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Meeting Review

BLAST 1995: International Conference on Bacterial Locomotion and Signal Transduction

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Overview

The third international conference on Bacterial Locomotion and Signal Transduction (BLAST III) was held January 12–16, 1995, in Austin, Texas. Participants were treated to a diverse array of 56 oral- and 104 poster presentations on current research in bacterial sensing and motility. Overall, this field continues to illustrate the power of combining genetic, biochemical, and physical methods to elucidate the fundamental mechanisms underlying cellular responses to sensory and metabolic stimuli. Together, such diverse approaches are generating what is likely to become the first truly molecular picture of cellular behaviour, in which the macroscopic movements and physiological responses of single bacterial cells can be described in terms of specific chemical and mechanical events within receptors, signalling proteins and motors of increasingly well-understood structure.

On the sensing and signalling front, diverse bacterial pathways with eukaryotic homologues continue to emerge. One focus of the meeting was the histidine kinase class of pathways which appears to be ubiquitous in bacteria, and widespread in eukaryotes. Pathways of this class possess up to four signalling components (Fig. 1): (i) a sensor or receptor, often containing putative membrane-spanning regions; (ii) a histidine kinase regulated by the sensor; (iii) a response regulator which the kinase targets phosphorylation on an aspartyl residue; and (iv) a protein which stimulates dephosphorylation of the kinase or response regulator, functionally termed a phosphatase. As discussed below, all four components have sparked new studies addressing fundamental structural, mechanistic, and functional questions. New evidence also points to the existence of prokaryotic G-protein and Ca^{2+} signalling pathways: *Myxococcus xanthus* possesses a 22 kDa G-protein required for motility and development which can be complemented by the SAR1 G-protein of yeast (Hartzell, University of Idaho); while intracellular Ca^{2+} signals of as yet unknown function have been detected by Ca^{2+}-sensitive fluors when *E. coli* cells are exposed to attractants and repellents (Tisa and Adler, University of Wisconsin).

On the motility front, new research continues to provide an increasingly molecular view of bacterial motors and propellers, including their structure, function, and assembly. These motility systems, together with their regulating sensory pathways, enable prokaryotic cells to exhibit an astonishing array of behavioural patterns and developmental processes. *Myxococcus xanthus*, for example, aggregates to form fruiting bodies of striking complexity (Sogaard-Andersen, Slack and Kaiser, Stanford University; Shi and Zusman, University of California, Berkeley). *Salmonella typhimurium* can swim alone in liquid media or zip down crowded bacterial highways on an agar surface (Harshey, University Texas). Marine *Escherichia coli* can, under proper conditions, form patterns in semi-solid agar as complicated and symmetric as grandmother's quilt (Budrene and Berg, Harvard University; see the cover figure). *Vibrio* cells can 'turn on the lights' of chemiluminescence in other cells of the same species (Bassler, Princeton University; Stevens, Dolan and Greenberg, University of Iowa). It is clear that many of these behaviours are regulated, in whole or in part, by histidine-kinase signalling pathways that control cellular motors or gene expression.

The remainder of this report describes new findings concerning the individual components of such signalling pathways, as well as the motility and gene expression systems that ultimately provide the appropriate response to changing environmental conditions. Most studies have focused on *E. coli*, *S. typhimurium*, *M. xanthus* or *Halobacterium salinarium*, but an array of other important organisms adds spice to the main courses.

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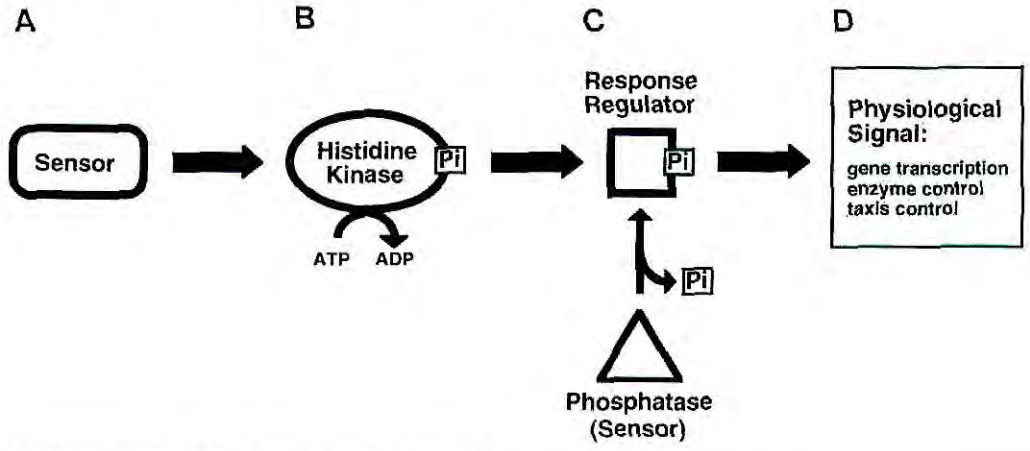

Fig. 1. The four components of histidine kinase signalling pathways.
A. The sensor protein monitors key environmental or intracellular stimuli. This protein is often, but not always, a receptor containing putative transmembrane segments.
B. The histidine kinase, which is regulated by one or more sensors, autophosphorylates an essential histidine residue.
C. The phosphate is transferred to a conserved aspartate residue of the response regulator, which subsequently triggers the appropriate physiological signal.
D. In some pathways, a fourth protein, functionally termed a phosphatase, stimulates dephosphorylation of the kinase or response regulator. Additional regulatory elements may modulate this phosphatase activity.

Receptors

Ligand recognition

Reported advances in the molecular understanding of bacterial receptors focused on three general areas: (i) ligand recognition, (ii) transmembrane signalling, and (iii) kinase regulation. In the chemosensory systems of *E. coli* and *S. typhiumurium*, schematically illustrated in Fig. 2, many ligands are first recognized by a class of periplasmic, water-soluble receptors termed binding proteins. Each binding protein engulfs a specific ligand in the deep cleft between its two domains. Following the association event, the occupied binding protein diffuses to the surface of the inner membrane, where it either activates a transmembrane chemotaxis receptor or passes the ligand to a transporter which carries it across the cytoplasmic membrane.

A general question regarding the function of cleft proteins and enzymes, including the binding proteins, concerns how the dynamics of cleft opening and closing are controlled. Such dynamics might play an important role in defining the kinetics of signal activation and inactivation, especially for the binding proteins which can exhibit quite long ligand-residency times ($\geqslant 1$ s). Crystallographic and small-angle X-ray scattering methods have been used to compare the open and closed conformations of the maltose-, ribose- and galactose-binding proteins, providing structural constraints on the open and closed forms in crystals and solution (Mowbray, Bjorkman, Flocco and Shilton, Upssala Biomedical Centre; Fig. 3A). The fraction of time spent in the open, empty form remains under investigation, but it has been proposed that the stability of this conformation in solution is controlled by interactions between the two domains.

Mutagenic studies continue to elucidate which faces of the binding proteins dock to the appropriate receptor or transporter, respectively (Eym, Park, Kim and Park, Korea Advanced Institute of Science and Technology (KAIST)). Recent work has employed a hybrid transmembrane receptor, in which the periplasmic domain of the receptor Trg is attached to the cytoplasmic kinase domain of the EnvZ receptor, permitting use of transcriptional reporters to detect interaction with ribose-binding protein. Studies of binding proteins may be relevant to other signalling systems using similar motifs to recognize ligands; for example, the ligand binding domain of the ionotropic glutamate receptor from vertebrate neurons is proposed to possess the same structural framework as the binding proteins (Sternback *et al.*, 1994, *Neuron* **13:** 1345).

Transmembrane signalling

The transmembrane chemotaxis receptors of *E. coli* and *S. typhimurium* are thought to be localized primarily at one end of the rod-shaped cytoplasmic membrane, although the cell orients this 'nose' either toward or against the direction of swimming (Turner and Berg, Harvard University). Chemotaxis receptors are homodimeric structures in which four α-helices, two provided by each subunit, span the membrane. The existing model for the packing of the transmembrane helices developed in several laboratories (Koshland, University of California, Berkeley; Simon, California Institute of Technology; Hazelbauer, Washington State University; Falke, University of

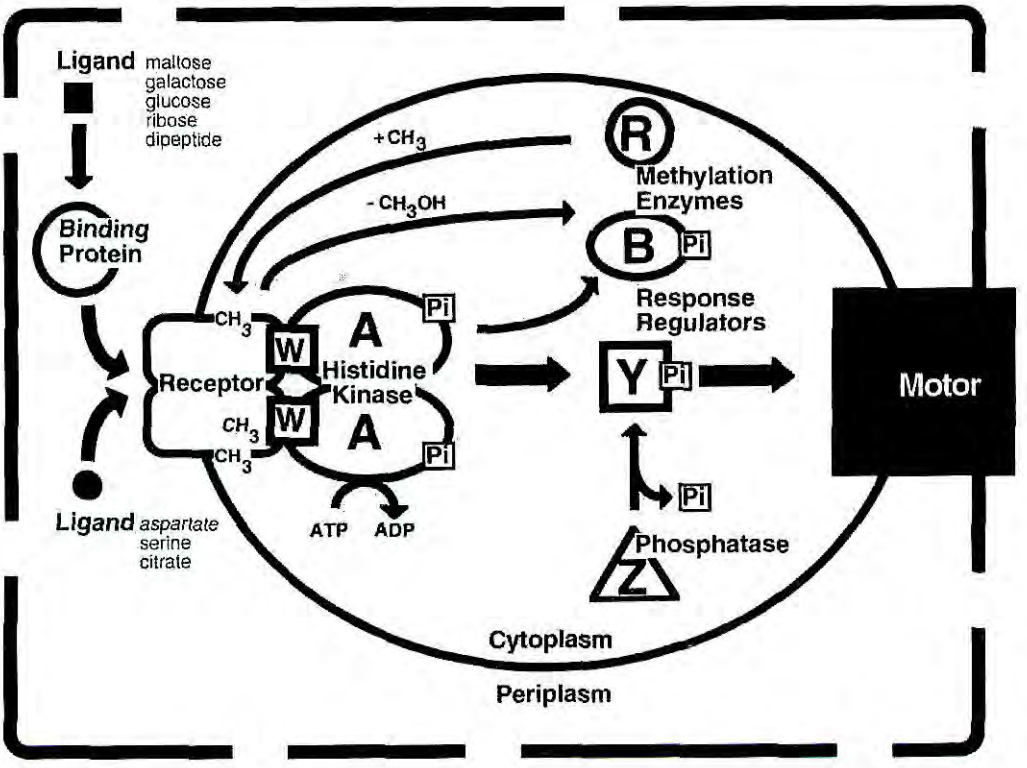

Fig. 2. The chemosensory pathway of *E. coli* and *S. typhimurium*. Chemosensing begins with a small-molecule ligand which enters the periplasmic compartment and activates a specific binding protein or transmembrane receptor. The receptors regulate a cytoplasmic histidine kinase pathway composed of proteins encoded by the *che* genes, as follows. CheW couples the receptor to the histidine kinase, CheA, providing a stable receptor–kinase complex. The kinase autophosphorylates an essential histidine residue, then the phosphate is transfered to a conserved aspartate residue of a response regulator, either CheY or CheB. The CheY protein regulates motor switching, while CheB controls the covalent modification of the receptors by an adaptive methylation pathway. Adaptation involves two competing reactions: methyl-esterification of specific receptor glutamates by CheR, and hydrolytic reversal of this methylation by phospho-CheB. Finally, the CheZ protein provides a specific phosphatase activity which facilitates the dephosphorylation of CheY. (*E. coli* lacks the citrate receptor, and *S. typhimurium* lacks a receptor for the maltose-binding protein.)

Colorado, Boulder) has been confirmed by randomization of the helical sequences, which reveals packing interfaces essential for function (Maruyama, Mikawa and Maruyama, Scripps Research Institute).

Recent work has focused on identification of the helices which transmit stimulus signals across the bilayer, using engineered inter-helix disulphides placed in the periplasmic and transmembrane regions of both the aspartate and ribose/galactose receptors (Chervitz and Falke, University of Colorado, Boulder; Hazelbauer, Washington State University). In general, disulphide bonds which crosslink the interface between the subunits do not block signal propagation, whereas intra- or inter-subunit cross-links, which lock the position of the second transmembrane helix (TM2), profoundly perturb function. Moreover, one disulphide has been observed to lock TM2 in a position that constitutively activates the CheA kinase (Chervitz and Falke, University of Colorado, Boulder). These findings implicate TM2 as the element that moves during signal transmission (Fig. 3B). The nature of that movement remains largely undefined.

Kinase regulation

The transmembrane signal generated by the chemotaxis receptors is passed to their cytoplasmic signalling domain, which forms a stable complex with the CheA histidine kinase and the linker protein CheW. Recent work suggests that kinase regulation may be provided by changing the subunit interaction within the receptor signalling domain (Surette and Stock, Princeton University; Cochran and Kim, Whitehead Institute; Weis, University of Massachusetts). One way to modulate that interaction is through the methylation sites flanking the signalling domain. These sites consist of specific glutamate residues targetted for methyl esterification by the adaptation branch of the chemotaxis pathway (Fig. 3C; Surette and Stock, Princeton University). Charge changes at the methylation sites, produced by glutamine substitutions, shift the monomer–dimer equilibrium of soluble signalling domain fragments (Cochran and Kim, Whitehead Institute). As charge neutralization (akin to methylation) also up-regulates the kinase (Lupas, Surette and Stock, Princeton University),

Fig. 3

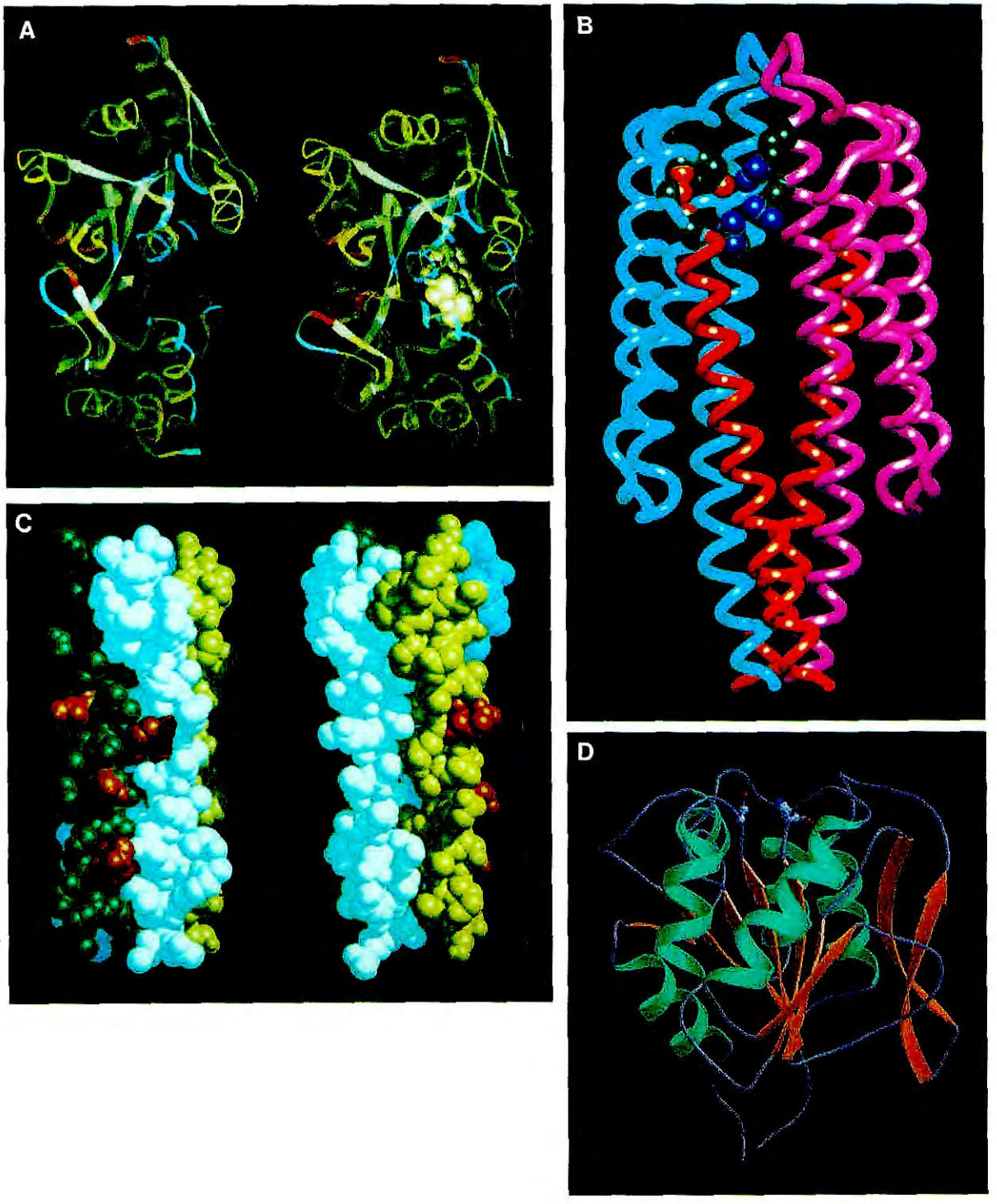

Fig. 3

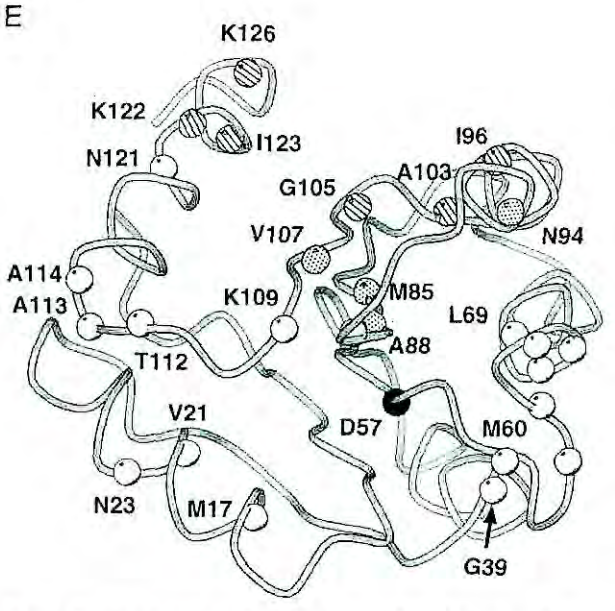

Fig. 3. Structural studies of the *E. coli* and *S. typhimurium* chemosensory pathways.
A. Crystal structures of the *E. coli* maltose-binding protein in its open (left) and closed (right) conformations (Mowbray, Bjorkman, Flocco Shilton, Upssala Biomedical Centre). Binding of maltose (Van der Waals (VDW) surface) within the inter-domain hinged cleft triggers its closure, trapping the sugar inside. Protein surfaces which are more buried in the apo- or sugar-occupied conformations are shown in red or blue, respectively, where the colour intensity indicates the degree of burial.
B. Crystal structure of the ligand-binding domain of the *S. typhimurium* transmembrane aspartate receptor (Prive, Milligan, Scott, Yeh, Jankarik, Koshland and Kim, University of California, Berkeley), highlighting, in red, the α4 helix proposed to transmit the membrane-spanning signal (Chervitz, Danielson and Falke, University of Colorado, Boulder; Lee, Lebert, Lilly and Hazelbauer, Washington State University; Biemann and Koshland, University of California, Berkelely). The identical subunits of the homodimer (pink and blue ribbons) and the co-ordinating residues of one aspartate binding site (VDW surfaces) are shown.
C. Two views of a model for the methylation region of the *S. typhimurium* aspartate receptor (Lupas, Surette and Stock, Princeton University), highlighting, in red, the methylation sites. The two identical subunits each possess a pair of methylation segments (MH1-green, yellow; MH2-white, blue) proposed to be α-helical. The model further proposes that the two anti-parallel MH1–MH2 coiled coils associate to form a four-helix bundle.
D. Crystal structure of the catalytic domain of the *S. typhimurium* response-regulator CheB, showing the active site Ser–His–Asp catalytic triad (top; West, Martinez-Hackert and Stock, University of Medicine and Dentistry of New Jersey (UMDNJ) and Rutgers University). This active site serves to hydrolyse the methyl esters formed by methylation of receptors during adaptation.
E. NMR-derived backbone structure of the *E. coli* response-regulator CheY (Lowry and Dahlquist, University of Oregon; Matsumura, University of Illinois, Chicago; Moy, Krywko and Domaille, DuPont Merck Pharmaceuticals). Highlighted are the backbone amide resonances shifted significantly by (i) phosphorylation of Asp-57 to yield the activated molecule (white spheres); (ii) CheY binding to the P2 fragment of the CheA kinase (hatched spheres); or (iii) both phosphorylation and kinase binding (dotted spheres; Lowry, Roth, Rupert, Deutschman and Dahlquist, University of Oregon; Moy and Domaille, DuPont Merck Pharmaceuticals; Matsumura, University of Illinois, Chicago).

these findings suggest a link between kinase activation and the subunit interactions within the signalling domain. Consistent with this idea, a leucine zipper introduced at the N-terminus of a signalling domain fragment substantially enhances its ability to activate the kinase (Surette and Stock, Princeton University; Cochran and Kim, Whitehead Institute). Moreover, the extent of activation is sensitive to the rotational register of the zipper (Cochran and Kim, Whitehead Institute), suggesting that the relative orientation of the receptor subunits may control their signalling activity.

An alternative view of kinase regulation comes from aspartate receptors engineered to have a monomeric signalling domain regulated by a single ligand binding site (Gardina and Manson, Texas A&M University; Oosawa, Teikyo University). These mutant receptor molecules support chemotaxis *in vivo*, implying that conformational changes within a single subunit of the receptor can regulate kinase activity. A three-dimensional structure of the signalling domain would help define the receptor–kinase interaction, and crystals have encouragingly been sighted (Sandgren, Shilton, Dunten and Mowbray, Uppsala University).

Receptor diversity

Transmembrane receptors in organisms distantly related to *E. coli* and *S. typhimurium*, such as *H. salinarium and Bacillus subtilis*, reveal interesting and informative variations on the enteric theme. The *Halobacterium* HtrI protein, which has a cytoplasmic signalling domain homologous to that of Gram-negative chemotaxis receptors, mediates phototactic responses. It receives light stimuli through interaction with sensory rhodopsin I (SR-I), a seven-helix retinal-containing photoreceptor. The mechanism of HtrI activation by SR-I has been probed with SR-I photoactive-site mutants, one of which reverses the red light attractant signals of HtrI. Moreover, in the absence of HtrI, SR-I photochemistry is altered and the protein becomes a light-driven proton transporter. Both findings point to a close connection between HtrI and the photocycle of SR-I (Olson, Zhang, Cervantes and Spudich, University of Texas).

Several chemoreceptor genes of *B. subtilis* have recently been sequenced, revealing candidate sites for the methylation events observed *in vivo* (Hanlon and Ordal, University of Illinois). Although the transmembrane organization and signalling domains of these receptors are similar to the Gram-negative examples, the methylation sites are not located in the corresponding sequence positions. Unlike Gram-negative organisms, but like *Halobacteria*, both attractants and repellents stimulate hydrolysis of methyl esters. Furthermore, receptor methyl groups seem to be transferred to and from other components of

the signalling pathway. The *B. subtilis* system may well reveal new mechanisms, and possibly new functions, for receptor methylation in prokaryotes.

A novel class of receptors, unrelated to *E. coli* chemoreceptors, has been discovered in *Pseudomonas putida*, a species which can migrate towards and degrade aromatic compounds. A 47 kDa integral membrane protein, termed PcaK, has recently been implicated as a receptor and transporter for the attractant 4-hydroxybenzoate (Harwood, Nichols and Ditty, University of Iowa). This protein, which is required for both chemotaxis toward, and import of, the attractant, exhibits a primary structure similar to the major facilitator class of membrane transport proteins. Overall, the available evidence suggests that the proposed receptor somehow links chemotaxis to transmembrane transport.

Kinases and phosphatases

Kinase organization: structure and function of domains

The prokaryotic and eukaryotic histidine kinases exhibit a simple modular structure assembled from conserved domains. Such a modular design confers both biological and experimental advantages, including (i) rapid evolution of new kinases through novel combinations of modular elements, and (ii) simplified structural determination of individual domains via a 'divide and conquer' approach. Recent work has continued to probe the structure and function of key domains. An important model has been the CheA histidine kinase of the *E. coli* and *S. typhimurium* chemosensory pathways.

The homodimeric CheA protein is an assembly of at least four functional domains (Jahreis, Morrison and Parkinson, University of Utah), the first two of which fold as independent structures characterized by multi-dimensional nuclear magnetic resonance (NMR).

(i) The N-terminal P1 domain, which contains the histidine target of the autokinase activity, consists of five α-helices dominated by an N-terminal four-helix bundle. The autophosphorylation site, His-48, is located near the end of the second helix (Zhou, Lowry, McEvoy and Dahlquist, University of Oregon; Swanson and Simon, Caltech).

(ii) The P2 domain, which binds the kinase substrate CheY, exhibits a 'split αβ sandwich' folding motif in which two α-helices lie on the same face of four antiparallel β-strands. CheY appears to bind to the helical face of the sandwich, perturbing the second helix and the loop following the first helix (McEvoy, Zhou, Roth, Lowry and Dahlquist, University of Oregon; Kay, University of Toronto; Morrison and Parkinson, University of Utah).

(iii) The catalytic region, comprising the middle section of primary structure, possesses ATP-binding elements and conserved active-site residues.

(iv) The receptor-coupling region is proposed to interact with CheW, and perhaps also directly with the receptor, during the formation of the stable receptor–kinase complex.

The regulation of CheA by the receptor complex has been probed using fragments of the serine receptor (Ames and Parkinson, University of Utah). When mutated receptor fragments are liberated from the signalling region between the two methylation helices, these fragments form homodimers capable of up- or down-regulating CheA activity *in vitro*. Kinase stimulation requires the coupling protein CheW. In contrast, kinase inhibition is CheW-independent, providing evidence for a direct receptor–kinase interaction. As a result of the modular design of histidine kinase and other signalling pathways, the domain-liberation approach should find wide application in elucidating the key protein–protein interactions.

Kinase diversity

Although many histidine kinases are regulated by a transmembrane receptor, there are clear examples of pathways in which regulation is provided by some other type of sensor protein. The FixL histidine kinase in the nitrogen-fixation pathway of *Rhizobia*, for example, is regulated by a soluble oxygen-sensing domain containing a haem site. The kinase on–off switch is proposed to be directly controlled by the spin state of the haem iron, rather than the oxidation state or ligand–protein interactions, (Gilles-Gonzalez, Ohio State University). The transition from low to high spin causes the iron to move out of the porphyrin plane, triggering a conformational change that activates kinase activity. It is not yet known whether the conformational changes which activate this and other histidine kinases are similar, or are instead specialized for individual sensor–kinase pairs.

Phosphatases

Similar to other phospho-signalling pathways, prokaryotic histidine kinase pathways often use a phosphatase as an additional regulatory element. The currently characterized phosphatases serve to speed the dephosphorylation of one or more specific response regulators. The CheY response regulator of *E. coli* and *S. typhimurium* chemotaxis, for example, binds to the CheZ phosphatase and appears to cause phosphatase oligomerization, perhaps providing some type of regulation (Blat and Eisenbach, Weizmann Institute). A clear example of phosphatase regulation is provided by the NRI response regulator of the *E. coli* nitrogen-assimilation pathway. The dephosphorylation of NRI, a transcriptional activator, is stimulated by the NRII phosphatase and the PII signal-transduction protein, where PII is part of the sensory apparatus that monitors

intracellular nitrogen (Kamberov, Atkinson and Ninfa, University of Michigan).

Phosphatases also provide multiple metabolic control points in the wonderfully complex sporulation pathway of *B. subtilis*, which is induced by nutrient deprivation and high cell density. The sporulation cycle is controlled by the phosphorelay, a signal transduction pathway based on phosphotransfers between histidine kinases and multiple response regulators. As the sporulation process is irreversible, multiple environmental and physiological cues are integrated and weighed before a decision to proceed is implemented. Part of this integration is provided by a group of newly discovered phosphatases which serve to drain the phosphorelay, lowering the concentration of the phosphorylated response-regulator Spo0A, and thereby preventing sporulation (Perego and Hoch, Scripps Research Institute). It follows that the sporulation phosphatases serve as negative regulators, counterbalancing the effects of multiple kinases.

Response regulators

Interactions with kinases and phosphatases

The targets of histidine kinases, termed the response regulators, are typically small, soluble proteins consisting of one or two stable domains. Their modular simplicity has facilitated structural studies by NMR and crystallography, which continue to progress rapidly. Much work has focused on CheY, the response regulator from the chemotaxis pathways of *E. coli* and *S. typhimurium*. CheY catalyses its own phosphorylation by phosphotransfer from the phospho-histidine of CheA, and the resulting phospho-CheY binds to and regulates the switching apparatus of the flagellar motor. Multi-dimensional NMR techniques have been used to map the binding surfaces of CheY and CheA that interact during the phosphotransfer reaction (Lowry, Roth, Rupert, Deutschman and Dahlquist, University of Oregon; Moy and Domaille, DuPont Merck Pharmaceuticals; Matsumura, University of Illinois, Chicago). P2, the CheY-binding domain in CheA, docks to a surface rotated approximately 90° from the site of phosphorylation on the roughly spherical CheY molecule (Fig. 3E). The CheY docking surface includes a set of lysine 'claws' proposed to grasp the P2 domain. As the P2–CheY contacts are not contiguous with the aspartate targetted for phosphorylation, additional interactions between CheY and CheA may direct the trajectory of the phosphotransfer reaction.

The probable CheY docking surface for the phosphatase protein CheZ has also been identified (Sanna, Swanson, Bourret and Simon, Caltech). Two CheY mutants that are dephosphorylated by CheZ at abnormally slow rates define a patch on the CheY surface that is non-contiguous with both the phosphorylation site and the CheA docking site (see the cover figure). This finding leaves open the possibility that CheZ, rather than catalysing the direct hydrolysis of the phospho-aspartyl side-chain, acts allosterically to stimulate the known auto-dephosphorylation activity of CheY. One potential advantage of an allosteric mechanism is that phospho-CheY bound to the motor could be dephosphorylated by CheZ even if the phospho-side-chain is buried at the motor interface.

Structural change induced by phosphorylation

Observation of the structural change induced in the CheY molecule by phosphorylation presents a considerable challenge because phospho-CheY is rapidly hydrolysed by an auto-dephosphorylation reaction. Nevertheless, multi-dimensional NMR studies have monitored the backbone resonances which shift upon CheY phosphorylation, and reveal a global conformational change which propogates through virtually all backbone positions (Lowry, Roth, Rupert, Deutschman and Dahlquist, University of Oregon; Moy and Domaille, DuPont Merck Pharmaceuticals; Matsumura, University of Illinois, Chicago; Fig. 3E). The conformational changes appear to involve small shifts of secondary-structure elements, which may provide long-range communication between the phosphorylation site and the surface regions that regulate interactions with the kinase, the phosphatase, and the motor. In the more complex two-domain response regulators, a similar global conformational change is presumably responsible for somehow regulating the function of the C-terminal effector domain.

To obtain a still higher-resolution view of the phospho-induced conformational change, other NMR studies are beginning to probe the response regulator Spo0F of the *B. subtilis* sporulation pathway, which yields a kinetically stable phosphorylated species. Initial characterization of the unphosphorylated protein reveals a fold very similar to that of CheY, with subtle structural differences localized to regions of CheY proposed to dock with CheA and the motor (Feher and Dahlquist, University of Oregon; Zapf, Hoch, Whiteley, and Cavanagh, Scripps Research Institute). Such differences may accommodate the different regulatory partners of CheY and Spo0F.

The effector domain: a structural example

The first structure of a response-regulator effector domain has been determined by crystallographic studies of the CheB protein, which serves as the methylesterase in the adaptation branch of *E. coli* and *S. typhiumurim* chemotaxis. CheB possesses an N-terminal domain homologous to CheY. Phosphorylation of this domain, by phosphotransfer from the CheA histidine kinase, greatly

stimulates the methylesterase activity of the C-terminal domain. The newly determined structure of the C-terminal catalytic domain exhibits an α/β folding motif in which a central seven-stranded parallel β-sheet is flanked on both sides by a total of six α-helices and an antiparallel β-hairpin motif (West, Martinez-Hackert and Stock, UMDNJ and Rutgers University; Fig. 3D). At the C-terminal edge of the sheet lies a cleft containing a Ser–His–Asp cluster reminiscent of, yet distinct from, the catalytic triads of serine proteases and hydrolases. This configuration of the active site is compatible with a proton shuttle mechanism generating a serine nucleophile for hydrolysis of receptor methyl esters. Future crystallographic and NMR studies should reveal how this active site is inhibited by interaction with the unphosphorylated N-terminal regulatory domain, and how this inhibition is relieved by phosphorylation.

Signal processing

Networkings in E. coli *chemotaxis*

Stepping back from the molecular mechanisms of specific signalling proteins, a new branch of research is monitoring the signalling characteristics of the fully assembled chemotaxis pathway in swimming *E. coli*. Caged compounds that release chemoeffectors upon photolysis reveal the kinetics of chemotactic responses to various stimuli (Khan, Albert Einstein Medical College; Corrie and Trentham, National Institute of Medical Research, London). This approach allows a stimulus (e.g., serine or protons) to be introduced at a precisely-timed moment, so that ensuing events can be monitored with accurate time resolution. When swimming cells are excited with two stimuli sensed by the same transmembrane receptor, the stimuli appear to be integrated immediately, at the level of the receptor itself. In contrast, simultaneous excitation of two different receptors gives a biphasic response, implying that integration occurs later in the signalling pathway, presumably at the level of phospho-CheY formation. The same approach has been used to measure the response time of chemotaxis via the phosphotransferase system (PTS). When caged glucose is used to trigger a PTS signal, a behavioural response is observed within 150 msec, roughly the same timescale observed for the chemotaxis pathway. The rapidity of this response must be accounted for in any proposed mechanism of PTS-sugar sensing.

In order to probe the chemotaxis pathway on a truly quantitative level, computer-based simulations are being used to model the chemotactic response. One approach focusses on the signalling parameters of the receptor-kinase complex, treating the rest of the pathway as a black box (Ford, Cummings and Middlebrooks, University of Virginia). The resulting simulation accurately reproduces the cellular migration experimentally observed in a controlled stimulus gradient. For two simultaneous stimuli the agreement is only approximate, suggesting that additional work is needed to understand the integration of multiple inputs. An even more ambitious approach explicitly uses the equilibrium constants and enzymatic rates of each step in the phosphosignalling pathway (Bray, University of Cambridge; Bourret, University of North Carolina). The resulting simulation accurately fits the observed response to a single chemotactic stimulus, and also explains the effects of specific pathway mutations on cellular behaviour. However, the values that must be assumed for the binding constants between pathway components are significantly higher than those measured *in vitro*. Additionally, in its present form, the model cannot account for the very high gain reported in some studies. These results suggest that some key elements might yet be missing from current descriptions of the chemotactic signalling machinery.

Related E. coli *signalling systems*

It remains to be seen whether Ca^{2+} signalling might somehow modulate the chemotaxis pathway. Although chemotactic behaviour in *E. coli* is believed to be regulated chiefly by histidine kinase signalling, Ca^{2+} signals are also reported to occur during the chemotactic response (Tisa and Adler, University of Wisconsin). Chemotaxis repellents cause an upward spike in the cytoplasmic Ca^{2+} concentration, while attractants generate a downward spike. Moreover, rapid release of caged Ca^{2+} in the cell causes motor reversal, consistent with the view that Ca^{2+} participates actively in signalling. Mutant analysis indicates that the CheA, CheY, and CheW proteins are needed for Ca^{2+} to stimulate motor switching, but its exact mode of action is not known.

Another mysterious area is the potential crosstalk between histidine kinase signalling and the metabolic formation of acetyl phosphate, which can directly activate certain response regulators by phosphotransfer. A mutation in acetate metabolism has been found which blocks asparate chemotaxis by an unknown mechanism (Wolfe, Prüß and Kumari, Loyola University). One possibility is that changes in the level of acetyl phosphate may modulate a histidine kinase pathway regulating the function or assembly of the taxis machinery.

Prokaryotes such as *E. coli* possess a variety of systems besides the chemotaxis pathway which contribute to the control of swimming behaviour. The PTS pathway, for example, controls migration up gradients of glucose and fructose. These sugars are phosphorylated by phosphenol-pyruvate (PEP)-dependent phosphotransfer during their entry into the cell. The machinery used to sense PTS sugars, and to relay this information to the flagellar motors, has remained unclear despite extensive

study. A new hypothesis has recently been proposed to explain this network (Lux and Lengeler, University of Osnabruck; Parkinson, University of Utah). The model centres on the cytoplasmic Enzyme I protein of the PTS phosphorylation pathway. In an *in vitro* assay, the dephosphorylated form of Enzyme I, but not the phosphorylated form, significantly reduces the rate of autophosphorylation of CheA, the central kinase of the chemotactic signalling pathway. This observation suggests a simple coupling between the PTS and chemotaxis phosphorylation networks, in which both regulate motor behaviour via modulation of CheA and the resulting phospho-CheY formation. Related studies also reveal that PEP alone slightly stimulates CheA autophosphorylation. This effect could further modulate phospho-CheY levels and swimming behaviour.

Chemotaxis in other prokaryotes

Comparisons of histidine kinase pathways in a variety of prokaryotes reveal a diversity of circuit diagrams, providing specialized types of crosstalk within each signalling network. Chemotaxis of *Rhodobacter sphaeroides* (Ward and Armitage, Oxford University) and the related *Rhizobium meliloti* (Schmitt, Sourjik and Platzer, University of Regensberg) appears to involve two distinct signalling pathways, one for sensing sugars and the other for sensing acetate and succinate (*Rhodobacter*) or amino acids (*Rhizobium*). The sugar-sensing pathway is proposed to involve a soluble sensor protein closely related to the *E. coli* chemotaxis receptors. This sensor, which contains both methylation and signalling domains homologous to the corresponding *E. coli* domains, probably controls a histidine kinase related to CheA. Moreover, in each species, two different CheY homologues have been found, which might be involved in distinct signalling pathways or interact with different sites on flagellar motors. Neither CheY1 nor CheY2 from *R. meliloti* can complement a CheY defect in *E. coli*, but both slightly impair chemotaxis when expressed in that species. An interesting new gene called *motX*, whose product, MotX, is essential for motility but not flagellar assembly, may be unique to *Rhizobium*. MotX has a signal sequence which indicates a periplasmic location, but no membrane-spanning segments. Thus, it is quite distinct from either the MotA or MotB motor proteins of *E. coli*, homologues of which are present in *R. meliloti* in addition to MotX.

The soil bacterium *Azospirillum brasilense* is able to undergo chemotaxis in gradients of chemical attractants, including molecular oxygen, despite its lack of receptors homologous to those used in *E. coli* chemotaxis (Zhulin, Sarmiento and Taylor, Loma Linda Medical School). Instead, chemotactic behaviour is proposed to depend on a functional redox chain and consequent changes in proton motive force (pmf). Evidence suggests that aerotaxis, in particular, directly senses pmf rather than a small molecule ligand such as oxygen. The pathway coupling the pmf sensor to the motor is yet to be described.

Motors and propulsion

Isolation of intact motor complexes

Bacterial flagella are rotated by reversible motors in the cell membrane, which are powered by the transmembrane gradient of protons or, in some species, sodium ions. A variety of ultrastructural, genetic and physiological methods are being applied to the study of these remarkable molecular machines. The proton-driven motors of *E. coli* and *S. typhimurium* illustrated in Fig. 4 have been the focus of many of these studies (as described below, except where another species is specifically mentioned). From electron micrographic studies, it has long been known that bacterial flagella have a structure at their base consisting of several rings mounted on a rod, which are thought to serve as a molecular 'bearing' for flagellar rotation. Owing to the harsh isolation procedures that

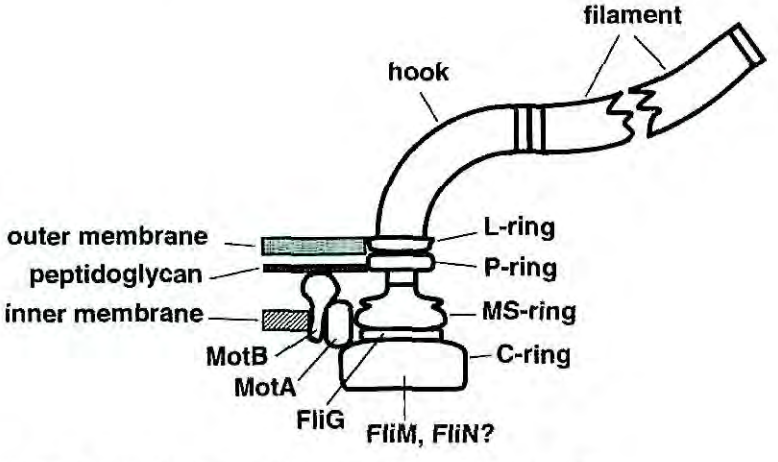

Fig. 4. Structure of the flagellar motor in *E. coli* and *S. typhimurium*. The classical flagellar basal structure consists of several rings (L, P, and MS) mounted on a rod, located in and between the cell membranes. The C-ring, located in the cytoplasm, is a comparatively recent discovery, found only in flagella isolated using gentle procedures. The basal structure is attached, via a flexible coupling called the hook, to the filament, which is a rigid helical structure that functions as a propellor. The five proteins indicated (MotA, MotB, FliG, FliM, and FliN) have been implicated in the process of torque generation. The locations suggested for these proteins are approximate; their precise positions in the structure are not known.

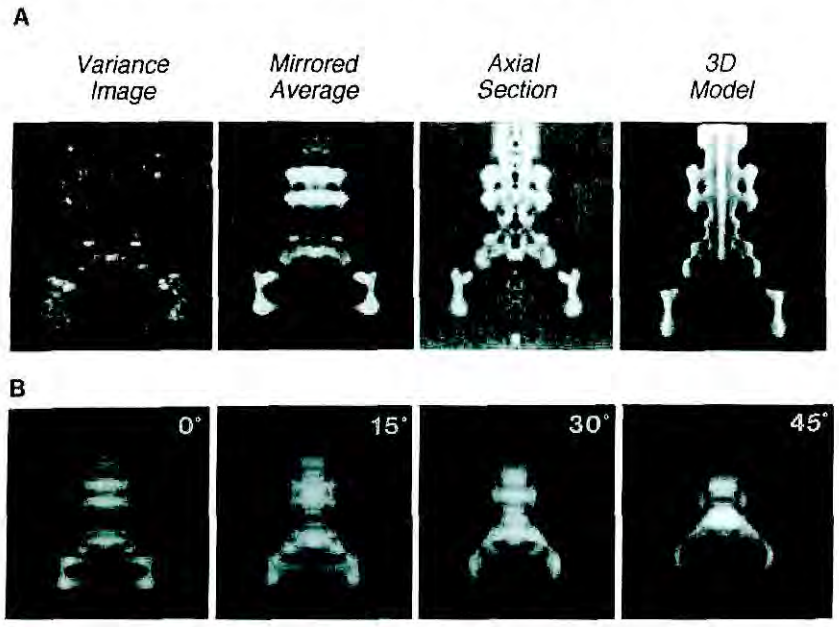

Fig. 5. Structure of the *S. typhimurium* flagellar motor.
A. Averaged electron-cryomicroscopic images of basal bodies retaining the putative C-ring complex (Thomas, Francis, Sosinsky and DeRosier, Brandeis University). Multiple images were combined using mirror symmetry to generate the illustrated averaged image and its variance (left two panels), as well as an axial section and a three-dimensional solid model (right two panels).
B. Different tilts of the model showing additional features of its three-dimensional profile. (Figures adapted from Francis, Sosinsky, Thomas and DeRosier (1994) *J Mol Biol* **235:** 1261–1270.)

have typically been used, however, the classical basal structure lacks several proteins known to be essential for torque generation.

One focus of recent work, therefore, has been the isolation and ultrastructural characterization of better-preserved flagellar basal structures. In electron micrographs of flagella isolated using relatively gentle procedures, two new studies have observed a large, ring- or bell-shaped structure attached to the cytoplasmic face of the basal body (Figs 4 and 5; Thomas, Francis, Sosinsky and DeRosier, Brandeis University; Zhao and Khan, Albert Einstein Medical College). This structure has been dubbed the C-ring to reflect its cytoplasmic location. Interestingly, these more intact basal structures contain the three 'switch-complex' proteins FliG, FliM, and FliN, known from previous studies to be important for flagellar assembly, rotation, and direction control. Current efforts are directed toward locating each of these proteins in the structure: high-resolution images of basal structures that contain FliG but lack FliM or FliN, exhibit a thickening of the MS ring, suggesting that FliG is attached to the cytoplasmic face of this ring (Thomas, Francis, Sosinsky and DeRosier, Brandeis University). Basal structures produced in various non-motile or non-chemotactic mutants yield a similar conclusion (Zhao and Khan, Albert Einstein Medical College). The precise composition of the C-ring is unknown; available evidence suggests that it contains FliM and FliN, but other proteins could also be present (Thomas, Francis, Sosinsky and DeRosier, Brandeis University, Zhao and Khan, Albert Einstein Medical College). The C-ring is quite large, and would contain approximately 100 copies of FliM and FliN, if formed from those proteins alone, in 1:1 stoichiometry. Using immunoblots calibrated by reference to the hook protein, whose stoichiometry in the flagellum is known, it has been estimated that FliM and FliN might each be present in 80 or more copies, suggesting that they could form most of the C-ring (Zhao and Khan, Albert Einstein Medical College). Additional studies should enable more precise estimation of the stoichiometries of these important motor parts.

Motor subunits involved in assembly and behavioural control

A long-standing puzzle has been to understand how assembly of the flagellar hook (Fig. 4) is controlled to give this structure a rather precise length. Extensive measurements of hook lengths in growing cells reveal that hooks vary in length by about 10% (55 ± 6 nm), somewhat more than was previously thought (Aizawa and Oosawa, Teikyo University; Yamaguchi, Meiji University; Williams and Macnab, Yale University). Control of hook length is known to involve the FliK protein, but its exact role is not known. One possibility is that FliK acts as a molecular ruler; however, a large number of *fliK* mutations have all led to the production of abnormally long hooks with similar phenotypes. This would suggest that FliK does not act as a ruler to measure hook length, as different mutations might in that case be expected to produce hooks of various lengths. Instead, it is now proposed that hook length might be regulated by structures at the base of the flagellum, possibly including the C-ring, that measure out an appropriate volume of hook subunits before delivering them to their site of assembly. Whatever the mechanism, it is likely to involve other proteins in addition to FliK,

since *fliK* mutations can be suppressed by certain mutations in another flagellar gene, *flhB*.

Although the FliG, FliM, and FliN proteins are known to be essential for flagellar assembly and rotation, their precise functions are still being probed. Early genetic studies suggested that these proteins might form a complex, and recent evidence obtained via the yeast two-hybrid approach has provided additional support for this idea (Marykwas and Berg, Harvard University). The approach reveals evidence for an interaction between FliM and FliG, enabling isolation and mapping of a number of *fliG* mutations that disrupt the interaction. The mutations cluster in parts of FliG thought to be important for controlling the direction of motor rotation, suggesting that the binding of FliM to FliG might be critical for switching.

Motor subunits involved in power production

Two flagellar proteins known to be essential for torque generation are MotA and MotB. These proteins are membrane bound, and are known to function in transmembrane proton conduction. Additionally, MotB might serve as an anchor which attaches the stator, or non-rotating part of the motor, to the cell wall. The interactions between these proteins have been successfully analysed by isolating intergenic suppressor mutations (Garza and Manson, Texas A&M University). Certain mutations in *motB* can be suppressed by mutations in *motA* and vice versa, strongly implying that MotA and MotB interact with each other. Suppressors of *motB* mutations are also found in *fliG*. In most cases suppression does not appear to involve direct contacts between mutated residues. The pattern of suppression effects suggests instead that the suppressible *motB* mutations cause a misalignment of MotB and MotA relative to other motor parts, probably including FliG, and that this defect can be corrected through compensating movements in those other parts.

New information regarding the molecular structures of MotA and MotB has been revealed by a mutational analysis of their membrane-spanning segments (Sharp, Zhou and Blair, University of Utah). Scanning tryptophan (Trp) mutagenesis, coupled with activity studies of the resulting mutants, suggests that the four membrane-spanning segments of MotA are α-helices, each with a face that is directed toward the lipid and can tolerate substitution of a bulky residue. MotB has only a single membrane-spanning segment, also proposed to be helical. Trp substitutions in this segment had effects which suggest that it interacts with the segments of MotA, but is tilted relative to them so that its cytoplasmic end is embedded deeply in a MotA/MotB complex while its periplasmic end is more exposed. In the hypothesized MotA/MotB channel structure, a conserved aspartate residue of MotB is directed toward the centre, where it could participate in proton transfer. A previous random mutagenesis showed that replacing this residue with asparagine abolishes function, so it is indeed critical for some aspect of channel structure or activity.

The analysis of MotA and MotB from various species promises to highlight functionally important conserved residues. In the deduced sequences of MotA and MotB homologues from *R. sphaeroides*, a number of key features are conserved (Shah and Sockett, Nottingham University). In the MotA homologue, these include four hydrophobic segments that could span the membrane, and some highly charged segments that are probably located in a cytoplasmic domain. The aforementioned aspartate residue of MotB (Asp-32 in *E. coli*) is conserved in *R. sphaeroides*; sequence comparisons also suggest that this residue is directed into the channel where it could have a direct role in proton transfer. In most species, the periplasmic domain of MotB contains a sequence motif found in several outer membrane proteins, which could be involved in binding to peptidoglycan. This sequence might form the point of attachment for MotB in its role as anchor for the stationary parts of the motor. Interestingly, MotB of *R. sphaeroides* lacks this sequence, exhibiting instead a heptad repeat of histidine residues. The significance of these sequence differences is not yet clear, but might be related to the fact that the motors of *R. sphaeroides* rotate unidirectionally, stopping occasionally but never reversing, while in other species that have been studied the motors alternate between clockwise and counter-clockwise rotation.

Alternative energy sources, and motor diversity

As noted, some flagellar motors are driven by a gradient of sodium ions rather than protons. Recent work has isolated the genes encoding components of the sodium channel from the flagellar motor of *Vibrio parahaemolyticus* (McCarter and Noack, Scripps Research Institute). One of these, called MotY, contains the peptidoglycan-binding sequence motif found in most homologues of MotB, and so is likely to function as an anchor for the channel. Like MotB, the MotY gene product has a single hydrophobic segment that probably traverses the membrane. The other component of the sodium channel, called MotX, is remarkable in that it also has only a single hydrophobic segment. As MotX and MotY are sufficient for sodium conduction, this suggests that the sodium channels in the *Vibrio* motor contain multiple copies of MotX and/or MotY. Continued comparisons of these channels with those from proton-driven motors will be informative.

While the assembly of bacterial motor complexes is quite complicated because of their large complement of subunits, some prokaryotic motors appear to pose even more formidable assembly problems. *Caulobacter crescentus*

differentiates into either an asymmetric, sessile stalked cell or a motile swarmer cell, requiring both spatial and temporal control of motor assembly. Immuno-localization has pinpointed the spatial targetting of the FliF protein, one of the earliest assembled motor subunits, to a specific pole of swarmer, but not stalked cells (Jenal, Meisenzahl and Shapiro, Stanford University). The FliF system may thus provide a useful model in which to probe the mechanisms underlying spatial and temporal targetting in prokaryotes. In the spirochete *Treponema pallidum*, the causative agent of syphilis, the flagellar assembly is astoundingly stable; for example, the hook protein FlgE resists depolymerization by heat, ionic detergents or 8 M urea (Limberger, Slivienski and Taflin, New York State Department of Health). It is proposed that this stability results from covalent crosslinking of the FlgE monomers, which could require a specialized crosslinking machinery.

Physiology and behaviour

Quorum sensing and light production

Together or separately, the sensory and motility systems of bacteria enable remarkably complex physiological and behavioural responses. The light-generating organs of certain fish contain *Vibrio fischeri*, providing a distinctive example of symbiosis regulated by physiological responses. In the light organ, these bacteria sense their own population density, and when it is high enough, luciferase production is induced and light is released. This sensing of a population theshhold is termed 'quorum sensing'. As the bacteria multiply, a homoserine lactone termed the autoinducer accumulates in the growth medium until its concentration reaches a critical level, whereupon *lux* gene expression is activated. The regulatory protein required for this response, LuxR, is of special interest because its homologues are found in many different organisms where they regulate a diverse array of genes. Recent studies reveal that the LuxR protein of *V. fischeri* has a two-domain structure (Stevens, Dolan and Greenberg, University of Iowa). The N-terminal domain, which is membrane associated, binds autoinducer and regulates the cytoplasmic C-terminal domain. If the regulatory domain is removed, the C-terminal domain can activate *lux* gene expression in the absence of the autoinducer. Furthermore, the C-terminal domain contains a helix-turn-helix motif, suggesting a direct interaction with DNA.

Vibrio harveyi, in contrast to its symbiotic relative, is free swimming. Nonetheless, it also secretes and responds to small molecule autoinducers that activate *lux* gene expression and produce light. However, unlike *V. fischeri*, *V. harveyi* has evolved elaborate, overlapping sensing mechanisms that appear to respond to two distinct external autodinducers (Bassler, Princeton University). Each of these cell density-dependent signalling pathways utilizes a membrane-bound sensor protein of the histidine kinase class. The pathways converge at the response regulator LuxO. It is proposed that the autoinducers activate the sensor kinases, triggering the phosphorylation of LuxO, which in turn relieves *lux* gene repression. It is interesting to note that besides LuxO, both sensor kinases contain an internal response regulator domain at their C-terminus. Such a modular design may allow signal amplification, or, at the other extreme, pseudo-substrate inhibition of the kinase.

Entry into stationary phase or virulence mode

Many bacteria, including *E. coli*, respond to nutrient deprivation and high cell density by entering stationary phase. Often this process triggers dramatic changes in the pattern of gene expression. Genetic analysis reveals the signalling pathway underlying the downregulation of *ompF*, which serves as a convenient marker for stationary phase. Genes identified in this mutant hunt include *rpoS* and a new gene termed *sprE* (Pratt and Silhavy, Princeton University). The RpoS protein is a stationary-phase sigma factor, while SprE regulates RpoS synthesis at the level of translation. Because SprE is homologous to response regulators, it is possible that histidine kinase signalling pathways play a central role in cell-cycle control.

It has long been thought that motility and chemotaxis were important for the virulence of *Vibrio cholerae*. A recent genetic analysis indicates, however, that virulence and motility are in fact oppositely regulated (Gardel and Mekalanos, Harvard Medical School). Several of the virulence factors produced by this organism are regulated by the transmembrane transcriptional activator ToxR. Mutants that lack ToxR show increased swarming ability on soft agar. Conversely, *motB* mutants exhibit constitutive expression of certain virulence factors, such as toxin and pili. It is proposed that swimming and chemotaxis are required for rapid migration of the bacteria to the intestinal surface. Correct attachment of the organism to the intestine may then trigger a pronounced change in the pattern of gene expression, leading to the appearance of virulence factors such as the toxin and haemolysin. One wonders whether this process might share certain features with the switch between swimming and swarming motility when certain Gram-negative bacteria, including *S. typhimurium* and *Proteus mirabilis*, are propogated on an agar surface (Harshey, University of Texas). Moreover, the model predicts that non-motile strains of *V. cholerae* might provide the basis for a long-awaited vaccine.

Gliding motility and pattern formation

Gliding remains a poorly understood type of locomotion, in

which the protein components and mechanical forces responsible for cellular propulsion remain to be identified. Two useful model organisms for the study of gliding motility are *Myxococcus xanthus* and *Cytophaga johnsonae*. *M. xanthus* contains, in addition to a multitude of genes that control its intricate developmental pattern, four groups of genes essential for gliding and chemotaxis: (i) group A genes produce 'adventurous', single-cell motility; (ii) group S genes yield 'social', many-cell motility; (iii) the *mglA* and *mglB* genes are needed for both single- and many-cell motilities; and (iv) the *frz* genes encode homologues of all the *E. coli* chemotaxis proteins with the exception of the phosphatase CheZ. Video-microscopy studies of gliding cells demonstrate that wild-type cells move significantly faster when in proximity to each other than when apart from one another (Spormann and Kaiser, Stanford University Medical School). Mutants in specific genes reveal that the group A genes are fundamentally important for gliding motility, while the group S genes determine gliding speed and the frequency of directional reversals. Moreover, group S genes encode pili used in social, but not adventurous motility, implying that these appendages may serve as a structural footing in social gliding.

The intricate relationships between *M. xanthus* gliding motility and development are highlighted by a new type of developmental mutant in the *psg* gene locus that, although it is a prototroph, requires proline for development (Youderian, University of Idaho). It appears that the intact *psg* gene is required for the proline signal involved in fruiting-body development and spore differentiation. Interestingly, gliding is inhibited by dissolved ammonia gas in the medium, although its site of action has not yet been identified.

Gliding in different organisms may utilize rather different chemical or physical mechanisms, as substantial variation is observed between the behaviours and cell-envelope characteristics of different gliding species. In contrast to *Myxococci*, the cell envelopes of the *Cytophaga* contain sulphonolipids in large proportion (20% of total lipid), as well as an unusual high-molecular-weight polysaccharide (Leadbetter and Godchaux, University of Connecticut). A sulphonolipid-deficient mutant exhibits neither colony spreading nor cell translocation on solid surfaces, but its motility is restored by the sulphur compound cysteate, a metabolic precursor to the sulphonolipid head group. Sulphonolipids are thus the first chemically defined component proposed to be necessary for gliding. Their importance raises the possibility that gliding results from an electrostatic interaction between the negatively charged sulphonolipid head groups and positive charges on the solid substrate.

The ultimate macroscopic example of complex bacterial behaviour is provided by the intricate patterns generated by *E. coli* swarms on agar plates (Budrene and Berg, Harvard University). These aesthetically striking swarm patterns, which may consist of concentric continuous or perforated rings, radiating features, spirals, or petals (see the cover figure), are formed in response to gradients of amino acid attractants that are secreted by the cells themselves. A qualitative model for the complex tactic and metabolic cell–cell interactions underlying pattern formation has been proposed, involving the sensing of secreted aspartate or glutamate by the standard histidine kinase pathway of *E. coli* chemotaxis.

Mechanisms of gene regulation

The OmpR protein of *E. coli* is a response regulator that controls expression of the porin genes in a histidine kinase pathway. The protein is activated by phosphorylation of its N-terminal domain, while the C-terminal domain binds DNA. A number of DNA-binding proteins share homology with the C-terminus of OmpR, but the interaction between these proteins and DNA is poorly understood. Recent careful studies of the binding of phospho-OmpR to synthetic DNA sites have revealed highly co-operative binding at adjacent sites (Huang, Lan and Igo, University of California, Davis). Such strong co-operativity could explain why OmpR footprints a large region of DNA at *ompF* in spite of the fact that the site immediately upstream of the RNA polymerase binding site has only weak affinity for OmpR. Such a view is also consistent with the extreme sensitivity of transcriptional activation to the level of OmpR phosphorylation.

Flagellin is a major protein produced by *B. subtilis*, but its expression requires a sigma factor (σ^{28}) that is present at low levels. The flagellin promoter is observed to contain an upstream activating sequence located between −42 and −65, resembling the upstream promoter (UP) elements first identified in *E. coli* at ribosomal RNA promoters which bind the α subunit of RNA polymerase (Fredrick, Chen and Helmann, Cornell University). It seems likely that such elements will be general features of highly expressed σ^{28}-dependent genes.

Concluding remarks

Studies of prokaryotic signalling and motility pathways continue to advance rapidly because of their experimental accessibility and relative simplicity. In many cases the power of classical and molecular genetics, coupled with advanced biochemical and biophysical methods, is revealing, at an astonishing pace, the molecular details of prokaryotic signalling, taxis and behaviour. Some ancient pathways, such as those controlled by histidine kinases, are found both in prokaryotic and eukaryotic systems, providing a direct link between studies of prokaryotes

and our understanding of higher organisms. Moreover, prokaryotes embody the same general principles shared by all biological signalling and motility systems. In short, the results presented at the 1995 BLAST meeting illustrate the rapidly advancing understanding at the molecular level of bacterial sensing and behaviour, and the many implications of these findings for eukaryotic organisms.

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