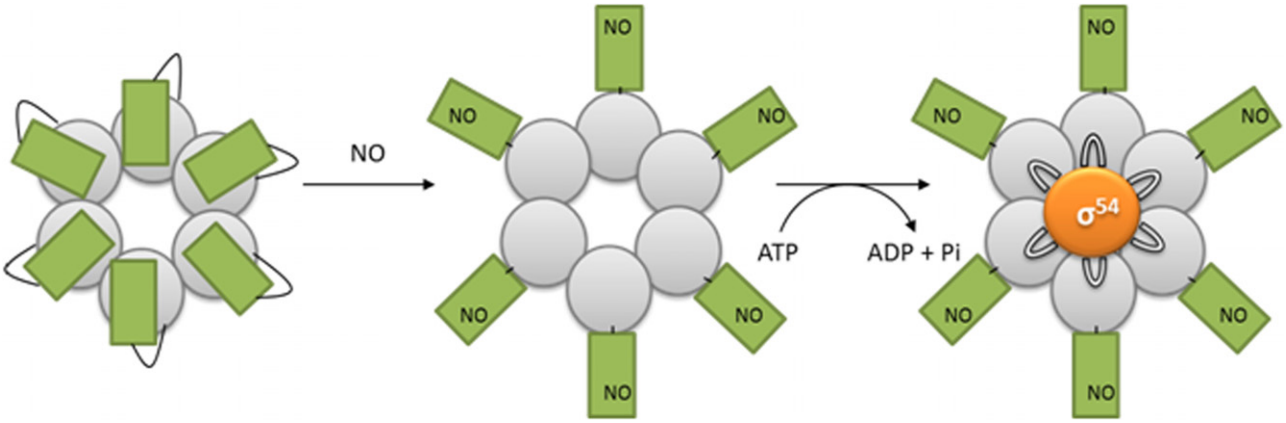

Fig. 2. Model for σ^{54}-dependent transcriptional regulation by NorR. Binding of NorR to the *norR-norVW* intergenic region containing the three NorR-binding sites (not shown) facilitates the formation of a higher-order oligomer, most likely a hexamer. In the 'off-state', the N-terminal GAF domains (green rectangles) negatively regulate the activity of the AAA+ domains (grey circles) by preventing L1 and L2 loop access to σ^{54} (left). In the 'on-state', NO binds to the iron centre in the GAF domain forming a mononitrosyl iron species. The repression of the AAA+ domain is relieved (centre), enabling ATP hydrolysis by NorR coupled to conformational changes in the AAA+ domain. During the nucleotide hydrolysis cycle, the surface-exposed loop (including the GAFTGA motif) moves into an extended conformation to allow σ^{54} interaction and remodelling (right). Figure adapted from Bush *et al.* (2010).

mediated repression of NorR activity. These substitutions were located either in the surface-exposed loops that engage σ^{54} during ATP hydrolysis, or in a highly conserved 'GAFTGA' motif that directly contacts σ^{54}. Further analysis using electron microscopy reconstructions of NorR variants in the active and inactive states led to the proposal of a novel regulatory mechanism, in which in the absence of the NO signal the inactive GAF domains occlude the σ^{54} interaction surface of the AAA+ domains (Bush *et al.*, 2010). Matthew also proposed that the pre-assembly of an inactive, DNA-bound NorR hexamer (Fig. 2) would enable the cell to more rapidly respond to NO-mediated stress.

Continuing on the theme of structure-function analyses, **Jake Malone** (John Innes Centre/University of East Anglia, UK), working in Urs Jenal's research group (Basel Biozentrum, Switzerland), analysed a series of mutations throughout the *P. aeruginosa* c-di-GMP signalling operon *yfiBNR*, and used them to define the regulation and activation mechanism of the YfiBNR proteins (Malone *et al.*, 2012). Jake and co-workers showed that control of the DGC effector YfiN is achieved via competitive binding of the repressor protein YfiR, either to the periplasmic YfiN PAS domain or to the outer-membrane lipoprotein activator YfiB, and defined the hydrophobic interaction surfaces of the three proteins (Fig. 1B). The clinical significance of this signalling pathway was underlined by the discovery of multiple cystic fibrosis (CF) lung isolates that harboured activating mutations throughout the *yfiBNR* locus.

Angelika Gründling (Imperial College, London, UK) discussed another recently discovered bacterial second messenger with her work on cyclic di-AMP (c-di-AMP) signalling in *Staphylococcus aureus*. Lipoteichoic acid (LTA), a *S. aureus* cell wall polymer, is crucial for normal bacterial growth and cell division. In a suppressor screening experiment, Angelika and co-workers used whole genome sequencing to isolate the *gdpP* (GGDEF domain protein containing PDE) gene, whose inactivation restores the ability of *S. aureus* to grow normally in the absence of LTA. GdpP contains two transmembrane helices, a PAS domain, a degenerate GGDEF domain and C-terminal DHH/DHHA1 domains that confer c-di-AMP PDE activity. Meanwhile, the GGDEF domain appears to have a regulatory function, and is required for protein activation. The Gründling group showed that the LTA suppressor phenotype was due to c-di-AMP accumulation, with levels in the *gdpP* mutant 10-fold higher than wild-type and *gdpP* complementation proving fatal in the LTA background. Furthermore, the *S. aureus* DacA protein has diadenylate cyclase activity, and is a tightly controlled, essential gene expressed in the stationary growth phase. In *S. aureus*, enhanced levels of cross-linked peptidoglycan and a reduction in cell size in the *gdpP* mutant suggest that c-di-AMP functions to control cell size and to resist membrane and cell wall stress (Corrigan *et al.*, 2011).

In a marked contrast to the preceding structural studies, **Cynthia Sharma** (University of Würzburg, Germany) described her transcriptomic analyses of the pathogenic epsilonproteobacterial species *Helicobacter pylori* and *Campylobacter jejuni*. Sharma and co-workers used a novel differential deep sequencing protocol (dRNA-seq) with enrichment for primary transcripts to identify transcriptional start sites (TSS) throughout the *H. pylori* genome (Sharma *et al.*, 2010). Over 1900 TSS were identified, including many both within operons or lying oppo-

site annotated genes, indicating that gene expression in *H. pylori* is extensively regulated by both uncoupling of polycistronic operons and by widespread antisense transcription. In addition, dRNA-seq revealed that *H. pylori* transcription initiates at conserved sites downstream of periodic AT-rich nucleotide stretches, and identified an unexpectedly large number of *H. pylori* small RNAs, as well as many mRNAs encoding small hydrophobic peptides. More recently, an ongoing comparative dRNA-seq analysis across four *C. jejuni* strains from different hosts has uncovered examples where point mutations have led to the selective loss of transcription in certain strains, consistent with their disparate environments.

Further transcriptomic research was presented in the poster session, with **Sathesh Sivasankaran** from Jay Hinton's group in Trinity College Dublin, Ireland, describing the use of RNA-seq and dRNA-seq to identify over 150 TSS in the three plasmids of the *Salmonella enterica* serovar Typhimurium ST4/74 genome. The Hinton lab's high-throughput cDNA sequencing studies identified 35 sRNA/asRNAs across the three plasmids, a subset of which is currently under further investigation. Meanwhile, **Charlotte Michaux** and co-workers, in the lab of Jean-Christophe Giard (University of Caen, France), analysed the genome of *Enterococcus faecalis*, a Gram-positive lactic acid bacterium and an important cause of nosocomial infections associated with high mortality. Charlotte and colleagues used customized DNA tiling arrays and RNA extractions from infection-mimicking cultures to identify 80 sRNA candidates in the intergenic regions of the *E. faecalis* V583 genome (Shioya *et al.*, 2011).

The bacterial cell envelope: biogenesis and transport

The bacterial cell envelope has been known for many years to provide bacterial cells with the structural support, protection and selective permeability barrier required for their survival and proliferation. However, many aspects of exactly how this sophisticated boundary structure is generated and maintained are far from being completely understood and remain the subject of active research, as reflected in several presentations at this meeting. The cytoplasmic membrane (CM), or inner membrane in Gram-negative bacteria, contains many integral membrane proteins essential for energy transduction and regulated transport. Bacteria utilize the same machineries to incorporate integral proteins into the CM and to transport other proteins fully across the membrane to an extracytoplasmic location, in both cases while maintaining the integrity of the ionically sealed barrier. Most proteins are exported across the CM via distinct transport systems called the general secretory (Sec) pathway and the twin arginine translocase. Sec substrates are kept in an unfolded conformation prior to transport and are targeted via an N-terminal signal peptide to the membrane-embedded Sec translocon. In contrast, proteins are transported through the twin arginine translocation (Tat) pathway in a folded state and targeted to the Tat apparatus by N-terminal signal peptides carrying a S-R-R-x-F-L-K consensus motif. Polytopic integral membrane proteins are synthesized in a co-translational manner. Once threaded into the Sec channel, membrane proteins are laterally released into the membrane. To achieve correct folding into the membrane, the protein YidC may cooperate as a folding assistant. YidC alone is able to insert small membrane proteins independently of the Sec pathway. A few membrane proteins, usually containing C-terminal anchor domains, are also transported by the Tat pathway. To date, it has never been reported that the Tat system cooperates with either the Sec pathway or the insertase YidC. At this meeting, **Rebecca Keller** (University of Dundee, UK) described a fascinating and unusual case revealing for the first time that these pathways can cooperate to assemble a single membrane protein. Using a combination of different *in vivo* and *in vitro* experimental approaches, it has been shown that the biogenesis of an important polytopic membrane protein requires the action of all the Sec, YidC and Tat systems. This important observation opens new mechanistic aspects for understanding the biogenesis of membrane proteins.

In Gram-negative bacteria a second membrane, the outer membrane (OM), surrounds the peptidoglycan cell wall, forming the periplasmic compartment and providing a vital permeability barrier around the outside of the cell. The OM is an asymmetric bilayer with lipopolysaccharides (LPS) on the outside and phospholipids on the inside. It contains specific proteins: integral β-barrel outer membrane proteins (OMPs), frequently porins, and lipid-anchored lipoproteins. The combination of LPS and proteins provides a key defensive layer against harsh environments, e.g. detergents or antibiotics, but also the ability to acquire required small molecules through the barrier. The OM is a complex structure and its correct assembly, in the absence of an energy source in the periplasm and while retaining the integrity of the barrier, represents a complex biological problem. Our current understanding of how *E. coli* solves this problem was described by **Tom Silhavy** (Princeton University, USA) in the American Society for Microbiology sponsored lecture. In particular, the talk focused on recent work deciphering the nature and mechanism of the β-barrel assembly machine (Bam) complex, which folds and inserts β-barrel OMPs (Hagan *et al.*, 2011; Rigel and Silhavy, 2012). As suggested by the title of the talk, 'Divide and conquer: genetic dissection of the β-barrel assembly machine', a key message, eloquently delivered, was the enduring power of genetics to solve biological problems. Specifically, Tom described how genetics had been used to

uncover and dissect the Bam pathway, from a screen to a gene to a protein, from a suppressor mutant to an interaction, or from synthetic lethality to overlapping function. These studies, integrated with structural and biochemical studies, have provided a model for how the machinery operates, as reviewed (Hagan *et al*., 2011; Rigel and Silhavy, 2012). Once unfolded OMPs have been chaperoned across the periplasm by Sur or Skp/DegP, they are delivered to the Bam complex in the OM, where they are folded and inserted. The Bam complex consists of BamA, itself a β-barrel, with five periplasmic POTRA domains which represent the site of interaction with the other four components, the OM lipoproteins BamB–E. BamD, like BamA, is essential, whereas mutants lacking BamB, BamC or BamE exhibit only minor OM defects. BamA and D likely participate directly in folding and inserting OMPs, whereas BamBCE may increase efficiency or have modulatory roles, and are perhaps more important in certain situations than others. An attractive model for how the Bam complex may facilitate β-barrel folding is known as 'β-strand augmentation', where the POTRA domains of BamA initiate and bind newly forming β-strands, whereas BamD may perform later steps of membrane insertion/release (Hagan *et al*., 2011).

Lipopolysaccharide molecules are not the only glycans found decorating the outside of bacterial cells. Cell envelope proteins and cell wall components may also be glycosylated in certain Gram-positive and Gram-negative bacteria. There is an enormous diversity of bacterial glycoproteins, which may be *O*- or *N*-linked, but to date these have been mostly studied in pathogens where they mediate specific interactions with host cells and receptors. **Hanne Tytgat** (University of Leuven, Belgium) described work to investigate protein glycosylation in probiotic organisms, in particular *Lactobacillus rhamnosus* GG. The major secreted protein 1 (Msp1) has recently been shown to be *O*-glycosylated in *L. rhamnosus* (Lebeer *et al*., 2012) and work is now underway to identify the mechanisms of glycosylation of Msp1 and other proteins in this organism. Several different approaches are being combined to try and achieve this aim: *in silico*, knockout mutants, glycoproteomics and functional studies. For the *in silico* analysis, new genome mining methodology has been developed to identify putative protein glycosyltransferases independently of any existing annotation (H.L. Tytgat and A. Sanchez-Rodriguez, unpublished). Mutants in several newly identified candidates showed altered adherence to intestinal epithelial cells, indicating that protein glycosylation is indeed of functional importance in this organism. Finally, Hanne proposed the intriguing idea that organisms such as *L. rhamnosus* GG might in the future be used as drug delivery vehicles for glycosylated molecules.

Considering the bacterial cell envelope from an alternative perspective, in addition to generating and maintaining this complex boundary structure, bacteria must also be able to move proteins across it. As mentioned previously, the Sec and Tat export machineries are used by both Gram-positive and Gram-negative bacteria to move proteins across the CM. For most Gram-positive bacteria this is sufficient for proteins to access the extracellular environment, whereas in Gram-negative bacteria specialized proteinaceous machineries known as secretion systems are required to move specific proteins across the OM to the exterior of the bacterial cell. Secretion systems and the proteins they secrete are critically important for bacterial virulence and interaction with host and other cells. Secretion systems fall into six classes (Type I–VI secretion systems) and secreted proteins vary widely, but include adhesins, toxins, hydrolytic enzymes and effector proteins which disrupt or manipulate host cell signalling or function (Gerlach and Hensel, 2007; Holland, 2010). They are best known for targeting eukaryotic cells or tissues, for example the three Type V, or two-partner, secretion systems involved in epithelial cell colonization and invasion by *Neisseria meningitidis*. **Sadeeq ur Rahman** (from the lab of Peter van Ulsen, Vrije University Amsterdam, the Netherlands) presented a poster describing studies to elucidate the specificity determinants of the OM TpsA translocator proteins for their cognate secreted TpsA exotoxins, identifying examples of possible redundancy and reduced specificity. However, secretion systems may also be used to target other, competitor bacterial cells. In the last two or three years, it has become apparent that certain Type VI secretion systems (T6SSs) can be used by bacteria to target competitor bacterial cells (although, intriguingly, many also or only target eukaryotic cells) (Hood *et al*., 2010; Schwarz *et al*., 2010). The T6SS is the most recently described of the Gram-negative protein secretion systems. It is a large multi-protein machine which spans the entire cell envelope and is believed to inject proteins directly into target cells, utilizing an extracellular cell-puncturing structure resembling a bacteriophage tail spike (Cascales and Cambillau, 2012). Reflecting significant current interest in this new system, several presentations at the meeting featured work on the T6SS. **Sarah Coulthurst** (University of Dundee, UK) described the potent antibacterial T6SS of the opportunistic enteric pathogen, *Serratia marcescens* (Murdoch *et al*., 2011), and the use of several experimental approaches aiming to identify and characterize its secreted antibacterial effector proteins. In a short presentation, **Frank Egan** from the O'Gara laboratory at University College Cork, described bioinformatic studies to assess diversity and predict function and candidate effectors within the multiple T6SSs encoded by *Pseudomonas* species (Barret *et al*., 2011).

Another way in which bacteria can kill competitor cells, this time specifically close relatives, is via bacteriocins, including colicins (bacteriocins targeting *E. coli*). Bacteri-

ocins are protein antibiotics which, in contrast with the outward movement of exported and secreted proteins, translocate into cells where various cytotoxic domains (typically nucleases or pore forming ionophores) cause highly efficient killing of a target cell (Kleanthous, 2010). Colicins translocate into the cell by parasitizing normal cellular transport pathways, initially binding to specific OM receptors and then utilizing OM translocators coupled with either the Ton or Tol systems to gain entry to the periplasm. This fascinating process, which also provides valuable insight into the envelope transport systems subverted by the colicins, was the subject of the talk by **Colin Kleanthous** (University of Oxford, UK). Colin described how one aspect of colicin translocation represents a new type of protein-mediated transmembrane signalling mechanism: 'directed epitope delivery' (Housden *et al*., 2010). Classically, ligand-mediated signalling across a membrane involves ligand-triggered conformational change or oligomerization of the receptor. In contrast, during the translocation of the colicin ColE9, and presumably other colicins, the signal itself, in the form of a peptide epitope in the colicin, moves through the porin to contact its cellular target in the periplasm (Housden *et al*., 2010). Colicins all comprise an N-terminal translocation (T) domain, a central receptor binding (R) domain and a C-terminal cytotoxic (C) domain. Initially, the R domain of ColE9 binds the BtuB receptor and then the T domain recruits the translocator OmpF using the intrinsically unstructured translocation domain (IUTD) within the T domain. The IUTD contains a TolB binding epitope which accesses the periplasm to bind TolB and thus recruit TolA. The Kleanthous lab has shown that the IUTD of ColE9 contains two OmpF binding sites (OBSs) flanking the TolB binding epitope and reported the crystal structure of an OmpF–OBS complex, revealing the colicin bound within the lumen of the porin (Fig. 3) (Housden *et al*., 2010). Therefore, the colicin IUTD is indeed delivered to the periplasm directly through the porin. Once the TolB binding epitope has been 'dangled through' the OmpF pore, TolB can bind the displayed epitope, promoting disorder in its own TolA box, in turn recruiting the TolA protein. Such studies are highlighting important similarities between the Ton and Tol systems, in particular how they communicate with activated OM receptors, a process subverted for each system by colicins (Kleanthous, 2010).

Sociomicrobiology and biofilms: microbe–microbe communication and interactions

Microbes in nature grow in communities that develop and are maintained by interactions between adjoining cells (Costerton *et al*., 1995; Davey and O'Toole, 2000; O'Toole *et al*., 2000). Many of these organisms have evolved systems to communicate and co-ordinate their behaviour

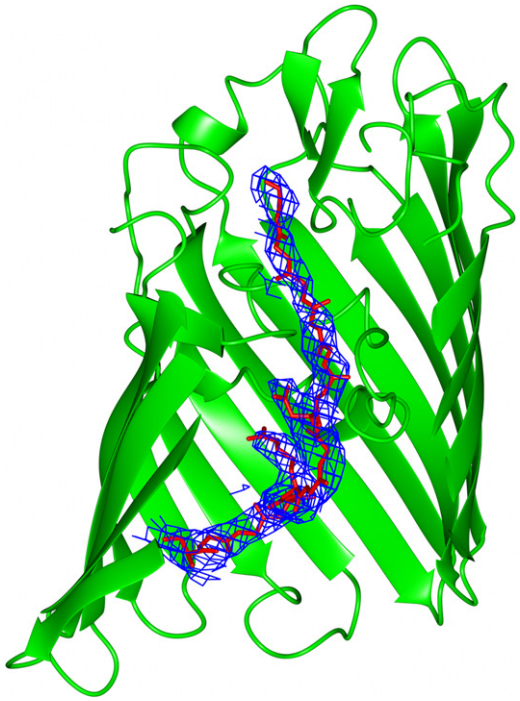

Fig. 3. Structure of OmpF complexed with a colicin peptide in the porin lumen. An OmpF monomer is shown as green ribbon, with the ColE9 OBS1 peptide (part of the IUTD within the N-terminal translocation domain) modelled into the electron density map within the lumen. Taken from Housden *et al*. (2010) with permission. ©2010 National Academy of Sciences.

through the use of diffusible signalling molecules or through physical interaction between bordering cells (Waters and Bassler, 2005). This section of lectures touched on various aspects of microbial interactions during the development of communities that included (i) the role of cell-to-cell signalling in development and morphogenesis of bacterial cells, (ii) interface between cell-to-cell signalling systems and second messenger signal transduction and (iii) how cell-to-cell signalling may contribute to interspecies and microbe–host relations.

How cell-to-cell signalling plays a role in morphological changes in the enterobacterial *Serratia* sp. ATCC 39006 was the main focus of **George Salmond**'s (University of Cambridge, UK) lecture. George explained how his group has studied the molecular biology of prodigiosin and carbapenem production for a number of years in this organism and shown how cell-to-cell signalling mediated by acyl-homoserine lactones (acyl-HSL) controls the generation of these specific antibiotics (Coulthurst *et al*., 2005). George further described how his team has recently demonstrated that these bacteria also develop gas vesicle structures under the control of acyl-HSL cell-to-cell signalling, the first description of cell-to-cell signal-dependent intracellular organelle development by a member of the Enterobacteriaceae (Ramsay *et al*., 2011;

Ramsay and Salmond, 2012). George went on to explain how the production of these gas vesicles appears to enable *Serratia* sp. ATCC 39006 to colonize the air–liquid interface, suggesting that the bacterium utilizes these vesicles to facilitate access to the oxygen-rich water surface. *Serratia* sp. ATCC 39006 encodes a 16.6 kb cluster of genes both necessary and sufficient for gas vesicle development, confirmed both through mutagenesis and the genetic engineering of buoyant, gas vesicle-expressing *E. coli*. Interestingly, *Serratia* 39006 encodes three distinct homologues of GvpA, a protein common to all gas vesicle-producing species discovered to date. Mutation in these *gvp* genes gives rise to vesicles of different widths and lengths, suggesting that it may be able to regulate vesicular dimensions, perhaps as a physiological and developmental response to environmental conditions. Consistent with this hypothesis, *Serratia* sp. ATCC 39006 gas vesicle morphogenesis is activated in cultures with reduced aeration. Consequently, it was suggested that gas vesicle biogenesis in *Serratia* represents a distinct mechanism of mobility, regulated by accessibility to oxygen and a distinct cell-to-cell signalling system (Ramsay *et al*., 2011; Ramsay and Salmond, 2012).

Yvonne McCarthy from the Dow lab at University College Cork, Ireland continued this theme by giving an example of how cell-to-cell signalling may interface with transduction systems that use second messenger signals. Yvonne's poster described the group's recent findings that explained how the *cis*-2-dodecenoic acid (BDSF) cell-to-cell signal controls expression of virulence factors in the opportunistic human pathogen *Burkholderia cenocepacia* by interfacing with the c-di-GMP effector protein BCAM1349 (McCarthy *et al*., 2010; Fazli *et al*., 2011). BDSF is a signal molecule of the DSF family that was originally described in the plant pathogen *X. campestris*. Mutation of *BCAM0581*, which encodes the BDSF synthase, gives rise to a reduction in biofilm formation, reduced cytotoxicity to Chinese hamster ovary cells and reduced virulence in both insect (*Galleria mellonella*) and agar-bead mouse models of pulmonary infection. Transcriptome profiling reveals that mutation of *BCAM0581* causes an alteration in expression of over 300 genes associated with a range of biological functions that include bacterial motility and attachment, stress tolerance, virulence, regulation, transport, multidrug resistance, detoxification and signal transduction. One of these genes, *BCAM1349* is predicted to encode a transcriptional regulator of the CRP/FNR superfamily. Analyses of purified full-length and truncated BCAM1349 protein demonstrated that it binds c-di-GMP *in vitro*. The BCAM1349 protein was shown to regulate the production of a number of components, including cellulose and fimbriae that are also regulated by BDSF. The BCAM1349 protein binds to the promoter region of the cellulose synthase operon, and this binding is enhanced by the presence of c-di-GMP. Thus, it was proposed that the BDSF system controls a downstream signal transduction cascade involving the c-di-GMP responsive element BCAM1349 that influences virulence and biofilm formation.

The lecture by **Sherry Kuchma** from George O'Toole's group based at Geisel School of Medicine at Dartmouth, USA built on the earlier theme of the intracellular signalling molecule c-di-GMP influencing surface-associated behaviours and virulence. Here Sherry explained how their group had begun to examine how the second messenger c-di-GMP controls the inverse regulation of biofilm formation and swarming motility in *P. aeruginosa*, where high levels of this signalling molecule encouraged cell aggregation and inhibited motility, and low levels of c-di-GMP promoted motile behaviours. Sherry went on to describe how the group recently reported that the BifA protein is a PDE, which catalyses the breakdown of c-di-GMP and inversely regulates biofilm formation and swarming motility (Kuchma *et al*., 2007). Meanwhile, SadC, a c-di-GMP generating DGC, functions with BifA to modulate cellular cyclic di-GMP levels and thus regulates biofilm formation and swarming motility (Fig. 1C) (Kuchma *et al*., 2007; Merritt *et al*., 2007). Consistent with a role for BifA as a c-di-GMP PDE, *bifA* mutants exhibit increased cellular levels of c-di-GMP relative to the wild-type. Phenotypically, *bifA* mutants form hyperbiofilms and are unable to swarm. In order to better understand how elevated c-di-GMP levels inhibit swarming motility, they deployed a genetic screen that exploits the swarming defect of the *bifA* mutant and identified suppressor mutants that restored the ability of *bifA* mutants to swarm. From this screen the PilY1 protein was identified (Kuchma *et al*., 2010). Taken together, the data point towards PilY1 functioning upstream of the c-di-GMP DGC SadC to regulate swarming motility in *P. aeruginosa*. Notably, PilY1 is also involved in regulating assembly of type IV pili responsible for a distinct form of surface motility known as twitching motility. The discovery of PilY1 as a regulator of both swarming and twitching suggests that *P. aeruginosa* is able to co-ordinately regulate these distinct motility systems when on a surface.

Several presentations also illustrated instances where cell-to-cell signalling may contribute to interspecies and microbe–host relations. **Kate Twomey** from the Ryan lab at University College Cork, Ireland explained how DSF signalling molecules (*cis*-2-unsaturated fatty acids), as well as functioning in intraspecies signalling, may also play a role in interspecies signalling during polymicrobial infections. Kate explained how DSF is synthesized by *B. cenocepacia* and *Stenotrophomonas maltophilia*, two species that are associated with *P. aeruginosa* in the lung infections of CF patients (Ryan and Dow, 2011). Although *P. aeruginosa* does not make DSF itself, previous work

from the group demonstrated that DSF alters *P. aeruginosa* biofilm formation and led to increased antimicrobial tolerance. The group's recent findings demonstrate that DSF is present in CF lung infections and that the presence of DSF in sputum correlates with *S. maltophilia* and/or *B. cenocepacia* colonization (Twomey *et al*., 2012). Interestingly, both model and clinical isolates of *P. aeruginosa* display increased antimicrobial tolerance when treated with DSF. Furthermore, DSF also increased *P. aeruginosa* persistence in a mouse lung infection model. Taken together, these data pointed towards DSF having a significant contribution to interspecies signalling in polymicrobial lung infection (Twomey *et al*., 2012).

In the case of beneficial bacteria, **Steven Christiaen** from Tom Coenye's team at Ghent University, Belgium described their investigations towards understanding how the interspecies signalling molecule autoinducer-2 (AI-2) functions in intestinal probiotic strains of Bifidobacteria. Steven explained how, as an approach towards understanding AI-2 signalling in these organisms, they carried out a transcriptome profile in the model strain *Bifidobacterium breve* UCC2003 where they made a comparison of the wild-type, an AI-2 non-producing strain (inactivated *luxS* gene) and an over-producing AI-2 strain. Overall, the data pointed towards AI-2 signalling having a significant role in *Bifidobacterium* physiology as changes in genes involved in transcription and stress response, carbohydrate transport and metabolism were described. These and other preliminary data presented by the group demonstrate a potential role for AI-2 in modulating beneficial bacteria behaviour, and for the described approach in unravelling the complex role that interspecies signalling may have *in vivo*.

Exogenous addition of cell-to-cell signalling inhibitors has been proposed as potential anti-biofilm agents. **Gilles Brackman** (also from Tom Coenye's lab) explained how they have begun to examine the utilization of this type of strategy in the treatment of polymicrobial chronic wounds that are recalcitrant to general clinical treatment (Brackman *et al*., 2011). Gilles explained how they have synthesized a selection of AI-2 inhibitors (containing a thiazolidinedione, pyrrolidinedione or oxazaborolidine backbone). These compounds were tested in combination with various antimicrobial agents for effects on chronic biofilm wound models of *S. aureus* and *Staphylococcus epidermidis*. Several thiazolidinedione derivatives displayed both biofilm inhibitory as well as antimicrobial potentiating activities. These data, in conjunction with other preliminary data presented, suggest this strategy may improve the success of treatment of chronically infected wounds by increasing the susceptibility of bacterial wound biofilms.

However, **Richard Allen** from Sam Brown's group at the University of Edinburgh, UK provided a cautionary note that many of these cell-to-cell signalling inhibitors are not 'evolution-proof' with resistance being strongly selected for under certain conditions. Richard detailed predictions of how a number of factors would influence selection of resistance to cell-to-cell signalling inhibitors as well as preliminary empirical data, testing these predictions in *P. aeruginosa*. These results show the importance of theoretical understanding in directing laboratory studies.

Microbe–host interactions: pathogenesis and commensalism

In order to colonize the host or to establish an infection, microbes must interact with host tissues and surfaces. The focus of this section was to highlight mechanisms and outcomes of bacterial interactions with the host. Particular emphasis was placed on those microbe–host interactions (i) required to establish infection and (ii) required for successful colonization by commensal organisms.

The ability of microbes to become internalized by host cells is often considered a crucial step in establishing a persistent infection. **Marta Zapotoczna** from Tim Foster's group in Trinity College Dublin, Ireland described a novel mechanism of *S. aureus* internalization into eukaryotic cells. IsdB, a cell wall-anchored surface protein, mediates adhesion to host cells and internalization of bacteria through direct interaction with integrins. The important role of IsdB was hitherto unrecognized because of the fact that it is only expressed under iron-restricted conditions such as those that prevail during growth *in vivo*.

Cystic fibrosis is characterized by chronic pulmonary infection by opportunistic pathogens such as *P. aeruginosa*. *P. aeruginosa* is primarily an environmental organism and must adapt to conditions encountered in the airways of CF patients during chronic infection (Smith *et al*., 2006; Yang *et al*., 2011). **Søren Molin** (Technical University of Denmark, Denmark) reported new insights into the adaptive evolution of *P. aeruginosa*. Monitoring long-term bacterial evolution *in vivo* (over more than 30 years) has revealed that bacteria acquire loss of function mutations in genes required for virulence (Yang *et al*., 2011). To establish a chronic infection, *P. aeruginosa* must transition from a non-mucoid to a mucoid, biofilm-forming phenotype. Bacteria in the biofilm are recalcitrant to antibiotics, making it difficult to treat chronic *P. aeruginosa* infection.

A novel approach to eliminating *P. aeruginosa* from the CF lung was described by **Jeremy Webb** (University of Southampton, UK), who showed that treatment of sputum from CF patients with NO disperses biofilm *in vitro*. A phase 2 clinical trial has shown that inhalation of NO increases the efficacy of antibiotics in CF patients. Novel systems are now being developed to allow delivery of NO upon contact with a biofilm *in vivo*.

Avril Monahan from Rebecca Ingram's lab in Queen's University, Belfast, UK continued the discussion of chronic *P. aeruginosa* infection by describing novel approaches to establishing a murine model of chronic bacterial infection. Existing animal models do not accurately represent chronic infection in humans as bacteria are either rapidly eliminated from the lung or the animals succumb to systemic infection. Preliminary data presented here suggest that selection of the most appropriate mouse strain and bacterial strain can promote sustained lung infection by *P. aeruginosa*. This may provide an exciting opportunity to study the interaction of *P. aeruginosa* with the host immune system over the course of an infection.

Beneficial microbe–host interactions occur in the gastrointestinal (GI) tract where commensal bacteria protect against pathogens. **Trevor Lawley** (Wellcome Trust Sanger Institute, UK) described how antibiotic treatment can disrupt the normal intestinal microbiota allowing the opportunistic pathogen *Clostridium difficile* to proliferate and cause disease. Murine models have been used to identify factors involved in *C. difficile* transmission and pathogenesis. In particular, highly resistant and infectious spores shed by infected mice can transmit infection to healthy mice mimicking the transmission route in humans (Lawley *et al*., 2009). A murine model was used to demonstrate that *C. difficile* infection can be treated by inoculating an infected mouse with faecal matter from a healthy mouse, allowing normal intestinal commensals to repopulate the GI tract and eliminate the pathogen. Lawley's group analysed the gut microbiome of healthy mice and identified the species of bacteria that they believe to be the most protective against *C. difficile* infection. Restoring just these bacteria to the GI tract of patients with *C. difficile* infection may be an effective treatment option.

Building on this theme of colonization of the GI tract, **Mary O'Connell Motherway** from Douwe van Sinderen's lab in University College Cork described an important factor that contributes to GI tract colonization by the gut commensal *Bifidobacterium*. Mary explained how whole genome sequencing of *B. breve* UCC2003 identified a gene cluster responsible for the assembly of sortase-dependent type IVb tight adherence (Tad) pili (O'Connell Motherway *et al*., 2011). These genes were highly upregulated during *in vivo* growth. Identification of the restriction barriers that prevent DNA uptake in *B. breve* (O'Connell Motherway *et al*., 2009) has allowed a genetic system to be established. This in turn allowed the *tadA* gene to be disrupted and the role of Tad pili in microbe–host interactions to be tested. Bacteria lacking Tad pili showed reduced colonization of normal healthy mice indicating that pili are required for *B. breve* to colonize the GI tract. However, pilus-deficient bacteria colonized germ-free mice to the same level as the wild-type suggesting that Tad pili are only necessary when the normal microbiota are present (O'Connell Motherway *et al*., 2011).

Concluding remarks

This symposium provided an excellent forum for those with an interest in microbiology to interact and learn about a wide range of topics. The aim of the meeting was to provide a platform for young microbiologists from around the world to communicate their research. The animated and exciting discussions between senior academics and scientists who are just beginning their careers suggested that the aim of the meeting had been fulfilled. In the final session of the meeting a number of awards were distributed that included a poster prize, sponsored by Biochemical Journal and awarded to **Victoria Marlow** (University of Dundee, UK) for her work on the role of DegU in *Bacillus subtilis* biofilm development. Victoria's work was selected from almost 80 posters. Meanwhile, the short talk prize, which was sponsored by Nature Reviews Microbiology, went to **Charlotte Michaux** (University of Caen, France) for her presentation on the functional characterization of small intergenic RNAs in *E. faecalis*. As well as the various scientific highlights of this meeting, there were also some unique social events including a conference dinner, held in the traditional Irish Pub Flannery's. Overall, the feedback from attendees was very positive; participants appreciated the quality of the scientific programme and the intimate atmosphere of a small conference, all of which bodes well for future Young Microbiologists Symposium meetings.

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References

Barret, M., Egan, F., Fargier, E., Morrissey, J.P., and O'Gara, F. (2011) Genomic analysis of the type VI secretion systems in *Pseudomonas* spp.: novel clusters and putative effectors uncovered. *Microbiology* **157:** 1726–1739.

Brackman, G., Cos, P., Maes, L., Nelis, H.J., and Coenye, T. (2011) Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics *in vitro* and *in vivo*. *Antimicrob Agents Chemother* **55:** 2655–2661.

Brown, M.T., Delalez, N.J., and Armitage, J.P. (2011) Protein dynamics and mechanisms controlling the rotational behaviour of the bacterial flagellar motor. *Curr Opin Microbiol* **14:** 734–740.

Bush, M., Ghosh, T., Tucker, N., Zhang, X., and Dixon, R. (2010) Nitric oxide-responsive interdomain regulation targets the sigma54-interaction surface in the enhancer binding protein NorR. *Mol Microbiol* **77:** 1278–1288.

Cascales, E., and Cambillau, C. (2012) Structural biology of type VI secretion systems. *Philos Trans R Soc Lond B Biol Sci* **367:** 1102–1111.

Corrigan, R.M., Abbott, J.C., Burhenne, H., Kaever, V., and Grundling, A. (2011) c-di-AMP is a new second messenger in *Staphylococcus aureus* with a role in controlling cell size and envelope stress. *PLoS Pathog* **7:** e1002217.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., and Lappin-Scott, H.M. (1995) Microbial biofilms. *Annu Rev Microbiol* **49:** 711–745.

Coulthurst, S.J., Barnard, A.M., and Salmond, G.P. (2005) Regulation and biosynthesis of carbapenem antibiotics in bacteria. *Nat Rev Microbiol* **3:** 295–306.

D'Autreaux, B., Tucker, N.P., Dixon, R., and Spiro, S. (2005) A non-haem iron centre in the transcription factor NorR senses nitric oxide. *Nature* **437:** 769–772.

Davey, M.E., and O'Toole, G.A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev* **64:** 847–867.

Delalez, N.J., Wadhams, G.H., Rosser, G., Xue, Q., Brown, M.T., Dobbie, I.M., *et al*. (2010) Signal-dependent turnover of the bacterial flagellar switch protein FliM. *Proc Natl Acad Sci USA* **107:** 11347–11351.

Fazli, M., O'Connell, A., Nilsson, M., Niehaus, K., Dow, J.M., Givskov, M., *et al*. (2011) The CRP/FNR family protein Bcam1349 is a c-di-GMP effector that regulates biofilm formation in the respiratory pathogen *Burkholderia cenocepacia*. *Mol Microbiol* **82:** 327–341.

Gerlach, R.G., and Hensel, M. (2007) Protein secretion systems and adhesins: the molecular armory of Gram-negative pathogens. *Int J Med Microbiol* **297:** 401–415.

Hagan, C.L., Silhavy, T.J., and Kahne, D. (2011) beta-Barrel membrane protein assembly by the Bam complex. *Annu Rev Biochem* **80:** 189–210.

Holland, I.B. (2010) The extraordinary diversity of bacterial protein secretion mechanisms. *Methods Mol Biol* **619:** 1–20.

Hood, R.D., Singh, P., Hsu, F., Guvener, T., Carl, M.A., Trinidad, R.R., *et al*. (2010) A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* **7:** 25–37.

Housden, N.G., Wojdyla, J.A., Korczynska, J., Grishkovskaya, I., Kirkpatrick, N., Brzozowski, A.M., and Kleanthous, C. (2010) Directed epitope delivery across the *Escherichia coli* outer membrane through the porin OmpF. *Proc Natl Acad Sci USA* **107:** 21412–21417.

Kleanthous, C. (2010) Swimming against the tide: progress and challenges in our understanding of colicin translocation. *Nat Rev Microbiol* **8:** 843–848.

Kuchma, S.L., Brothers, K.M., Merritt, J.H., Liberati, N.T., Ausubel, F.M., and O'Toole, G.A. (2007) BifA, a cyclic-Di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. *J Bacteriol* **189:** 8165–8178.

Kuchma, S.L., Ballok, A.E., Merritt, J.H., Hammond, J.H., Lu, W., Rabinowitz, J.D., and O'Toole, G.A. (2010) Cyclic-di-GMP-mediated repression of swarming motility by *Pseudomonas aeruginosa*: the *pilY1* gene and its impact on surface-associated behaviors. *J Bacteriol* **192:** 2950–2964.

Kuchma, S.L., Griffin, E.F., and O'Toole, G.A. (2012) Minor pilins of the type IV pilus system participate in the negative regulation of swarming motility. *J Bacteriol* doi: 10.1128/JB.00899-12.

Lawley, T.D., Clare, S., Walker, A.W., Goulding, D., Stabler, R.A., Croucher, N., *et al*. (2009) Antibiotic treatment of *Clostridium difficile* carrier mice triggers a supershedder state, spore-mediated transmission, and severe disease in immunocompromised hosts. *Infect Immun* **77:** 3661–3669.

Lebeer, S., Claes, I.J., Balog, C.I., Schoofs, G., Verhoeven, T.L., Nys, K., *et al*. (2012) The major secreted protein Msp1/p75 is O-glycosylated in *Lactobacillus rhamnosus* GG. *Microb Cell Fact* **11:** 15.

McCarthy, Y., Yang, L., Twomey, K.B., Sass, A., Tolker-Nielsen, T., Mahenthiralingam, E., *et al*. (2010) A sensor kinase recognizing the cell-cell signal BDSF (cis-2-dodecenoic acid) regulates virulence in *Burkholderia cenocepacia*. *Mol Microbiol* **77:** 1220–1236.

Malone, J.G., Jaeger, T., Manfredi, P., Dotsch, A., Blanka, A., Bos, R., *et al*. (2012) The YfiBNR signal transduction mechanism reveals novel targets for the evolution of persistent *Pseudomonas aeruginosa* in cystic fibrosis airways. *PLoS Pathog* **8:** e1002760.

Merritt, J.H., Brothers, K.M., Kuchma, S.L., and O'Toole, G.A. (2007) SadC reciprocally influences biofilm formation and swarming motility via modulation of exopolysaccharide production and flagellar function. *J Bacteriol* **189:** 8154–8164.

Murdoch, S.L., Trunk, K., English, G., Fritsch, M.J., Pourkarimi, E., and Coulthurst, S.J. (2011) The opportunistic pathogen *Serratia marcescens* utilizes type VI secretion to target bacterial competitors. *J Bacteriol* **193:** 6057–6069.

O'Connell Motherway, M., O'Driscoll, J., Fitzgerald, G.F., and Van Sinderen, D. (2009) Overcoming the restriction barrier to plasmid transformation and targeted mutagenesis in *Bifidobacterium breve* UCC2003. *Microb Biotechnol* **2:** 321–332.

O'Connell Motherway, M., Zomer, A., Leahy, S.C., Reunanen, J., Bottacini, F., Claesson, M.J., *et al*. (2011) Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. *Proc Natl Acad Sci USA* **108:** 11217–11222.

O'Toole, G., Kaplan, H.B., and Kolter, R. (2000) Biofilm for-

mation as microbial development. *Annu Rev Microbiol* **54:** 49–79.

Porter, S.L., Wadhams, G.H., and Armitage, J.P. (2011) Signal processing in complex chemotaxis pathways. *Nat Rev Microbiol* **9:** 153–165.

Ramsay, J.P., and Salmond, G.P. (2012) Quorum sensing-controlled buoyancy through gas vesicles: intracellular bacterial microcompartments for environmental adaptation. *Commun Integr Biol* **5:** 96–98.

Ramsay, J.P., Williamson, N.R., Spring, D.R., and Salmond, G.P. (2011) A quorum-sensing molecule acts as a morphogen controlling gas vesicle organelle biogenesis and adaptive flotation in an enterobacterium. *Proc Natl Acad Sci USA* **108:** 14932–14937.

Rigel, N.W., and Silhavy, T.J. (2012) Making a beta-barrel: assembly of outer membrane proteins in Gram-negative bacteria. *Curr Opin Microbiol* **15:** 189–193.

Roberts, M.A., Wadhams, G.H., Hadfield, K.A., Tickner, S., and Armitage, J.P. (2012) ParA-like protein uses non-specific chromosomal DNA binding to partition protein complexes. *Proc Natl Acad Sci USA* **109:** 6698–6703.

Ryan, R.P., and Dow, J.M. (2011) Communication with a growing family: diffusible signal factor (DSF) signaling in bacteria. *Trends Microbiol* **19:** 145–152.

Ryan, R.P., Fouhy, Y., Lucey, J.F., Crossman, L.C., Spiro, S., He, Y.W., *et al*. (2006) Cell-cell signaling in *Xanthomonas campestris* involves an HD-GYP domain protein that functions in cyclic di-GMP turnover. *Proc Natl Acad Sci USA* **103:** 6712–6717.

Ryan, R.P., Romeo, T., De Keersmaecker, S.C., and Coulthurst, S.J. (2009) Nurturing scientific mutualism: a report from the 'Young Microbiologists Mini-Symposium on microbe signalling, organisation and pathogenesis'. *Mol Microbiol* **73:** 760–774.

Ryan, R.P., McCarthy, Y., Andrade, M., Farah, C.S., Armitage, J.P., and Dow, J.M. (2010) Cell-cell signal-dependent dynamic interactions between HD-GYP and GGDEF domain proteins mediate virulence in *Xanthomonas campestris*. *Proc Natl Acad Sci USA* **107:** 5989–5994.

Ryan, R.P., McCarthy, Y., Kiely, P.A., O'Connor, R., Farah, C.S., Armitage, J.P., and Dow, J.M. (2012) Dynamic complex formation between HD-GYP, GGDEF and PilZ domain proteins regulates motility in *Xanthomonas campestris*. *Mol Microbiol* doi:10.1111/mmi.12000

Schwarz, S., West, T.E., Boyer, F., Chiang, W.C., Carl, M.A., Hood, R.D., *et al*. (2010) Burkholderia type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. *PLoS Pathog* **6:** e1001068.

Sharma, C.M., Hoffmann, S., Darfeuille, F., Reignier, J., Findeiss, S., Sittka, A., *et al*. (2010) The primary transcriptome of the major human pathogen *Helicobacter pylori*. *Nature* **464:** 250–255.

Shioya, K., Michaux, C., Kuenne, C., Hain, T., Verneuil, N., Budin-Verneuil, A., *et al*. (2011) Genome-wide identification of small RNAs in the opportunistic pathogen *Enterococcus faecalis* V583. *PLoS ONE* **6:** e23948.

Smith, E.E., Buckley, D.G., Wu, Z., Saenphimmachak, C., Hoffman, L.R., D'Argenio, D.A., *et al*. (2006) Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc Natl Acad Sci USA* **103:** 8487–8492.

Thompson, S.R., Wadhams, G.H., and Armitage, J.P. (2006) The positioning of cytoplasmic protein clusters in bacteria. *Proc Natl Acad Sci USA* **103:** 8209–8214.

Twomey, K.B., O'Connell, O.J., McCarthy, Y., Dow, J.M., O'Toole, G.A., Plant, B.J., and Ryan, R.P. (2012) Bacterial cis-2-unsaturated fatty acids found in the cystic fibrosis airway modulate virulence and persistence of *Pseudomonas aeruginosa*. *ISME J* **6:** 939–950.

Waters, C.M., and Bassler, B.L. (2005) Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* **21:** 319–346.

Yang, L., Jelsbak, L., Marvig, R.L., Damkiaer, S., Workman, C.T., Rau, M.H., *et al*. (2011) Evolutionary dynamics of bacteria in a human host environment. *Proc Natl Acad Sci USA* **108:** 7481–7486.

Youle, M., Rohwer, F., Stacy, A., Whiteley, M., Steel, B.C., Delalez, N.J., *et al*. (2012) The microbial Olympics. *Nat Rev Microbiol* **10:** 583–588.