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Bacterial Motility and Signal Transduction

Meeting Review

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How is recognition of an extracellular stimulus transduced into an intracellular signal, and how does such a signal evoke an appropriate cellular response? These questions are the focus of intensive research in many areas of biology. A notably active and productive field is the study of signal transduction in bacteria. Some 180 researchers gathered for the second meeting on Bacterial Locomotion and Signal Transduction (BLAST II; Austin, Texas, January 1993). This meeting, like its predecessor in 1991, aimed to bring together the growing number of scientists studying bacterial taxis and motility or other bacterial signal transduction systems. An important goal was to give younger researchers a chance to present their work. In this review, we hope to convey some of the excitement and enthusiasm evident at that meeting, emphasizing work at the molecular level.

Bacterial Signal Transduction

Work in recent years has revealed a common mechanism of bacterial signal transduction in which a kinase transfers the γ-phosphate of ATP to one of its own histidinyl residues and then transfers that phosphate to an aspartyl residue of a response regulator protein (Bourret et al., 1991; Stock et al., 1991). This process is involved in an impressive diversity of responses for a wide array of bacterial species; such systems are called "two-component signaling (or regulatory) systems." The first identified examples were the components controlling nitrogen assimilation in Escherichia coli (Ninfa and Magasanik, 1986) and subsequently the chemotaxis system of the same organism (Hess et al., 1987). Pairs of protein components in many other bacterial sensory systems exhibit substantial sequence identity with biochemically characterized kinase–regulator pairs, and conserved motifs have been defined by Parkinson and Kofoid (1992).

Receptors (sensors), which are often transmembrane proteins, contain a C-terminal transmitter motif of roughly 240 residues that includes the kinase active site and the phosphorylated histidine. Response regulators have an N-terminal receiver motif of approximately 120 residues that contains the phosphorylated aspartate. These modules are linked to various unrelated domains that function as "input" and "output" elements. The great majority of the two-component systems act to control specific gene expression in response to environmental signals. However, the most extensively studied system controls motile behavior, mediating chemotaxis by E. coli and its close relative, Salmonella typhimurium (Figure 1).

The system directs migration of swimming cells in chemical gradients by controlling the direction of flagellar rotation. The flagellar rotary motor is thought to turn clockwise upon interaction with the phosphorylated response regulator CheY but otherwise to turn counterclockwise. CheY is phosphorylated by a soluble kinase, CheA, and its dephosphorylation is accelerated by CheZ. Kinase activity is controlled by interaction with the cytoplasmic domain of transmembrane chemoreceptors in a process that involves CheW. Chemotactic behavior exhibits sensory adaptation, which is mediated by covalent modification of the cytoplasmic domain of stimulated receptors; the effect of receptor occupancy is offset by an appropriate level of modification. A methyltransferase catalyzes formation of carboxyl methyl esters, and a specific methylesterase hydrolyzes them. Thus, chemoreceptors are also called methyl-accepting chemotaxis proteins.

Chemoreceptors

Substantial information about structure and dynamics is being obtained for the chemoreceptors of E. coli and S. typhimurium (reviewed by Hazelbauer, 1992). These receptors share a number of features with eukaryotic receptors for growth factors. Both are organized with substantial hydrophilic domains on each side of the membrane and only a few transmembrane segments (one per subunit in eukaryotes, two in prokaryotes), both form dimers, and both recognize polypeptide ligands. X-ray crystallographic analysis of the ligand-binding domain of Tar_s (Milburn et al., 1991), the aspartate receptor of S. typhimurium, and of the receptor for human growth factor (DeVos et al., 1992) revealed a common dimeric structure in which a single ligand was bound in a site created across the subunit interface and located distal to the membrane in an

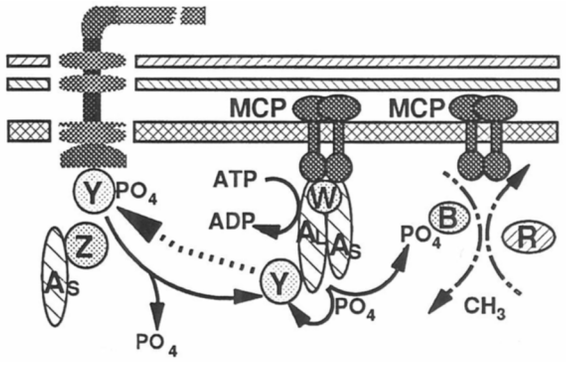

Figure 1. Circuitry of Signaling in the Chemotaxis System of E. coli
See text for details.

elongated structure. The Tar_s ligand-binding domain is a dimer of two four-helix bundles (Figure 2). Two longer helices in each bundle form a quasi-four-helix bundle at the interface between the two subunits and connect to the two pairs of transmembrane segments.

Studies of oxidative crosslinking of cysteine-substituted Tar_E (the E.coli homolog of Tar_s) suggested that the four transmembrane segments of this chemoreceptor were organized as α helices in a loose four-helix bundle (Pakula and Simon, 1992). M. Lebert from G. Hazelbauer's laboratory (Washington State University) showed that extensive cross-linking studies using cysteine-substituted Trg (a chemoreceptor that recognizes ligand-occupied galactose- and ribose-binding proteins) defined a very similar model for the transmembrane segments, even though the two receptors have different amino acid sequences in that region. Thus it seems likely that chemoreceptor dimers have four long continuous helices extending from the membrane-distal, ligand-binding site through the membrane (Figure 2).

How do chemoreceptors signal across the membrane? Since the proteins are dimers under all sensory states examined (Milligan and Koshland, 1988), the mechanism of signaling is unlikely to be simply dimer formation. However, the subunit interface appears to be important, since the structures of the Tar_s ligand-binding domain with and without ligand differed only by a subtle shift in packing between subunits, not in monomer structure (Milburn et al., 1991). One suggestion is that the small shift in subunit packing at the ligand-binding site is amplified by movement of the extended helices to cause a change in the cytoplasmic domain (Milburn et al., 1991). In fact, oxidative cross-linking experiments provided evidence for limited conformational change along the subunit interface both within the transmembrane region (Lynch and Koshland, 1991) and at the cytoplasmic extension of the transmembrane helices (Stoddard et al., 1992). Yet the issue is controversial, since studies with heterodimers containing truncated monomers indicated that at least some signaling occurs in receptors with only one subunit spanning the membrane (Milligan and Koshland, 1991).

Does signaling involve conformational changes within subunits, between subunits, or both? One approach to the issue is mutational analysis. P. Gardina and M. Manson (Texas A&M University) showed that some mutational substitutions in Tar_E, initially identified as causing defects in recognition of the ligands (aspartate or occupied maltose-binding protein), mimicked the signaling effect of ligand occupancy. All but one of these substitutions were near the subunit interface, as were all occupancy-mimicking substitutions in a study of Trg (Yaghmai and Hazelbauer, 1992). These observations provide in vivo evidence that perturbations at the subunit interface can initiate transmembrane signaling. Lebert showed that among 53 Trg proteins with single different cysteine substitutions in the two transmembrane segments, only a limited number were defective and those (partially) defective receptors exhibited less efficient or occupancy-mimicking signaling. The perturbing substitutions occurred along identified packing faces between the subunits (see above), indicating that perturbation of dimeric packing in the membrane can reduce or induce signaling to the cytoplasmic domain in vivo. R. Weis (University of Massachusetts) reported that in vitro studies of a 30 kd fragment of the cytoplasmic domain of Tar_E revealed that interaction between subunits was strongly affected by mutations that locked the domain into a signaling state corresponding to ligand occupancy. It may be that stronger interaction between subunits is a consequence of transmembrane signaling.

A higher level of chemoreceptor organization was addressed by J. Maddock from L. Shapiro's laboratory (Stanford University). She presented evidence from immunoelectron microscopy and immunofluorescence that the receptors, in complex with CheA and CheW, were not distributed randomly over the membrane surface, but rather occurred in clusters, most of which were at the cell poles. Polar localization was dependent on the presence of CheA and CheW. A role for this unanticipated clustering in the functioning of the chemosensory system would be of great interest.

Signaling Circuitry

S. Schuster and R. Swanson (laboratory of M. Simon, California Institute of Technology, Pasadena) used the BIAcore surface plasmon resonance device (Malmqvist, 1993) to demonstrate a complex of receptor, the kinase CheA, the accessory protein CheW, and the response regulator CheY—a result consistent with in vitro binding studies (Gegner et al., 1992) as well as with the in vivo colocalization observed by Maddock. Addition of ATP, presumably resulting in phosphorylation of CheY, released CheY from the complex. Receptor occupancy affected release appropriately. Relatively stable complexes of transmembrane receptors and cytoplasmic signaling components may be a feature of many histidine kinase–based sensory systems. T. Morrison from J. Parkinson's laboratory (University of Utah) and Swanson have both identified a small domain of CheA between the phosphorylation and kinase domains as central for interaction with CheY.

R. Stewart (McGill University, Montreal) reported on collaborative work with A. Wolfe (Loyola University, Chicago) investigating the short form of CheA produced in wild-type cells as the result of an alternative in-frame translational initiation site that creates a product lacking 97 N-terminal residues including the phosphorylated histidinyl residue. They showed that this truncated protein possesses kinase activity capable of phosphorylating the appropriate histidinyl residue on the long form of CheA. This cross-phosphorylation, which appeared to occur in heterodimers, parallels results obtained by Yang and Inouye (1991) for the kinase domain of the transmembrane receptor EnvZ. H. Wang from P. Matsumura's laboratory (University of Illinois, Chicago) found by coimmunoprecipitation that CheZ interacted specifically with the short form but not the long form of CheA and that the complexed CheZ had increased activity in accelerating hydrolysis of CheY–phosphate. This may represent a sophisticated addition to the basic control circuits of two-component signaling.

The calcium ion is involved in many eukaryotic signaling

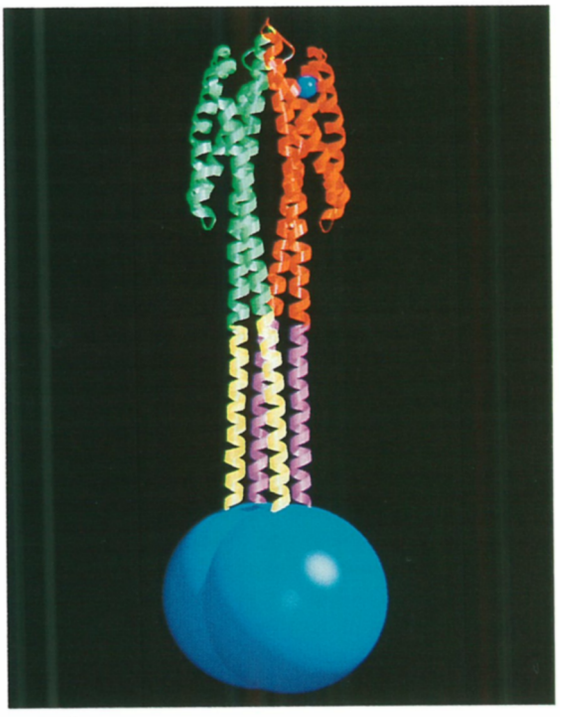

Figure 2.

Figure 2. A Three-Dimensional Model of the Dimeric Chemoreceptor Protein

The green and orange ribbons trace the α-carbon backbone of the two subunits of the ligand-binding domain of the aspartate receptor as determined by X-ray crystallography (Milburn et al., 1991), and the yellow and purple ribbons depict modeled transmembrane helices. The cytoplasmic signaling domains, for which no detailed structural information is available, are represented as blue balls. The position of the single aspartate bound to the receptor dimer is shown as a small blue ball. Figure provided by J. Yeh, M. Milburn, and S.-H. Kim.

Figure 3. Three-Dimensional Structure of E. coli CheY

Two stereo views of the α-carbon backbone (white) are shown.
(A) Residues shown as space-filling models are Asp-57 (yellow), the site of phosphorylation, and residues exhibiting large chemical shifts in NMR analysis upon phosphorylation (red). Blue stippling indicates positions of substitutions for suppressors of mutations in the flagellar switch genes *fliG* and *fliM*.
(B) Residues shown as space-filling models are Phe-111 (yellow) at which a parafluorophenylalanine label was used to monitor movement at Lys-109 (blue) and Phe-8, Phe-30, Phe-53, and Phe-124 (red) at which parafluorophenylalanine labels showed large changes in chemical shift upon phosphorylation.

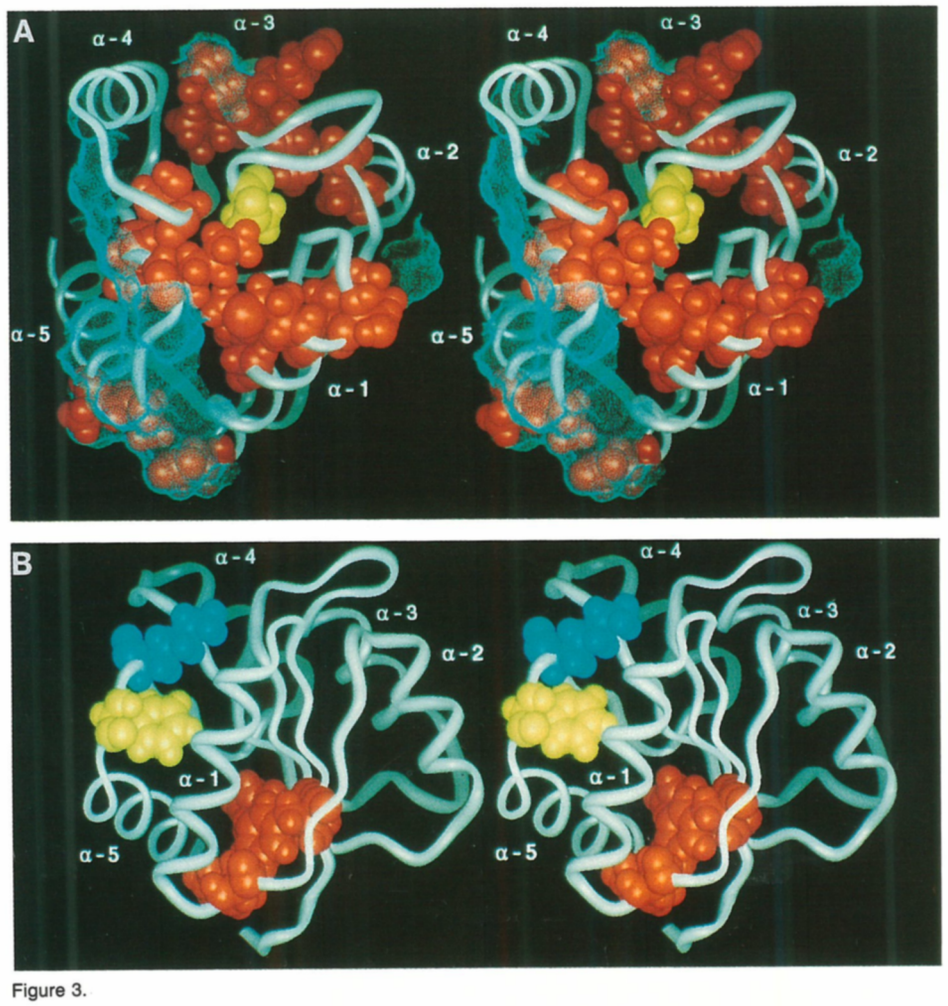

Figure 3.

systems, but early reports of its involvement in bacterial sensory systems have not resulted in a defined role for this ion in taxis mechanisms. L. Tisa (University of Wisconsin, Madison) has reinvestigated the issue in E. coli (Tisa and Adler, 1992) and found tantalizing indications that internal free Ca^{2+} could influence motile behavior and that the level of free Ca^{2+} appeared to change transiently upon sensory stimulation. It will be particularly interesting to see how these observations are related to the known components of the signaling pathway.

CheY: Molecular Details of a Response Regulator

The response regulator CheY is a receiver domain without a separate output domain. Information about its structure and activation are likely to be applicable to receiver domains of all response regulators. The three-dimensional structure of CheY from both S. typhimurium (Stock et al., 1989) and E. coli (Volz and Matsumura, 1991) shows an α/β organization with the phospho-accepting Asp-57 in an "acid pocket" at one end of the protein (Figure 3). Phosphorylation is thought to induce an active conformation that allows CheY to interact with the flagellar motor along a face removed from the site of phosphorylation (Figure 3A). Available evidence argues that CheY catalyzes its own phosphorylation and dephosphorylation, and thus the protein can be viewed as a phosphatase that removes the phosphoryl group from histidinyl phosphate to create a phosphorylated enzyme intermediate that exhibits delayed hydrolysis. Like eukaryotic G proteins, the prokaryotic response regulators can be viewed as molecular switches. In fact, there are significant structural similarities between CheY and the Ras family of signal-transducing proteins (for further consideration of these issues see Parkinson and Kofoid, 1992; Stock et al., 1991).

F. Dahlquist (University of Oregon) reported results from ongoing multidimensional nuclear magnetic resonance (NMR) studies aimed at defining the conformation in solution of CheY and CheY–phosphate. The short lifetime of the phosphorylated species has precluded crystallographic studies, but Dahlquist exploited the observation by Lukat et al. (1992) that some phosphorylated small molecules serve as donors to CheY and supplied sufficient acetyl phosphate to maintain CheY in a phosphorylated state long enough to collect useful data. Substantial changes in the backbone conformation were detected in three regions of CheY (Figure 3A), one in the immediate vicinity of the phosphorylation site and the others overlapping regions where mutational analysis had placed the motor interaction face (Sockett et al., 1992; Roman et al., 1992). Complementary ^{19}F NMR studies of fluorine-labeled side chains by J. Falke (University of Colorado) detected changes upon phosphorylation at a cluster of positions distant from the modified residue (Figure 3B). Taken together, the NMR data are consistent with a substantial conformational change triggered by phosphorylation and provide the first structural information about the activated state of CheY. The results imply that the motor-interaction face identified genetically undergoes a phosphorylation-induced conformational change that allows binding to specific motor components. M. Welch of M. Eisenbach's laboratory (Weizmann Institute, Rehovot, Israel) used immobilized CheY–phosphate (generated using acetyl phosphate) to assess binding to the three switch proteins FliG, FliM, and FliN. Genetic studies had indicated that both FliM and FliG interacted with CheY (Parkinson et al., 1983), but Welch's in vitro studies detected strong binding of the former and weak binding of the latter, suggesting that FliM is the primary interaction site.

The magnesium ion is required for both phosphorylation and dephosphorylation of CheY. A. West (laboratory of A. Stock, University of Medicine and Dentistry, Piscataway, New Jersey) described localized but potentially important differences between the structure of CheY lacking bound Mg^{2+} and the newly determined crystal structure of CheY complexed to the ion. Magnesium binds at the active site with octahedral coordination to three protein oxygens (from the carboxylates of Asp-13 and Asp-57 and the backbone carbonyl of Asn-59) and three molecules of H_2O. Thus, the highly conserved aspartyl residues are crucial in positioning the divalent ion for catalysis of phosphoryl transfer. Lys-109 is an invariant residue in receiver domains. Mutational substitutions there have dramatic effects, including uncoupling of phosphorylation from activation. In the structure of CheY without bound Mg^{2+}, there is a strong ionic interaction between Lys-109 and the phosphorylation target, Asp-57 (Volz and Matsumura, 1991). Phosphorylation might displace Lys-109 and thus initiate the conformational change. Monitoring a ^{19}F-labeled Phe-111 (Figure 3B) by NMR, Falke examined six mutant CheY proteins in which single substitutions had created constitutively active proteins (Bourret et al., 1990) and found spectral changes in the Phe-111 side chain corresponding to those generated by substitutions that eliminated interaction between Lys-109 and Asp-57. However, phosphorylation of wild-type CheY had little effect on the probe at position 111. This could mean that the amino group of Lys-109 maintained an ionic interaction in the phosphorylated protein, shifting from the carboxylate of Asp-57 to the acyl phosphate of the same residue after phosphorylation. To complicate matters further, in the magnesium-bound form of CheY described by West, the side chain of Asp-57 adopts a conformation that places the carboxylate too distant for interaction with Lys-109. This is clearly an area that requires further studies in the solution state.

Signaling Components in Other Organisms

Much information is emerging from the sequencing of genes from other organisms, many of which are related to a known gene in E. coli or S. typhimurium. The most extensive sequence information available is for Bacillus subtilis, in which G. Ordal (University of Illinois, Urbana) has identified homologs to all the *che* genes of E. coli, except *cheZ*, as well as to many flagellar genes. In addition, his laboratory has identified, within an enormous 26 kb operon devoted to chemotaxis and motility, open reading frames that do not correspond to any known sensory components in E. coli. The products of these genes may be involved in features of the B. subtilis sensory system

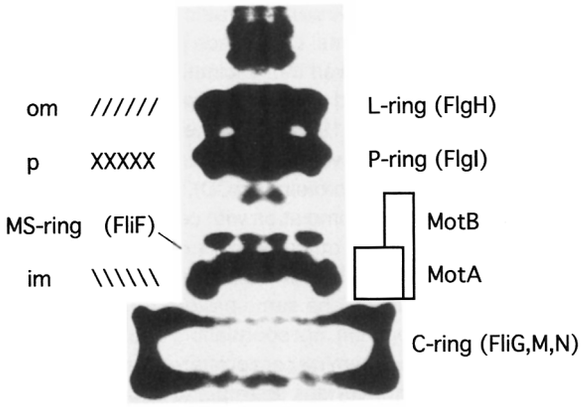

Figure 4. A Mockup of the Flagellar Rotary Motor of a Gram-Negative Bacterium

The photograph represents averaged images of basal bodies of S. typhimurium (provided by N. Francis, G. Sosinsky, D. Thomas, and D. J. DeRosier). Added to this structure are outlines of one set of force-generating elements (MotA and MotB) and parts of the cell wall (om, outer membrane; p, peptidoglycan layer; im, inner or cytoplasmic membrane), together with names for other components and their gene products. The rod (not visible in this high contrast image) passes from the MS ring through the L and P rings and attaches to the hook, part of which is shown at the top of the figure. The hook connects to the filament via hook-associated proteins 1 and 3, and the distal end of the filament is capped by hook-associated protein 2 (not shown). Scale, the L and P rings are about 26 nm in diameter.

apparently different from E. coli (Bischoff and Ordal, 1992). Other homologous genes were described by L. McCarter (University of Wisconsin, Madison; two motility and four flagellar genes from Vibrio parahaemolyticus), R. Schmitt (University of Regensburg, Federal Republic of Germany; five *che* genes, two unusual *mot* genes and a gene encoding a protein with a chemoreceptor-like signaling region from Rhizobium meliloti), and W. Deakin and C. Shaw (University of Durham, England; flagellar genes from Agrobacterium tumefaciens, including three genes for flagellar filament proteins corresponding to the set of genes from the related species R. meliloti).

Bacterial Flagella and Motility

Bacterial flagella are powered by the only known biological rotary motor. Elucidation of the structure and mechanism of such a motor, with dimensions of a few hundred angstroms, is a major intellectual and technical challenge. The axial core of the basal body of the flagellum of a gram-negative bacterium (Figure 4) comprises four rings on a rod: the M, S, P, and L rings, named for the components of the cell wall with which they are associated (membrane/cytoplasmic, supramembrane, peptidoglycan, and lipopolysaccharide/outer membrane, respectively) (DePamphilis and Adler, 1971). The M and S rings, now called the MS ring, because they are products of a single gene, *fliF* (Ueno et al., 1992), are thought by most to turn rigidly with the rod (the drive shaft), which drives the hook (a flexible coupling) and thence the flagellar filament (a helical propeller). According to this model, the L and P rings serve as a bushing that gets the rod through the outer membrane (Berg, 1974). DePamphilis and Adler (1971) visualized these components by negative staining, following solubilization of cells in lysozyme-EDTA and Triton X-100. Elements necessary for force generation, MotA and MotB, were visualized later by freeze fracture and appeared as transmembrane studs surrounding a doughnut-shaped depression, believed to arise through collapse of the MS ring (Coulton and Murray, 1978; Khan et al., 1988).

A new structural element, the C ring (Figure 4), was described by D. DeRosier (Brandeis University, Waltham, Massachusetts) and by I. Khan and S. Khan (Albert Einstein College of Medicine, New York). This ring is much larger in diameter than the MS ring and extends into the cytoplasm. The Brandeis group prepared basal bodies by avoiding exposure to extremes of pH or high ionic strength and produced a three-dimensional reconstruction from averaged images of ice-imbedded specimens. The Einstein group purified basal bodies by Percoll density-gradient centrifugation and Sephacryl S-1000 gel filtration, and then visualized them by negative staining (Khan et al., 1992). Immunoblots of the Brandeis preparations revealed the presence of the switch proteins—FliG, FliM, and FliN—known to control the direction of flagellar rotation, and antibodies to these proteins decorated the C ring/MS ring region of the structure. The Einstein group found that the C ring was missing in preparations made from nonmotile alleles of *fliG*, *fliM*, and *fliN* but present in preparations made from motile, nonchemotactic alleles. Thus, the switch proteins reside in the C ring or at the C ring/MS ring interface.

This interpretation was reinforced by R. Macnab (Yale University), whose group has sequenced a large number of switch-gene mutations and identified in-frame fusions between *fliF* (the MS ring gene) and *fliG* (Francis et al., 1992) and between *fliM* and *fliN*. Cells expressing these fusions are motile and can change the direction of flagellar rotation. The simplest interpretation of these results is that FliG is normally attached to FliF, while FliM is normally attached to FliN. (Flagellar structure has been reviewed by Macnab, 1992.) S.-I. Aizawa and K. Oosawa (Teikyo University, Japan) have purified the MS ring and switch proteins and determined which proteins interact in vitro. Both FliG and FliM interact with the MS ring—G strongly, M more weakly. FliN fails to interact with the MS ring, but it does interact with FliM. So the best guess now is that FliG and FliM are bolted onto the MS ring, while FliN is attached to FliM. If this is true, the switch is part of the rotor, not the stator.

F. Dailey and H. Berg (Harvard University) reported that enzymes involved in periplasmic disulfide bond formation (products of the *dsbA* and *dsbB* genes) are required for assembly of the P ring, with *dsbB* defects readily suppressible by cystine. D. Blair (University of Utah) has attempted to restore normal flagellar rotation to cells containing little or no functional FliM or FliN. For FliM, induced production of the normal protein resulted in little improvement, with many cells functioning at the "one-piston" level. (The strategy of such experiments is described by Blair and Berg, 1988.) With FliN, restoration was eventually complete, but along the way there were dramatic fluctuations in speed.

The stoichiometry appeared to be 8, suggesting that FliN might be more closely associated with the motility proteins MotA and MotB than with the MS ring.

A variety of other motors and motility systems were discussed. E. Baeuerlein (Max Planck Institut, Martinsried, Federal Republic of Germany) described an anaerobic gram-negative bacterium Wolinella succinogenes, which has a C ring and also a large disk associated with the L ring and the outer membrane, comprising 2300 subunits arrayed in an Archimedean spiral. Y. Imae (Nagoya University, Japan) described alkalophilic bacilli and a marine vibrio whose flagella are driven by a sodium-motive rather than a proton-motive force (see Imae and Atsumi, 1989). This motility is inhibited competitively by amiloride. With 6-iodoamiloride and exposure to ultraviolet light, the inhibition is irreversible. With Bacillus firmus, the inhibition appeared to be stepwise, suggesting the presence of several (5 to 9) independent force-generating units. J. Armitage (Oxford University) spoke about the photosynthetic purple bacterium Rhodospirillum sphroides, which either spins its motors clockwise or stops. These motors are proton driven, even at pH values as high as 10. Klinokinesis (modulation of stopping frequency) and orthokinesis (change in swimming speed) both occur on exposure to chemoattractants, which are not recognized by methyl-accepting chemotaxis proteins.

Behavior and Development in Myxobacteria

Myxobacteria are relatively closely related to E. coli by molecular taxonomy, but they exhibit developmental behavior of a complexity rarely observed in prokaryotes. Mutational analysis of motility (a very slow gliding at rates of 2–4 μm per minute) and fruiting body formation in Myxococcus xanthus led to isolation of several genes important in these processes (for reviews see Shimkets, 1990; Zusman and McBride, 1991). Many of the deduced gene products are related to sensory or other proteins of more conventional prokaryotes. P. Hartzell (University of California, Los Angeles) discussed *mglA* and *mglB*, genes that are crucial for both adventurous (single cell) and social (many cell) gliding in M. xanthus. The MglB protein contained a putative Ca^{2+}-binding site similar to those in calmodulin, and MglA had a near-consensus GTP-binding site.

L. Plamann (Texas A&M) is studying *asg* mutants. These are defective in cell–cell interactions required for fruiting body formation because of defects in release of A signal, a particular group of amino acids believed to be generated by extracellular proteolysis. The deduced sequence of AsgB suggested that it was a DNA-binding protein, while the sequence of AsgA contained both a putative histidine kinase domain and a response regulator domain typical of two-component regulatory systems. H. Kaplan (University of Texas Medical School, Houston) described the *sasA* locus, which is required for fruiting body formation and sporulation and is involved in negative regulation of the gene expressed earliest after stimulation by A signal. Two of the three identified open reading frames coded for products with strong sequence similarity to members of the large family of transport systems, present in both eukaryotes and prokaryotes, known as traffic ATPases or ATP-binding cassette systems. D. Zusman (University of California, Berkeley) reviewed the fascinating information his laboratory has amassed about the *frz* genes of M. xanthus (Zusman and McBride, 1991). These genes encode homologs of all the *che* genes of E. coli except *cheZ*, as well as a methyl-accepting protein (FrzCD), the methylation of which changes upon stimulation with certain compounds. As observed for some other systems (see AsgA above), the histidine kinase and the phosphorylated response regulator were domains of the same protein. *frz* mutants are defective in aggregation, not sporulation, and have altered patterns of reversals reminiscent of altered behavior of *che* mutants in E. coli. Previous attempts to assay migration in chemical gradients had led to the conclusion that M. xanthus did not exhibit chemotaxis, but Zusman described a new procedure that demonstrated positive and negative chemotaxis and a requirement for methylation of FrzCD in the process.

Tactic Behavior in an Archaebacterium

The archaebacterium Halobacterium halobium is phototactic and chemotactic. It lives in very high salt concentrations and produces four photoactive proteins similar to visual pigments, all of which exhibit a common organization of seven transmembrane helices. Two are light-driven ion pumps—bacteriorhodopsin, which pumps protons, and halorhodopsin, which pumps chloride ions—and two are sensory rhodopsins known to mediate phototaxis.

S. Bibikov (Max-Planck-Institut, Martinsried) showed that in a strain lacking the sensory rhodopsins, bacteriorhodopsin mediated photoresponses by a mechanism involving changes in membrane potential. Those photoresponses were linked to the methylation system of this organism. V. Yao and J. Spudich (University of Texas Medical School, Houston) reported the sequence of *htrI*, the gene coding for a methyl-accepting protein functionally linked to sensory rhodopsin I (SR-I) (Yao and Spudich, 1992). This protein was clearly related to the chemoreceptors of E. coli: it contained two putative transmembrane segments and two candidate methyl-accepting regions that bracketed a region of highest identity with the chemoreceptors, a segment implicated in E. coli in interaction with the soluble kinase, CheA. Conserved sequences in this latter region imply that signaling in H. halobium is also via a histidine kinase. HtrI lacked the ligand-binding domain of the chemoreceptors but contained additional sequence in the cytoplasmic domain. Altered spectroscopic and kinetic properties of SR-I in cells lacking HtrI, determined by E. Spudich, implied that the two proteins are in physical contact, perhaps involving the additional sequence in HtrI. W. Marwan (laboratory of D. Oesterhelt, Max-Planck-Institut, Martinsried) reported the independent sequencing of *htrI* and analysis of the link between HtrI and SR-I, coming to the same conclusions as the Spudich laboratory. Marwan noted that signal transduction from H. halobium photoreceptors to the flagellar motor exhibited substantial amplification and reviewed evidence

that implicated fumarate as a factor that influences the flagellar motor.

A. Schimz (Research Center, Jülich, Federal Republic of Germany) discussed evidence for higher order control of motile behavior in H. halobium. Cells submitted to certain frequency ranges of periodic light stimulation or to an inhibitor of phosphodiesterase exhibited nonrandom distribution of times between reversals, a behavior consistent with the involvement of an endogenous oscillator influencing reversal frequency.

Crosstalk and Integrated Regulation

E. coli may contain as many as 50 transmitter–receiver pairs, raising issues of specificity and cross-talk. Phosphotransfer between heterologous pairs of kinases and response regulators has been observed under certain circumstances in vivo and in vitro. B. Wanner (Purdue University, Indiana) described a well-documented example of cross regulation for the response regulator PhoB, involved in expression of the phosphate regulon, which occurred under physiological conditions and could well have physiological significance. In the absence of its cognate sensor kinase, the regulator could become phosphorylated by interaction with a noncognate kinase and thus activate gene expression. Another possibility for alternative regulation arises from the observation that various small molecules including acetyl phosphate can serve as donors in phosphorylation of response regulators (Lukat et al., 1992), suggesting that such phosphorylation might occur in vivo and thus provide a pathway by which gene expression could be influenced by cellular levels of metabolic intermediates. A number of laboratories reported observations consistent with this notion. W. McCleary and J. Stock (Princeton University, New Jersey) measured intracellular acetyl phosphate and found substantial differences depending on carbon source and growth phase. These different levels affected production of outer membrane porins, a process controlled by a two-component system, presumably via phosphorylation of the response regulator, OmpR. A. Ninfa (Wayne State University, Detroit) found that the Ntr system responded to cross-talk phosphorylation via acetyl phosphate. C. Park (Korea Advanced Institute of Science and Technology, Taejon) provided evidence for a global regulatory network by showing that mutations in the metabolic pathway for acetyl phosphate altered production of flagella and of an outer membrane porin.

Concluding Remarks

In recent years, the number of laboratories involved in the study of signal transduction in bacteria has grown rapidly, in part because the well-characterized components of the chemotaxis system of E. coli and S. typhimurium are attractive subjects for studies at the molecular level. Also, however, a surprisingly large number of seemingly unrelated aspects of prokaryotic cell biology appear to involve a common signaling mechanism based on a particular type of phosphotransfer. The combination of unifying mechanism and diversity of system (and thus of informative variations) has great promise for producing insights into fundamental principles of signal transduction.

Acknowledgments

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