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MicroMeeting Report

Bacterial moving and shaking: the 11th BLAST meeting

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Summary

Since their inception 20 years ago, the biennial BLAST (Bacterial Locomotion and Signal Transduction) meetings instantly became the place to be for exchanging and sharing the latest developments in the field of bacterial motility and signalling. At the 11th edition, held last January in New Orleans, LA, researchers reported on the myriad of mechanisms involved in bacterial movement, sensing and adaptation, ranging from the molecular level to multicellular behaviour. New insights into bacterial signalling phenomena were gained, revealing previously unsuspected layers of complexity, particularly in mechanisms ensuring signal transduction fidelity and novel links to metabolic processes.

Introduction

The 11th Bacterial Locomotion and Signal Transduction meeting gathered 156 scientists from 16 different countries in New Orleans' historical and cultural landmark French Quarter from 16 to 21 January 2011, to exchange and share the latest developments in the fields of chemotaxis, motility and sensory perception. BLAST XI was chaired by Bob Bourret (University of North Carolina, Chapel Hill) and Urs Jenal (Biozentrum, Basel, Switzerland) and organized by Sandy Parkinson (University of Utah), Joe Falke (University of Colorado), Philip Matsumura (University of Illinois-Chicago) and Michael Manson (Texas A&M University). One of the hallmarks of these meetings is the priority given to talks by graduate students and postdoctoral fellows, 33 of whom presented their work on this occasion, with a total of 49 talks given over 4 days.

Life is no bed of roses for bacteria, and their constant 'fight or flight' struggle to survive requires the ability to rapidly adapt their gene expression and behaviour to their surroundings. Among bacterial adaptive responses, chemotaxis is one of the most well-studied, and for several decades scientists have been unravelling the complex mechanisms involved in co-ordinating bacterial movement up or down nutrient and other attractant/repellant gradients. Two-component systems (TCSs), first discovered and defined as such 25 years ago (Ninfa and Magasanik, 1986; Nixon *et al*., 1986; Hess *et al*., 1987), play a central role in procaryotic signal transduction. TCSs relay signals via phosphorylation from a sensor kinase to a response regulator, thereby ultimately regulating the response output (e.g. a change in the direction of flagellar rotation, gene expression, protein stability, enzymatic activity, etc.) (Hoch and Silhavy, 1995; Bourret and Silversmith, 2010). Because they contribute to cellular adaptation to changes in their surroundings, these signal transduction systems constitute attractive targets for antimicrobial development.

The bacterial chemotaxis signal transduction pathway is arguably one of the best studied signalling systems in biology, coupling extracellular ligand sensing to flagellar rotation and bacterial movement (Fig. 1). Initially characterized using genetics, biochemistry and a set of motility assays to quantify the chemotactic response under various conditions, the function and interaction between most component signalling proteins of the enteric chemotaxis signalling pathway have been described and their structures are now known at atomic resolution (Hazelbauer *et al*., 2008). This has provided not only a fertile ground for mathematical modelling of enteric motility behaviour but has also stimulated further advances in

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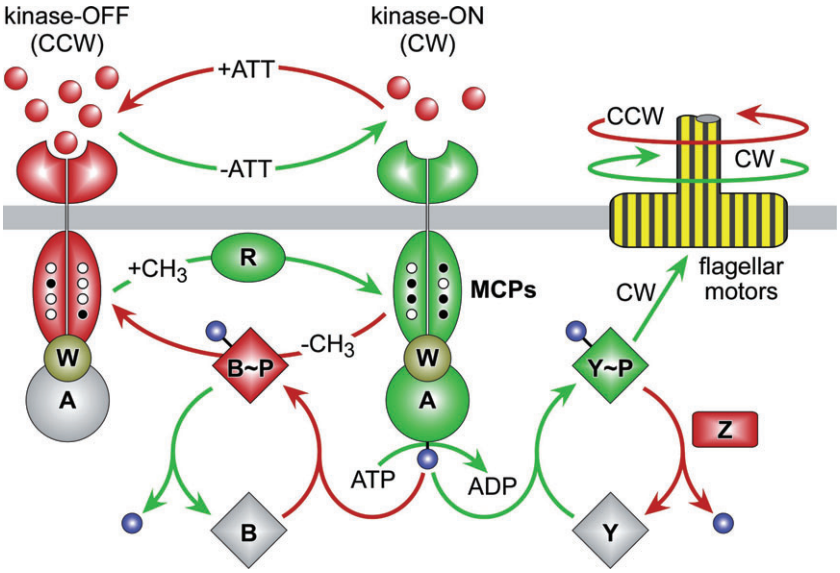

Fig. 1. Chemotaxis signal transduction in *Escherichia coli* and the two-state model for chemoreceptor signalling.
The activity of the chemotaxis signal transduction pathway ultimately regulates the direction of rotation of the flagellar motors by modulating the phosphorylation state of the CheY response regulator, which has increased affinity for the flagellar switch complex in the phosphorylated form. CheY is phosphorylated by activated CheA histidine kinase, which is coupled to the chemoreceptors (methyl accepting chemotaxis proteins or MCPs) via CheW. Attractant (+ATT) binding to MCPs has an inhibitory effect on the kinase activity of CheA (Kinase OFF), lowering the phospho-CheY pool, thereby promoting counterclockwise rotation (CCW) of the flagellar motors. Methylation of the cytoplasmic domains of MCPs by the CheR methyltransferase promotes the activating state of the CheA kinase (Kinase ON), increasing the pool of phospho-CheY and thus clockwise (CW) rotation of the flagella motors. Activated phospho-CheA also phosphorylates CheB, thereby promoting its methylesterase activity, allowing the MCPs to be reset to background methylation state. (Courtesy of Sandy Parkinson, University of Utah).

understanding of chemotaxis signal transduction in non-model microorganisms. The analysis of completely sequenced microbial genomes has revealed tremendous diversity in chemotaxis signal transduction with the number of pathways and receptors encoded within each genome being highly variable. In fact, such variability is a hallmark of two-component signal transduction systems in completely sequenced microbial genomes. At BLAST XI, Michael Y. Galperin (NCBI, NIH) reported that the total number of signal transduction proteins increases with the square of the genome size, correlating mostly with the lifestyle of the organism, with gene loss and duplications being the evolutionary driving forces for this distribution.

One of the greatest challenges in bacterial signalling lies in identifying the subtle cues that initiate the signalling cascade. In chemotaxis signal transduction systems, signals are not directly perceived by the histidine kinase, but instead by receptors that are usually embedded in the membrane, the methyl accepting chemotaxis proteins (MCPs) (Hazelbauer *et al.*, 2008). These MCPs form polar clusters of ordered arrays of hundreds of interacting receptors at the leading end of the moving cell that have been likened to a bacterial 'nose' (Parkinson and Blair, 1993). Although over three decades of research have established the sensory specificity of the five *Escherichia coli* chemotaxis receptors, Aer (aerotaxis and redox/energy taxis), Tsr (serine and energy taxis), Tar (aspartate and maltose taxis), Tap (dipeptide and pyrimidine taxis) and Trg (ribose and galactose taxis), the sensory specificity of MCPs encoded within the genome of most bacterial species remains unknown. At this year's BLAST meeting, the characterization of several receptors functioning in chemotaxis or two-component signal transduction was presented.

Signal transduction proteins contain several types of conserved domains involved in detecting and/or transferring information through conformational changes. Among these, PAS input domains (PER, ARNT, SIM) and HAMP (Histidine kinases, Adenylyl cyclases, Methyl-acceptingchemotaxis proteins, Phosphatases) transduction domains, found in several types of sensory proteins, have attracted considerable interest during the past few years due to their important roles in linking input signals to output responses (Taylor and Zhulin, 1999; Parkinson, 2010). The BLAST meeting focuses not only on signal transduction systems and their input signals, but also on the regulatory outputs, with the bacterial flagellar motor being a prime research focus. Despite decades of research, several fundamental questions regarding how flagellar motor rotation and proton flow through the stator generates the torque that ultimately propels the cells remain to be elucidated. For example, while flagellar motor torque has been suggested to be generated by a discrete stepping mechanism, the exact molecular events that

underlie these processes have yet to be clearly established. Several BLAST XI presentations reported the implementation of a series of methods and approaches that are providing valuable insight toward a detailed understanding of the underlying molecular mechanisms. In addition to signal processing and motor functions, several talks focused on intracellular signals and second messenger signalling that modulate the forward flow of phosphorylation, highlighting the emerging view of the complex contribution of metabolic signals in modulating phosphorylation-dependent signal transduction pathways.

Starting the engine: signal perception and acquisition

Our understanding of the molecular mechanism of signal detection by chemoreceptor proteins continues to develop. Mingshan Li from Jerry Hazelbauer's group (University of Missouri-Columbia) reported on the characterization and purification of core units of chemotaxis signalling complexes using Nanodiscs, plugs of lipid bilayers rendered soluble by a belt of amphipathic membrane scaffold protein (Bayburt and Sligar, 2003; Alami *et al*., 2007), which are proving to be very useful tools in biochemical characterization of chemoreceptors and signalling complexes (Boldog *et al*., 2006). By purifying Nanodiscs that contain a sufficient number of chemoreceptors to activate the chemotaxis kinase, M. Li was able to determine the extent and affinities of binding to receptors of the soluble components of signalling complexes. Using these Nanodisc-embedded receptors, he was able to purify kinase-activating complexes. These contained two trimers of Tar dimers, a single CheA dimer, and two CheW molecules (Fig. 2).

Ariane Briegel of Grant Jensen's group (California Institute of Technology, Pasadena) reported on recent developments using cryo-EM tomography to study bacterial chemoreceptor complexes. Annette Erbse from Joe Falke's group (University of Colorado) reported the development of a one-sample FRET protocol, which enables one to monitor conformational changes in an intact membrane-associated chemoreceptor complex at physiological conditions (Fig. 3). Using this method, they were able to demonstrate ligand-induced conformational change in the core signalling complex in which FRET label pairs on CheW and the P5 domain of CheA move apart without CheW release (Erbse *et al*., 2011).

Most of the diversity in chemotaxis signalling resides in the component protein repertoire, with the greatest variability seen in the number of receptors and the structure of the ligand binding domains involved in signal perception. Many sensory domains found in chemotaxis or sensor kinase receptors are conserved; however, most sensory cues detected by receptors remain to be identified. This

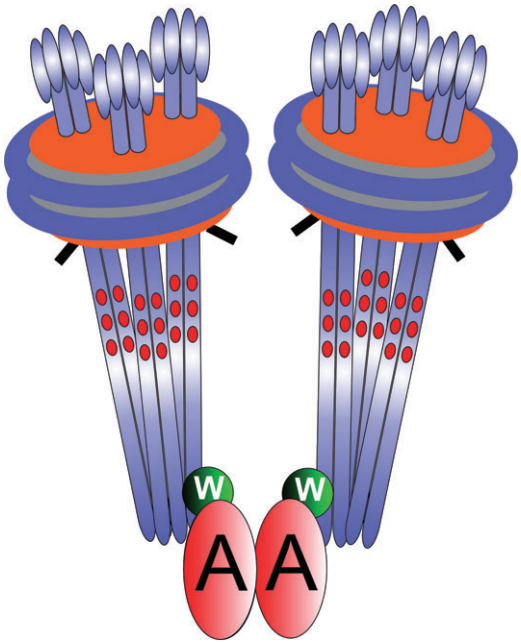

Fig. 2. Model of the core unit chemotaxis signalling complex purified with Nanodisc-inserted chemoreceptors.
The cartoon shows diagrammatically the stoichiometry and deduced organization of purified, kinase-activating core units of MCP/CheW/CheA, formed with Nanodisc-embedded Tar chemoreceptors, organized as trimers of dimers.

represents a significant knowledge gap as such information is a prerequisite to understanding the conditions that modulate the adaptation of microorganisms to changes in their surroundings. Tino Krell (Estación Experimental del Zaidín, Granada, Spain) reported the biochemical characterization and X-ray crystal structure of the ligand binding domain (LBD) of the McpS chemotaxis receptor from *Pseudomonas putida*. The TCA cycle intermediates succinate, fumarate, malate, oxaloacetate, citrate and isocitrate (but not structurally related compounds like maleate, aspartate, itaconate or tricarballylate) were bound by McpS, albeit with variable affinities ranging from 8–300 μM (Lacal *et al*., 2010; 2011). The X-ray crystal structure of McpS-LBD in complex with malate and succinate was also obtained and revealed two-stacked 4-helical bundles. Malate or succinate were both found to bind to the proximal bundle, whereas acetate to the distal bundle (Pineda-Molina, E. *et al*., submitted). Ligand binding occurs at the dimer interface and was found to stabilize the dimeric form of McpS-LBD, suggesting a signal transduction mechanism involving dimers, similar to that of *E. coli* chemoreceptors. Analysis of the distribution of McpS-LBD-like domains in completely sequenced genomes suggested that homologues are present in many different receptors found in a large number of bacterial species (Pineda-Molina, E. *et al*., submitted).

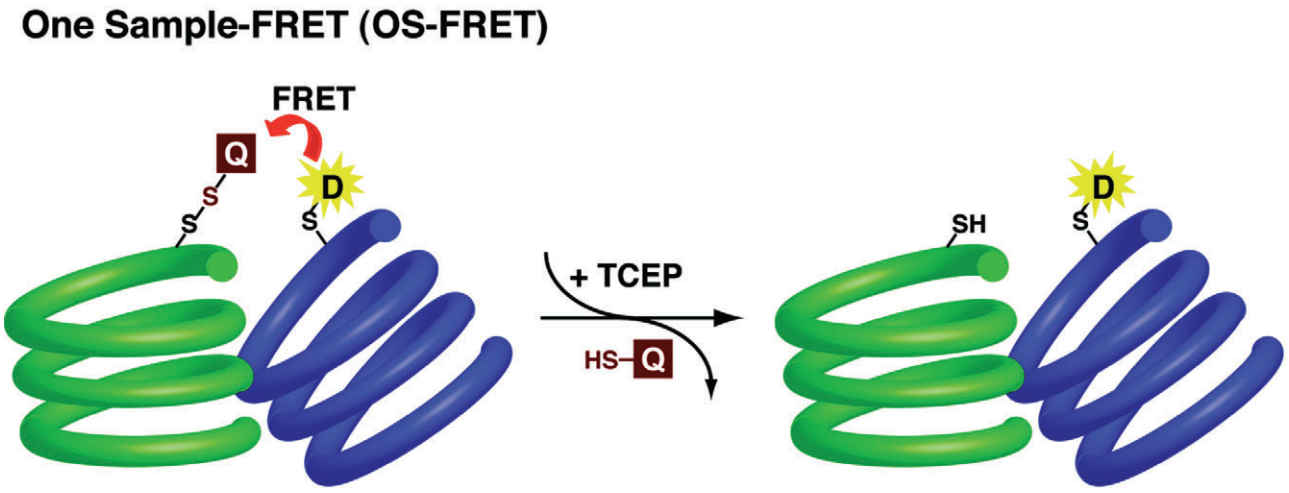

Fig. 3. One-sample FRET technique (OS-FRET).
The new OS-FRET method utilizes a fluorescent donor (D) and a novel non-fluorescent FRET acceptor NFQ1 (Q). NFQ1 is reversibly coupled to one protein and the donor is irreversibly linked to the second protein in the complex. After the first measurement in the presence of quencher and donor, reducing agent is added to quantify NFQ1 release and a second measurement is done on the same sample in the absence of FRET (Courtesy of Annette Erbse and Joe Falke, University of Colorado).

Using genetic and biochemical approaches, Matthew Russell (Gladys Alexandre's laboratory, University of Tennessee) described the role of the *Azospirillum brasilense* Tlp2 chemoreceptor in sensing nitrogenous compounds, including nitrate and nitrite, with apparent high affinity (350–460 nM range for both ligands). The *A. brasilense* Tlp2 sensory domain has no apparent similarity with other characterized nitrate sensors. Tlp2 orthologues are exclusively present in the genome of a subgroup of alphaproteobacteria, suggesting recent evolutionary divergence. In *Helicobacter pylori*, there are only four MCPs that all seem to be involved in colonization and infection of the gastric mucus. One of these receptors, TlpD, is a cytoplasmic protein, with no N-terminal sensory domain (Schweinitzer *et al*., 2008). TlpD has previously been shown to contribute to taxis responses to changes in cell's energy levels (e.g. proton motive force, or fluxes through the electron chain) (collectively termed 'energy taxis') (Schweinitzer *et al*., 2008); however, the mechanisms underlying the ability of TlpD to monitor such metabolic changes remain unknown. Karen M. Ottemann (University of California, Santa Cruz) presented data from her group indicating that the *Helicobacter pylori* TlpD chemoreceptor possesses a conserved C-terminal motif that was shown to tightly bind zinc. Genome analyses reveal that this domain is widespread in chemoreceptors and other signalling proteins, however, its function, if any, in TlpD energy taxis or other tactic behaviours remains to be determined. One hypothesis for the energy sensing mechanism mediated by the *H. pylori* TlpD intracellular taxis sensor is its interaction with enzymes involved in bacterial metabolic activities (Schweinitzer *et al*., 2008). This interaction is suggested to relay changes in enzyme function and conformation relating to alterations in energy production to the taxis core components (based on the concept of 'moonlighting' enzymes; for recent reviews, see (Jeffery, 2003; Huberts and van der Klei, 2010). Wiebke Behrens (Christine Josenhans's laboratory, Hannover Medical School, Germany), winner of the Robert J. Kadner Award for an outstanding poster presentation by a young investigator, presented data suggesting that TlpD interacts with several enzymes related to metabolism and the TCA cycle, including catalase (KatA) and aconitate hydratase (AcnB). Interestingly, *H. pylori* mutants lacking KatA or AcnB displayed changes in energy taxis responses, indicating a connection between TlpD – enzyme interactions and the energy sensing process.

In addition, the function of prototypical *E. coli* receptors in sensing unexpected chemical cues was presented. Hai The Pham (Sandy Parkinson's laboratory, University of Utah), reported the intriguing role of the Tsr and Tar chemoreceptors in mediating phenol taxis. Tsr senses phenol as a repellent while Tar senses phenol as an attractant (Imae *et al*., 1987; Yamamoto *et al*., 1990). A combination of approaches, including site-specific mutagenesis, characterization of chimeric receptors where the periplasmic domain of Tar or Tsr is replaced with that of other foreign receptors and soft agar plate assays suggested that the periplasmic ligand binding domain (LBD) of Tar or Tsr were not required for phenol sensing. This hypothesis was supported by the successful selection of non-LBD Tar mutants that could sense phenol as an attractant. On the other hand, a number of mutants where the Tsr HAMP domain is modified displayed positive taxis responses toward phenol instead of the expected repellent taxis response and in addition, the

second transmembrane and HAMP domain regions of the receptors were found to contribute to taxis responses. Together, the results obtained suggest that Tsr- and Tar-mediated phenol sensing occurs through stability changes in the HAMP domain of these receptors, thereby modulating the kinase and motility response.

Mike Manson (Texas A&M University) described another unexpected role for the *E. coli* Tsr chemoreceptor in sensing AI-2, a furanosyl borate diester autoinducer (Chen *et al*., 2002), which functions as a general quorum-sensing signal. AI-2 is bound by the LsrB periplasmic component of the Lsr ABC transporter system. Interestingly, AI-2 acts as a chemoattractant for both *E. coli* and *Salmonella typhimurium*, a finding that was confirmed in three independent spatial gradient assays, including a flow-based microfluidic device (Englert *et al*., 2010). Both Δ*tsr* and Δ*lsrB* mutants were found to be impaired for AI-2 chemotaxis. However, a Δ*lsrC* mutant defective for AI-2 uptake was still competent for AI-2 chemotaxis, indicating that AI-2 transport is not required for chemotaxis (Hegde *et al*., 2011). These results suggest that AI-2 chemotaxis may occur via a direct Tsr-LsrB interaction in the periplasm, a possibility supported by the existence of point mutations in *lsrB* that eliminate chemotaxis to AI-2 without affecting AI-2 uptake.

Ina Haneburger (Kirsten Jung's laboratory, Ludwig-Maximilians-University, Munich, Germany) presented the structure-function analysis, including the 1.8 Å resolution crystal structure, of the pH-responsive membrane-integrated transcriptional activator CadC from *E. coli*. CadC is a ToxR-like protein mediating *E. coli* adaptation to acidic stress, and its periplasmic domain ($CadC_{pd}$) is required for signal sensing and transduction. The crystal structure showed that the C-terminal domain of $CadC_{pd}$ is alpha-helical whereas the N-terminal domain is a mixed parallel and antiparallel β-sheet in contact with three α-helices. CadC likely functions as a dimer (Eichinger *et al*., 2011). Site-directed mutagenesis allowed the identification of several amino acids essential for pH sensing, clustered in a negatively charged surface patch, suggesting that changes in protonation of these residues and subsequent conformational changes provide the signal for activation of CadC (Haneburger *et al*., 2011).

Loo Chien Wang from Ganesh Anand's group (National University of Singapore), in collaboration with Linda Kenney's group (University of Illinois at Chicago), presented fascinating results using amide hydrogen/deuterium exchange mass spectrometry (HDXMS) to investigate conformational changes reflecting intramolecular signalling in the EnvZ histidine kinase osmosensor of *E. coli*. Their results indicate that the cytoplasmic soluble domain of the kinase is in itself sufficient to respond to changes in osmolality, and that these changes are first integrated at the four-helix bundle containing the His_{243} autophosphorylation site. ATP binding is not coupled to the osmosensing function. Their results suggest that in the case of EnvZ, contrary to the established dogma for membrane-bound histidine kinases, the transmembrane domains and extracellular loop may in fact be vestigial and that they are not required for its function as an osmosensor.

Tarek Msadek (Institut Pasteur, Paris, France) presented work from his group characterizing a novel TCS, BraS/BraR, which they showed to be involved in resistance of *Staphylococcus aureus* to bacitracin and nisin. The BraS histidine kinase belongs to the recently defined 'intramembrane-sensing kinase' (IMSK) subfamily, conserved in low G+C % Gram-positive bacteria and characterized by a very short amino-terminal sensing domain, composed of two transmembrane helices separated by a small loop of only a few amino acids, that is thought to be buried in the cytoplasmic membrane (Mascher, 2006). Several of these kinases have been shown to require an associated ABC transporter in order to effectively sense environmental signals (Coumes-Florens *et al*., 2011). Tarek's group showed that in response to bacitracin or nisin, BraSR controls the synthesis of two ABC transporters playing distinct and original roles in antibiotic resistance: BraDE is involved in bacitracin sensing and signalling through BraSR, whereas VraDE acts specifically as a detoxification module and is sufficient to confer bacitracin and nisin resistance when produced on its own (A. Hiron *et al*., submitted). In both cases, a functional ABC transporter nucleotide-binding domain appears to be required, and they showed that the long extracellular loop of the VraE permease confers its specificity in bacitracin sensing (A. Hiron *et al*., submitted).

Haifeng Geng reported on work from Robert Belas' laboratory (University of Maryland, Baltimore County), characterizing the mechanism involved in controlling biosynthesis of tropodithietic acid (TDA) by the marine bacterium *Silicibacter* sp. TM1040. This tropolone compound may play an important role in symbiosis between *Silicibacter* and the dinoflagellate phytoplankton *Pfiesteria piscicida*. Genetic studies allowed Haifeng to show that TDA acts as an auto-inducing quorum sensing signal among a subgroup of the marine *Roseobacter* clade. Furthermore, he discovered that TdaA, a LysR-family transcriptional regulator, directly binds to the promoter region of the *tdaCDE* TDA biosynthesis operon to activate its expression (Geng and Belas, 2010).

Putting it in gear: signal transduction

Ben Hall, from the groups of Judith Armitage and Mark Sansom (University of Oxford, UK), reported on molecular dynamic studies of the Tsr serine receptor. Using coarse grain simulations to access microsecond time scales, Ben presented evidence that the Tsr HAMP domain acts as a

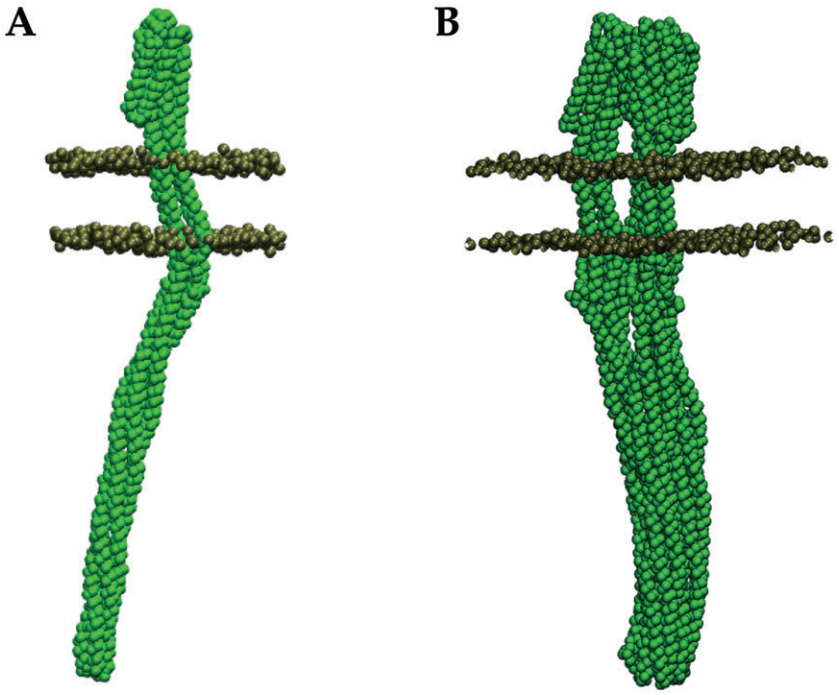

Fig. 4. Images of coarse grain models of a bacterial chemoreceptor in a membrane. Backbone particles are shown as green spheres and phosphate head groups as brown spheres.
A. Models of isolated Tsr dimers show a pronounced kink just below the HAMP domain.
B. Models of the trimer of dimers show a rod-like structure and reduced kinking.

conformational hinge point through which signals may be transduced across the cell membrane (Fig. 4).

Work (biophysical techniques, including crystallography) on the multi-HAMP containing structure of *Pseudomonas aeruginosa* Aer2 presented by Nattakan Sukomon from Brian Crane's laboratory (Cornell University) reveals two distinct HAMP conformations in serially arranged HAMP domains. This is consistent with Ben Hall's predictions, and provides more detailed molecular insight into HAMP function in signal transduction. Notably, each conformation appears to favour a specific direction of flagellar rotation. Kylie Watts (Barry Taylor's group, Loma Linda University) reported on an extensive biochemical analysis of PAS-HAMP interactions in *E. coli* Aer. Her study demonstrated interaction of a conserved HAMP helix with the β-scaffold region of the PAS structure. Her data are consistent with the emerging model in PAS signalling whereby signals are transduced from the core of the PAS domain across the conserved β-scaffold, often to a bound helix (Möglich *et al*., 2009).

Spinning the wheels: driving the flagellar motor

Smooth, directional bacterial swimming requires precise co-ordination of flagellar rotation. The flagellar motor is the actuator of swimming motility, generating mechanical torque to drive the rotation of flagellar filaments, the output of chemotaxis signalling, controlling this rotation in response to the intracellular concentration of phosphorylated CheY, [CheY-P]. Fresh ideas and novel experiments were presented, allowing a detailed understanding of both of these functional aspects, namely torque generation and control. On the control side, in a remarkable convergence, Hajime Fukuoka and Michael Sneddon presented new results from two groups on co-ordinated responses among the multiple flagellar motors of the peritrichously flagellated bacterium *E. coli*. Hajime Fukuoka (Akihiko Ishijima's laboratory, Tohoku University, Japan) reported on experiments in which the timing of reversals in rotation direction (switching) was monitored simultaneously for three motors, two on the same cell, and the third on another cell. No correlation of rotation direction was found between motors belonging to different cells, but rotation directions of flagellar motors on the same cell were found to be significantly correlated. This result is in stark contrast to an earlier result in filamentous cells, where no cross-correlation was found between motors separated by 3–47 μm (Ishihara *et al*., 1983). H. Fukuoka further noted in his cross-correlation data a characteristic lag of one motor relative to the other. The motor closer to the polarly localized receptor cluster tends to switch earlier than the motor further from it. H. Fukuoka proposed an interpretation in which both motors are responding to changes in [CheY-P], generated by the receptor clusters, and propagating through the cytoplasm as wave-like perturbations in [CheY-P].

What could be the practical implications of such multiple-motor co-ordination? An intriguing answer to this question was suggested by Michael Sneddon (Thierry Emonet's group, Yale University), who presented numeri-

cal simulations in which noise-driven co-ordination of motor switching, similar in principle to the Fukuoka proposal described above, could enhance chemotaxis despite the fact that the noise degrades chemotactic signal transduction. The key idea is that the distribution of run durations would be extended if motors tended to switch at the same time, rather than independently (the termination of a run requires only one flagellum to come out of the bundle in peritrichously flagellated *E. coli*) (Turner *et al*., 2000). The occasional long runs enhance the sensing of shallow chemoeffector gradients by allowing a cell to make comparisons of chemoeffector concentrations over longer distances, up to a threshold steepness that is determined by the swimming speed and adaptation timescale. The noise required for such motor co-ordination could plausibly arise from the 'intrinsic noise' in the adaptation system driven by CheR and CheB (Korobkova *et al*., 2004; Emonet and Cluzel, 2008).

In addition to control through [CheY-P], motor switching can be modulated by physical parameters, such as temperature (Turner *et al*., 1996) and mechanical load (Yuan *et al*., 2009). At this meeting, Masayoshi Nishiyama (Kyoto University, Japan) presented the effect of hydrostatic pressure on the switching behaviour of the flagellar motor. Working with a *cheY* mutant strain, which rotates exclusively counterclockwise (CCW) at atmospheric pressure and room temperature, M. Nishiyama showed that high pressure can induce clockwise (CW) rotation, with this rotational bias increasing progressively with the ambient hydrostatic pressure. At 20°C, the midpoint of this transition was found to be ~ 1200 bar, but this threshold decreased with temperature, down to ~ 300 bar at 5°C. Noting that the effect of increasing pressure closely resembles the effect of decreasing temperature, M. Nishiyama proposed that the thermodynamics of switching involves a free-energy difference between the CW and CCW states that is proportional to the ratio of pressure to temperature.

Switching from the thermodynamics of control to the mechanics of torque generation, Junhua Yuan (Howard Berg's group, Rowland Institute, Harvard University) presented experiments that highlight an asymmetry in rotation mechanisms between the CW and CCW states of the motor. The torque-speed relationship is important in discriminating between possible molecular mechanisms for motor rotation, and from previous work in *cheY* mutants (Chen and Berg, 2000), it is known that torque is a nonlinear function of speed for CCW rotation. CCW torque declines only slightly as the speed rises from zero to a characteristic 'knee speed', but it falls rapidly towards zero at higher speeds. Surprisingly, J. Yuan's new measurements using laser-darkfield microscopy in mutants overexpressing CheY revealed that the torque decreases linearly with speed for CW rotation. J. Yuan remarked that these differences in mechanics could reflect optimization for different purposes. CCW rotation determines swimming velocity, so the knee in the torque-speed relation could have arisen from selection for faster swimmers. CW rotation, on the other hand, is responsible for generating tumbles, the efficacy of which might be less dependent on the rotation speed.

Yuichi Inoue (Akihiko Ishijima's laboratory, Tohoku University, Japan) presented results of experiments that probed temperature effects on torque generation. Working with a chimeric motor powered by Na^+-driven stator units from *Vibrio alginolyticus* in *E. coli* (the native stator units of *E. coli* are H^+-driven), Y. Inoue found that transient heating over 40°C can induce stepwise changes in torque, suggesting dynamic changes in the number of stator units associated with the motor. When the heating was sustained for longer periods (10–20 min), similar stepwise changes in torque were observed, but with smaller step sizes. The maximum number of torque steps remained constant, however, in the range 10–13, consistent with previous estimates for the torque-generating stator units of ~ 11 (Reid *et al*., 2006).

Murray Tipping (Judith Armitage's group, Oxford University, UK) presented a new experimental approach for studying properties of the flagellar motor mechanics in which the proton-motive force across the plasma membrane, which drives flagellar rotation, can be controlled by light. This system of light-powering the cell is analogous to that of Walter *et al*. (Walter *et al*., 2007), but M. Tipping and colleagues have adapted the system for improved studies of the flagellar motor by combining it with an optical trap for probing motor output at high resolution. Using this system M. Tipping has been able to perform 'resurrection' experiments (restoration of rotation by incorporation of torque-generator stator units) on wild-type flagellar motors.

Recent discoveries on the molecular mechanisms of flagellar motor regulation were reported by several groups. Li Na of Michio Homma's group (Nagoya University, Japan) reported on a structure/function analysis of the PomB sodium-driven stator component from *Vibrio alginolyticus*. Her data provide evidence for a functional role of the periplasmic domain of PomB in assembly as well as in ion flux regulation of the PomA/PomB complex. Gabriel Zarbiv from Michael Eisenbach's group (Weizmann Institute, Israel) presented evidence that the F_0F_1 ATP synthase interacts with FliG of the *E. coli* flagellar motor and that this association affects rotary switching.

Bdellovibrio bacteriovorus are predatory bacteria that are unable to replicate as free-swimming cells, but grow within the periplasm of Gram-negative prey after infection. These bacteria are highly motile by means of a single polar flagellum and their rapid motility (swimming at

speeds of up to 160 μm s^{-1}) plays a major role in survival of free-swimming cells as well as during infection and release from the bdelloplast (the structure formed when the *Bdellovibrio* infecting the prey bacterium). The genome sequence of *B. bacteriovorus* contains multiple copies of flagellar biosynthesis and motility genes, providing further evidence for the essential role for motility in its predatory lifestyle (Lambert *et al*., 2006). Laura Hobley (Liz Sockett's laboratory, University of Nottingham, UK) described the functions of the three copies of MotAB motor pairs in *B. bacteriovorus* motility. Each of the three pairs of *motAB* genes encodes for proton-driven motors and while singly deleted cells were still motile, swimming speeds were reduced. Interestingly, slower motility impacted the overall predation ability of *B. bacteriovorus*, but each single *motAB* deletion strain was still capable of predation. These results indicate that all three gene pairs, some of which were likely acquired by lateral gene transfer, contribute to flagellar motor function (Morehouse *et al*., 2011). Further analysis of the genome sequence has revealed additional genes encoding GGDEF domain proteins that may also modulate flagellar motility.

Beyond the paradigm: variations on a theme

Recurrent themes during BLAST XI were second messenger signalling and the realization not only that multiple pathways for response regulator phosphorylation exist, through small metabolic phosphodonors (e.g. acetyl~P) rather than by kinases, but also that other post-translational modifications can be equally important in modulating response regulator activity, including protein acetylation. Among several bacterial nucleotide-based second messengers, bis-(3′-5′)-cyclic dimeric GMP (c-di-GMP) has recently emerged as a major modulator of numerous signal transduction pathways, and is a key determinant in controlling the switch between planktonic and sedentary bacterial lifestyles (Schirmer and Jenal, 2009). C-di-GMP has been recently shown to directly affect *E. coli* swimming speed and to slow flagellar rotation by binding to YcgR, a protein that interacts with the flagellar motor and/or switch proteins (Boehm *et al*., 2010; Fang and Gomelsky, 2010; Paul *et al*., 2010). However, the exact molecular mechanisms by which c-di-GMP bound to YcgR acts to slow down flagellar rotation remains to be elucidated. Working in *Salmonella*, Vincent Nieto of Rasika Harshey's group (University of Texas at Austin), reported that the 'backstop brake' effects of YcgR on the flagellar rotation that lead to decreased swimming speed occur in a series of events: the motor first shifts to a CCW bias followed by a reduction in speed before coming to a complete halt. This would support the model proposed by this group whereby YcgR bound to c-di-GMP interacts with the flagellar switch protein FliM, before disrupting motor function, perhaps via an effect on the FliG/MotA interaction.

Varisa Huangyutitham (Caroline Harwood's group, University of Washington) presented data on the subcellular localization of the *P. aeruginosa* WspR hybrid response regulator/diguanylate cyclase. Phosphorylation of WspR, controlled by an alternative chemotaxis-like pathway, occurs in response to a surface-associated signal and increases its catalytic activity. Their results indicate that phosphorylated WspR is preferentially localized as subcellular non-polar cytoplasmic clusters, which may serve to enhance its activity, leading to increased c-di-GMP production and consequent biofilm formation. Claudine Baraquet (Caroline Harwood's group, University of Washington) received the Nucleic Acids Research Award for outstanding poster presentation by a young investigator for her ongoing work on the molecular mechanism by which c-di-GMP modulates activity of the *P. aeruginosa* FleQ transcriptional regulator at the *pel* operon promoter. Previous work has shown that FleQ represses expression of the Pel polysaccharide genes and that this repression is relieved by c-di-GMP (Hickman and Harwood, 2008). Using DNase I footprinting and site-directed mutagenesis, she showed that FleQ is not only a repressor of *pel* expression in the absence of c-di-GMP, but also an activator of *pel* expression in the presence of c-di-GMP. They propose that in the absence of c-di-GMP FleQ represses *pel* expression by impairing RNA-polymerase binding, but that the association of FleQ with c-di-GMP induces a conformational change which switches FleQ from a repressor to an activator, allowing recruitment of RNA polymerase and *pel* gene expression.

Joseph Boll (David Hendrixson's group, University of Texas Southwestern) presented a detailed analysis of the *Campylobacter jejuni* NtrC-like FlgR response regulator. He showed that the C-terminal domain (CTD) of FlgR, usually involved in DNA-binding activity, was surprisingly not required *in vivo* for activation of σ^{54}-dependent flagellar gene expression. Indeed, $FlgR_{\Delta CTD}$, lacking the C-terminal domain, was as effective as the full-length response regulator in activating transcription and furthermore did not require the presence of the cognate histidine kinase FlgS, in contrast to intact FlgR. Instead, their genetic results indicate that in the absence of the CTD, $FlgR_{\Delta CTD}$ is phosphorylated by intracellular pools of the small molecule phosphodonor acetyl phosphate, and that the CTD in fact acts unconventionally as a specificity determinant, ensuring FlgR is only phosphorylated *in vivo* by FlgS, effectively preventing phosphorylation by metabolic phosphodonors or non-cognate histidine kinases.

Protein N-acetylation has recently emerged as a significantly widespread post-translational modification of bacterial proteins, whose importance had previously been

considerably underestimated (Hu *et al*., 2010). Indeed, over 140 *E. coli* proteins have been shown to be acetylated (Yu *et al*., 2008; Zhang *et al*., 2009), including the CheY response regulator (Yan *et al*., 2008; Li *et al*., 2010). Linda Hu (Alan Wolfe's laboratory, Loyola University Chicago) presented studies aimed at understanding *in vivo* mechanisms that control activity of the *E. coli* RcsB response regulator. RcsB, a member of the RcsC/RcsD/RcsB phosphorelay, is required for the expression of the small RNA gene *rprA*, which increases *rpoS* translation (Majdalani *et al*., 2002; Majdalani and Gottesman, 2005). L. Hu presented results showing that RcsB can activate *rprA* transcription in a RcsC sensor kinase-independent manner. Using genetic and biochemical approaches, including mass spectrometry, she presented evidence supporting the hypothesis that acetylation of RcsB inhibits activation of *rprA* transcription. She showed that RcsB is acetylated *in vivo* on at least three lysine residues. She also showed that overexpression of the SpeG acetyltransferase or reduced activity of the CobB deacetylase diminished RcsC-independent *rprA* transcription. Finally, she proposed a mechanism by which two different posttranslational modifications (phosphorylation and acetylation) could calibrate RcsB function.

For better or for worse: ensuring partner fidelity in signal transduction interactions

One of the challenges in studying two-component signal transduction in microorganisms sometimes lies simply in determining which cognate histidine kinase is responsible for phosphorylation of a given response regulator. Conserved genomic gene order often suggests the corresponding proteins interact (Dandekar *et al*., 1998). Indeed, in most bacteria, genes encoding the histidine kinase and response regulator of a TCS are adjacent and usually organized as operons. In *Myxococcus xanthus* however, over half of the response regulator and histidine kinase genes are so-called 'orphans' as they are not colocalized (Shi *et al*., 2008). In an elegantly designed study, Tobias Petters (Lotte Søgaard-Andersen's laboratory, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany) presented experiments aimed at addressing this question in *M. xanthus*. They began by studying the SgmT hybrid kinase, which they showed to be required for *M. xanthus* S-motility and extracellular matrix accumulation. Their results suggested that signal detection via the SgmT GAF domain is the primary sensory input controlling kinase activity. In parallel, they studied the DigR orphan response regulator, also required for extracellular matrix formation, showing that Δ*digR* mutant phenotypes mirrored those of the Δ*sgmT* mutant. Finally, using a combination of transcriptome analyses of strains lacking either SgmT or DigR, and *in vitro* protein phosphorylation, their results indicated that SgmT is the cognate kinase for the DigR response regulator.

In a related approach, Sonja Pawelczyk (George Wadham's laboratory, University of Oxford, UK) sought to identify the cognate response regulator for the *Rhodobacter sphaeroides* orphan histidine kinase RSP_0203. Noting that this kinase presented similarities in its response regulator specificity residues and carboxy-terminal domain with EnvZ of *E. coli*, they demonstrated *in vitro* phosphotransfer between the purified kinase domain of RSP_0203 and RSP_1138, a *R. sphaeroides* orphan response regulator sharing similarities with OmpR of *E. coli*. They also showed that interspecies cross-talk can occur between the *E. coli* EnvZ/OmpR TCS and RSP_0203/RSP_1138 of *R. sphaeroides*, both *in vitro* and *in vivo*.

Putting on the brakes: phosphatase activity and switching off the response

Just as important as triggering a response are the mechanisms involved in switching it off and ensuring it does not persist beyond the appropriate response time frame. This was the topic of several reports during BLAST XI. Tu-Anh Huynh (Valley Stewart's laboratory, University of California, Davis), winner of the BLAST Board of Directors' Award for outstanding presentation, reported a detailed genetic and biochemical analysis of kinase-directed response regulator phosphatase activity in the NarX/NarL system of *E. coli* (Huynh *et al*., 2010). They demonstrated that a conserved DXXXQ motif, immediately adjacent to the phosphorylation site of the HisKA_3 subfamily of histidine kinases, plays an essential role in NarX-dependent dephosphorylation of NarL, and extended their conclusions to other members of this subfamily.

Paphavee Lertsethtakarn of Karen Ottemann's group (University of California, Santa Cruz) reported that the remote CheZ orthologue, $CheZ_{HP}$, plays a critical role in *Helicobacter pylori* chemotaxis; its function as a regulator of motility requires structural regions outside of the conserved active site. Moreover, $CheZ_{HP}$ localizes to the cell pole by a mechanism that differs from *E. coli* CheZ. Analysing the diversity within CheY response regulator sequences retrieved from prokaryotic genomes led Kristin Wuichet (Igor Zhulin's group, University of Tennessee) to highlight that their diversity is reflected in the experimentally characterized CheY proteins. Furthermore, CheY phylogenies appear to correlate with functional diversification, with some of the CheY groups suggested to be associated with specific phosphatases.

Biophysics/mathematical modelling

Bacterial motility and chemotactic signalling has long been fertile ground for the development of novel biophysi-

cal experiments and theories, and the wealth of genetic, biophysical and structural data accumulated over the past decades has made this system particularly amenable to mathematical modelling (reviewed in Tindall *et al.*, 2008a,b). This year's BLAST meeting featured, in addition to the many detailed physical studies of the flagellar motor (see above), an array of experiments and theories that provide a 'bigger picture' view of motility and chemotaxis.

Laurence Wilson (Rowland Institute at Harvard) described two new microscopy techniques he developed for high-throughput characterization of the motility of microorganisms at the population level. These methods are based on simple instrumentation: a high-speed camera (> 1000 fps) mounted on a standard microscope, plus a computer for offline image analysis. Differential dynamic microscopy (DDM) is similar in principle to light scattering techniques commonly used in physics to study colloidal suspensions or polymer solutions. But DDM is better suited for studying microbial motion (mean and variance of velocities) as the use of a camera (instead of a photomultiplier tube) provides access to motion information over a greater range of length scales ($\sim 0.63\ \mu m < l < \sim 630\ \mu m$), and also has the advantage of simpler instrumentation (it uses brightfield illumination, instead of a laser beam). Darkfield flicker microscopy (DFM) uses the same instrumentation, but with darkfield illumination instead of brightfield, and can be used to simultaneously measure rotation rates of flagella and the cell bodies. Both DDM and DFM are very fast: measurements of a few seconds, plus up to 10 min of offline image processing by the software suffice to characterize a population of ~ 5000 bacteria.

In another study that addressed population-level phenomena, Yuhai Tu (IBM T. J. Watson Research Center) presented results of *E. coli* chemotaxis experiments in microfluidic devices that allow temporal modulation of spatial chemoeffector gradients. By periodically modulating the gradient over time and observing the migratory behaviour of swimming cells, it was found that the chemotactic response at the population level is highly frequency dependent – at low rates of temporal modulation, the cells can fully respond to the gradient, but at high modulation rates, the gradient is reversed before the cells can adjust their behaviour appropriately. The half-maximal population response, which defines a cut-off for this low-pass behaviour of chemotactic *E. coli* populations, was found to occur at a modulation period of ~ 200 s. Using simulations of swimming cells that behave according to a coarse-grained models of intracellular signalling (Tu *et al.*, 2008), Y. Tu showed that this frequency-dependent behaviour can only be achieved if the correct time scale of receptor methylation in the adaptation system, as measured by FRET (Shimizu *et al.*, 2010), is included in a population-level model of chemotaxis (Keller and Segel, 1971).

Further insights on adaptation were presented by Ganhui Lan (Yuhai Tu's laboratory, IBM T. J. Watson Research Center), who presented results on the energetic costs of sensory adaptation. Observing that reducing errors in the precision of adaptation requires a 'regulatory flux' within a non-equilibrium cycle driven by energy input, G. Lan proposed a simple mathematical expression $\varepsilon = \varepsilon_0 e^{-\alpha \dot{W} \tau / k_B T}$, which he calls the energy–speed–accuracy (ESA) relationship. It describes the trade-offs that exist for systems that adapt by maintaining regulatory fluxes at steady state – to achieve a low adaptation error, ε, one needs high energy dissipation, $\dot{W} / k_B T$, slow adaptation (i.e. a long adaptation time scale, τ), or both. The relation suggests that maintaining the same accuracy under reduced energy dissipation requires adaptation to slow down. This prediction was tested in FRET experiments in *E. coli* – when cells starved of energy sources were repeatedly exposed to step stimulations, the adaptation rate decreased, while the precision of adaptation was unaffected.

Pushkar Lele (Howard Berg's laboratory, Rowland Institute, Harvard University) won the Robert M. McNab Award for an outstanding poster presentation by a young investigator, in which he described experiments using optical traps to study how the dynamics of hydrodynamically interacting microscopic objects are affected by nearby surfaces. P. Lele's measurements indicate that contrary to what was previously believed, these interactions are long-range even when close to a wall. These effects can prove non-trivial, especially when measuring the dynamics of multiple, closely spaced flagellar motors with beads. Such experiments can form the basis for studying more complex behaviour of motile bacteria near surfaces.

Slip sliding away: other modes of bacterial motility

Bacterial flagella rotate to propel the cells forward in liquid environments. What happens when motile cells contact a surface? The observation most often is that cells tend to swim for longer periods of time in contact with the surface and Guanglai Li (Jay Tang's laboratory, Brown University) presented results suggesting that near-surface accumulation of swimming bacteria is mediated by rotational Brownian motion and low Reynolds number hydrodynamic collision of cells with the surface. The collision initiates the accumulation, while the rotational Brownian motion relaxes it. The combined effect of these two factors is the observed cell density distribution. These results led G. Li to propose that rotational Brownian motion, which has long been ignored, likely plays a critical role in bacterial adhesion (Li and Tang, 2009).

Once in contact with surfaces, many flagellated bacteria are capable of moving across them using flagellar motility in a manner called swarming (Kearns, 2010).

Swarming cells move within a micron-thick fluid film that covers the entire colony (Zhang *et al*., 2010). Using an ingenious approach where microbubbles (formed by explosion of the non-ionic water-insoluble surfactant Span83) were made on agar surfaces ahead of a swarm, Yilin Wu and his colleagues (Howard C. Berg laboratory, Rowland Institute, Harvard University) were able to follow the motion of the swarm fluid. They observed an extensive stream (or river) flowing CW along the advancing edge of an *E. coli* swarm, at speeds on the order of 10 μm/s, about three times faster than the swarm expansion. Moreover, the fluid was swirling CCW in between multiple cells but circulating CW around individual cells. Similar chiral flow patterns were observed in swarming *Bacillus subtilis* and *Serratia marcescens*, suggesting that the patterns were general features of fluid dynamics in flagellated bacteria swarms. Data from combined phase contrast and epifluorescent microscopy suggest that the observed flow patterns are generated by the action of CCW rotating flagella of cells stuck to the substratum. The results provide a mechanistic insight into the hydrodynamics that contribute to swarm expansion and suggest an avenue for long-range communication in the swarming colony by fluid flows (Wu *et al*., 2011).

Myxococcus xanthus are predatory bacteria capable of self-organizing in travelling density waves of motile cells, called ripples. The ripples form when cells are starved (developmental ripples) (Sager and Kaiser, 1994; Igoshin *et al*., 2001) or move on top of prey bacteria (predatory ripples), such as *E. coli*. In ripples, gliding *M. xanthus* cells reverse direction of movement when they come in contact with other cells and side-by-side contact was shown to increase the probability of these reversals (Mauriello *et al*., 2010). However, how these contacts generate co-ordinated travelling waves of cells in ripples (Berleman *et al*., 2006; 2008) was not completely clear. Haiyang Zhang (Oleg Igoshin's Laboratory, Rice University) presented an agent–based model that could reproduce experimental observations of individual and group behaviours of *M. xanthus* cells during rippling. The model provided further quantitative support for the hypothesis that rippling behaviour allows *M. xanthus* cells to cover the prey faster and thus to ingest nutrients released from lysis of the prey for longer period of times (Berleman *et al*., 2006; 2008).

Both the social (S)-motility and the adventurous (A)-motility of *M. xanthus* contribute to the many facets of the complex lifestyle of this soil bacterium. While S-motility involves the retraction of type IV pili and groups of cells moving together, A-motility allows the movement of isolated cells (Mauriello *et al*., 2010). Large multiprotein complexes that span the membrane and the periplasmic space have been shown to be involved in A-motility, and unexpectedly, these complexes appear stationary, during forward movement of cells (Mignot *et al*., 2007; Nan *et al*., 2010). These and other observations have led to the proposal that gliding *M. xanthus* cells move by A-motility by rotating large mutliprotein complexes along internal helical tracks (Mignot *et al*., 2007). However, the motor for A-motility has remained enigmatic. By analysing the subcellular dynamic localization of an essential A-motility protein, AgmU, Beiyan Nan (David R. Zusman laboratory, University of California, Berkeley) showed that membrane-associated AgmU assembles along helical structures that rotate as the cell glides and reverse their rotation when cells reverse direction of gliding. Using metabolic inhibitors, including proton motive force inhibitors, Nan further showed that the rotation of the AgmU helix did not depend on ATP but on the DpH component of the proton motive force (Nan *et al*., 2011). This rotation also requires functional MreB cytoskeletal filaments (Nan *et al*., 2011). When integrated into a mechanochemical model, the data suggest that PMF-driven motors similar to the MotAB bacterial flagella stator complexes move along helical tracks, creating surface waves that generate sufficient drag for gliding A-motility (Nan *et al*., 2011). Cell-to-cell interactions are also involved in both A- and S-motility in *M. xanthus* and lipoproteins (TgI and CglB) were previously shown to be transferred between cells and are required for motility. Daniel Wall (University of Wyoming) reported the identification of type II signal sequences, found in TgI or CglB, as being sufficient for heterologous protein transfer between cells. Interestingly, lipoprotein transfer occurred exclusively between aligned cells, suggesting specific cell orientation contacts were required for transfer. The identification of a *trans* factor required for protein transfer was also reported.

Another non-flagellated, non-piliated gliding bacterium, *Flavobacterium johnsoniae*, of the phylum Bacteroidetes crawl over surfaces by an apparently novel mechanism. Mark McBride and Ryan Rhodes (University of Wisconsin-Milwaukee) presented a model to explain *F. johnsoniae* gliding motility (Fig. 5). In this model adhesins, such as SprB, are propelled rapidly along the cell surface by motors, composed of Gld proteins, embedded in the cell envelope (Nelson *et al*., 2008). Most of the known components of the gliding machinery are unique to members of the phylum Bacteroidetes. A dedicated protein secretion system (PorSS) unrelated to Type I-VII protein secretion systems, is required for surface localization of SprB (Rhodes *et al*., 2010; 2011). A related PorSS is required for secretion of virulence factors in the non-motile bacteroidetes, *Porphyromonas gingivalis* (Sato *et al*., 2010). Transposon mutagenesis and analysis of strains with multiple gene deletions revealed apparent redundancies in some cell surface components of the motility machinery. SprB exhibits partial redundancy with the cell surface lectin RemA (redundant motility protein A). SprB

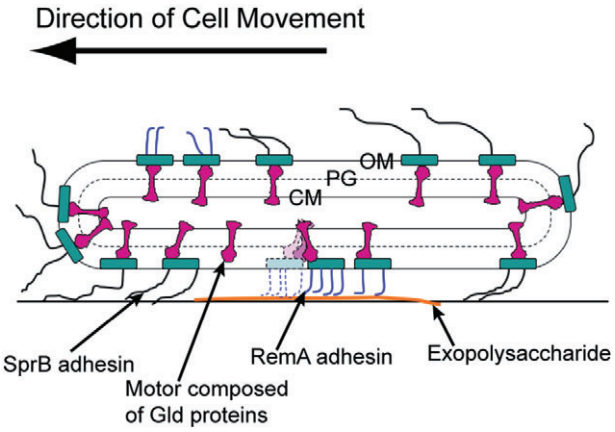

Fig. 5. Working model of *Flavobacterium johnsoniae* gliding motility.
Gld proteins in the cell envelope are thought to form the motor (pink) that propels adhesins, such as SprB (black) and RemA (blue), along the cell surface. Exopolysaccharides (orange) enhance motility by coating the substratum and interacting with specific adhesins. OM, outer membrane; CM, cytoplasmic membrane; PG, peptidoglycan (Courtesy of Mark McBride, University of Wisconsin).

and RemA may function as mobile adhesins that allow movement over different surfaces. Mutations in genes involved in polysaccharide synthesis and secretion resulted in motility defects that were similar to those of *remA* mutants. Exopolysaccharides may interact with RemA lectin to facilitate movement over some surfaces.

Global regulatory networks

Several groups presented data from recent studies on global regulators of cellular development and physiology. Maren Schniederberend of Barbara Kazmierczak's group (Yale University) presented a genetic and biochemical analysis of the flagellar assembly regulator, FlhF, of *P. aeruginosa*. Her study provides evidence that FlhF is a slow GTPase that is inhibited, rather than activated, by Mg^{2+}.

David Hendrixson (University of Texas Southwestern) reported results demonstrating that the *C. jejuni* FlhF GTPase and FlhG ATPase control flagellar number and placement, with FlhG performing a second role in cell division. FlhG negatively controls the activity of FlhF, which acts positively on flagellar biosynthesis, and together they co-ordinate production of a single flagellum at the new pole following cell division. David's group has shown that Δ*flhG* mutants produce minicells reminiscent of mutations in the *min* genes of *E. coli* or *B. subtilis*. Their results indicate that FlhG may act similarly to MinD in *C. jejuni*, and that polar flagellum biosynthesis could be involved in proper septum placement and cell division.

Penelope Higgs (Max Planck Institute for Terrestrial Microbiology, Marburg, Germany) presented studies aimed at understanding the roles of several histidine kinases that negatively control progression through the *M. xanthus* developmental program: the EspC and EspA hybrid kinases, TodK, an orphan histidine kinase and RedE, associated with a stand-alone receiver domain response regulator. Their results indicate that these negative regulators play an important role by preventing inappropriately rapid accumulation of MrpC and FruA, two transcriptional regulators necessary for promoting development. The resulting gradual accumulation of MrpC and FruA ensures that the cells complete aggregation into fruiting bodies before inducing sporulation.

Birgit Prüß (North Dakota State University) reported transcriptome analyses of the FlhC regulon in the O157 : H7 enterohemorrhagic strain of *E. coli*. Seeking to establish physiologically relevant conditions to study the role of this pleiotropic regulator, with an aim to develop effective treatments to limit transmission through contaminated meat products, they carried out microarray experiments using cDNA prepared from RNA isolated from the parental and Δ*flhC* strains grown on a meat surface. Their results indicate that FlhC controls expression of 287 genes, affecting functions including virulence, biofilm development, metabolism, transport, cell division or proteolysis, and that the Δ*flhC* mutant reached higher cell densities than the parental strain and displayed significantly increased biofilm formation and virulence.

Don Walthers (Linda Kenney's group, University of Illinois at Chicago), in collaboration with Jie Yan's group (National University of Singapore), used DNaseI footprinting and *in vitro* transcription to show that the *Salmonella enterica* SsrB response regulator directly activates transcription of the *Salmonella* pathogenicity island 2 type three secretion system effector genes *sifA*, *sifB* and *sseJ*, which are subject to H-NS-dependent gene silencing. Through single molecule experiments with magnetic tweezers, they showed that in addition to acting as a positive regulator of transcription, SsrB also acts by relieving gene silencing through displacement of H-NS bound in polymerization mode, but not when it is bound in bridged form (Walthers *et al.*, 2011).

Conclusions/future directions

Research on bacterial signal transduction is providing deep molecular and quantitative insights regarding general principles of cellular information processing. As illustrated by the presentations at BLAST XI, traditional genetic and biochemical approaches combined with novel analytical and imaging technologies promise to push us to new heights of understanding, from the molecular to the systems level.

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