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Diversity in Chemotaxis Mechanisms among the Bacteria and Archaea

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INTRODUCTION

The discovery of microbes by Antonie van Leeuwenhoeik (59) was aided by their ability to swim, clearly indicating that they are living organisms. Not surprisingly the mechanism controlling this behavior has since then been studied extensively. Arguably, chemotaxis is the best understood of all signal transduction systems that control movement. While the motility apparatus differs among organisms, the general control mechanism is conserved throughout all bacteria and archaea. The centerpiece of this control mechanism is the "two-component" system in which phosphorylation of a response regulator reflects phosphorylation of a histidine autokinase that senses environmental parameters (117). This is the most common type of signal transduction system in bacteria and controls diverse processes such as gene expression, sporulation, and chemotaxis. In chemotaxis, events at the receptors control autophosphorylation of the CheA histidine kinase, and the phosphohistidine is the substrate for the response regulator CheY, which catalyzes the transfer of the phosphoryl group to a conserved aspartate (for a recent review, see reference 250). The resulting CheY-P can interact with the switch mechanism in the motor (42, 149, 186, 193, 234). This interaction causes a change in behavior, such as in direction or speed of rotation of flagella. Thus, for example, in *Bacillus subtilis*, binding of the attractant asparagine to the receptor McpB quickly increases the levels of CheA-P and CheY-P, as the excitation event, and produces increased counterclockwise (CCW) rotation of the flagella (265). The receptors undergo adaptation, a feature that allows the mechanism to reset so that bacteria can progress up concentration gradients of attractants or down concentration gradients of repellents (152). In general, the

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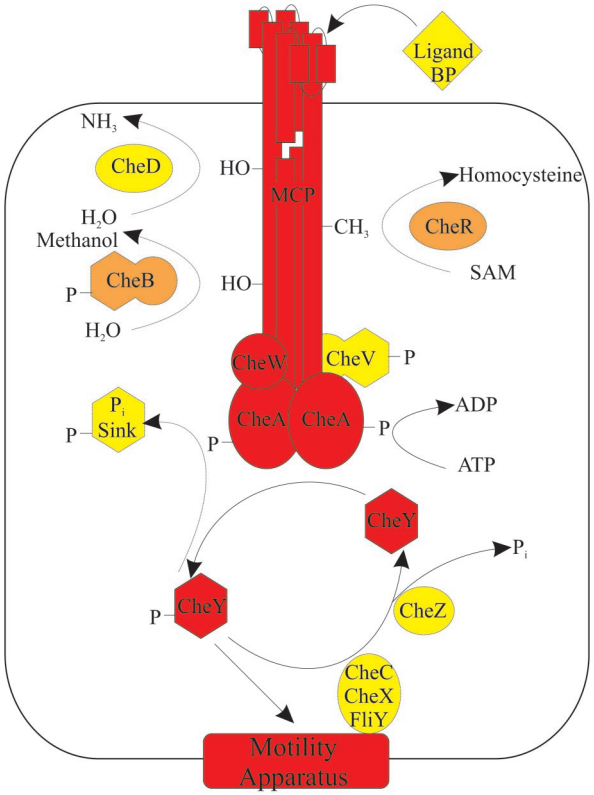

FIG. 1. General chemotaxis model. A schematic of the biochemical processes in the two-component chemotaxis pathway is shown. Hexagons represent response regulator domains. The universal components are in red; almost universal components are in orange; optional components are in yellow.

excitation process is highly conserved and there is considerable variety in the adaptation process (Fig. 1).

Besides these two core proteins, many other proteins contribute to making the process work. Chemotaxis proteins can be ordered into four groups—a signal recognition and transduction group, an excitation group, an adaptation group, and a signal removal group (to dephosphorylate CheY-P). The signal recognition and transduction group includes the receptors (9, 81, 118) and ligand binding proteins (4, 86), which are capable of binding effectors outside the cell; a few receptors, however, are cytoplasmic (92, 93, 229). The signal, i.e., changing concentrations of a chemical, is then transduced to the excitation proteins, CheA and CheY (34, 90). Adaptational proteins alter CheA activity to reset the system. This can be done either by influencing CheA activity directly or through the receptors. Lastly, the signal removal proteins ensure that CheY-P levels can be adjusted to prestimulus levels quickly (the roles of the chemtaxis proteins are summarized in Table 1).

All biochemical processes described here were first discovered in the enteric bacteria *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. Fortunately, the enteric chemotaxis system turned out to be comparatively simple. Since then, chemotaxis in many diverse organisms has been studied and many, often complex, variations have been found. This review aims to summarize and compare the different chemotactic systems that have been studied to date. The main focus is not *E. coli* chemotaxis, since many review articles have dealt with its chemotaxis, but *Bacillus subtilis*, which is arguably the second best understood chemotactic bacterium. Other than *cheZ*, it possesses at least one copy of each chemotaxis protein found to date (although some fusions of chemotaxis proteins exist that are not found in *B. subtilis*).

As will become apparent, the chemotaxis mechanism in *B. subtilis* is probably close to that of the ancestral organism from which the bacteria and archaea descended, so that understanding this mechanism should provide considerable insights into mechanisms used in the diverse species of motile bacteria and archaea alive today. To appreciate the divergence in chemotaxis, we tried to include in this review some information about at least one representative of each phylum of bacteria and archaea in which a CheA homolog could be found, indicating the existence of a chemotaxis pathway. Of course, many organisms have not yet been studied in detail, and the available information is often based only on the genomic sequence of those organisms. We have made a special effort to include information about organisms whose chemotaxis mechanism appears to diverge from the *E. coli* paradigm. Other reviews, most of which emphasize the *E. coli* mechanism, include references 13, 39, 61, 224, and 227. The review by Berg (20) does justice to the rather considerable literature dating from the late 19th and early 20th centuries.

SIGNAL RECOGNITION AND TRANSDUCTION

Receptors

Understanding how receptors control the CheA kinase is at the heart of understanding chemotaxis. In *E. coli*, binding of attractant inhibits the CheA kinase (34), whereas in *B. subtilis*, binding of attractant stimulates the CheA kinase (64, 67). It is important to understand the structure of receptors and how binding of attractant (or repellent) changes the activity of the associated CheA kinase.

Classes of receptors. The receptors are usually transmembrane proteins with an extramembrane sensing domain that binds attractant across the dimeric interface (253), two transmembrane (TM) regions (TM1, between the N terminus and the sensory region, and TM2, between the sensory region and the cytoplasmic regions), and several cytoplasmic regions. These include the signaling region, where the CheA kinase and the CheW coupling protein and analogs bind, and the methylation region, where methylation/demethylation of the receptors occurs to compensate for changes in CheA kinase activity caused by binding attractant (Fig. 2). The enzymes catalyzing these reactions, the CheR methyltransferase and the CheB methylesterase, are described below. Between the methylation region and the membrane is the HAMP (histidine kinase, adenylyl cyclase, methyl-accepting chemotaxis protein, and phosphatase) linker, which conveys the signal of attractant binding to the rest of the cytoplasmic region (see below). Based on crystal structures of the extramembrane N-terminal part of Tar, the aspartate receptor of *E. coli*, and of the cytoplasmic C-terminal part of Tsr, the serine receptor, virtually the entire receptor is thought to consist mainly of α-helix (51, 108, 109).

TABLE 1. List and description of proteins involved in chemotaxis

Protein by category	Activity or role	Comment	Where found	References
Signal recognition and transduction				
Methyl-accepting chemotaxis proteins (MCPs)	Receptors	Binds chemoeffectors and transduces signal to CheA. Is methylated and demethylated on glutamate residues.	Universal among all chemotactic bacteria and archaea	9, 33, 46, 56, 75, 104, 109, 110, 118, 119, 128, 148, 171, 179, 197, 199, 208, 213, 215, 229, 237, 241, 243, 245, 247, 264, 265
CheD	Glutamine deamidase	Role in receptor maturation by deamidation of particular glutamine residues	All chemotactic archaea, gram-positive bacteria, *Thermatoga*, and some proteobacteria	67, 122, 182
Ligand binding proteins	Ligand recognition	Binds chemoeffectors and transduces signal to the receptors.	*E. coli*, not yet known for other organisms	1, 3, 4, 24, 28, 36, 40, 53, 65, 85, 86, 88, 118, 141, 157, 158, 165, 166, 256, 266
Excitation				
CheA	Histidine kinase	Autophosphorylates on histidine residue; substrate for CheY and other response regulators.	Universal among all chemotactic bacteria and archaea	23, 33, 64, 67, 70, 90, 137, 151, 156, 185, 218, 233, 264
CheW	Coupling protein	Couples CheA to the receptors.	Universal among all chemotactic bacteria and archaea	37, 70, 78–80, 131, 144, 187
CheY	Response regulator	Primary response regulator. Interacts with the motility apparatus to induce change of swimming behavior when phosphorylated.	Universal among all chemotactic bacteria and archaea	16, 25, 26, 35, 49, 209, 211, 218, 244, 259
Adaptation				
CheR	Methyl transferase	Methylates glutamate residues on MCPs; role in adaptation.	Almost universal among chemotactic bacteria and archaea (exception, *H. pylori*)	47, 48, 116, 182, 214, 264, 265
CheB	Methyl esterase	Hydrolyzes methyl glutamate residues on MCPs; role in adaptation. Usually has response regulator domain.	Almost universal among chemotactic bacteria and archaea (exception, *H. pylori*)	10, 45, 57, 73, 74, 90, 102, 103, 106, 108, 113, 115, 130, 134, 145, 160, 217, 219, 220, 226, 264
CheV	Coupling and adaptational protein	Couples CheA to the receptors; response regulator domain can be phosphorylated; role in adaptation.	Many bacteria including the *E. coli* close relative *S. enterica* serovar Typhimurium; however not in the archaea or *E. coli*	63, 99, 181
Signal removal				
CheC	Phosphatase and adaptational protein	Hydrolyzes CheY-P; also probable role in adaptation.	All chemotactic archaea, gram-positive bacteria, *Thermatoga*, and some proteobacteria	112, 182, 183, 190
CheX	Probable phosphatase	Homologous to CheC, probably has the same function.	The spirochetes, some archaea, some gram-positive bacteria, *Thermatoga*, and some proteobacteria	69, 77, 137
CheZ	Phosphatase	Hydrolyzes CheY-P.	The β- and γ-proteobacteria	29–32, 43, 49, 90, 146, 188, 189, 195, 223, 257, 261
FliY	Phosphatase at the flagellar switch	N terminus homologous to CheC; hydrolyzes CheY-P; integral part of the flagellar switch.	The gram-positive bacteria, some spirochetes, and *Thermatoga*	26, 95, 112, 234
CheY*	Phosphate sink	Alternative CheY that lowers primary CheY-P levels by acting as a phosphate sink.	The α-proteobacteria, possibly others	172, 191, 196, 212

Le Moual and Koshland (124) showed that the chemotaxis receptors in the bacteria and archaea fell into three classes, based on the presence or absence of two pairs of insertions (called "indels," for insertions/deletions) of 14 amino acids (four turns of the α-helix). The class III receptors are likely to be the ancestral receptors (124). The locations of these pairs in *B. subtilis* McpB are illustrated in Fig. 2. They lie on the membrane-proximal side of the signaling regions and methylation regions. The class III receptors have both pairs, the class II receptors have only the pair between the signaling and methylation regions, and the class I receptors have neither. More recent analysis of sequences in the database (Fig. 3) gives some additional perspective. The original receptors are obviously the class III receptors, but they have undergone modifications early in the lines of descent. One modification, which presumably occurred after the gram-positive bacterial line had diverged from the original line and before the proteobacterial line had diverged, was the deletion of the first and fourth indels to produce the class II receptors. The class I receptors, which involve deletion of indels 2 and 3, may have arisen several times, once during formation of the α-proteobacterial line (see *Rhodobacter sphaeroides*), once during formation of the δ-proteobacterial line (see *Myxococcus xanthus*), once during formation of the β-proteobacterial line (see *Ralstonia solanacearum*), and once after the γ-line had been formed (see *E. coli* and *Pseudomonas aeroginosa*) (Fig. 3). Alternatively, and more probably, the class I receptors may have arisen fewer times and, early during the evolution of a particular line of descent, may have entered by gene transfer and displaced the original receptors. One obvious case of gene transfer is in *Clostridium acetobutylicum*, a gram-positive bacterium having 38 class III receptors (all the rest of

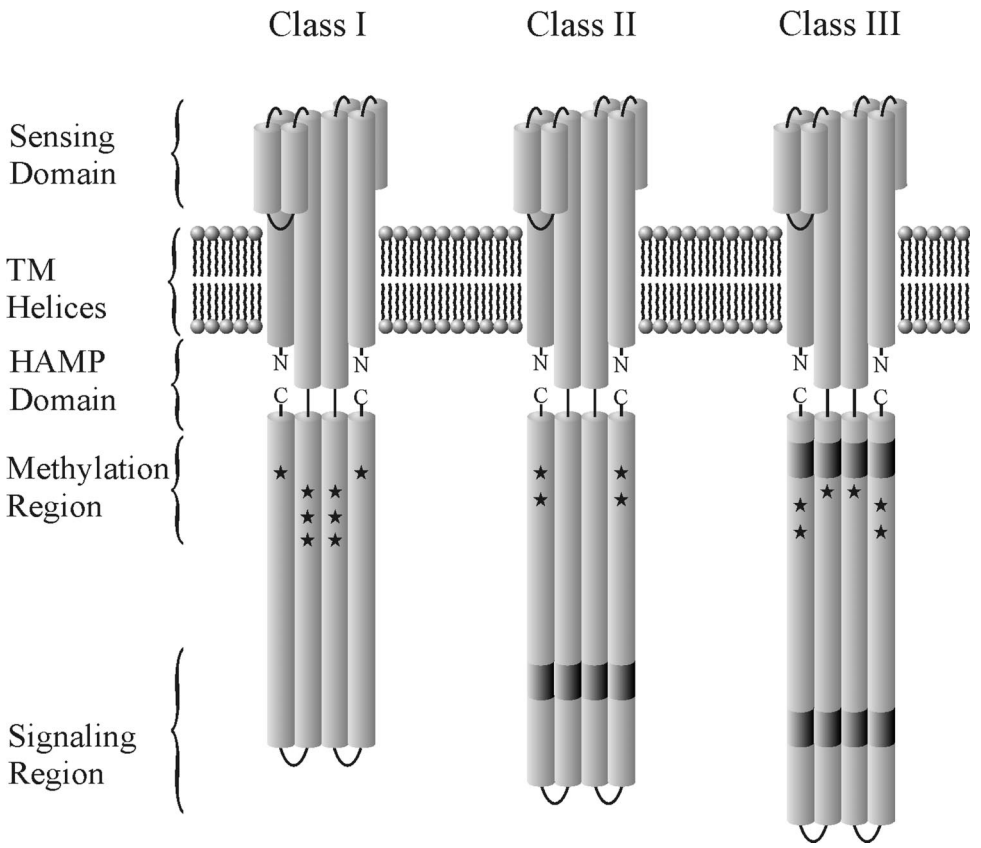

FIG. 2. Schematic of the three classes of chemotaxis receptors. Shown is a representative dimer for each class of chemotaxis receptors. Insertion/deletion regions (indels) are shaded in dark gray. Stars indicate sites of methylation for (from left to right) *E. coli* Tar, *M. xanthus* FrzCD, and *B. subtilis* McpB.

the gram-positive bacterial receptors are class III) and one class I receptor (Fig. 3). The receptors in the spirochete *Borrelia burgdorferi*, here termed class O, are a special case: these receptors are missing indels 1 and 4 but are missing pairs of 21 amino acids where the loss of indels 2 and 3 would produce a loss of only a pair of 14 amino acids.

HAMP domain. As implied above, the HAMP domain or linker is found in a number of different types of proteins. Although there is little sequence identity among HAMP domains, they generally have two segments of hydrophobic aminoacyl residues in a heptameric arrangement characteristic of amphipathic α-helices joined by unstructured amino acids (11, 12). The α-helices are probably in a coiled coil (206). The purpose of HAMP domains is generally to convey signals from input domains to output modules. Mutations in the *E. coli* receptor Tsr HAMP domain caused locked signal output (that is, persistently clockwise [CW] or CCW or in between, but switching rarely) (8).

Methylation of class I and III receptors. Considerable work has been done on McpB from *B. subtilis*, which might be considered a prototype for the class III receptors. The first difference noticed between the class I and III receptors was that methanol was released in response to all stimuli in *B. subtilis* (111, 240) and *Halobacterium salinarum* (163, 216) whereas it was released from *E. coli*, the prototype organism for class I receptors, only after the application of negative stimuli; methanol evolution in this species was suppressed below background levels after the application of positive stimuli (105, 241, 242). Interestingly, methanol is released from the class I receptors of *R. spheroides* on addition of attractant (which, as in *E. coli*, inhibits CheA [196]) (145). There is no consequence on methanol formation of removing the attractant. However, as described below, this organism has multiple copies of chemotaxis genes. The principal methyltransferase appears to be CheR2; deletion of *cheR1* causes methanol to be produced after both addition and removal of attractant (145). The related organism, *Rhodospirillum centenum*, however, behaved as might have been anticipated from the *E. coli* precedent: a reduction of light intensity, which would cause inhibition of the CheA, caused methanol formation, and increase of light intensity did not (97).

What is the reason for this difference in methanol formation? In *E. coli*, methylation of receptors increases CheA kinase activity, an adaptational mechanism to compensate for the decreased activity caused by attractant, and it does not appear to matter which sites become methylated (106, 197, 238). By contrast, each of the sites in McpB, the one class III receptor that has been characterized in some detail, appear to have a different function. Glu630 is demethylated both after addition and removal of attractant (265) (provided that this site is in the methylated form). When it is changed to Asp630, which cannot be methylated (198), the resulting mutant has a

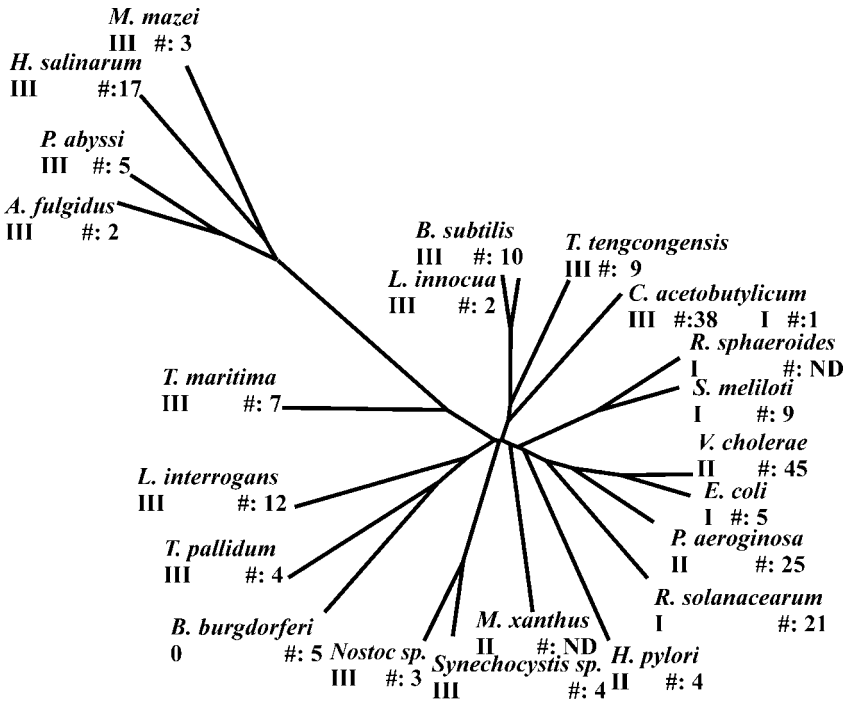

FIG. 3. Phylogenetic tree of chemotactic bacteria and archaea. The phylogenetic tree was generated from the 16S rRNA sequences by using the programs CLUSTALW and DRAWTREE. Included is information regarding the number and class of chemoreceptors for each respective organism. ND, not determined. Organisms, by phylum, are as follows: *Archaea*: *Archaeoglobus fulgidus*, *Halobacterium salinarum*, *Methanosarcina mazei*, and *Pyrococcus abyssi*; *Thermotogales*: *Thermotoga maritima*; spirochetes: *Borrelia burgdorferi*, *Leptospira interrogans*, and *Treponema pallidum*; cyanobacteria: *Nostoc* and *Synechocystis* spp.; gram-positive bacteria: *Bacillus subtilis*, *Clostridium acetobutylicum*, *Listeria innocua*, and *Thermoanaerobacter tengcongensis*; proteobacteria (α-subgroup): *Rhodobacter sphaeroides* and *Sinorizhobium meliloti*; proteobacteria (β-subgroup): *Ralstonia solanacearum*; proteobacteria (δ-subgroup): *Myxococcus xanthus*; proteobacteria (ε-subgroup): *Helicobacter pylori*; proteobacteria (γ-subgroup): *Escherichia coli*, *Pseudomonas aeroginosa*, and *Vibrio cholerae*.

low prestimulus bias; when attractant is added, it adapts only to the higher wild-type bias, and when attractant is removed, it hardly adapts at all (265). Glu637 seems crucial for causing adaptation to attractants, and only on removal of attractants does methanol evolve from that site (provided that this site is in the methylated form). Glu371 (encoded as Gln371 but deamidated (probably by CheD [see below]) does not play a role during adaptation to addition or removal of attractant but may in some way be involved in being a regulator of methylation, since an *mcpB* Q371A mutant shows poor taxis to high concentrations of asparagine, similar to a *cheB* mutant (see below) (M. A. Zimmer and G. W. Ordal, unpublished data). Methanol arises from Glu371 only on addition of attractant (provided that this site is in the methylated form). Thus, in *B. subtilis*, each of the positions from which methanol arises has a different function, and these functions, in the case of McpB (as stated above), cause methanol production from Gln(Glu)371 and Glu630 on addition of attractant and from Glu630 and Glu637 on removal (provided that these sites are in the methylated form). The extent to which this rule about particular sites having particular functions applies generally is not known; further experiments are required, and one obvious organism with which to explore this question would be *R. spheroides* (see previous paragraph). In any case, what had seemed until now the general rule that class III receptors produce methanol in response to all stimuli and class I receptors do so only in response to negative stimuli does not appear to be true (the case of *R. spheroides* contradicts this rule). No work of this type has been done for any class II receptors.

Structure of receptors. The *E. coli* receptors are stable dimers (153) arranged as trimers of dimers (9). The *B. subtilis* receptors are also similarly arranged, and, in fact, the trimers of dimers themselves interact near the outer part of the cytoplasmic membrane (46), as predicted by Kim et al. (110). X-ray structures of the extramembrane ligand binding domain of the *E. coli* receptor Tar (194) and the cytoplasmic domain of the *E. coli* receptor Tsr (109) are available, as well as of the ligand binding domain of the soluble, cytoplasmic *B. subtilis* receptor HemAT, in the presence and absence of the natural ligand O_2. The dimer that binds O_2 (the form that stimulates the CheA kinase) is very symmetrical, and the dimer that is free of O_2 shows a distinct conformational change in the Tyr70 of one of the two subunits of the dimer (254, 255). As described in detail below, the CheR methyltransferase and the CheB methylesterase appear to be especially active on receptors that have just bound or released attractant and before the compensating methylation changes have occurred that would help bring about adaptation. The structural basis of this conformation of increased susceptibility is still unknown (45).

Effect of attractant on structure. One longstanding question has been how attractant induces the receptors to change CheA kinase activity. In *E. coli*, attractant causes diminished CheA activity (35). Based on evidence from work on Cys-substituted receptors, whose cross-linking is accelerated by oxidant, there

appears to be "downward piston" movement of TM2 of one monomer of the receptor dimer (50, 94, 123). Based on experiments where the nitroxide spin label was attached to Cys residues in Cys-substituted receptors, the extent of movement was deduced to be small, only about 1 Å (171). The effect of repellent on these receptors and the effect of attractant or repellent on class III receptors, such as McpB of *B. subtilis*, where attractant activates rather than inhibits the receptor (67), is unknown.

Maturation by CheD. CheD is a deamidase that deamidates particular glutamines in the *B. subtilis* receptors (122), a function carried out by CheB in *E. coli*. Most chemotactic bacteria and archaea carry *cheD*. Therefore CheD is probably the ancestral mechanism of glutamine deamidation. The role of this function is not yet fully understood. However without CheD the receptors are undermethylated and activate the kinase poorly, indicating that deamidation is necessary for activation of the receptors (67, 182).

Binding proteins. Although in most instances chemoeffectors are thought to interact directly with the receptors, in some particular instances, specifically dedicated binding proteins bind the chemoeffector and the complex then binds the receptor. Thus, in *E. coli*, galactose binds to the galactose binding protein (86), ribose binds to the ribose binding protein (4), and the complex binds to the receptor Trg (83, 87). Maltose binds to the maltose binding protein (85), and the complex binds to the aspartate chemoreceptor Tar (176). It is suspected that the *B. subtilis* receptor McpC binds attractants indirectly, via binding proteins, since it mediates taxis to all amino acids except asparagine, some (such as proline and alanine) at very low concentrations (159, 168); however, no mutants in any such putative binding proteins have been identified.

Oxygen sensing. The oxygen sensor in *B. subtilis* is HemAT, which is homologous to myoglobin (92, 93). It is similar to the repellent oxygen sensor, also termed HemAT, in the archeon *H. salinarum*. It is a soluble receptor, having no transmembrane region, and hence senses the internal oxygen concentration. *H. salinarum* has another receptor for oxygen as an attractant. However, this receptor is homologous to cytochrome oxidase of mitochondria (44) and has six membrane-spanning regions and may be a heme protein that also senses oxygen directly. Conversely, Aer, the oxygen sensor of *E. coli*, binds flavin adenine dinucleotide (FAD) (21, 22, 177), and the signal caused by changing oxygen concentrations is probably mediated by changes in the level of reduction/oxidation of this FAD (21, 177, 235, 236), a process involving a PAS domain in the receptor (178). *Pseudomonas putida* would appear to use the same mechanism (161). Tsr in *E. coli* also mediates oxygen taxis, perhaps by sensing changes in the proton motive force across the cytoplasmic membrane (177, 204) but certainly not by binding oxygen directly (Fig. 4).

Many other organisms perform aerotaxis (reviewed in reference 236). *Azospirillum brasilense*, an α-proteobacterium, accumulates in an oxygen gradient at 3 to 5 μM. At both lower and high oxygen tensions, the proton motive force is lower, so that it is assumed that both positive and negative aerotaxis, which causes accmulation of bacteria at the optimum oxygen tension, is due to sensing of changes in the proton motive force (262). *R. sphaeroides* also accumulates at an optimum oxygen concentration. It shows negative aerotaxis due to interaction between the chemotaxis machinery and the Prr system, which monitors electron flow through the alternative high-affinity cytochrome oxidase, cbb_3, and positive aerotaxis by interaction with another, unknown sensor, both of which may operate through one of the chemotaxis kinases of the cell, $CheA_2$ (180). Some species, for example *Sinorhizobium meliloti*, do not seem to respond to oxygen in the same way that *E. coli* and *B. subtilis* do (by modulating the frequency of CCW versus CW rotation) but, rather, change their swimming speed in response to oxygen gradients (263).

Phosphotransferase System

The phosphotransferase system (PTS) helps mediate taxis to a number of sugars and sugar alcohols in *B. subtilis* (68, 121, 169). Transport is required, but metabolism is not. Unlike taxis to PTS sugars in *E. coli*, which does not require a specific receptor and works even when the methylation system is inactivated by mutation (162) (but works poorly unless some receptor is present [136]), chemotaxis to PTS substrates in *B. subtilis* requires the C-terminal part of McpC (Fig. 5). In these experiments, chimeras between the asparagine receptor McpB and the proline receptor McpC revealed that the N-terminal, extramembrane part of the receptors mediated amino acid taxis, as expected, but only the C-terminal part of McpC could mediate taxis to PTS substrates and, in particular, the methylation region appeared to be involved. The data were best interpreted by a model in which unphosphorylated enzyme I interacted with McpC to bring about increased CheA activity and adaptation occurred through the normal means (121). Indeed, methanol was produced on addition of glucose, a sign that CheB was stimulated to help bring about adaptation (239). In *E. coli*, since unphosphorylated enzyme I interacts with CheA (135), it is suspected that interaction of CheA with unphosphorylated enzyme I inhibits CheA. Large changes in the levels of unphosphorylated enzyme I compared with phosphorylated enzyme I occur during chemotactic excitation (136). It would seem likely that the requirement for receptor found by Lux et al. (136) might be due to the inherent low activity of CheA in the absence of receptors (34) rather than the interaction with a specific receptor, as found for *B. subtilis*.

EXCITATION

With the exeption of *Mycoplasma* gliding motility, it appears that bacterial and archaeal motility is universely controlled by the two-component system of the CheA kinase and the CheY response regulator.

CheA Kinase

The central enzyme that mediates input, usually as sensed by the receptors, and creates an appropriate signal for the motor is the CheA kinase. Attractants inhibit it in *E. coli* (34), *S. meliloti* (192), and *R. spheroides* (196) and stimulate it in *B. subtilis* (67). As described in "CheY response regulator" (below), it is likely that for the archaea and the spirochetes, positive stimuli (for instance, chemoattractants or attractant light), decrease CheA activity. Thus, *B. subtilis* would appear to be the

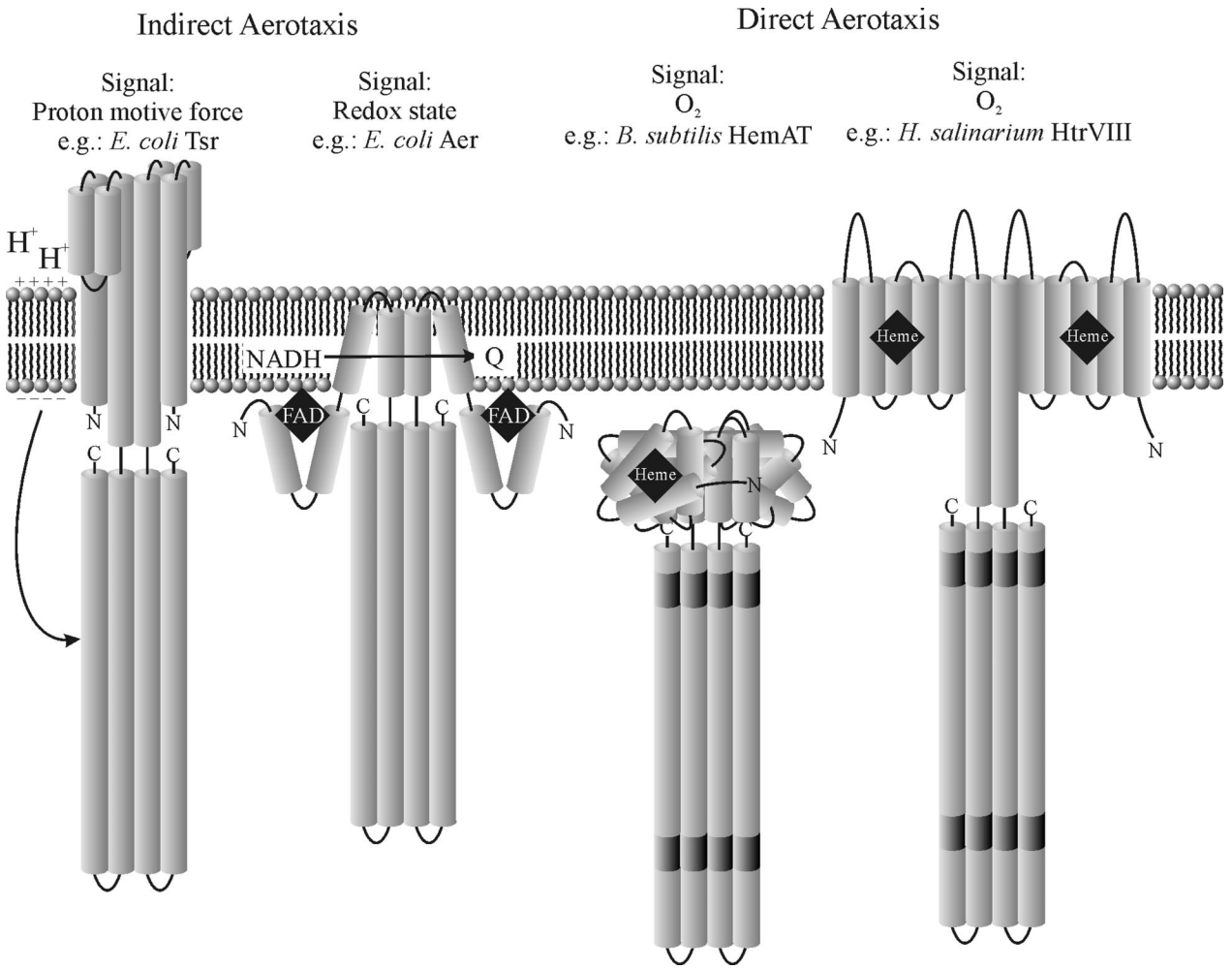

FIG. 4. Aerotaxis receptors. Shown is a schematic of the four known types of aerotaxis receptors. Indirect aerotaxis defines receptors that detect oxygen levels by the proton motive force or redox state of the cell. Direct aerotaxis defines receptors that detect levels directly by interacting with oxygen. Black diamonds represent the indicated receptor cofactors.

exception to this rather incompletely verified hypothesis that positive stimuli decrease CheA activity.

On activation, CheA becomes phosphorylated on a particular histidine residue (His46 in *B. subtilis* and His48 in *E. coli* [258]). CheA has five domains (described in detail in reference 23), and this His residue is located within the first, P1 or Hpt, domain (156). The fourth (P4) domain is where ATP binds and catalysis occurs. The third (P3) domain is the dimerization domain, important since CheA is a dimer that transphosphorylates (232) (the P4 region of one monomer phosphorylates a His residue in the P1 domain of the other monomer). The P5 domain is where CheA contacts the receptors and the coupling protein, CheW. The P2 domain is where CheY and CheB, which receive phosphoryl groups from CheA-P, dock (23, 89, 233). The exact mechanism of CheA autophosphorylation is not yet known; however, several conserved regions within the P4 domain—the N-box, G1-box, F-box, G2 box, and GT-block—are essential for catalysis in *E. coli* and are thought to be involved in positioning of ATP into the active site (Fig. 6) (91).

CheA-P from *B. subtilis* differs from its *E. coli* counterpart in being of considerably lower energy ($K_{eq} = 1.2 \times 10^4$ instead of 1 in the reaction CheA + ATP $\rightarrow$ CheA-P + ADP) (66). When *E. coli* becomes somewhat deenergized, it becomes smooth swimming (107), since CheA cannot be phosphorylated, and thus the bacterium has a larger "diffusion constant" so that it will leave the local environment by rapid translational movement. However, when *B. subtilis* becomes somewhat deenergized, the chemotaxis system still functions. Thus, the bacteria do not become tumbly (the condition in the absence of CheY-P [27]) and thus unable to move away from the unfavorable environment but instead use chemotaxis to depart, a much more effective process than unregulated smooth swimming. In terms of phylogeny, CheA from *B. subtilis* clusters with CheAs from archaea and spirochetes, apart from CheAs of the proteobacteria (2).

Coupling to receptors. CheA is coupled to the receptors via CheW. CheW is present in all bacteria that have chemotaxis or phototaxis receptors. A second protein, CheV, which is a CheW-CheY fusion protein, is also capable of coupling the kinase to the receptors and is described below. CheV is present

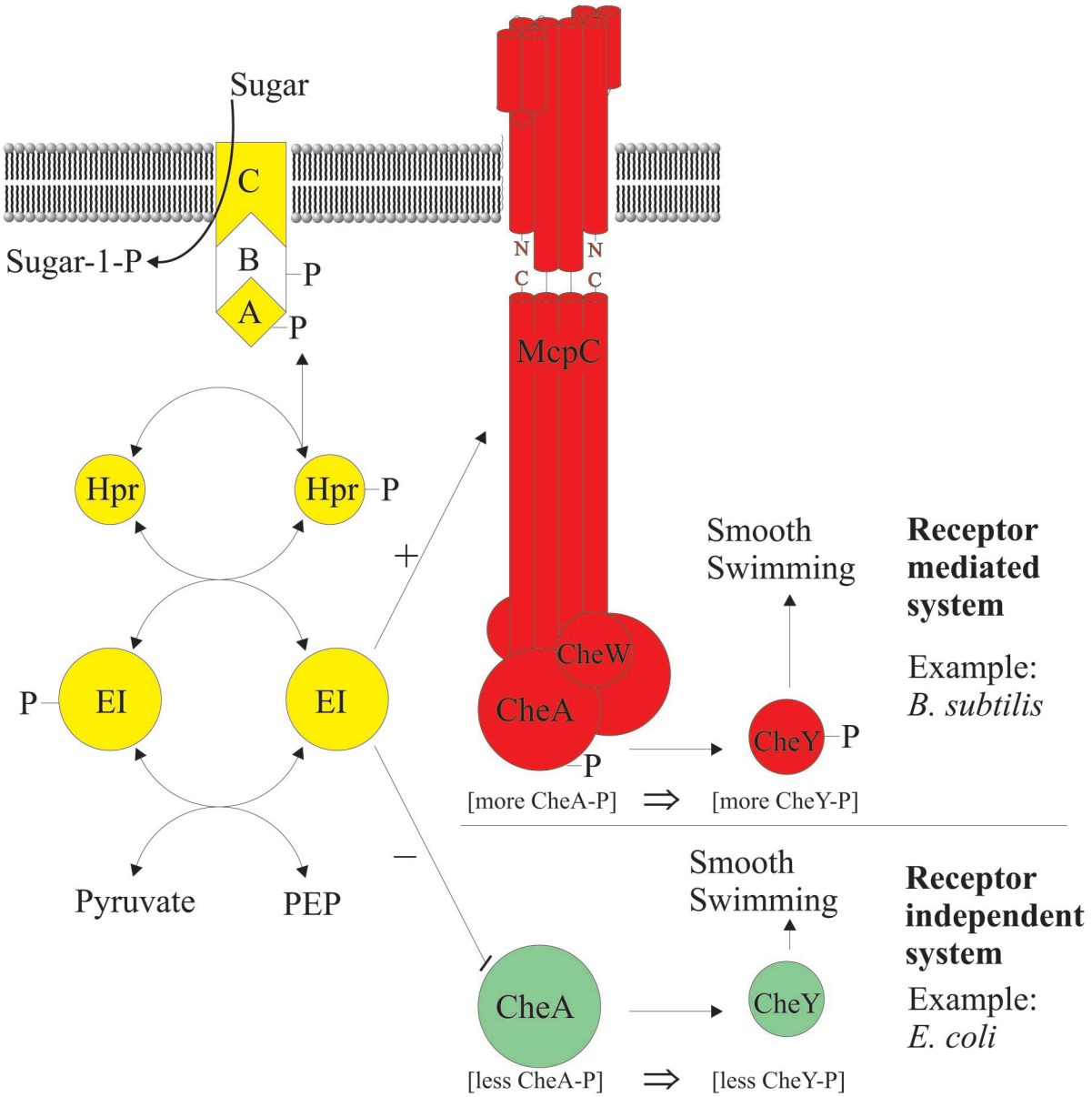

FIG. 5. PTS in chemotaxis. The two known chemotaxis pathways for PTS sugars are shown. Transport of PTS sugars increases the concentration of unphosphorylated enzyme I (EI) that can either directly interact and inhibit CheA (receptor-independent system), as is the case in *E. coli*, or indirectly stimulate CheA through the receptors (receptor-dependent system), as thought to be the case in *B. subtilis*. The letters A, B, and C represent components of an ABC transporter. For better understanding, PTS proteins are in yellow, *B. subtilis* chemotaxis proteins are in red, and *E. coli* chemotaxis proteins are in green.

in many eubacteria, including *S. enterica* serovar Typhimurium, a close relative of *E. coli*, but not in *E. coli* and not in any archaea. In *E. coli*, CheW is required for activation of CheA but not for its inhibition (7, 35). CheW is homologous to the P5 or regulator domain of CheA (23).

CheY Response Regulator

The primary response regulator that governs the direction of flagellar rotation is CheY-P (16, 25, 90) and, as implied above, it causes CCW flagellar rotation in *B. subtilis* and CW flagellar rotation in *E. coli*. It catalizes its own phosphorylation on a conserved aspartate residue by using CheA-P as a substrate. This process is thought to involve several conserved residues—two aspartates, which position an essential Mg^{2+} ion, a lysine, and a threonine (Fig. 6) (132, 244, 260). In flagellated bacteria, CheY-P interacts with FliM, shown for *E. coli* (248) and *B. subtilis* (26, 234). In the spirochete *Treponema denticola*, mutation of *cheA* blocked chemotaxis and caused the bacteria to have few reversals of motion (137), implying that CheY-P causes reversals of motion. To achieve this, it probably binds to FliM, which is present in the spirochetes. The archeon *H. salinarum* similarly showed no reversals of motion when *cheA* or *cheY* was deleted and, indeed, showed preferential forward

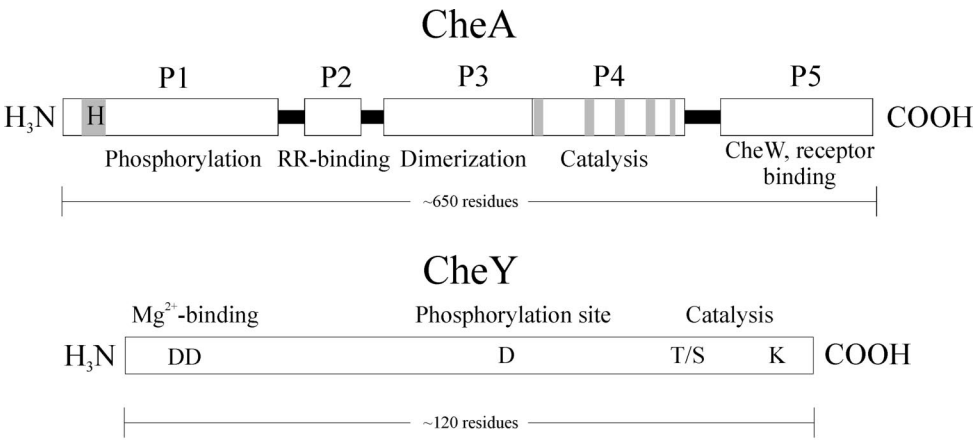

FIG. 6. Schematic of CheA and CheY. Shown are the histidine kinase CheA and the general response regulator CheY. For CheA, the five domains are labeled P1 through P5. The phosphoreceiving histidine in P1 is highlighted in gray, and conserved regions within domain P4 that are thought to play an active role in catalysis are also highlighted in gray. For CheY, conserved residues that participate in catalysis are positioned as indicated.

swimming (chronic CW rotation of the flagella at each end of the archeon) (184). Thus, in this organism, CheY-P also causes reversals of motion and is required for CCW rotation of the flagella. (Note: these are right-handed flagella [5], not the more common left-handed flagella [139] found in *E. coli* and *B. subtilis*, and the flagella themselves are more similar to type IV pili [52] than to the flagella of bacteria.) There are no FliM homologs in the archaea, and the site of the interaction of CheY-P to control the direction of flagellar rotation is unknown. The implication of these findings is that the flagella of *Halobacterium* and spirochetes have a default direction of rotation in the absence of CheY-P, as do the flagella in the peritrichous bacteria like *E. coli* and *B. subtilis*, and that CheY-P not only facilitates rotation in the opposite direction but also facilitates switching between the two directions. How this might occur is mentioned below (see "CheC dephosphorylating and adaptational protein").

Signal Amplification

Binding of one or two receptors by attractant can lead to a behavioral response in *B. subtilis* (113) and *E. coli* (195). Using photoreleased aspartate, Jasuja et al. (96) found that nanomolar asparate (1.2 μM K_D) could evoke a response and that the response times were proportional to changes in receptor occupany near the threshold, irrespective of prior occupancy. (Therefore, adaptation is complete.) In experiments with *E. coli*, using fluorescence resonance energy transfer to measure CheY-P levels (rather than CCW/CW rotation, which is a complex function of CheY-P levels), Sourjik and Berg (210) found that the *cheB* mutant was very insensitive to attractant compared to both the wild type and a *cheR* mutant. Using photoreleased asparate, Kim et al. (108) also found that the *cheB* mutant was far less sensitive than was the wild type. Both groups found that absence of CheZ, which catalyzes the dephosphorylation of CheY-P, had little effect on amplification; therefore, accelerated loss of CheY-P is not the cause of signal amplification; it must be sought in signal generation. How CheB might be involved in this is described in "CheB methylesterase" (below). Besides this, it seems likely that organization of the receptors into a lattice could lead to amplification; in this arrangement, judicious methylation of receptors to increase CheA activity (after reduction of activity from attractant [in *E. coli*]) would allow this amplification to occur over a broad range of attractant concentrations (201, 202). However, it would appear that this alleged lattice quickly forms and disappears, according to circumstances (see "Localization of chemotaxis proteins," below).

ADAPTATION

To sense ever higher concentrations of attractant and to move toward favorable environments, chemotaxis systems have to be able to adapt to existing stimuli. Additionally, the nature of bacterial motion requires the ability to recognize when the bacterium is moving in the wrong direction, i.e., away from higher attractant concentrations. To do that, a "memory" is required that is able to indicate whether higher or lower concentrations are being reached (120). This is achieved by the adaptational mechanisms. The methylation system of CheR and CheB is the only adaptational mechanism in *E. coli* that has been studied, although another, undescribed mechanism may exist (162, 228). However, other organisms, e.g., *B. subtilis*, have at least partly characterized adaptational systems, namely, CheV and CheC (Fig. 7), in addition to the mechanism involving CheR and CheB (99, 112).

Methylation

CheR methyltransferase. CheR methyltransferase transfers methyl groups from *S*-adenosylmethionine to particular glutamate residues (102, 164, 238) on the receptors (82, 164), with production of *S*-adenosylhomocysteine (47, 48, 214). In *B. subtilis*, it is required for adaptation to repellents. In its absence, *B. subtilis* is very tumbly (with predominantly CW rotation of the flagella) (116). In *E. coli*, it is required for adaptation to attractants (76, 119) and binds both to a flexible tether at the C-terminal end of the receptor and to the methylation region

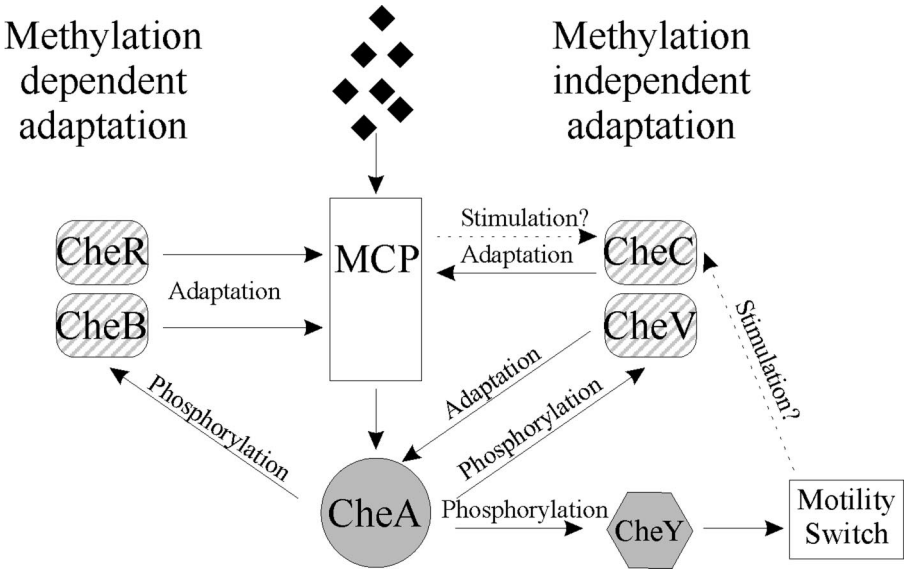

FIG. 7. Adaptation systems. A flowchart of the possible means of adaptation is shown. The almost universal methylation-dependent adaptation system is shown on the left. The less highly conserved methylation-independent pathways are on the right. We speculate here that CheV might directly influence CheA activity following phosphorylation, as shown, while the means of CheC activation and adaptational action are not yet understood. Black diamonds represent a chemoeffector. Adaptational proteins are hatched; excitatory proteins are shaded.

of the same or a nearby receptor and so moves through a receptor cluster in a hand-over-hand fashion (58, 127, 252). This property, as might be expected, allows CheR bound to one receptor to methylate another (125, 126, 129), and receptors lacking the C-terminal binding site are poorly methylated unless receptors containing it are present in the same cell (19). The properties of *E. coli* CheR and *B. subtilis* CheR are likely to be similar since receptors of either organism can be methylated by CheR from either organism (47). CheR is present in virtually all bacteria and archaea showing chemotaxis, since it is important for adaptation; the exception is *Helicobacter pylori*, which has CheV, whose phosphorlation, as described below, is known to help bring about adaptation in *B. subtilis* (99).

CheB methylesterase. Unlike CheR, CheB is usually regulated; it has an N-terminal response regulator domain, subject to phosphorylation, and a C-terminal enzymatic domain (57, 90, 134, 221). The *E. coli* enzyme is 10-fold more active when phosphorylated (134). The *B. subtilis* enzyme can satisfy the requirement for methylesterase for chemotaxis in *E. coli* (115), and, in vitro, both enzymes were able to demethylate both *B. subtilis* and *E. coli* receptors (160). One interesting possible difference is that CheB deamidates particular *E. coli* receptors, after which the site can be methylated and demethylated (102). In the instances where *B. subtilis* receptors are deamidated (for instances where the reaction has been characterized), this reaction is catalyzed by CheD, not by CheB (122). Perhaps, then, it is not surprising that the *B. subtilis* CheB appears to be more closely related to CheB in the archaea, which have CheD and class III receptors, than to CheB in most bacteria, some of which lack CheD and, in the case of the proteobacteria, have class I and class II receptors (2).

The isolated enzymatic domain of CheB catalyzes receptor demethylation in *B. subtilis* (45) and in *E. coli* (134), although not as effectively as does the phosphorylated whole enzyme (10, 57). However, in *B. subtilis*, a truncated *cheB* encoding the enzymatic domain complements a null *cheB* mutant, and this strain (null *cheB* mutant having truncated *cheB* on a plasmid) releases enhanced levels of methanol on both addition and removal of attractant. This result implies that the demethylation reaction involves primarily the existence of a suitable substrate. The value of phosphorylation of CheB would then be to increase the rate of receptor demethylation and thus speed up adaptation and to minimize unnecessary receptor demethylation, since loss of a methyl group is equivalent to hydrolyzing 11 to 14 ATP molecules to ADP and P_i (45, 225). Interestingly, CheB from *Campylobacter jejuni* lacks a response regulator domain (142). It is assumed that the time during which enhanced methanol formation occurs is the time between addition or removal of attractant and the resulting compensating (to bring about adaptation) methylation events on the receptor. This particular susceptible conformation does not require the coupling proteins CheW or CheV for events after the addition of attractant but does require them for events after the removal of attractant (45). The fact that the receptors that have bound attractant are more susceptible to methylesterase was shown in vitro many years ago (160).

The methylation system, involving CheR and CheB, is important for chemotaxis to high concentrations of attractant and only peripherally for chemotaxis to low concentrations of attractant in *B. subtilis* (113, 115). The reason is thought to be that at low concentrations of attractant, signal amplification occurs since binding attractant to a receptor activates neighboring receptors (203). At high concentrations of attractant, without CheB to generate charge-charge repulsion, there are no available free receptors that can activate the kinase on binding attractant (113). At low concentrations of attractant, methylation-independent systems suffice to bring about adaptation, such as the CheV system (see below). Thus, it may not be surprising that the amount of methanol evolved increases exponentially with receptor occupancy by attractant (111).

CheB may play another role as well. Under certain conditions, it appears to be required for a response to removal of attractants (113), and the first characterized *cheB* (then termed *cheL*) mutant (OI1130) was tumbly and unresponsive to stimuli (167). The implication is that this mutant CheB prevents normal functioning of the receptor-CheA complex. No similar mutants have been characterized in other organisms, such as *E. coli*, but absence of CheB results in an *E. coli* strain that is far less sensitive to the addition of attractant than is the wild type (108, 210), as described in more detail in "Signal amplification" (above). As stated above, attractants inhibit CheA in *E. coli*; this experiment implies that CheB is needed to amplify the process leading to low CheA-P and thus CheY-P levels (18). One way of achieving this is to inactivate the CheA associated with a receptor complex and have CheB molecules, which undergo very rapid autodephosphorylation (90), diffuse to neighboring complexes and inactivate them.

Methylation-Independent Adaptation

CheV adaptational and coupling protein. CheV has two domains, a N-terminal domain homologous to CheW and a response regulator C-terminal domain (63), and can substitute for CheW in coupling receptors to CheA in *B. subtilis* (181). Insight into its function has come from experiments using a mutant in which the phosphorylated aspartatyl residue was replaced with an alanyl residue (CheV *D235A* strain) and the whole response regulator domain was deleted. Both mutant strains showed poor adaptation to the addition of attractants, a result implying that the purpose of CheV phosphorylation is to bring about adaptation (99). Interestingly, mutants lacking *cheV* altogether did adapt normally in the tethered-cell assay. Thus, it would appear that the conformation of the coupling ("CheW") domain of CheV is such as to strongly favor receptors bound with attractant in the conformation to activate CheA, since other adaptation systems like the methylation system are unable to restore the prestimulus bias. Since the CheV *D235A* strain adapts poorly, it would seem that adaptation requires phosphorylation of D235, probably so that the regulator domain can interact with the coupling domain to affect the conformation of the coupling domain and allow the attractant-bound receptors to reassume their prestimulus conformation.

CheV may be the only adaptation system in *H. pylori*, since CheR and CheB are absent. However, there are three CheVs, of which only CheV1 appeared to be required for chemotaxis, and none could substitute for CheW (172).

CheC dephosphorylating and adaptational protein. As mentioned below CheC has CheY-P hydrolyzing activity (234a). However it is hard to explain the tethered cell phenotype of a *B. subtilis* Δ*cheC* mutant, other than by assuming that it also plays a role in adaptation. While the prestimulus rotational bias of Δ*cheC* is approximately that of the wild type, cells do not adapt to the addition of attractant (112, 182). This can be explained by the presence of persistently elevated CheA-P levels. CheC was shown to bind to McpB and CheA and so might either directly or indirectly influence CheA activity (112).

In addition, mutants with mutations in *cheC* have a lower frequency of switching the direction of rotation (from CCW to CW and from CW to CCW), implying that CheC lowers the energy of transition of switching (thus, the wild type, which has CheC, has a higher switching frequency than does the *cheC* mutant). Mutants with mutations in *cheB* have the opposite phenotype, an increased frequency of switching (190). It is hard to imagine that CheB binds to the switch but not so far-fetched to imagine that CheC does, since it is homologous to most of the FliM and FliY proteins (two of the three proteins comprising the switch). The state of two proteins being homologous does not necessarily imply that they bind each other; however, such binding does occur, for instance between CheA and CheW, the P5 domain of CheA being homologous to CheW (see above). These results can be accounted for by assuming that CheC has minimal affinity for overmethylated receptors so that in a *cheB* mutant, there would be more CheC bound at the switch. Very interestingly, a *cheB* mutant of *H. salinarum* shows increased frequency of reversals, with no effect on the ratio of CW and CCW rotation of the flagella, compared with the wild type (184). Similarly, the *cheB* mutant of *B. subtilis* has a normal bias (i.e., the same ratio of CW and CCW rotation of the flagella as in the wild type). Thus, it is not hard to imagine that reversal frequency in *H. salinarum* and *B. subtilis* is controlled by the same mechanism, namely, the amount of CheC bound at the switch. Presumably, in *H. salinarum*, the mechanism by which CheY-P produced by repellents or repellent light would cause increased reversals would involve inducing increased CheC binding at the switch. A similar situation may exist for the spirochetes, which undergo reversals of motion, except that there the CheC homolog is CheX, which is smaller than CheC (137).

CheC is not the only substance, however, that affects the switching frequency. Fumarate also promotes increased switching frequency in *E. coli* (17, 155, 175) and also increases the probability of CW rotation (154) by binding at the flagellar switch (175). As a central metabolite, fumarate would not be expected to be a chemotaxis signal whose concentration changes on a timescale of seconds, as does CheY-P, but might, instead, somehow be a barometer of the metabolic state of the cell. However, the way in which it would facilitate cell survival by reducing bias and switching frequency when present at low concentration and increasing bias and switching frequency when present at high concentration is, at this point, unknown.

SIGNAL REMOVAL

One of the unique challenges faced by chemotaxis systems is the necessity for quick responses (on a timescale of seconds) to ever changing environments. This is in constrast to most other two-component signal transduction systems that control gene expression and act over minutes to hours. To cope with this problem, the half-lives of CheY-Ps, are brief, shorter than 1 min (38, 66). CheY is thought to actively catalyze autodephosphorylation, a process involving several conserved residues (two aspartates, a lysine, and a threonine) and a Mg^{2+} ion (133, 205, 222). However, the half-lives still appear to be too long. To further speed the signal removal, the enteric organisms as well as some other γ- and β-proteobacteria express *cheZ*. The protein further destabilizes CheY-P (257). In these organisms, CheZ is essential for chemotaxis. It was puzzling that most chemotactic bacteria and archaea do not carry a

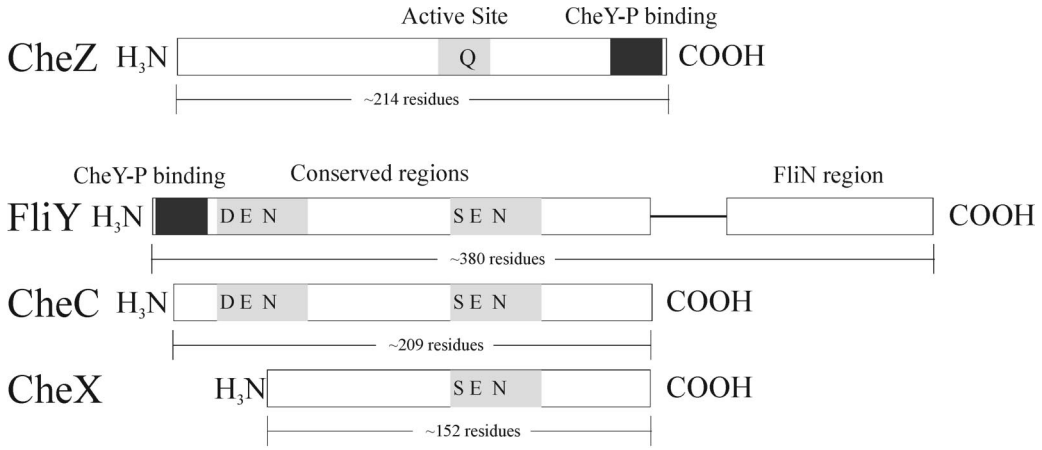

FIG. 8. Schematic of CheY-P-hydrolyzing proteins. For CheZ, the C-terminal CheY-P binding region is shown in black and the area including what is thought to be the active site is shaded in gray. For FliY, the CheY-P binding site is shown in black. For FliY, CheC, and CheX, conserved regions are in gray, with highly conserved residues positioned as indicated.

cheZ gene. Based on data available for *B. subtilis*, it is now apparent that in many if not most of these organisms a combination of CheC, FliY, and/or possibly CheX perform this function (234, 234a). These three proteins are homologous but have no sequence similarity to CheZ (Fig. 8). A third mechanism of signal removal has been suggested for *S. meliloti* and other α-proteobacteria where an alternative CheY is thought to act as a phosphate sink and possibly support signal removal (212). The methods of signal removal for different bacteria are summarized in Fig. 9. Interestingly, some organisms do not express a *cheZ* or *cheC* homologue or an alternative *cheY*. Other means of signal removal in these organisms could in-

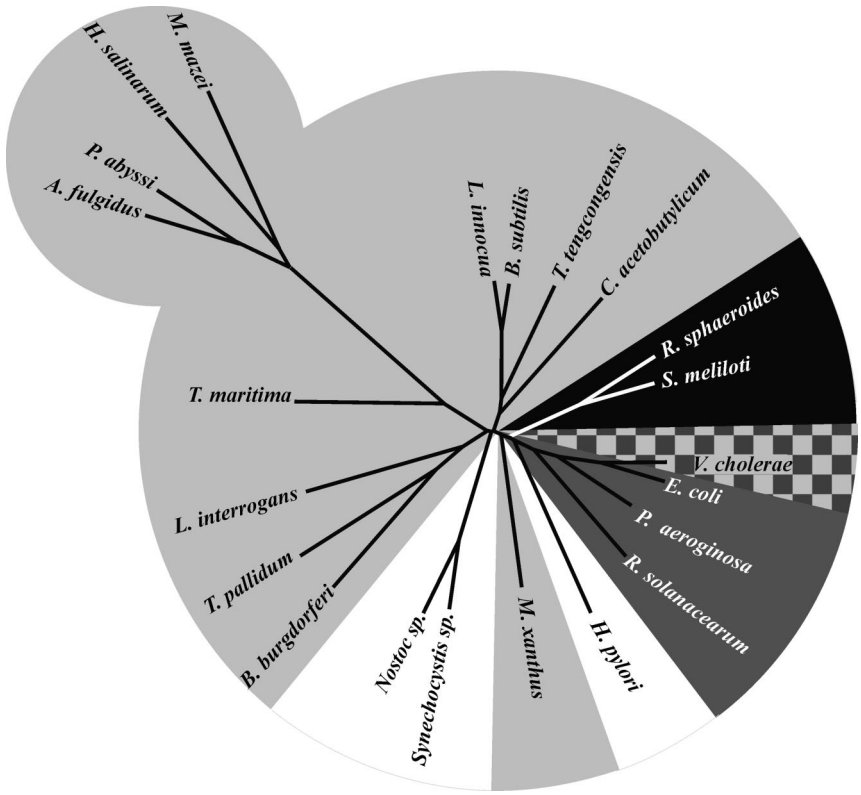

FIG. 9. Means of CheY-P hydrolysis in chemotactic organisms. The tree was generated as described for Fig. 3. Chemotactic organisms that encode a CheC homolog are highlighted in light gray; dark gray represents organisms that encode a CheZ; black represents organisms that encode an alternative CheY that acts as a phosphate sink; white represents organisms with no known mechanism of CheY-P hydrolysis. *V. cholerae* encodes both CheZ and CheC homologs and is therefore indicated by dark and light gray squares.

clude the presence of a response regulator domain fused to other chemotaxis components acting as phosphate sink.

CheZ Phosphatase

CheZ is found exclusivly in the β- and γ-proteobacteria (see the legend to Fig. 3 for type species). From its limited spread among bacteria it can be concluded that it evolved relatively late, and that the original chemotactic organism had other means of signal removal.

As part of the *E. coli* chemotaxis system, CheZ is by far the best studied CheY-P phosphatase and the only one for which an X-ray structure is available (257). CheZ is localized to the receptor complexes, in the enteric bacteria, via CheA-short (a form of CheA that lacks part of the N-terminal sequence, including the site of phosphorylation [251]) (209). However, the meaning of this localization is not yet clear, since a mutant not capable of making CheA-short does not have a chemotactic phenotype (150). At one time, it was thought that enhancement of CheZ activity might be the means by which CheY-P could be rapidly hydrolyzed following addition of attractant to generate the excitatory signal. However, CheZ does not appear to play any excitatory role (108, 210). Studies of fragments of the protein identified the C terminus of CheZ to be the CheY-P binding domain (29). However, insight into the mechanism of CheZ action remained elusive until the X-ray structure of CheZ in complex with activated CheY was solved recently. Based on this structure, it has been proposed that CheZ residue Gln147 is actively involved in increasing the rate of CheY-P hydrolysis by positioning and activating a water molecule in the active site of CheY-P (257).

CheC/FliY/CheX Phosphatase

Most chemotactic bacteria and archaea do not encode a CheZ homolog and so must cope with the problem of fast signal removal in some other way. A recent study found that the flagellar switch protein FliY in *B. subtilis* is able to increase the rate of CheY-P hydrolysis. The C-terminal region of FliY is homologous to *E. coli* FliN. The N-terminal domain is homologous to two other chemotaxis proteins, CheC and CheX (234). While FliY is exclusive to gram-positive bacteria, some spirochetes, and *Thermatoga*, CheC and/or CheX can be found in almost all phyla of chemotactic organisms, including some proteobacteria. Indeed, *B. subtilis* CheC shares the ability of FliY to hydrolyze CheY-P (234a). Therefore, conserved residues between CheC and FliY are possibly involved in the chemistry. Six residues—Asp39, Glu43, Asn46, Ser136, Glu140, and Asn143 (following *B. subtilis* FliY numbering)—are conserved among these proteins, and any could play a similar role to residue Gln147 in *E. coli* CheZ. Interestingly, the latter three residues are also conserved among CheX proteins, which appear to be truncated versions of CheC. Most chemotactic bacteria and all chemotactic archaea have a CheC homolog. Whether the mechanism of dephosphorylation of CheY-P is similar to that of CheZ will ultimately be shown only by obtaining an X-ray refraction structure of any of these proteins in complex with CheY.

Phosphate Sink

Work on the α-proteobacteria *S. meliloti* and *R. spheroides* suggests a third mechanism of signal removal (174, 212). In each of these organisms, deletion of at least two CheY homologs causes a defect in chemotaxis. In *S. meliloti*, CheY2 is the main response regulator that interacts with the flagellar switch and causes the reversal of flagellar rotation. CheY1 does not interact with the switch, although, like CheY2, it is rapidly phosphorylated by CheA-P. CheY2, however, is capable of transferring its phosphoryl group back to CheA and subsequently to CheY1, so that CheY1 may act as a phosphate sink (212). In *R. spheroides* this mechanism is more complex, since this organism contains six *cheY* genes; some CheY proteins are thought to act as phosphate sinks (174). Since many chemotactic organisms have more than one *cheY*, one can imagine that the phosphate sink mechanism may be widespread. In addition, some organisms do not encode any of the known signal-removing proteins and, we speculate, may use response regulator domains fused to other chemotaxis proteins. However, no data suggesting this have yet been reported.

MULTIPLE COPIES OF CHEMOTAXIS GENES

As mentioned above, the *E. coli* chemotaxis system is simple in comparison to most other chemotaxis systems. This is because there is only one copy for each chemotaxis protein. *B. subtilis* already proves more complex since partially redundant proteins like CheW and CheV or like CheC and FliY make phenotypes less severe and conclusions about them less obvious (99, 181, 234). However, still more complex systems can be found. Some organisms contain multiple sets of chemotaxis genes, some of which may have functions other than controlling motility. *P. aeruginosa* has five clusters of chemotaxis genes (62, 230). Two of these clusters (I and V) are required for chemotaxis (100, 146). Another (IV) is required for chemotaxis by twitching motility (55, 101), which involves extension and retraction of type IV pili (207). This type of movement is thought to facilitate movement across surfaces and formation of biofilms (170); it usually involves rafts of cells rather than individual cells (147). It seems reasonable that the apparent redundancy of chemotaxis genes in this organism is due to genes within a cluster being devoted to a particular function, such as chemotaxis involving flagella or twitching motility, and is not actual redundancy.

Another organism with multiple copies of chemotaxis-type genes is *M. xanthus*. *M. xanthus* has two types of motility, A-motility and S-motility (98, 200). S-motility is homologous to twitching motility in *P. aeruginosa* and involves extension and retraction of type IV pili (231). *M. xanthus* has nine clusters of chemotaxis-type genes (14), of which the Frz genes mediate chemotaxis by controling reversals of (gliding) cells, the Dif genes are involved in fibril formation (necessary for S-motility), and the Che4 cluster is also involved in S-motility. However, another set, the Che3 cluster, affects the entry of *M. xanthus* into the developmental program to produce spores, and the output would appear to be the response regulator protein CrdA (whose cognate histidine kinase appears to be CheA3), predicted to be the transcriptional activator for σ^{54}-dependent promoters (114). Thus, in this case, what must have

originally been a chemotaxis-type set of genes controlling motility evolved into a set of genes controlling transcriptional activation. The main difference between ordinary transcriptional activation and this type might be that the latter would undergo adaptation so that the time derivative of the input signal, rather than the magnitude of the input signal itself, would control transcription. Such an arrangement might provide for sensitivity to changes over many orders of magnitude, as in the case for bacterial chemotaxis (41, 54). A detailed account of the issues involved may be found in reference 14.

The α-proteobacterium *R. spheroides* has three sets of chemotaxis genes. *che*Op$_1$ contains *cheY*$_1$, *cheA*$_1$, *cheW*$_1$, *cheR*$_1$, and *cheY*$_2$. *che*Op$_2$ contains *cheY*$_3$, *cheA*$_2$, *cheW*$_2$, *cheW*$_3$, *cheR*$_2$, *cheB*$_1$, and *tlpC*. *che*Op$_3$ contains *cheA*$_4$, *cheR*$_3$, *cheB*$_2$, *cheW*$_4$, *slp*, *tlpT*, *cheY*$_6$, and *cheA*$_3$. Besides these chemotaxis genes, there is one encoding a fusion protein of CheBRA and 13 encoding receptors, including 4 cytoplasmic receptors (lacking a membrane-spanning region) and *cheY*$_4$ (www.jgi.doe.gov/JGI microbial/html/rhodobacter). Deletion of *cheA*$_2$ prevents aerotaxis, phototaxis, and chemotaxis, but deletion of *cheA*$_1$ has little effect. Deletion of *cheW*$_2$ has a much bigger effect on localization (see below) of *cheA*$_2$ at the poles of the cell than does deletion of *cheW*$_3$, which marginally affects *cheA*$_2$ localization. CheA2 causes the phosphorylation of CheY4, and CheY3 facilitates signal temination, possibly acting as a phosphate sink (196). CheA1, with its cognate response regulator CheY5, mediates a repellent ("inverted") response (196). The function of the genes in *che*Op$_3$ is unknown. In this organism, the response to negative stimuli is to stop (rather than rotate the single polar flagellum CW, as does *E. coli* for its peritrichous flagella). On stopping, the flagellum goes from helical to coiled (15). This transition, coupled with rotational Brownian motion, reorients the bacterium (173) so that the next smooth swim will take a new direction.

LOCALIZATION OF CHEMOTAXIS PROTEINS

Polar localization of chemotaxis proteins was first explored in *Caulobacter*, a natural organism with which to investigate polarity since it undergoes differentiation in which a stalk cell produces a swarmer cell with a single polar flagellum that is made shortly before cell division in every generation (249). Later, the flagellum is discarded and is replaced by a new stalk (60). The receptor is located at the pole (6). This expected finding led to an unexpected one, namely, that the receptors of *E. coli* are also located at the poles of the cell (140). This finding has led to a considerable body of research that has documented that chemotaxis receptors generally are clustered, usually at the pole but, for cytoplasmic receptors as in *R. spheroides*, at an apparently random place in the cytoplasm (84, 143, 246). The *B. subtilis* asparagine receptor, McpB (and presumably the other receptors spanning the membrane) is also located at the poles of cells (113). However, the significance of clustering for signal amplification is uncertain, since it was unaltered in *E. coli* strains lacking CheR or CheB (138), but strains lacking CheB are very impaired in sensitivity to attractants, although strains lacking CheR are still very sensitive (108, 210). Moreover, addition of a multivalent ligand that can bind two receptors simultaneously greatly increases the sensitivity of heterologous receptors to their ligands, and this sensitivity is diminished when other heterologous receptors are deleted (71, 72). Thus, it would appear that clustering of receptors may facilitate taxis since receptors are close to each other but active signaling must require a particular arrangement of the receptors, a goal that is hard to achieve when they are fully methylated.

CONCLUSIONS AND PROSPECTS

One great achievement in our understanding of bacterial chemotaxis in the 1970s was the discovery of the methylation system as foundational for bringing about adaptation to stimuli. In the 1980s and early 1990s came the discovery of the two-component system involving phosphotransfer as mediating excitation. Now, during the past decade, there has been a growing appreciation of the diversity of chemotactic mechanisms used in the broad sweep of bacteria and archaea. In this review we have emphasized this diversity. We have acknowledged that many of the principles have been worked out in the *E. coli-S. enterica* chemotaxis system and that great progress in elucidating that system is still occurring. However, it has become clear that the *E. coli* system is streamlined and lacks or has significantly modified some basic features of the primordial mechanism that existed when the bacteria and archaea separated during evolution. It seems that many of these features exist in the *B. subtilis* mechanism, and the elucidation of this mechanism has, accordingly, been one of the features of this review. The processes used to restore behavioral conditions to their prestimulus conditions have changed the most during the streamlining that has led to the *E. coli* mechanism. However, evolution in other organisms has not stood still, and a lot of changes in other directions have occurred since the primordial mechanism was widely used; we have tried to do justice to these. A lot of information, however, is still at the genome-sequencing level, and more behavioral, genetic, and biochemical work is needed on these organisms. Some of the most interesting and unanticipated advances are occurring in research on organisms that have multiple copies of chemotaxis genes and those that have employed, for controlling development, proteins that once had a chemotaxis function. These new areas, as well as the dynamics of receptor-receptor interactions in bringing about extreme sensitivity to the slightest changes in attractant concentrations over many orders of magnitude, are promising areas of future investigation.

Understanding the structural changes that underlie this remarkable capability is a major challenge; however, we believe that the talented cadre of investigators are up to meeting this challenge. All of these new investigations should serve to make the study of bacterial chemotaxis as exciting during the next 30 years as it has been during the past 30.

ACKNOWLEDGMENT

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REFERENCES

1. **Adler, J., G. L. Hazelbauer, and M. M. Dahl.** 1973. Chemotaxis toward sugars in *Escherichia coli*. J. Bacteriol. **115:**824–847.
2. **Aizawa, S. I., Zhulin, I. B., L. Marquez-Magana, and G. W. Ordal.** 2002. Chemotaxis and motility, p. 437–452. *In* A. L. Sonenshein, R. Losick, and J. A. Hoch (ed.), *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, D.C.

3. **Aksamit, R., and D. E. Koshland, Jr.** 1972. A ribose binding protein of *Salmonella typhimurium*. Biochem. Biophys. Res. Commun. **48:**1348–1353.
4. **Aksamit, R. R., and D. E. Koshland, Jr.** 1974. Identification of the ribose binding protein as the receptor for ribose chemotaxis in *Salmonella typhimurium*. Biochemistry **13:**4473–4478.
5. **Alam, M., M. Claviez, D. Oesterhelt, and M. Kessel.** 1984. Flagella and motility behaviour of square bacteria. EMBO J. **3:**2899–2903.
6. **Alley, M. R., J. R. Maddock, and L. Shapiro.** 1992. Polar localization of a bacterial chemoreceptor. Genes Dev. **6:**825–836.
7. **Ames, P., and J. S. Parkinson.** 1994. Constitutively signaling fragments of Tsr, the *Escherichia coli* serine chemoreceptor. J. Bacteriol. **176:**6340–6348.
8. **Ames, P., and J. S. Parkinson.** 1988. Transmembrane signaling by bacterial chemoreceptors: *E. coli* transducers with locked signal output. Cell **55:**817–826.
9. **Ames, P., C. A. Studdert, R. H. Reiser, and J. S. Parkinson.** 2002. Collaborative signaling by mixed chemoreceptor teams in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **99:**7060–7065.
10. **Anand, G. S., P. N. Goudreau, and A. M. Stock.** 1998. Activation of methylesterase CheB: evidence of a dual role for the regulatory domain. Biochemistry **37:**14038–14047.
11. **Appleman, J. A., L. L. Chen, and V. Stewart.** 2003. Probing conservation of HAMP linker structure and signal transduction mechanism through analysis of hybrid sensor kinases. J. Bacteriol. **185:**4872–4882.
12. **Appleman, J. A., and V. Stewart.** 2003. Mutational analysis of a conserved signal-transducing element: the HAMP linker of the *Escherichia coli* nitrate sensor NarX. J. Bacteriol. **185:**89–97.
13. **Armitage, J. P.** 1999. Bacterial tactic responses. Adv. Microb. Physiol. **41:**229–289.
14. **Armitage, J. P.** 2003. Taxing questions in development. Trends Microbiol. **11:**239–242.
15. **Armitage, J. P., and R. M. Macnab.** 1987. Unidirectional, intermittent rotation of the flagellum of *Rhodobacter sphaeroides*. J. Bacteriol. **169:**514–518.
16. **Barak, R., and M. Eisenbach.** 1992. Correlation between phosphorylation of the chemotaxis protein CheY and its activity at the flagellar motor. Biochemistry **31:**1821–1826.
17. **Barak, R., I. Giebel, and M. Eisenbach.** 1996. The specificity of fumarate as a switching factor of the bacterial flagellar motor. Mol. Microbiol. **19:**139–144.
18. **Barkai, N., U. Alon, and S. Leibler.** 2001. Robust amplification in adaptive signal transduction networks. C. R. Acad Sci. Paris **IV:**1–7.
19. **Barnakov, A. N., L. A. Barnakova, and G. L. Hazelbauer.** 1998. Comparison in vitro of a high- and a low-abundance chemoreceptor of *Escherichia coli*: similar kinase activation but different methyl-accepting activities. J. Bacteriol. **180:**6713–6718.
20. **Berg, H. C.** 1975. Chemotaxis in bacteria. Annu. Rev. Biophys. Bioeng. **4:**119–136.
21. **Bibikov, S. I., L. A. Barnes, Y. Gitin, and J. S. Parkinson.** 2000. Domain organization and flavin adenine dinucleotide-binding determinants in the aerotaxis signal transducer Aer of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **97:**5830–5835.
22. **Bibikov, S. I., R. Biran, K. E. Rudd, and J. S. Parkinson.** 1997. A signal transducer for aerotaxis in *Escherichia coli*. J. Bacteriol. **179:**4075–4079.
23. **Bilwes, A. M., L. A. Alex, B. R. Crane, and M. I. Simon.** 1999. Structure of CheA, a signal-transducing histidine kinase. Cell **96:**131–141.
24. **Binnie, R. A., H. Zhang, S. Mowbray, and M. A. Hermodson.** 1992. Functional mapping of the surface of *Escherichia coli* ribose-binding protein: mutations that affect chemotaxis and transport. Protein Sci. **1:**1642–1651.
25. **Bischoff, D. S., R. B. Bourret, M. L. Kirsch, and G. W. Ordal.** 1993. Purification and characterization of *Bacillus subtilis* CheY. Biochemistry **32:**9256–9261.
26. **Bischoff, D. S., and G. W. Ordal.** 1992. Identification and characterization of FliY, a novel component of the *Bacillus subtilis* flagellar switch complex. Mol. Microbiol. **6:**2715–2723.
27. **Bischoff, D. S., and G. W. Ordal.** 1991. Sequence and characterization of *Bacillus subtilis* CheB, a homolog of *Escherichia coli* CheY, and its role in a different mechanism of chemotaxis. J. Biol. Chem. **266:**12301–12305.
28. **Bjorkman, A. J., R. A. Binnie, H. Zhang, L. B. Cole, M. A. Hermodson, and S. L. Mowbray.** 1994. Probing protein-protein interactions. The ribose-binding protein in bacterial transport and chemotaxis. J. Biol. Chem. **269:**30206–30211.
29. **Blat, Y., and M. Eisenbach.** 1996. Conserved C-terminus of the phosphatase CheZ is a binding domain for the chemotactic response regulator CheY. Biochemistry **35:**5679–5683.
30. **Blat, Y., and M. Eisenbach.** 1996. Mutants with defective phosphatase activity show no phosphorylation-dependent oligomerization of CheZ. The phosphatase of bacterial chemotaxis. J. Biol. Chem. **271:**1232–1236.
31. **Blat, Y., and M. Eisenbach.** 1996. Oligomerization of the phosphatase CheZ upon interaction with the phosphorylated form of CheY. The signal protein of bacterial chemotaxis. J. Biol. Chem. **271:**1226–1231.
32. **Boesch, K. C., R. E. Silversmith, and R. B. Bourret.** 2000. Isolation and characterization of nonchemotactic CheZ mutants of *Escherichia coli*. J. Bacteriol. **182:**3544–3552.
33. **Borkovich, K. A., L. A. Alex, and M. I. Simon.** 1992. Attenuation of sensory receptor signaling by covalent modification. Proc. Natl. Acad. Sci. USA **89:**6756–6760.
34. **Borkovich, K. A., N. Kaplan, J. F. Hess, and M. I. Simon.** 1989. Transmembrane signal transduction in bacterial chemotaxis involves ligand-dependent activation of phosphate group transfer. Proc. Natl. Acad. Sci. USA **86:**1208–1212.
35. **Borkovich, K. A., and M. I. Simon.** 1990. The dynamics of protein phosphorylation in bacterial chemotaxis. Cell **63:**1339–1348.
36. **Bornhorst, J. A., and J. J. Falke.** 2003. Quantitative analysis of aspartate receptor signaling complex reveals that the homogeneous two-state model is inadequate: development of a heterogeneous two-state model. J. Mol. Biol. **326:**1597–1614.
37. **Boukhvalova, M. S., F. W. Dahlquist, and R. C. Stewart.** 2002. CheW binding interactions with CheA and Tar. Importance for chemotaxis signaling in *Escherichia coli*. J. Biol. Chem. **277:**22251–22259.
38. **Bourret, R. B., J. F. Hess, and M. I. Simon.** 1990. Conserved aspartate residues and phosphorylation in signal transduction by the chemotaxis protein CheY. Proc. Natl. Acad. Sci. USA **87:**41–45.
39. **Bourret, R. B., and A. M. Stock.** 2002. Molecular information processing: lessons from bacterial chemotaxis. J. Biol. Chem. **277:**9625–9628.
40. **Brass, J. M., and M. D. Manson.** 1984. Reconstitution of maltose chemotaxis in Escherichia coli by addition of maltose-binding protein to calcium-treated cells of maltose regulon mutants. J. Bacteriol. **157:**881–890.
41. **Bray, D., M. D. Levin, and C. J. Morton-Firth.** 1998. Receptor clustering as a cellular mechanism to control sensitivity. Nature **393:**85–88.
42. **Bren, A., and M. Eisenbach.** 1998. The N terminus of the flagellar switch protein, FliM, is the binding domain for the chemotactic response regulator, CheY. J. Mol. Biol. **278:**507–514.
43. **Bren, A., M. Welch, Y. Blat, and M. Eisenbach.** 1996. Signal termination in bacterial chemotaxis: CheZ mediates dephosphorylation of free rather than switch-bound CheY. Proc. Natl. Acad. Sci. USA **93:**10090–10093.
44. **Brooun, A., J. Bell, T. Freitas, R. W. Larsen, and M. Alam.** 1998. An archaeal aerotaxis transducer combines subunit I core structures of eukaryotic cytochrome *c* oxidase and eubacterial methyl-accepting chemotaxis proteins. J. Bacteriol. **180:**1642–1646.
45. **Bunn, M. W., and G. W. Ordal.** 2004. Receptor conformational changes enhance methylesterase activity during chemotaxis by *Bacillus subtilis*. Mol. Microbiol. **51:**721–728.
46. **Bunn, M. W., and G. W. Ordal.** 2003. Transmembrane organization of the *Bacillus subtilis* chemoreceptor McpB deduced by cysteine disulfide crosslinking. J. Mol. Biol. **331:**941–949.
47. **Burgess-Cassler, A., and G. W. Ordal.** 1982. Functional homology of *Bacillus subtilis* methyltransferase II and *Escherichia coli* CheR protein. J. Biol. Chem. **257:**12835–12838.
48. **Burgess-Cassler, A., A. H. Ullah, and G. W. Ordal.** 1982. Purification and characterization of *Bacillus subtilis* methyl-accepting chemotaxis protein methyltransferase II. J. Biol. Chem. **257:**8412–8417.
49. **Cantwell, B. J., R. R. Draheim, R. B. Weart, C. Nguyen, R. C. Stewart, and M. D. Manson.** 2003. CheZ phosphatase localizes to chemoreceptor patches via CheA-short. J. Bacteriol. **185:**2354–2361.
50. **Chervitz, S. A., and J. J. Falke.** 1996. Molecular mechanism of transmembrane signaling by the aspartate receptor: a model. Proc. Natl. Acad. Sci. USA **93:**2545–2550.
51. **Chi, Y. I., H. Yokota, and S. H. Kim.** 1997. Apo structure of the ligand-binding domain of aspartate receptor from *Escherichia coli* and its comparison with ligand-bound or pseudoligand-bound structures. FEBS Lett. **414:**327–332.
52. **Cohen-Krausz, S., and S. Trachtenberg.** 2002. The structure of the archeabacterial flagellar filament of the extreme halophile *Halobacterium salinarum* R1M1 and its relation to eubacterial flagellar filaments and type IV pili. J. Mol. Biol. **321:**383–395.
53. **Dahl, M. K., and M. D. Manson.** 1985. Interspecific reconstitution of maltose transport and chemotaxis in *Escherichia coli* with maltose-binding protein from various enteric bacteria. J. Bacteriol. **164:**1057–1063.
54. **Dahlquist, F. W.** 2002. Amplification of signaling events in bacteria. Sci. STKE **2002:**PE24.
55. **Darzins, A.** 1994. Characterization of a *Pseudomonas aeruginosa* gene cluster involved in pilus biosynthesis and twitching motility: sequence similarity to the chemotaxis proteins of enterics and the gliding bacterium *Myxococcus xanthus*. Mol. Microbiol. **11:**137–153.
56. **DeFranco, A. L., and D. E. Koshland, Jr.** 1980. Multiple methylation in processing of sensory signals during bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **77:**2429–2433.
57. **Djordjevic, S., P. N. Goudreau, Q. Xu, A. M. Stock, and A. H. West.** 1998. Structural basis for methylesterase CheB regulation by a phosphorylation-activated domain. Proc. Natl. Acad. Sci. USA **95:**1381–1386.
58. **Djordjevic, S., and A. M. Stock.** 1998. Chemotaxis receptor recognition by protein methyltransferase CheR. Nat. Struct. Biol. **5:**446–450.

59. **Dobell, C.** 1958. Antony van Leeuwenhoek and his "little animals". Russell and Russell, New York, N.Y.
60. **Driks, A., P. V. Schoenlein, D. J. DeRosier, L. Shapiro, and B. Ely.** 1990. A *Caulobacter* gene involved in polar morphogenesis. J. Bacteriol. **172:**2113–2123.
61. **Falke, J. J., and G. L. Hazelbauer.** 2001. Transmembrane signaling in bacterial chemoreceptors. Trends Biochem. Sci. **26:**257–265.
62. **Ferrandez, A., A. C. Hawkins, D. T. Summerfield, and C. S. Harwood.** 2002. Cluster II *che* genes from *Pseudomonas aeruginosa* are required for an optimal chemotactic response. J. Bacteriol. **184:**4374–4383.
63. **Fredrick, K. L., and J. D. Helmann.** 1994. Dual chemotaxis signaling pathways in *Bacillus subtilis*: a sigma D-dependent gene encodes a novel protein with both CheW and CheY homologous domains. J. Bacteriol. **176:**2727–2735.
64. **Fuhrer, D. K., and G. W. Ordal.** 1991. *Bacillus subtilis* CheN, a homolog of CheA, the central regulator of chemotaxis in *Escherichia coli*. J. Bacteriol. **173:**7443–7448.
65. **Gardina, P., C. Conway, M. Kossman, and M. Manson.** 1992. Aspartate and maltose-binding protein interact with adjacent sites in the Tar chemotactic signal transducer of *Escherichia coli*. J. Bacteriol. **174:**1528–1536.
66. **Garrity, L. F.** 1996. Biochemistry and regulation of cheA in *Bacillus subtilis* chemotaxis. University of Illinois, Urbana.
67. **Garrity, L. F., and G. W. Ordal.** 1997. Activation of the CheA kinase by asparagine in *Bacillus subtilis* chemotaxis. Microbiology **143:**2945–2951.
68. **Garrity, L. F., S. L. Schiel, R. Merrill, J. Reizer, M. H. Saier, Jr., and G. W. Ordal.** 1998. Unique regulation of carbohydrate chemotaxis in *Bacillus subtilis* by the phosphoenolpyruvate-dependent phosphotransferase system and the methyl-accepting chemotaxis protein McpC. J. Bacteriol. **180:**4475–4480.
69. **Ge, Y., I. G. Old, I. Saint Girons, and N. W. Charon.** 1997. Molecular characterization of a large *Borrelia burgdorferi* motility operon which is initiated by a consensus sigma70 promoter. J. Bacteriol. **179:**2289–2299.
70. **Gegner, J. A., D. R. Graham, A. F. Roth, and F. W. Dahlquist.** 1992. Assembly of an MCP receptor, CheW, and kinase CheA complex in the bacterial chemotaxis signal transduction pathway. Cell **70:**975–982.
71. **Gestwicki, J. E., and L. L. Kiessling.** 2002. Inter-receptor communication through arrays of bacterial chemoreceptors. Nature **415:**81–84.
72. **Gestwicki, J. E., L. E. Strong, S. L. Borchardt, C. W. Cairo, A. M. Schnoes, and L. L. Kiessling.** 2001. Designed potent multivalent chemoattractants for *Escherichia coli*. Bioorg. Med. Chem. **9:**2387–2393.
73. **Goldman, D. J., D. O. Nettleton, and G. W. Ordal.** 1984. Purification and characterization of chemotactic methylesterase from *Bacillus subtilis*. Biochemistry **23:**675–680.
74. **Goldman, D. J., and G. W. Ordal.** 1984. In vitro methylation and demethylation of methyl-accepting chemotaxis proteins in *Bacillus subtilis*. Biochemistry **23:**2600–2606.
75. **Gomes, S. L., and L. Shapiro.** 1984. Differential expression and positioning of chemotaxis methylation proteins in *Caulobacter*. J. Mol. Biol. **178:**551–568.
76. **Goy, M. F., M. S. Springer, and J. Adler.** 1978. Failure of sensory adaptation in bacterial mutants that are defective in a protein methylation reaction. Cell **15:**1231–1240.
77. **Greene, S. R., and L. V. Stamm.** 1999. Molecular characterization of a chemotaxis operon in the oral spirochete, *Treponema denticola*. Gene **232:**59–68.
78. **Griswold, I. J., and F. W. Dahlquist.** 2002. The dynamic behavior of CheW from *Thermotoga maritima* in solution, as determined by nuclear magnetic resonance: implications for potential protein-protein interaction sites. Biophys. Chem. **101–102:**359–373.
79. **Griswold, I. J., H. Zhou, M. Matison, R. V. Swanson, L. P. McIntosh, M. I. Simon, and F. W. Dahlquist.** 2002. The solution structure and interactions of CheW from *Thermotoga maritima*. Nat. Struct. Biol. **9:**121–125.
80. **Hamblin, P. A., N. A. Bourne, and J. P. Armitage.** 1997. Characterization of the chemotaxis protein CheW from *Rhodobacter sphaeroides* and its effect on the behaviour of *Escherichia coli*. Mol. Microbiol. **24:**41–51.
81. **Hanlon, D. W., and G. W. Ordal.** 1994. Cloning and characterization of genes encoding methyl-accepting chemotaxis proteins in *Bacillus subtilis*. J. Biol. Chem. **269:**14038–14046.
82. **Hanlon, D. W., C. Ying, and G. W. Ordal.** 1993. Purification and reconstitution of the methyl-accepting chemotaxis proteins from *Bacillus subtilis*. Biochim. Biophys. Acta **1158:**345–351.
83. **Harayama, S., P. Engstrom, H. Wolf-Watz, T. Iino, and G. L. Hazelbauer.** 1982. Cloning of *trg*, a gene for a sensory transducer in *Escherichia coli*. J. Bacteriol. **152:**372–383.
84. **Harrison, D. M., J. Skidmore, J. P. Armitage, and J. R. Maddock.** 1999. Localization and environmental regulation of MCP-like proteins in *Rhodobacter sphaeroides*. Mol. Microbiol. **31:**885–892.
85. **Hazelbauer, G. L.** 1975. Maltose chemoreceptor of *Escherichia coli*. J. Bacteriol. **122:**206–214.
86. **Hazelbauer, G. L., and J. Adler.** 1971. Role of the galactose binding protein in chemotaxis of *Escherichia coli* toward galactose. Nat. New Biol. **230:**101–104.
87. **Hazelbauer, G. L., and S. Harayama.** 1979. Mutants in transmission of chemotactic signals from two independent receptors of *E. coli*. Cell **16:**617–625.
88. **Hazelbauer, G. L., S. Harayama, and P. Engstrom.** 1982. Special features of chemotaxis towards maltose. Ann. Microbiol. (Paris) **133A:**191–194.
89. **Hess, J. F., R. B. Bourret, K. Oosawa, P. Matsumura, and M. I. Simon.** 1988. Protein phosphorylation and bacterial chemotaxis. Cold Spring Harbor Symp. Quant. Biol. **53:**41–48.
90. **Hess, J. F., K. Oosawa, N. Kaplan, and M. I. Simon.** 1988. Phosphorylation of three proteins in the signaling pathway of bacterial chemotaxis. Cell **53:**79–87.
91. **Hirschman, A., M. Boukhvalova, R. VanBruggen, A. J. Wolfe, and R. C. Stewart.** 2001. Active site mutations in CheA, the signal-transducing protein kinase of the chemotaxis system in *Escherichia coli*. Biochemistry **40:**13876–13887.
92. **Hou, S., T. Freitas, R. W. Larsen, M. Piatibratov, V. Sivozhelezov, A. Yamamoto, E. A. Meleshkevitch, M. Zimmer, G. W. Ordal, and M. Alam.** 2001. Globin-coupled sensors: a class of heme-containing sensors in *Archaea* and *Bacteria*. Proc. Natl. Acad. Sci. USA **98:**9353–9358.
93. **Hou, S., R. W. Larsen, D. Boudko, C. W. Riley, E. Karatan, M. Zimmer, G. W. Ordal, and M. Alam.** 2000. Myoglobin-like aerotaxis transducers in *Archaea* and *Bacteria*. Nature **403:**540–544.
94. **Hughson, A. G., and G. L. Hazelbauer.** 1996. Detecting the conformational change of transmembrane signaling in a bacterial chemoreceptor by measuring effects on disulfide cross-linking in vivo. Proc. Natl. Acad. Sci. USA **93:**11546–11551.
95. **Irikura, V. M., M. Kihara, S. Yamaguchi, H. Sockett, and R. M. Macnab.** 1993. *Salmonella typhimurium fliG* and *fliN* mutations causing defects in assembly, rotation, and switching of the flagellar motor. J. Bacteriol. **175:**802–810.
96. **Jasuja, R., J. Keyoung, G. P. Reid, D. R. Trentham, and S. Khan.** 1999. Chemotactic responses of *Escherichia coli* to small jumps of photoreleased L-aspartate. Biophys. J. **76:**1706–1719.
97. **Jiang, Z. Y., and C. E. Bauer.** 2001. Component of the Rhodospirillum centenum photosensory apparatus with structural and functional similarity to methyl-accepting chemotaxis protein chemoreceptors. J. Bacteriol. **183:**171–177.
98. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76:**5952–5956.
99. **Karatan, E., M. M. Saulmon, M. W. Bunn, and G. W. Ordal.** 2001. Phosphorylation of the response regulator CheV is required for adaptation to attractants during *Bacillus subtilis* chemotaxis. J. Biol. Chem. **276:**43618–43626.
100. **Kato, J., T. Nakamura, A. Kuroda, and H. Ohtake.** 1999. Cloning and characterization of chemotaxis genes in *Pseudomonas aeruginosa*. Biosci. Biotechnol. Biochem. **63:**155–161.
101. **Kearns, D. B., J. Robinson, and L. J. Shimkets.** 2001. Pseudomonas aeruginosa exhibits directed twitching motility up phosphatidylethanolamine gradients. J. Bacteriol. **183:**763–767.
102. **Kehry, M. R., M. W. Bond, M. W. Hunkapiller, and F. W. Dahlquist.** 1983. Enzymatic deamidation of methyl-accepting chemotaxis proteins in *Escherichia coli* catalyzed by the *cheB* gene product. Proc. Natl. Acad. Sci. USA **80:**3599–3603.
103. **Kehry, M. R., and F. W. Dahlquist.** 1982. Adaptation in bacterial chemotaxis: CheB-dependent modification permits additional methylations of sensory transducer proteins. Cell **29:**761–772.
104. **Kehry, M. R., and F. W. Dahlquist.** 1982. The methyl-accepting chemotaxis proteins of *Escherichia coli*. Identification of the multiple methylation sites on methyl-accepting chemotaxis protein I. J. Biol. Chem. **257:**10378–10386.
105. **Kehry, M. R., T. G. Doak, and F. W. Dahlquist.** 1985. Sensory adaptation in bacterial chemotaxis: regulation of demethylation. J. Bacteriol. **163:**983–990.
106. **Kehry, M. R., T. G. Doak, and F. W. Dahlquist.** 1984. Stimulus-induced changes in methylesterase activity during chemotaxis in *Escherichia coli*. J. Biol. Chem. **259:**11828–11835.
107. **Khan, S., and R. M. Macnab.** 1980. The steady-state counterclockwise/clockwise ratio of bacterial flagellar motors is regulated by protonmotive force. J. Mol. Biol. **138:**563–597.
108. **Kim, C., M. Jackson, R. Lux, and S. Khan.** 2001. Determinants of chemotactic signal amplification in *Escherichia coli*. J. Mol. Biol. **307:**119–135.
109. **Kim, K. K., H. Yokota, and S. H. Kim.** 1999. Four-helical-bundle structure of the cytoplasmic domain of a serine chemotaxis receptor. Nature **400:**787–792.
110. **Kim, S. H., W. Wang, and K. K. Kim.** 2002. Dynamic and clustering model of bacterial chemotaxis receptors: structural basis for signaling and high sensitivity. Proc. Natl. Acad. Sci. USA **99:**11611–11615.
111. **Kirby, J. R., C. J. Kristich, S. L. Feinberg, and G. W. Ordal.** 1997. Methanol production during chemotaxis to amino acids in *Bacillus subtilis*. Mol. Microbiol. **24:**869–878.
112. **Kirby, J. R., C. J. Kristich, M. M. Saulmon, M. A. Zimmer, L. F. Garrity, I. B. Zhulin, and G. W. Ordal.** 2001. CheC is related to the family of

flagellar switch proteins and acts independently from CheD to control chemotaxis in *Bacillus subtilis*. Mol. Microbiol. **42:**573–585.
113. **Kirby, J. R., T. B. Niewold, S. Maloy, and G. W. Ordal.** 2000. CheB is required for behavioural responses to negative stimuli during chemotaxis in *Bacillus subtilis*. Mol. Microbiol. **35:**44–57.
114. **Kirby, J. R., and D. R. Zusman.** 2003. Chemosensory regulation of developmental gene expression in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **100:**2008–2013.
115. **Kirsch, M. L., P. D. Peters, D. W. Hanlon, J. R. Kirby, and G. W. Ordal.** 1993. Chemotactic methylesterase promotes adaptation to high concentrations of attractant in *Bacillus subtilis*. J. Biol. Chem. **268:**18610–18616.
116. **Kirsch, M. L., A. R. Zuberi, D. Henner, P. D. Peters, M. A. Yazdi, and G. W. Ordal.** 1993. Chemotactic methyltransferase promotes adaptation to repellents in *Bacillus subtilis*. J. Biol. Chem. **268:**25350–25356.
117. **Kofoid, E. C., and J. S. Parkinson.** 1988. Transmitter and receiver modules in bacterial signaling proteins. Proc. Natl. Acad. Sci. USA **85:**4981–4985.
118. **Kondoh, H., C. B. Ball, and J. Adler.** 1979. Identification of a methyl-accepting chemotaxis protein for the ribose and galactose chemoreceptors of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **76:**260–264.
119. **Kort, E. N., M. F. Goy, S. H. Larsen, and J. Adler.** 1975. Methylation of a membrane protein involved in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **72:**3939–3943.
120. **Koshland, D. E., Jr.** 1977. A response regulator model in a simple sensory system. Science **196:**1055–1063.
121. **Kristich, C. J., G. D. Glekas, and G. W. Ordal.** 2003. The conserved cytoplasmic module of the transmembrane chemoreceptor McpC mediates carbohydrate chemotaxis in *Bacillus subtilis*. Mol. Microbiol. **47:**1353–1366.
122. **Kristich, C. J., and G. W. Ordal.** 2002. *Bacillus subtilis* CheD is a chemoreceptor modification enzyme required for chemotaxis. J. Biol. Chem. **277:**25356–25362.
123. **Lee, G. F., M. R. Lebert, A. A. Lilly, and G. L. Hazelbauer.** 1995. Transmembrane signaling characterized in bacterial chemoreceptors by using sulfhydryl cross-linking in vivo. Proc. Natl. Acad. Sci. USA **92:**3391–3395.
124. **Le Moual, H., and D. E. Koshland, Jr.** 1996. Molecular evolution of the C-terminal cytoplasmic domain of a superfamily of bacterial receptors involved in taxis. J. Mol. Biol. **261:**568–585.
125. **Le Moual, H., T. Quang, and D. E. Koshland, Jr.** 1998. Conformational changes in the cytoplasmic domain of the *Escherichia coli* aspartate receptor upon adaptive methylation. Biochemistry **37:**14852–14859.
126. **Le Moual, H., T. Quang, and D. E. Koshland, Jr.** 1997. Methylation of the *Escherichia coli* chemotaxis receptors: intra- and interdimer mechanisms. Biochemistry **36:**13441–13448.
127. **Levin, M. D., T. S. Shimizu, and D. Bray.** 2002. Binding and diffusion of CheR molecules within a cluster of membrane receptors. Biophys. J. **82:**1809–1817.
128. **Levit, M. N., and J. B. Stock.** 2002. Receptor methylation controls the magnitude of stimulus-response coupling in bacterial chemotaxis. J. Biol. Chem. **277:**36760–36765.
129. **Li, J., G. Li, and R. M. Weis.** 1997. The serine chemoreceptor from *Escherichia coli* is methylated through an inter-dimer process. Biochemistry **36:**11851–11857.
130. **Li, J., R. V. Swanson, M. I. Simon, and R. M. Weis.** 1995. The response regulators CheB and CheY exhibit competitive binding to the kinase CheA. Biochemistry **34:**14626–14636.
131. **Liu, J. D., and J. S. Parkinson.** 1989. Role of CheW protein in coupling membrane receptors to the intracellular signaling system of bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **86:**8703–8707.
132. **Lukat, G. S., B. H. Lee, J. M. Mottonen, A. M. Stock, and J. B. Stock.** 1991. Roles of the highly conserved aspartate and lysine residues in the response regulator of bacterial chemotaxis. J. Biol. Chem. **266:**8348–8354.
133. **Lukat, G. S., A. M. Stock, and J. B. Stock.** 1990. Divalent metal ion binding to the CheY protein and its significance to phosphotransfer in bacterial chemotaxis. Biochemistry **29:**5436–5442.
134. **Lupas, A., and J. Stock.** 1989. Phosphorylation of an N-terminal regulatory domain activates the CheB methylesterase in bacterial chemotaxis. J. Biol. Chem. **264:**17337–17342.
135. **Lux, R., K. Jahreis, K. Bettenbrock, J. S. Parkinson, and J. W. Lengeler.** 1995. Coupling the phosphotransferase system and the methyl-accepting chemotaxis protein-dependent chemotaxis signaling pathways of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **92:**11583–11587.
136. **Lux, R., V. R. Munasinghe, F. Castellano, J. W. Lengeler, J. E. Corrie, and S. Khan.** 1999. Elucidation of a PTS-carbohydrate chemotactic signal pathway in Escherichia coli using a time-resolved behavioral assay. Mol. Biol. Cell **10:**1133–1146.
137. **Lux, R., J. H. Sim, J. P. Tsai, and W. Shi.** 2002. Construction and characterization of a cheA mutant of *Treponema denticola*. J. Bacteriol. **184:**3130–3134.
138. **Lybarger, S. R., and J. R. Maddock.** 1999. Clustering of the chemoreceptor complex in *Escherichia coli* is independent of the methyltransferase CheR and the methylesterase CheB. J. Bacteriol. **181:**5527–5529.
139. **Macnab, R. M.** 1977. Bacterial flagella rotating in bundles: a study in helical geometry. Proc. Natl. Acad. Sci. USA **74:**221–225.
140. **Maddock, J. R., and L. Shapiro.** 1993. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. Science **259:**1717–1723.
141. **Manson, M. D., W. Boos, P. J. Bassford, Jr., and B. A. Rasmussen.** 1985. Dependence of maltose transport and chemotaxis on the amount of maltose-binding protein. J. Biol. Chem. **260:**9727–9733.
142. **Marchant, J., B. Wren, and J. Ketley.** 2002. Exploiting genome sequence: predictions for mechanisms of *Campylobacter* chemotaxis. Trends Microbiol. **10:**155–159.
143. **Martin, A. C., U. Nair, J. P. Armitage, and J. R. Maddock.** 2003. Polar localization of CheA2 in *Rhodobacter sphaeroides* requires specific Che homologs. J. Bacteriol. **185:**4667–4671.
144. **Martin, A. C., G. H. Wadhams, and J. P. Armitage.** 2001. The roles of the multiple CheW and CheA homologues in chemotaxis and in chemoreceptor localization in *Rhodobacter sphaeroides*. Mol. Microbiol. **40:**1261–1272.
145. **Martin, A. C., G. H. Wadhams, D. S. Shah, S. L. Porter, J. C. Mantotta, T. J. Craig, P. H. Verdult, H. Jones, and J. P. Armitage.** 2001. CheR- and CheB-dependent chemosensory adaptation system of *Rhodobacter sphaeroides*. J. Bacteriol. **183:**7135–7144.
146. **Masduki, A., J. Nakamura, T. Ohga, R. Umezaki, J. Kato, and H. Ohtake.** 1995. Isolation and characterization of chemotaxis mutants and genes of *Pseudomonas aeruginosa*. J. Bacteriol. **177:**948–952.
147. **Mattick, J. S.** 2002. Type IV pili and twitching motility. Annu. Rev. Microbiol. **56:**289–314.
148. **McBride, M. J., T. Kohler, and D. R. Zusman.** 1992. Methylation of FrzCD, a methyl-accepting taxis protein of *Myxococcus xanthus*, is correlated with factors affecting cell behavior. J. Bacteriol. **174:**4246–4257.
149. **McEvoy, M. M., A. Bren, M. Eisenbach, and F. W. Dahlquist.** 1999. Identification of the binding interfaces on CheY for two of its targets, the phosphatase CheZ and the flagellar switch protein FliM. J. Mol. Biol. **289:**1423–1433.
150. **McNamara, B. P., and A. J. Wolfe.** 1997. Coexpression of the long and short forms of CheA, the chemotaxis histidine kinase, by members of the family *Enterobacteriaceae*. J. Bacteriol. **179:**1813–1818.
151. **Mehan, R. S., N. C. White, and J. J. Falke.** 2003. Mapping out regions on the surface of the aspartate receptor that are essential for kinase activation. Biochemistry **42:**2952–2959.
152. **Mesibov, R., G. W. Ordal, and J. Adler.** 1973. The range of attractant concentrations for bacterial chemotaxis and the threshold and size of response over this range. Weber law and related phenomena. J. Gen. Physiol. **62:**203–223.
153. **Milligan, D. L., and D. E. Koshland, Jr.** 1988. Site-directed cross-linking. Establishing the dimeric structure of the aspartate receptor of bacterial chemotaxis. J. Biol. Chem. **263:**6268–6275.
154. **Montrone, M., M. Eisenbach, D. Oesterhelt, and W. Marwan.** 1998. Regulation of switching frequency and bias of the bacterial flagellar motor by CheY and fumarate. J. Bacteriol. **180:**3375–3380.
155. **Montrone, M., D. Oesterhelt, and W. Marwan.** 1996. Phosphorylation-independent bacterial chemoresponses correlate with changes in the cytoplasmic level of fumarate. J. Bacteriol. **178:**6882–6887.
156. **Mourey, L., S. Da Re, J. D. Pedelacq, T. Tolstykh, C. Faurie, V. Guillet, J. B. Stock, and J. P. Samama.** 2001. Crystal structure of the CheA histidine phosphotransfer domain that mediates response regulator phosphorylation in bacterial chemotaxis. J. Biol. Chem. **276:**31074–31082.
157. **Mowbray, S. L.** 1992. Ribose and glucose-galactose receptors. Competitors in bacterial chemotaxis. J. Mol. Biol. **227:**418–440.
158. **Mowbray, S. L., R. D. Smith, and L. B. Cole.** 1990. Structure of the periplasmic glucose/galactose receptor of *Salmonella typhimurium*. Receptor **1:**41–53.
159. **Muller, J., S. Schiel, G. W. Ordal, and H. H. Saxild.** 1997. Functional and genetic characterization of *mcpC*, which encodes a third methyl-accepting chemotaxis protein in *Bacillus subtilis*. Microbiology **143:**3231–3240.
160. **Nettleton, D. O., and G. W. Ordal.** 1989. Functional homology of chemotactic methylesterases from *Bacillus subtilis* and *Escherichia coli*. J. Bacteriol. **171:**120–123.
161. **Nichols, N. N., and C. S. Harwood.** 2000. An aerotaxis transducer gene from *Pseudomonas putida*. FEMS Microbiol. Lett **182:**177–183.
162. **Niwano, M., and B. L. Taylor.** 1982. Novel sensory adaptation mechanism in bacterial chemotaxis to oxygen and phosphotransferase substrates. Proc. Natl. Acad. Sci. USA **79:**11–15.
163. **Nordmann, B., M. R. Lebert, M. Alam, S. Nitz, H. Kollmannsberger, D. Oesterhelt, and G. L. Hazelbauer.** 1994. Identification of volatile forms of methyl groups released by *Halobacterium salinarium*. J. Biol. Chem. **269:**16449–16454.
164. **Nowlin, D. M., J. Bollinger, and G. L. Hazelbauer.** 1987. Sites of covalent modification in Trg, a sensory transducer of *Escherichia coli*. J. Biol. Chem. **262:**6039–6045.
165. **Ordal, G. W., and J. Adler.** 1974. Isolation and complementation of mutants in galactose taxis and transport. J. Bacteriol. **117:**509–516.
166. **Ordal, G. W., and J. Adler.** 1974. Properties of mutants in galactose taxis and transport. J. Bacteriol. **117:**517–526.
167. **Ordal, G. W., H. M. Parker, and J. R. Kirby.** 1985. Complementation and

characterization of chemotaxis mutants of *Bacillus subtilis*. J. Bacteriol. **164:**802–810.
168. **Ordal, G. W., D. P. Villani, and K. J. Gibson.** 1977. Amino acid chemoreceptors of *Bacillus subtilis*. J. Bacteriol. **129:**156–165.
169. **Ordal, G. W., D. P. Villani, and M. S. Rosendahl.** 1979. Chemotaxis towards sugars by *Bacillus subtilis*. J. Gen. Microbiol. **115:**167–172.
170. **O'Toole, G. A., and R. Kolter.** 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. **30:**295–304.
171. **Ottemann, K. M., W. Xiao, Y. K. Shin, and D. E. Koshland, Jr.** 1999. A piston model for transmembrane signaling of the aspartate receptor. Science **285:**1751–1754.
172. **Pittman, M. S., M. Goodwin, and D. J. Kelly.** 2001. Chemotaxis in the human gastric pathogen *Helicobacter pylori:* different roles for CheW and the three CheV paralogues, and evidence for CheV2 phosphorylation. Microbiology **147:**2493–2504.
173. **Poole, P. S., and J. P. Armitage.** 1988. Motility response of *Rhodobacter sphaeroides* to chemotactic stimulation. J. Bacteriol. **170:**5673–5679.
174. **Porter, S. L., and J. P. Armitage.** 2002. Phosphotransfer in *Rhodobacter sphaeroides* chemotaxis. J. Mol. Biol. **324:**35–45.
175. **Prasad, K., S. R. Caplan, and M. Eisenbach.** 1998. Fumarate modulates bacterial flagellar rotation by lowering the free energy difference between the clockwise and counterclockwise states of the motor. J. Mol. Biol. **280:** 821–828.
176. **Reader, R. W., W. W. Tso, M. S. Springer, M. F. Goy, and J. Adler.** 1979. Pleiotropic aspartate taxis and serine taxis mutants of *Escherichia coli*. J. Gen. Microbiol. **111:**363–374.
177. **Rebbapragada, A., M. S. Johnson, G. P. Harding, A. J. Zuccarelli, H. M. Fletcher, I. B. Zhulin, and B. L. Taylor.** 1997. The Aer protein and the serine chemoreceptor Tsr independently sense intracellular energy levels and transduce oxygen, redox, and energy signals for *Escherichia coli* behavior. Proc. Natl. Acad. Sci. USA **94:**10541–10546.
178. **Repik, A., A. Rebbapragada, M. S. Johnson, J. O. Haznedar, I. B. Zhulin, and B. L. Taylor.** 2000. PAS domain residues involved in signal transduction by the Aer redox sensor of *Escherichia coli*. Mol. Microbiol. **36:**806–816.
179. **Rice, M. S., and F. W. Dahlquist.** 1991. Sites of deamidation and methylation in Tsr, a bacterial chemotaxis sensory transducer. J. Biol. Chem. **266:**9746–9753.
180. **Romagnoli, S., H. L. Packer, and J. P. Armitage.** 2002. Tactic responses to oxygen in the phototrophic bacterium *Rhodobacter sphaeroides*. WS8N. J. Bacteriol. **184:**5590–5598.
181. **Rosario, M. M., K. L. Fredrick, G. W. Ordal, and J. D. Helmann.** 1994. Chemotaxis in *Bacillus subtilis* requires either of two functionally redundant CheW homologs. J. Bacteriol. **176:**2736–2739.
182. **Rosario, M. M., J. R. Kirby, D. A. Bochar, and G. W. Ordal.** 1995. Chemotactic methylation and behavior in *Bacillus subtilis:* role of two unique proteins, CheC and CheD. Biochemistry **34:**3823–3831.
183. **Rosario, M. M., and G. W. Ordal.** 1996. CheC and CheD interact to regulate methylation of *Bacillus subtilis* methyl-accepting chemotaxis proteins. Mol. Microbiol. **21:**511–518.
184. **Rudolph, J., and D. Oesterhelt.** 1996. Deletion analysis of the che operon in the archaeon *Halobacterium salinarium*. J. Mol. Biol. **258:**548–554.
185. **Rudolph, J., N. Tolliday, C. Schmitt, S. C. Schuster, and D. Oesterhelt.** 1995. Phosphorylation in halobacterial signal transduction. EMBO J. **14:** 4249–4257.
186. **Sagi, Y., S. Khan, and M. Eisenbach.** 2003. Binding of the chemotaxis response regulator CheY to the isolated, intact switch complex of the bacterial flagellar motor: lack of cooperativity. J. Biol. Chem. **278:**25867–25871.
187. **Sanders, D. A., B. Mendez, and D. E. Koshland, Jr.** 1989. Role of the CheW protein in bacterial chemotaxis: overexpression is equivalent to absence. J. Bacteriol. **171:**6271–6278.
188. **Sanna, M. G., and M. I. Simon.** 1996. In vivo and in vitro characterization of *Escherichia coli* protein CheZ gain- and loss-of-function mutants. J. Bacteriol. **178:**6275–6280.
189. **Sanna, M. G., and M. I. Simon.** 1996. Isolation and in vitro characterization of CheZ suppressors for the *Escherichia coli* chemotactic response regulator mutant CheYN23D. J. Biol. Chem. **271:**7357–7361.
190. **Saulmon, M. M., E. Karatan, and G. W. Ordal.** 2004. Effect of loss of CheC and other adaptational proteins on chemotactic behavior in *Bacillus subtilis*. Microbiology **150:**581–589.
191. **Scharf, B., and R. Schmitt.** 2002. Sensory transduction to the flagellar motor of *Sinorhizobium meliloti*. J. Mol. Microbiol. Biotechnol. **4:**183–186.
192. **Schmitt, R.** 2002. Sinorhizobial chemotaxis: a departure from the enterobacterial paradigm. Microbiology **148:**627–631.
193. **Schuster, M., R. Zhao, R. B. Bourret, and E. J. Collins.** 2000. Correlated switch binding and signaling in bacterial chemotaxis. J. Biol. Chem. **275:** 19752–19758.
194. **Scott, W. G., D. L. Milligan, M. V. Milburn, G. G. Prive, J. Yeh, D. E. Koshland, Jr., and S. H. Kim.** 1993. Refined structures of the ligand-binding domain of the aspartate receptor from *Salmonella typhimurium*. J. Mol. Biol. **232:**555–573.
195. **Segall, J. E., S. M. Block, and H. C. Berg.** 1986. Temporal comparisons in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **83:**8987–8991.
196. **Shah, D. S., S. L. Porter, A. C. Martin, P. A. Hamblin, and J. P. Armitage.** 2000. Fine tuning bacterial chemotaxis: analysis of *Rhodobacter sphaeroides* behaviour under aerobic and anaerobic conditions by mutation of the major chemotaxis operons and *cheY* genes. EMBO J. **19:**4601–4613.
197. **Shapiro, M. J., I. Chakrabarti, and D. E. Koshland, Jr.** 1995. Contributions made by individual methylation sites of the *Escherichia coli* aspartate receptor to chemotactic behavior. Proc. Natl. Acad. Sci. USA **92:**1053–1056.
198. **Shapiro, M. J., and D. E. Koshland, Jr.** 1994. Mutagenic studies of the interaction between the aspartate receptor and methyltransferase from *Escherichia coli*. J. Biol. Chem. **269:**11054–11059.
199. **Shi, W., and D. R. Zusman.** 1994. Sensory adaptation during negative chemotaxis in *Myxococcus xanthus*. J. Bacteriol. **176:**1517–1520.
200. **Shi, W., and D. R. Zusman.** 1993. The two motility systems of *Myxococcus xanthus* show different selective advantages on various surfaces. Proc. Natl. Acad. Sci. USA **90:**3378–3382.
201. **Shimizu, T. S., S. V. Aksenov, and D. Bray.** 2003. A spatially extended stochastic model of the bacterial chemotaxis signalling pathway. J. Mol. Biol. **329:**291–309.
202. **Shimizu, T. S., and D. Bray.** 2002. Modelling the bacterial chemotaxis receptor complex. Novartis Found. Symp. **247:**162–177; discussion, 177–181, 198–206, 244–252.
203. **Shimizu, T. S., N. Le Novere, M. D. Levin, A. J. Beavil, B. J. Sutton, and D. Bray.** 2000. Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis. Nat. Cell Biol. **2:**792–796.
204. **Shioi, J., and B. L. Taylor.** 1984. Oxygen taxis and proton motive force in *Salmonella typhimurium*. J. Biol. Chem. **259:**10983–10988.
205. **Silversmith, R. E., J. L. Appleby, and R. B. Bourret.** 1997. Catalytic mechanism of phosphorylation and dephosphorylation of CheY: kinetic characterization of imidazole phosphates as phosphodonors and the role of acid catalysis. Biochemistry **36:**14965–14974.
206. **Singh, M., B. Berger, P. S. Kim, J. M. Berger, and A. G. Cochran.** 1998. Computational learning reveals coiled coil-like motifs in histidine kinase linker domains. Proc. Natl. Acad. Sci. USA **95:**2738–2743.
207. **Skerker, J. M., and H. C. Berg.** 2001. Direct observation of extension and retraction of type IV pili. Proc. Natl. Acad. Sci. USA **98:**6901–6904.
208. **Slocum, M. K., and J. S. Parkinson.** 1985. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli:* null phenotypes of the *tar* and *tap* genes. J. Bacteriol. **163:**586–594.
209. **Sourjik, V., and H. C. Berg.** 2000. Localization of components of the chemotaxis machinery of *Escherichia coli* using fluorescent protein fusions. Mol. Microbiol. **37:**740–751.
210. **Sourjik, V., and H. C. Berg.** 2002. Receptor sensitivity in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **99:**123–127.
211. **Sourjik, V., and R. Schmitt.** 1996. Different roles of CheY1 and CheY2 in the chemotaxis of *Rhizobium meliloti*. Mol. Microbiol. **22:**427–436.
212. **Sourjik, V., and R. Schmitt.** 1998. Phosphotransfer between CheA, CheY1, and CheY2 in the chemotaxis signal transduction chain of *Rhizobium meliloti*. Biochemistry **37:**2327–2335.
213. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli:* two complementary pathways of information processing that involve methylated proteins. Proc. Natl. Acad. Sci. USA **74:**3312–3316.
214. **Springer, W. R., and D. E. Koshland, Jr.** 1977. Identification of a protein methyltransferase as the *cheR* gene product in the bacterial sensing system. Proc. Natl. Acad. Sci. USA **74:**533–537.
215. **Spudich, E. N., C. A. Hasselbacher, and J. L. Spudich.** 1988. Methyl-accepting protein associated with bacterial sensory rhodopsin I. J. Bacteriol. **170:**4280–4285.
216. **Spudich, E. N., T. Takahashi, and J. L. Spudich.** 1989. Sensory rhodopsins I and II modulate a methylation/demethylation system in *Halobacterium halobium* phototaxis. Proc. Natl. Acad. Sci. USA **86:**7746–7750.
217. **Stewart, R. C.** 1993. Activating and inhibitory mutations in the regulatory domain of CheB, the methylesterase in bacterial chemotaxis. J. Biol. Chem. **268:**1921–1930.
218. **Stewart, R. C.** 1997. Kinetic characterization of phosphotransfer between CheA and CheY in the bacterial chemotaxis signal transduction pathway. Biochemistry **36:**2030–2040.
219. **Stewart, R. C., and F. W. Dahlquist.** 1988. N-terminal half of CheB is involved in methylesterase response to negative chemotactic stimuli in *Escherichia coli*. J. Bacteriol. **170:**5728–5738.
220. **Stewart, R. C., A. F. Roth, and F. W. Dahlquist.** 1990. Mutations that affect control of the methylesterase activity of CheB, a component of the chemotaxis adaptation system in *Escherichia coli*. J. Bacteriol. **172:**3388–3399.
221. **Stock, A., D. E. Koshland, Jr., and J. Stock.** 1985. Homologies between the *Salmonella typhimurium* CheY protein and proteins involved in the regulation of chemotaxis, membrane protein synthesis, and sporulation. Proc. Natl. Acad. Sci. USA **82:**7989–7993.
222. **Stock, A. M., E. Martinez-Hackert, B. F. Rasmussen, A. H. West, J. B. Stock, D. Ringe, and G. A. Petsko.** 1993. Structure of the Mg^{2+}-bound form of CheY and mechanism of phosphoryl transfer in bacterial chemotaxis. Biochemistry **32:**13375–13380.

223. **Stock, A. M., and J. B. Stock.** 1987. Purification and characterization of the CheZ protein of bacterial chemotaxis. J. Bacteriol. **169:**3301–3311.
224. **Stock, J., and M. Levit.** 2000. Signal transduction: hair brains in bacterial chemotaxis. Curr. Biol. **10:**R11–R14.
225. **Stock, J., and S. Simms.** 1988. Methylation, demethylation, and deamidation at glutamate residues in membrane chemoreceptor proteins. Adv. Exp. Med. Biol. **231:**201–212.
226. **Stock, J. B., and D. E. Koshland, Jr.** 1978. A protein methylesterase involved in bacterial sensing. Proc. Natl. Acad. Sci. USA **75:**3659–3663.
227. **Stock, J. B., M. N. Levit, and P. M. Wolanin.** 2002. Information processing in bacterial chemotaxis. Sci. STKE **2002:**PE25.
228. **Stock, J. B., A. M. Maderis, and D. E. Koshland, Jr.** 1981. Bacterial chemotaxis in the absence of receptor carboxylmethylation. Cell **27:**37–44.
229. **Storch, K. F., J. Rudolph, and D. Oesterhelt.** 1999. Car: a cytoplasmic sensor responsible for arginine chemotaxis in the archaeon *Halobacterium salinarum*. EMBO J. **18:**1146–1158.
230. **Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrener, M. J. Hickey, F. S. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K. Wong, Z. Wu, I. T. Paulsen, J. Reizer, M. H. Saier, R. E. Hancock, S. Lory, and M. V. Olson.** 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature **406:** 959–964.
231. **Sun, H., D. R. Zusman, and W. Shi.** 2000. Type IV pilus of *Myxococcus xanthus* is a motility apparatus controlled by the frz chemosensory system. Curr. Biol. **10:**1143–1148.
232. **Surette, M. G., M. Levit, Y. Liu, G. Lukat, E. G. Ninfa, A. Ninfa, and J. B. Stock.** 1996. Dimerization is required for the activity of the protein histidine kinase CheA that mediates signal transduction in bacterial chemotaxis. J. Biol. Chem. **271:**939–945.
233. **Swanson, R. V., S. C. Schuster, and M. I. Simon.** 1993. Expression of CheA fragments which define domains encoding kinase, phosphotransfer, and CheY binding activities. Biochemistry **32:**7623–7629.
234. **Szurmant, H., M. W. Bunn, V. J. Cannistraro, and G. W. Ordal.** 2003. *Bacillus subtilis* hydrolyzes CheY-P at the location of its action, the flagellar switch. J. Biol. Chem. **278:**48611–48616.
234a. **Szurmant, H., T. J. Muff, and G. W. Ordal.** 27 January 2004. *Bacillus subtilis* CheC and FliY are members of a novel class of CheY-P hydrolyzing proteins in the chemotactic signal transduction cascade. J. Biol. Chem. 10.1074/jbc.M311497200.
235. **Taylor, B. L., and I. B. Zhulin.** 1998. In search of higher energy: metabolism-dependent behaviour in bacteria. Mol. Microbiol. **28:**683–690.
236. **Taylor, B. L., I. B. Zhulin, and M. S. Johnson.** 1999. Aerotaxis and other energy-sensing behavior in bacteria. Annu. Rev. Microbiol. **53:**103–128.
237. **Terwilliger, T. C., E. Bogonez, E. A. Wang, and D. E. Koshland, Jr.** 1983. Sites of methyl esterification on the aspartate receptor involved in bacterial chemotaxis. J. Biol. Chem. **258:**9608–9611.
238. **Terwilliger, T. C., J. Y. Wang, and D. E. Koshland, Jr.** 1986. Kinetics of receptor modification. The multiply methylated aspartate receptors involved in bacterial chemotaxis. J. Biol. Chem. **261:**10814–10820.
239. **Thoelke, M. S., J. M. Casper, and G. W. Ordal.** 1990. Methyl transfer in chemotaxis toward sugars by *Bacillus subtilis*. J. Bacteriol. **172:**1148–1150.
240. **Thoelke, M. S., J. R. Kirby, and G. W. Ordal.** 1989. Novel methyl transfer during chemotaxis in *Bacillus subtilis*. Biochemistry **28:**5585–5589.
241. **Thoelke, M. S., H. M. Parker, E. A. Ordal, and G. W. Ordal.** 1988. Rapid attractant-induced changes in methylation of methyl-accepting chemotaxis proteins in *Bacillus subtilis*. Biochemistry **27:**8453–8457.
242. **Toews, M. L., M. F. Goy, M. S. Springer, and J. Adler.** 1979. Attractants and repellents control demethylation of methylated chemotaxis proteins in Escherichia coli. Proc. Natl. Acad. Sci. USA **76:**5544–5548.
243. **Ullah, A. H., and G. W. Ordal.** 1981. In vivo and in vitro chemotactic methylation in *Bacillus subtilis*. J. Bacteriol. **145:**958–965.
244. **Volz, K., and P. Matsumura.** 1991. Crystal structure of *Escherichia coli* CheY refined at 1.7-A resolution. J. Biol. Chem. **266:**15511–15519.
245. **Wadhams, G. H., A. C. Martin, and J. P. Armitage.** 2000. Identification and localization of a methyl-accepting chemotaxis protein in *Rhodobacter sphaeroides*. Mol. Microbiol. **36:**1222–1233.
246. **Wadhams, G. H., A. C. Martin, S. L. Porter, J. R. Maddock, J. C. Mantotta, H. M. King, and J. P. Armitage.** 2002. TlpC, a novel chemotaxis protein in *Rhodobacter sphaeroides*, localizes to a discrete region in the cytoplasm. Mol. Microbiol. **46:**1211–1221.
247. **Ward, M. J., D. M. Harrison, M. J. Ebner, and J. P. Armitage.** 1995. Identification of a methyl-accepting chemotaxis protein in *Rhodobacter sphaeroides*. Mol. Microbiol. **18:**115–121.
248. **Welch, M., K. Oosawa, S. Aizawa, and M. Eisenbach.** 1993. Phosphorylation-dependent binding of a signal molecule to the flagellar switch of bacteria. Proc. Natl. Acad. Sci. USA **90:**8787–8791.
249. **Wheeler, R. T., J. W. Gober, and L. Shapiro.** 1998. Protein localization during the *Caulobacter crescentus* cell cycle. Curr. Opin. Microbiol. **1:**636–642.
250. **Wolanin, P. M., P. A. Thomason, and J. B. Stock.** 2002. Histidine protein kinases: key signal transducers outside the animal kingdom. Genome Biol. **3:**REVIEWS3013.
251. **Wolfe, A. J., B. P. McNamara, and R. C. Stewart.** 1994. The short form of CheA couples chemoreception to CheA phosphorylation. J. Bacteriol. **176:** 4483–4491.
252. **Wu, J., J. Li, G. Li, D. G. Long, and R. M. Weis.** 1996. The receptor binding site for the methyltransferase of bacterial chemotaxis is distinct from the sites of methylation. Biochemistry **35:**4984–4993.
253. **Yeh, J. I., H. P. Biemann, G. G. Prive, J. Pandit, D. E. Koshland, Jr., and S. H. Kim.** 1996. High-resolution structures of the ligand binding domain of the wild-type bacterial aspartate receptor. J. Mol. Biol. **262:**186–201.
254. **Zhang, W., and G. N. Phillips, Jr.** 2003. Crystallization and X-ray diffraction analysis of the sensor domain of the HemAT aerotactic receptor. Acta Crystallogr. Ser. D **59:**749–751.
255. **Zhang, W., and G. N. Phillips, Jr.** 2003. Structure of the oxygen sensor in *Bacillus subtilis:* signal transduction of chemotaxis by control of symmetry. Structure **11:**1097–1110.
256. **Zhang, Y., C. Conway, M. Rosato, Y. Suh, and M. D. Manson.** 1992. Maltose chemotaxis involves residues in the N-terminal and C-terminal domains on the same face of maltose-binding protein. J. Biol. Chem. **267:** 22813–22820.
257. **Zhao, R., E. J. Collins, R. B. Bourret, and R. E. Silversmith.** 2002. Structure and catalytic mechanism of the E. coli chemotaxis phosphatase CheZ. Nat. Struct. Biol. **9:**570–575.
258. **Zhou, H., D. F. Lowry, R. V. Swanson, M. I. Simon, and F. W. Dahlquist.** 1995. NMR studies of the phosphotransfer domain of the histidine kinase CheA from *Escherichia coli:* assignments, secondary structure, general fold, and backbone dynamics. Biochemistry **34:**13858–13870.
259. **Zhu, X., C. D. Amsler, K. Volz, and P. Matsumura.** 1996. Tyrosine 106 of CheY plays an important role in chemotaxis signal transduction in *Escherichia coli*. J. Bacteriol. **178:**4208–4215.
260. **Zhu, X., J. Rebello, P. Matsumura, and K. Volz.** 1997. Crystal structures of CheY mutants Y106W and T87I/Y106W. CheY activation correlates with movement of residue 106. J. Biol. Chem. **272:**5000–5006.
261. **Zhu, X., K. Volz, and P. Matsumura.** 1997. The CheZ-binding surface of CheY overlaps the CheA- and FliM-binding surfaces. J. Biol. Chem. **272:** 23758–23764.
262. **Zhulin, I. B., V. A. Bespalov, M. S. Johnson, and B. L. Taylor.** 1996. Oxygen taxis and proton motive force in *Azospirillum brasilense*. J. Bacteriol. **178:** 5199–5204.
263. **Zhulin, I. B., A. F. Lois, and B. L. Taylor.** 1995. Behavior of *Rhizobium meliloti* in oxygen gradients. FEBS Lett. **367:**180–182.
264. **Zimmer, M. A., H. Szurmant, M. M. Saulmon, M. A. Collins, J. S. Bant, and G. W. Ordal.** 2002. The role of heterologous receptors in McpB-mediated signalling in *Bacillus subtilis* chemotaxis. Mol. Microbiol. **45:**555–568.
265. **Zimmer, M. A., J. Tiu, M. A. Collins, and G. W. Ordal.** 2000. Selective methylation changes on the *Bacillus subtilis* chemotaxis receptor McpB promote adaptation. J. Biol. Chem. **275:**24264–24272.
266. **Zukin, R. S., P. G. Strange, R. Heavey, and D. E. Koshland.** 1977. Properties of the galactose binding protein of *Salmonella typhimurium* and *Escherichia coli*. Biochemistry **16:**381–386.