Interaction and Signalling Networks: a report from the fourth 'Young Microbiologists Symposium on Microbe Signaling, Organisation and Pathogenesis'

Clare L. Kirkpatrick[1], Olivier Lesouhaitier[2], Jacob G. Malone[3,4], Shi-Qi An[5] and Delphine L. Caly[6]

[1]Department of Microbiology & Molecular Medicine, Institute of Genetics & Genomics in Geneva (iGE3), Faculty of Medicine/CMU, University of Geneva, Switzerland

[2]Laboratory of Microbiology Signals and Microenvironnement LMSM, EA 4312, Normandie Université, Université de Rouen Evreux, France

[3]John Innes Centre, Norwich Research Park, Norwich, United Kingdom

[4]University of East Anglia, Norwich, United Kingdom

[5]Division of Molecular Microbiology, College of Life Sciences, University of Dundee

[6]Univ. Lille, EA 7394, ICV - Institut Charles Viollette, Lille, France.

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Abstract

At the end of June, over 120 microbiologists from 18 countries gathered in Dundee, Scotland for the fourth edition of the Young Microbiologists Symposium on "Microbe Signalling, Organisation and Pathogenesis". The aim of the symposium was to give early career microbiologists the opportunity to present their work in a convivial environment and to interact with senior world-renowned scientists in exciting fields of microbiology research. The meeting was supported by the Microbiology Society, the Society of Applied Microbiology, the American Society for Microbiology with further sponsorship from the European Molecular Biology Organisation and The Royal Society of Edinburgh. In this report, we highlight some themes that emerged from the many interesting talks and poster presentations, and some of the other activities that were on offer at this energetic meeting.

Introduction

The fourth Young Microbiologists Symposium (YMS2016) took place at the Apex City Quay Hotel in Dundee, Scotland on the 29th and 30th June 2016. The conference gathered 126 scientists coming from 18 countries and was organized by **Helge Dorfmueller** and **Robert Ryan**, from University of Dundee, and **Delphine Caly** from University of Lille in France. The main objective of the YMS2016 was to bring together early career microbiologists. The symposium programme covered several hot topics in microbiology and touched on current areas of interest to microbiologists including intracellular signalling, antibiotic resistance, bacterial secretion and host-microbe interactions. Renowned experts, who led sessions, and the many junior microbiologists who attended provided insight and new findings into these exciting areas. A novelty to this year's meeting was that participants were given the opportunity to attend a PLOS Pathogens writing and publishing workshop, chaired by **Neil Mabbott** from

the Roslin Institute and University of Edinburgh in Scotland, which provided valuable advice for PhD students and junior post-docs on how to write scientific papers and achieve successful publication.

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Sensing, transduction and intracellular signalling

The YMS2016 kicked off with the FEMS keynote lecture from **Ute Römling** (Karolinska Institute, Sweden), who discussed her work mapping the distribution and prevalence of the *Pseudomonas aeruginosa* PAO clone C strain cluster in clinics worldwide. As part of this research, Ute discussed how her group identified the PACGI-1 genomic island in this cluster, and showed that it contributes to stress tolerance and heat-shock resistance by encoding a protein quality-control system that functions in response to environmental stresses (Lee *et al.*, 2015). Next, Ute described her group's work on the ubiquitous bacterial second messenger signal cyclic-di-GMP in *Salmonella typhimurium*, which controls biofilm formation and virulence as part of a complex regulatory network involving the transcriptional regulator CsgD. Ute explained how her lab have identified and characterised several key players in this network, including the diguanylate cyclase AdrA, the cellulose synthase dinucleotide-binding protein BcsE, and the degenerate phosphodiesterase STM1697, which controls flagellar gene transcription through binding to the master regulator FlhDC (Le Guyon *et al.*, 2015).

These themes were built upon in the first session, which was opened by **Max Dow** (University College Cork, Ireland). Max discussed the structure-function relationship of HD-GYP domains that function to turn over the second messenger cyclic-di-GMP. Max began with a summary of his lab's work on the protein RpfG, which contains a HD-GYP domain and controls virulence and motility in the plant pathogen *Xanthomonas campestris* (Ryan *et al.*, 2010). Recently, Max and collaborators examined the structures of different HD-GYP proteins from *Pseudomonas*

aeruginosa, identifying a relationship between protein activity and the number of bound metal ions in the active site (Bellini *et al.*, 2014). Max explained that full c-di-GMP to GMP phosphodiesterase activity is mediated by HD-GYP domains with three metal-ion cofactors, while a second class containing two metal-binding sites appears to degrade the dinucleotide to its linear form, pGpG.

Lisa Bowman (Imperial College London, UK) described a second, equally interesting dinucleotide second messenger; cyclic-di-AMP. Pioneering work from the Gründling lab has shown that cyclic-di-AMP controls potassium uptake and cation proton antiporter activity in *Staphylococcus aureus*, and is produced by the membrane bound cyclase DacA (Corrigan *et al.*, 2011). Lisa discussed her work to expand on the existing model for cyclic-di-AMP signalling by explaining her inventive use of a BioLog phenotypic microarray to determine the function of YbbR, an uncharacterised component of the DacA membrane protein complex. Based on this screen and suppressor mutagenesis, Lisa proposed that YbbR localises to DacA at the membrane, increasing osmolyte uptake under stress conditions.

In the final talk in this session, **Francesca D'Angelo** (University Roma Tre, IT) attracted significant interest and many audience questions with her talk on the generation of synthetic cells. These synthetic cells consist of liposomes containing biological molecules, and represent an ambitious new approach to drug delivery (Stano *et al.*, 2012). After demonstrating that the HSL signal could be produced *in vitro*, Francesca built on this by encapsulating the functional HSL production system in her synthetic cells, protecting the HSL pathway from externally added inhibitors. The next step for this project will be to generate synthetic cells that can sense signals as well as produce an output.

Symbiosis, pathogenesis and mechanisms of host interaction

The ASM keynote lecture was presented by **Scott Hultgren** (Washington University, USA). Scott gave a fantastic and informative overview of his research into urinary tract infections (UTIs) by *E. coli*, which seem to be mediated by the activities of type I pili. Building on structural biology models of pili from the lab of Gabriel Waksman, Scott showed that high and low-affinity mannose-binding forms of the terminal FimH adhesin exist in equilibrium, with both states required for effective infection (Hospenthal *et al.*, 2016). He then moved on to a discussion of the clinical aspects of UTI, showing that bladder cells are remodelled by sensitisation to UTI, and thereafter are significantly more likely to become re-infected. Scott's talk finished with a description of several promising lines of research into UTI treatment, including an anti-pilus vaccine, and drugs targeting both pili and the FimH adhesin.

The host-microbe interactions session covered a large spectrum of topics introduced in the ASM lecture including polymicrobial infection, the use of new tools for studying host-microbe interactions in real time, and the impact of both host communication signals and small metabolic compounds.

Marvin Whiteley (University of Texas, USA) showed that microbe-microbe interactions increase bacterial resistance to host defences (Ramsey & Whiteley, 2009) and allow synergistic effects for some pathogenic bacteria (Turner *et al.*, 2015), using various examples of interactions, such as *P. aeruginosa* and *S. aureus* in the cystic fibrosis lung or *Aggregatibacter actinomycetemcomitans* and *Streptococcus gordonii* that form biofilms in the oral cavity. The highly organised wound communities and the precise spacing between bacteria during polymicrobial infection are required for infectious success (Stacy *et al.*, 2015), and Marvin explained why understanding this process could help in improving therapeutic strategies. The following talk was given by **Andrew Roe** (University of Glasgow, UK) who presented a new tool for studying protein interactions specifically dedicated to the host-pathogen interaction research field. This tool, named LOV for light-oxygen-voltage sensing domain, enables the

visualisation of bacterial cells attached to host cells. In parallel, Andrew showed how the LOV tool could be very suitable to study the direct translocation of bacterial type III effectors into host cells. Andrew's talk was illustrated by amazing images obtained by the fusion of a LOV-based reporter with the *Shigella flexneri* effector IpaB, demonstrating its interaction with the host cell actin network (Gawthorne *et al.*, 2016).

The use of mass spectrometry imaging in microbiology was discussed by **Heather Hulme** (University of Glasgow, UK), who showed that it could be a valuable tool for identifying biomarkers during an infection process. Using the example of mesenteric lymph node infection by *Salmonella*, Heather showed that palmitoylcarnitine (PalC), which is localised and accumulates in the damaged infected tissue, could be measured and used as a potential biomarker of infection.

The host environment encountered by bacteria plays a role in the success of infections. In this context, **Tuuli Ahlstrand** (University of Turku, Finland) showed that biofilms formed by the opportunistic pathogen *A. actinomycetemcomitans* could disrupt the host inflammation response by binding and internalising interleukin-1β (Paino *et al.*, 2012), through interaction with a specific bacterial sensor named bacterial interleukin receptor I (BilRI) (Paino *et al.*, 2013). In the same vein, **James Connolly** (University of Glasgow, UK) demonstrated how pathogenic *E. coli* integrates host signals in order to regulate its ability to colonize the urinary tract. More precisely, James demonstrated how D-serine influences both gene content and virulence factor expression in pathogenic *E. coli* (Connolly *et al.*, 2015) and how bacteria use a D-serine sensing system to adapt to their environment (Connolly *et al.*, 2016). Another way to prevent bacterial infection, using inhibitors of multivalent adhesion molecule 7 (MAM7), was described by **Daniel Stones** (University of Birmingham, UK) who described a bead-coupled recombinant MAM7 that not only prevented bacterial adhesion and infection in mice,

but also did not affect IL-1 release and wound healing, suggesting a promising drug to counteract infection (Krachler *et al.*, 2011).

Bacterial shape, secretion and development

This session began and ended with a review of new developments in our understanding of the operation of the bacterial type VI secretion system (T6SS). This multi-protein complex is a delivery system for protein-based toxins targeted at other bacteria or at eukaryotic cells, while the bacteria that are the source of the toxins also express specific immunity proteins to protect themselves against them. **Alain Filloux** (Imperial College London, UK) presented two complementary projects from his lab describing one of the *Pseudomonas* T6SS. The first, a recently published structural study (Planamente *et al.*, 2016), focused on a previously uncharacterised component of the complex, the TssA baseplate and demonstrated that TssA forms a circular baseplate-like structure that assembles onto the membrane-facing end of the TssBC sheath, sharing structural and functional homology with the gp6 baseplate of T4 bacteriophage, and is essential for T6SS activity. The second project concerned a genetic strategy to identify novel toxin-immunity protein pairs that could be delivered by the T6SS, using an ultra-high density transposon mutagenesis approach. With this innovative approach, several candidates for novel toxin-immunity protein pairs were identified. One, named Tse8, was further characterised and found to have an unusual mode of action, targeting the GatA component of the GatABC transamidosome (a protein complex responsible in certain bacterial species for correcting wrongly charged tRNAs). This work should open up highly interesting new avenues of investigation into whether the T6SS-using bacteria have specific sensors for certain classes of bacteria, since it would make no sense to deploy these toxins unless a sensitive host strain is present in the environment.

Bacterial lifestyle changes often require remodelling of the cell envelope, whether to permit the entry of extracellular DNA during competence or to generate a spore that will be more resistant to the external environment than the mother cell from which it develops. **Emma Denham** (University of Warwick, UK) presented her group's ongoing work on the role of small RNAs in bacterial lifestyle transition using *Bacillus subtilis* as their model system. This talk focused on one notable sRNA-controlled process, the AbrB-dependent transition from exponential to stationary phase (Mars *et al.*, 2015), where the protein expression "noise" of AbrB is regulated by the small RNA S1022, in such a way as to create phenotypic heterogeneity in terms of exponential phase growth rate suggesting a novel sRNA-regulated bet-hedging strategy.

Tessa Quax (University of Freiburg, Germany) provided the conference's only talk on Archaea, specifically on archaellum-mediated motility in these organisms. Named "archaellum" due to its extreme structural difference to the bacterial flagellum, this substructure resembles the type IV pili seen in bacteria in terms of its components and assembly mechanism. Surprisingly, Tessa showed it can also interact with a CheY-like component of a chemotaxis system as the bacterial flagellum does, despite the extreme evolutionary divergence between these two kingdoms of life and the completely different composition of their respective motility organelles. Finally, **Francesca Cianfanelli** from the Coulthurst group (University of Dundee, UK) presented her work on the T6SS of *Serratia marcescens* and the specific interactions of VgrG and PAAR proteins at the tip of the T6SS "spike". This showed that PAAR proteins are not only important but in some cases essential for T6SS function and that particular VgrG-PAAR combinations are required for full T6SS-dependent antibacterial activity, including activity mediated by cargo adaptors that are not normally considered dependent on specific VgrG proteins (Cianfanelli *et al.*, 2016).

Bacterial inter-species and inter-kingdom interactions

The final session covered the topic of inter-species and inter-kingdom interactions, which included talks regarding interactions within complex communities, between microbes, and the various host signals/triggers that shape the interactions within these communities. A captivating example of the former was presented by **Christoph Tang** (University of Oxford, UK) who delivered the EMBO lecture. Christoph described that temperature is one of the most important environmental cues that act on regulatory networks of pathogenic microbes. His group discovered and characterised the RNA thermometer CssA from *Neisseria meningitidis*, an elegant mechanism that this microbe uses to adapt to different temperature changes. Christoph explained how using NMR spectroscopy and SHAPE (Selective 2′-OH acylation analysed by primer extension) assays, the group discovered that at low temperature (4°C) all base pair regions of CssA are stably formed, and the ribosome cannot access the RBS which is fully occluded (Barnwal *et al.*, 2016). As the temperature is raised toward 30°C, the RNA structure starts to unfold but the RBS is still inaccessible and protein synthesis is still inhibited. By 42°C, the thermometer structure is fully open, leading to efficient translation. Taken together, it suggests that CssA acts as a rheostat, whose stability is optimized to respond in a small temperature range such as occurs within the upper airways during infection.

Continuing with the theme of environmental cues altering the response of the microbial community during infection, **Vanessa Sperandio** (UT Southwestern Medical Center, USA) showed that enterohaemorrhagic *E. coli* (EHEC) senses fucose cleaved from the mucus layer in the colon by *Bacteroides thetaiotaomicron* through the histidine kinase FusK. It then rewires its transcription, repressing the expression of the LEE and fucose utilisation genes (Pacheco *et al.*, 2012). However, without mucus as a carbon source, *B. thetaiotaomicron* starts to secrete succinate, which upon being taken up by EHEC is sensed by the Cra transcription factor as a clue to a gluconeogenic environment. Cra binds to another transcription factor, KdpE, which is an RR phosphorylated by the QseC adrenergic sensor, to integrate adrenergic and sugar

sensing to activate virulence gene expression at the interface with the intestinal epithelium. Through interaction with another RR; QseB, QseC also represses the expression of the *fusKR* genes, further derepressing the virulence regulon. These data suggest a new layer of complexity in the inter-kingdom signalling that underlies EHEC pathogenicity.

Given what is now known regarding the contribution of the host microbiota to health there is an urgent need for relevant animal models. **Beckie Ingram** (Queens College Belfast, UK) gave an inspiring talk about her group's work on developing appropriate murine models for understanding the pathophysiology of lung inflammation and the pathogenesis of lung disease in cystic fibrosis. These approaches will become crucial in improving our understanding of microbial community interactions in the field of infectious diseases. Finally, **Clare Kirkpatrick** (University of Geneva, Switzerland) discussed the role of toxin-antitoxin (TA) systems in bacterial interactions and how they can shape the community. Clare discussed her recent work on the HigBA system from *Caulobacter crescentus* and revealed that this TA system acts as a switch to regulate bacterial growth and induce cell death upon antibiotic-induced DNA damage (Kirkpatrick *et al.*, 2016). This novel regulatory mechanism could potentially be used to develop new treatments to clear bacterial infections.

Conclusions

This symposium, like previous meetings (Caly *et al.*, 2012, 2014; Ryan *et al.*, 2009), covered many fascinating areas of microbiology. As always the forum allowed the attendees to gain many insights into up and coming areas and techniques in bacteriology, and provided junior microbiologists the opportunity to present and discuss their work. This was successfully achieved judging by the numerous interactions between junior and senior scientists observed during and between scientific sessions.

After the final session, a number of awards were distributed. These included the Frontiers in Microbiology short talk prize that went to **Fang-Fang Wang** (Chinese Academy of Sciences Beijing, China) for her excellent presentation entitled, "Receptor histidine kinase directly binds plant chemical to promote bacterial adaptation in host plant". The Nature Reviews in Microbiology, Trends in Microbiology, Biochemical Journal and Molecular Microbiology poster prizes went to several PhD students working on outstanding projects. The meeting finished on a fun and friendly note with a Ceilidh organised in the Apex hotel following the conference dinner.

Overall, the feedback from attendees was very positive; participants appreciated the quality of the scientific programme and the intimate atmosphere of the small conference. A post-meeting survey reported that 71% of the survey participants (n=68) found the scientific programme 'very good' and 83% were interested in attending a future YMS conference (n=65). One of the participants, who gave a talk as a PhD student at the YMS2012 and is now setting up her lab, used this opportunity to advertise for positions in her new lab and made several promising contacts. This bodes well for further iterations of the meeting in the future.

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