Molecular Components of Bacterial Chemotaxis

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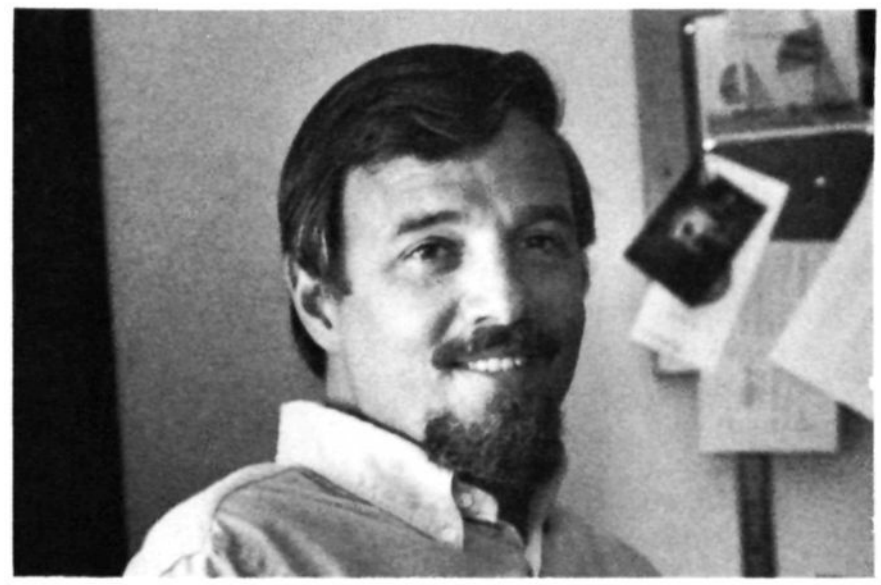

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I. Introduction

Bacterial chemotaxis is a complex phenomenon in which bacterial cells detect temporal changes in concentrations of specific chemicals, behaviorally respond

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to these changes, and then adapt to the new concentration of the chemical stimulus. In recent years, the molecular events that allow this remarkable series of events to occur have been partially elucidated.

As the various components of the system have been revealed, there appear to be at least two complex molecular machines involved in the process. One of these is the molecular device that allows measurement of temporal gradients of chemical stimuli, and the other is the bacterial flagellar motor. In addition, there must be some mechanism that allows the gradient measuring device to communicate with the flagellar motor.

The behavioral response itself involves modulation of the sense of rotation of the bacterial flagellar motor.[1-4] This rotary motor is driven by proton motive force and has the capacity to change its sense of rotation while the proton motive force remains fixed in polarity.[5-11] This raises a number of fascinating questions. For instance, how does the motor convert proton motive force into rotary motion? How does the molecular "gear box" allow the sense of rotation to be changed?

For us, the most perplexing and interesting aspect of bacterial chemotaxis concerns the biochemical events that allow the temporal gradient sensing device to function. The device must possess a component that responds rapidly to the presence of chemical stimuli, a second component that responds more slowly to such stimuli, and a means of comparing the two. We believe that the rapidly responding component is the binding of chemicals to the appropriate receptors and that at least part of the slower responding component involves the reversible covalent modification of the receptors. The detailed means of comparing the two remains unknown, although some qualitative features of the comparison device are becoming clear. At the end of this review we present a model for how the comparison could be made.

Here, we present our view of bacterial chemotaxis with emphasis on the nature of the signal(s) that couples the receptors to the flagellar motors. We have been selective in choosing and emphasizing topics for discussion, but we hope we have included all points of view expressed by our colleagues in the field for those selected topics. There is excellent work in the field that we do not discuss here, and we refer interested readers to several excellent reviews that may not share our emphasis.[12-26]

A. Overview of Chemotaxis

In the absence of spatial or temporal gradients of chemoattractants, bacteria such as *Escherichia coli* and *Salmonella typhimurium* propel themselves through their liquid environment in a series of smooth, rather straight "runs" that are terminated by a turning maneuver, a "tumble" or "twiddle".[27] The runs involve rotation of all or most of the bacterial flagellar motors in the counterclockwise sense (CCW). A tumble occurs when reversal of the sense of rotation to clockwise (CW) occurs.[28,29] In *E. coli* and *S. typhimurium*, about six to eight flagellar motors are located uniformly over the cell surface.[30] It is not yet clear if reversal of all, a majority, or a single motor is required to produce a tumble.[26,31] Tumbling events are usually short-lived (less than 1 s) and result in a nearly random reorientation of the cell.[27] In the absence of stimuli, the probability of undergoing a tumble is essentially independent of when the cell last tumbled.[27,32] As a result, the cell undergoes a classic random walk in three dimensions.[27,33-35]

In the presence of gradients of chemical stimuli, the probability per unit time (the rate) of undergoing a tumble now depends on the direction in which the cell is moving in the gradient. For a cell moving in a favorable direction, the tumbling rate is less than in the absence of a gradient. A cell moving in a less favorable direction has a tumbling rate approximately the same as that observed in the absence of a gradient.[27,32,36] As a result of this asymmetry of tumbling probabilities there is a net flux of the cells in the favorable direction. Bacteria are unable to directly turn toward the favorable direction.[27] Rather, they increase the time spent going in favorable directions by suppressing tumbling.

S. thyphimurium and *E. coli* sense gradients of chemoattractants in time rather than in space. This was elegantly demonstrated by Macnab and Koshland,[36] who applied sudden and large increases of the chemoattractant serine to *S. typhimurium* cells and observed a complete inhibition of tumbling for a period of a minute or so. After that time, the cells returned to the prestimulus swimming pattern of runs terminated by tumbles. Thus, it appears that large temporal increases of chemoattractant are detected by the bacteria as positive gradients, and tumbles are suppressed. After a minute or so, the cells adapt to the new high-attractant environment. From this we conclude that the cells maintain a record of their chemical environment over the recent past. If the current environment is detected to be "better" than the previous recorded one, tumbles are suppressed. If the opposite is detected, tumbling is enhanced. The record is continually updated. It takes minutes to update the memory if a massive chemostimulus is applied.[36-38] After this time, a comparison of the immediate environment with the record shows no difference, and behavior returns to the random swimming pattern. The adaptation time for smaller gradients of stimuli is shorter; for typical gradients of physiological importance the adaptation time is on the seconds time scale.[36-38]

It is useful to describe the chemotactic response to temporal stimuli as consisting of two phases; an excitatory phase that leads to modification of the rate of tumbling, followed by an adaptive phase corresponding to cells' updating their record of the chemical environment during the recent past. The excitatory phase is fast but not instantaneous; it takes *E. coli* a fraction of a second to initiate the response (CCW flagellar rotation).[39-41] The adaptive phase is slow, ranging from seconds to minutes depending on the size of the stimulus.[36-38]

B. Components of the Chemotaxis System

There are a discrete number of chemoattractant molecule species that are detected by a discrete number of receptor proteins. The types of receptors vary somewhat with bacterial species. The situation for repellent molecules is more complex. Some chemorepellents appear to interact directly with a receptor protein, while others appear to be detected by a more esoteric mechanism such as a monitor of the membrane potential.

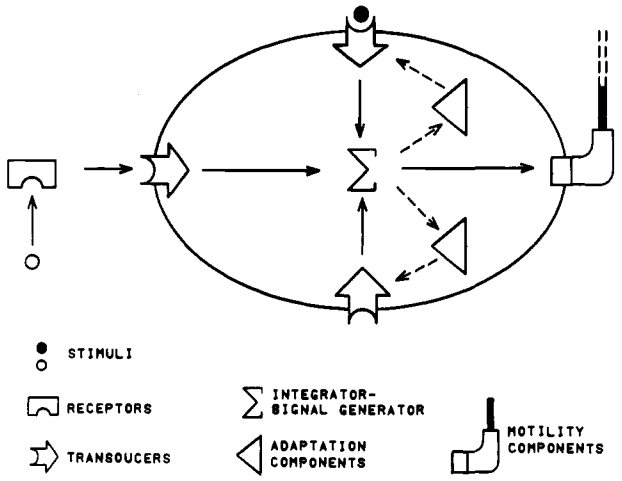


Figure 1. Pathway of information flow through components of the chemotaxis machinery of *E. coli*. The schematic representation of the cell shows the subcellular location (periplasmic, membrane bound, or cytosolic) of general groups of chemotaxis components as well as their locations in the flow of information (indicated by arrows). Solid arrows trace the flow of information from the stimulus–receptor interactions to the components of the flagellar motor. Dashed arrows follow the transfer of information in the feedback mechanism that enables sensory adaptation.

The known chemoreceptors fall into three distinct categories. One of these includes certain of the soluble periplasmic binding proteins such as the galactose, ribose, and maltose binding proteins of *E. coli* and *S. typhimurium*. In addition to their role(s) in transport, these proteins have a distinct role as chemoreceptors.[42-46] Interestingly, not all periplasmic binding proteins are chemoreceptors, even though they share considerable structural similarity with those that do function as receptors.[47]

The periplasmic binding proteins do not communicate directly with the cytoplasm but must interact with a second class of receptor proteins, which are also sometimes called transducer proteins.[48-52] These span the inner membrane and appear to provide the pathway for transmembrane signal production.[19-22,53-56] In addition to acting as secondary receptors for the periplasmic binding proteins, these membrane proteins can also function as primary receptors. Thus, the Tar protein of *E. coli* mediates the signals from the periplasmic maltose binding protein,[51] and it directly binds aspartate,[56,61] one of the more potent chemoattractants. These fascinating membrane proteins also serve as the sites of reversible methylation reactions, which play an important role in behavioral adaptation to some stimuli.[19-22,56,62-65]

A third type of chemoreceptor appears to be involved in responses to sugars such as glucose that are transported by the phosphotransferase system. Here the receptors are the Enzyme II proteins involved in binding and phosphorylation of the sugars.[66-68] Adaptation to the stimuli mediated by these receptors appears to be methylation independent.[68,69]

The molecular events that give rise to the signal that allows communication of the receptors with the flagella are not yet known. At least some of the proteins involved in this process have been identified by using various genetic and biochemical methods. These appear to include the products of the *cheA*, *cheW*, *cheY*, and *cheZ* genes. These genes encode proteins that seem to be distinct from the flagellar components or the receptors. In addition, the *cheR* and *cheB* genes code for the methyltransferase[65] and methylesterase,[70] respectively, needed for the methylation and demethylation events associated with some forms of sensory adaptation.

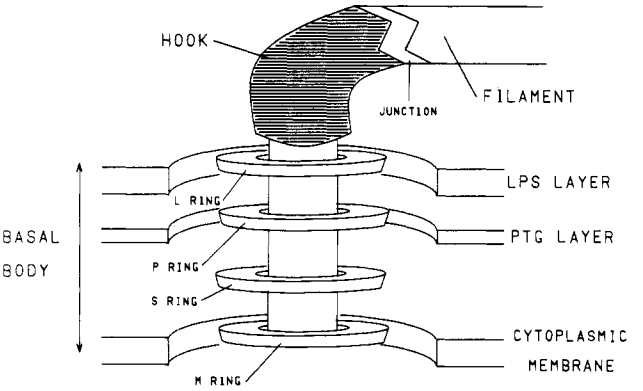


Figure 2. Schematic representation of the flagellar–basal body complex of *S. typhimurium* and *E. coli*. The major components of the filament–HBB complex and their orientations with respect to the inner and outer membranes are shown. These morphological features have been defined through EM images of wild-type and mutant HBB complexes.[71,74,75,95,96] Abbreviations: LPS, lipopolysaccharide layer of the outer membrane; PTG, peptigoglycan layer of the outer membrane.

This scheme is shown diagramatically in Figure 1. We discuss the various components of the system in more detail below.

II. Structure and Function of Bacterial Flagella

A. Morphology and Surmised Function of Flagellar Components

The small motors (diameter ~300 Å[5]) responsible for the propeller-like rotation of bacterial flagella reside at the base of each flagellum.[3] The motors are reversible and are powered by transmembrane proton motive force.[5-11] As depicted in Figure 2, electron microscopy of the flagella in intact cells and in various cell membrane preparations indicates that each flagellar organelle is comprised of three distinct substructures referred to as the flagellar filament, the flagellar hook, and the basal body.[71-74] More detailed images of these organelles are obtained by dissolving the inner and outer membranes of such preparations, leaving so-called "intact flagella".[71,75] The general morphology of the filament–hook–basal body complex and the orientation of these components with respect to the cell membrane are also shown in Figure 2.

The flagellar filament is a long (length ~10 μm), thin (diameter ~20 nm),[71] relatively rigid structure. In *E. coli*, *S. typhimurium*, and numerous other bacteria,[76] the filament is comprised of identical subunits of a single protein, flagellin, which are capable of assembling themselves into the characteristic cylindrical filament structure in vitro.[77] In vivo assembly of the filament occurs by the addition of flagellin subunits to the distal end of the growing filament.[78] Transport of the flagellin molecules from the site of their synthesis (the cytosol) to the tip of the growing filament may occur through the hollow central core of the filament.[79] Although the shape of the filament can be altered by subjecting it to varying external load[31] or pH,[80] its role appears to be that of a semirigid propeller. When all or most of the

flagella on a single bacterium rotate in the CCW direction, favorable hydrodynamic and mechanical interactions[81] among the filaments organize them into a flagellar bundle that propels the cell at speeds ranging from 20 to 60 $\mu m\ s^{-1}$ (up to 30 body lengths/s).[26] When some or all of the filaments switch from CCW to CW rotation, the favorable interactions among filaments are disrupted; the flagellar bundle comes apart.[26,31] Under these circumstances filament rotation no longer propels the cell; instead, it causes the tumbling behavior that points the cell in a random direction.[3,82] When the cell resumes smooth swimming (CCW filament rotation), it explores a new, randomly selected region of its environment. Because the filament constitutes over 95% of the mass of isolated intact flagella,[16] its presence complicates biochemical investigations of the remainder of the flagellar organelle. It has therefore been useful to prepare hook–basal body (HBB) complexes lacking most or all of the filament by treating intact flagella with denaturants that selectively dissociate the filaments[83] or by isolating the organelles from mutants lacking filaments.[84]

The flagellar hook is a short (~90 nm), curved structure that connects the base of the filament to the basal body; its diameter is slightly greater than that of the filament.[71] The hook is thought to serve as a flexible "universal joint".[1,13,14,26] In other words, it transmits the rotational motion of the motor (residing in the membrane plane) to the filament so that the filament rotates about an axis appropriate for propelling the cell. In *E. coli* and *S. typhimurium* the flagellar hook is assembled primarily from identical 42-kDa polypeptide subunits.[85–87]

The basal body is the most complex known part of the flagellar organelle. Electron micrographs[71] show four ringlike structures and a central rod that terminates at the flagellar hook. As shown in Figure 2, the four rings (designated as L, P, S, and M) appear to have specific orientations within the layers comprising the cell wall and membrane. Although the HBB complex contains only a subset of the flagellar motor components,[26] its morphology has inspired speculation on the functional roles of the basal body components.[4,26,88] In Berg's model[4,88] of the flagellar rotary motor, the M ring serves as the rotor; it can rotate freely in the cytoplasmic membrane. Attached to the M ring is the rod that serves as the driveshaft; it ends at the universal joint (hook). The S ring is mounted rigidly in the cell wall proximal to the M ring and can therefore function as the stator (stationary part of the motor). The motor is driven by generating torque between the M and S rings.

B. Molecular Biology of Flagellar Components

Over 30 genes are necessary for flagellar assembly and function.[26] As shown in Figure 3, the organization of these genes in the *E. coli* (*S. typhimurium*) genome has been defined by complementation analysis[13,89] and other procedures (e.g., sequencing). These genes are located at three major regions of the *E. coli* chromosome. Region I is located near the *pyrC* locus (23 min); region II lies between *aroD* and *uvrC* at 43 min; and region III falls between *uvrC* and *supD* at 43 min.[90] A mutation at any of these genes results in one of three distinct phenotypes: Fla$^-$, Mot$^-$, or Che$^-$. Mutations or deletions at most of these loci result in nonflagellated cells (Fla$^-$ phenotype; genes designated as *fla*, *flb*, or *hag*). In most cases, such mutants lack any detectable flagellar precursors. Mutants exhibiting a Mot$^-$ phenotype (genes designated *mot*) have morphologically normal flagella, but they are incapable of rotating them. Che$^-$ mutants (genes designated *che*) are motile but have abnormal bias in the direction of flagellar rotation and are incapable of chemotactic response to any of the receptor-mediated stimuli. A mutation in a structural gene for one of the transducers (*tsr*, *tar*, *tap*, *trg*) generally affects responsiveness only to those stimuli mediated by that specific transducer.

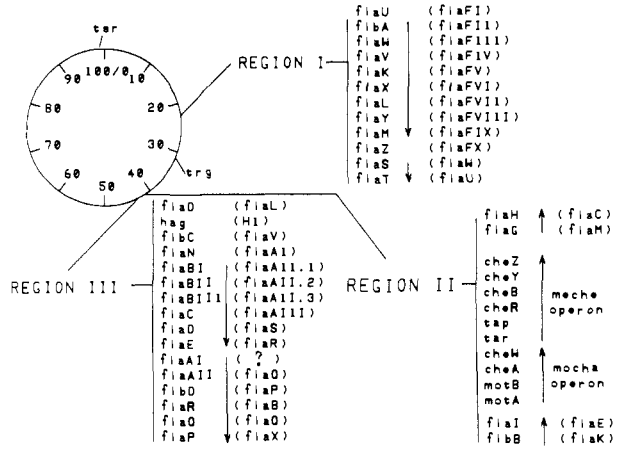


Figure 3. Location of flagellar and chemotaxis genes on the 100-min maps of *E. coli* and *S. typhimurium*. *E. coli* genes are shown first, followed by the corresponding genes for *S. typhimurium* in parentheses. The *mocha* and *meche* operon genes have the same names in *E. coli* and *S. typhimurium*. Operons are indicated by vertical arrows. This scheme is based on that presented by Parkinson[19] with additions to accommodate recent data.

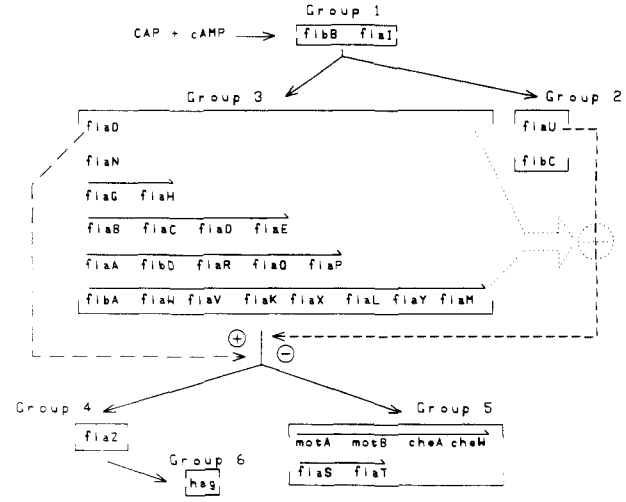


Figure 4. Regulatory cascade governing expression of flagellar and chemotaxis genes in *E. coli*. Based on the results of Komeda,[91–93] this scheme indicates that *flbB* and *flaI* direct expression of two groups of genes, which are required for expression of two additional groups of genes. The *flaD* and *flaU* gene products function as positive and negative effectors, respectively, as indicated in the figure and as discussed in the text. Operons are indicated by horizontal arrows above the corresponding genes.

1. Regulation of Expression

Transcription of the flagellar and chemosensory genes is regulated in a hierarchical manner as depicted in

Figure 4.[91-94] In this scheme, each gene occupies a position on one of at least four levels in the regulatory cascade. Transcription of the *flaI* and *flbB* genes appears to be directly controlled by catabolite repression.[94] Transcription of all other flagellar genes requires expression of *flaI* and *flbB*; therefore, catabolite repression indirectly regulates expression of all these genes. The cascade defined by Komeda[91-93] indicates that expression of *flaI* and *flbB* (group 1) is directly required for expression of genes in groups 2 and 3. Group 3 gene products are required for expression of group 4 and group 5 genes; and group 4 (*flaZ*) must be expressed to obtain *hag* (flagellin) expression. It seems unreasonable to propose that each of the group 3 genes serves as a positive effector of group 4 gene expression. Silverman[16] suggested that intermediate assemblages of these gene products may affect transcription either directly (acting at the flagellar gene promoters) or indirectly (by sequestering or altering key flagellar gene products that would otherwise be free to function as negative effectors). Komeda[93] recently demonstrated that the *flaD* and *flaU* gene products serve as direct positive and negative effectors, respectively, of the genes located beneath them in the regulatory cascade. The ability of these key elements to affect transcription is influenced by the other group 3 gene products. According to Komeda's scheme,[93] under normal circumstances the *flaU* gene product facilitates assembly of the group 3 proteins (acting either as a catalyst or as a component of the assembly product). As this assembly proceeds successfully, the *flaD* gene product turns on transcription of the operons in groups 4, 5 and 6, and flagellar assembly can proceed to completion. However, any defect in the group 3 genes that disables assembly will lead to accumulation of the *flaU* product, which then functions as a repressor of transcription of the operons comprising groups 4, 5 and 6. Mutations in genes of groups 4, 5 and 6 that disrupt assembly could also lead to accumulation of *flaU* product and thereby shut down expression of these genes.

2. Regulation of Flagellar Assembly

Formation of the flagellar organelle is also regulated by the nature of the assembly process itself. The components appear to be assembled in a specific order. Extensive searches for incomplete flagellar structures in *S. typhimurium*[95] and *E. coli*[96] mutants have yielded electron micrographs of probable intermediates along the normal assembly pathway. Such a pathway is shown in Figure 5. Such studies have also enabled researchers to make associations between groups of gene products and specific morphological features in the electron micrographs (see Figure 5). Unfortunately, definitive 1:1 correspondences cannot be made following this approach. Although the order of the observable intermediates along the proposed assembly pathway has not been established, it seems likely that the simpler a structure is, the earlier it lies along the pathway. Assembly of the HBB complex appears to proceed progressively from the interior of the cell toward the exterior. Each successive addition of a morphological feature requires correct assembly of all of the structures that normally precede it in the assembly pathway. Perhaps the most striking result obtained from such studies is that over half of the known *fla* genes are required to obtain the simplest (first) intermediate HBB structure.[95,96]

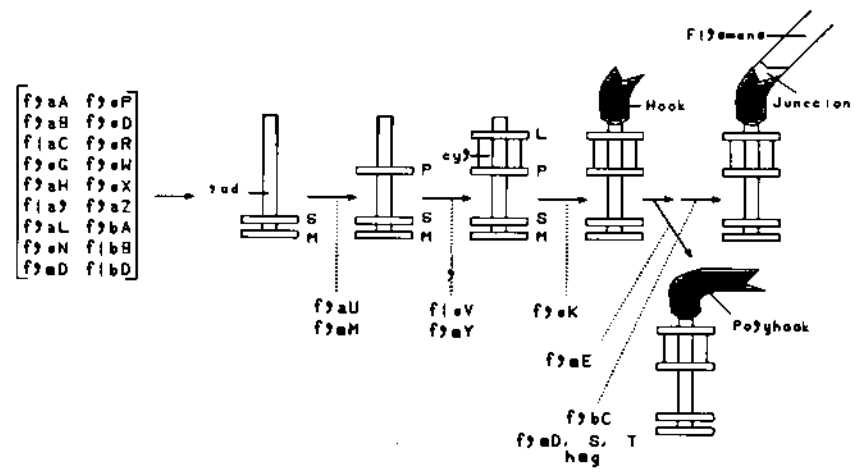


Figure 5. Proposed assembly pathway of the filament–basal body complex in *E. coli*. The pathway is based on that proposed by Suzuki and Komeda.[96] Shown below the arrows leading from one intermediate to another are the genes necessary for formation of the additional features in successive stages of morphogenesis. A similar series of intermediates has been identified in *S. typhimurium*.[95] Abbreviation: cyl, cylinder.

3. Structural Components

a. The Filament. The filament is composed of subunits of a single protein, flagellin.[76] In *E. coli* this 54-kDa protein is the product of the *hag* gene.[97-99] *S. typhimurium*, however, has two flagellin genes, H1 and H2, which encode similar but distinct proteins.[100-103] H1 is analogous to the *E. coli hag* gene, but H2 has no *E. coli* counterpart. A single *S. typhimurium* bacterium expresses only one of these flagellin genes at any time but can switch to express the other H gene (frequency $\sim 10^{-3}$–10^{-5}/cell per generation[13,16,104]). This switch alters the structure of the flagellar filament, changing the H serotype of the bacterium, and enabling the cell to evade the immunological response of the host. The molecular mechanism responsible for the switch has been uncovered by Zeig et al.[105] and Iino's group.[106]

b. Flagellar Hook. The flagellar hook is composed primarily of a single 42-kDa protein[85-87,107] encoded by the *flaK* gene.[86] The length of the hook (~90 nm) is regulated by the *flaE* gene product;[108] mutants in *flaE* produce abnormally long superhooks. Three additional gene products, the hook-associated proteins (HAPs), appear to function in defining proximal and distal ends of the flagellar filament.[84] HAP1 (59 kDa), HAP2 (48 or 53 kDa, depending on the strain), and HAP3 (31 kDa) are apparently encoded by the *flaW* (*flaS*), *flaV* (*flaC*), and *flaU* (*flaT*) genes, respectively, of *S. typhimurium* (*E. coli*). HAP1 and HAP3 are localized at the hook–filament junction. HAP2 is localized at the tip (distal end) of the flagellar filament and appears to function in trapping and assembling monomeric flagellin subunits transported from the cytosol through the hollow core of the growing filament.[84] In the absence of a functional HAP2 this flagellin leaks out into the external medium.[84]

c. Basal Body. The basal body complex is comprised of approximately nine proteins. In *S. typhimurium* (and by analogy in *E. coli*), most of these proteins can now be assigned to specific genes and to specific morphological features of the basal body.[83,84,89] These assignments are shown in Table I. Many of these assignments were made and/or confirmed by Aizawa et al.,[83] who developed procedures enabling fa-

TABLE I. Assignments of HBB Proteins and Morphological Features to Corresponding Genes in *S. typhimurium*[83,84,109]

gene	protein kDa	assoc morphol feature
flaAII.1	65	M ring and associated structures
flaFV	42	hook
flaFVI	32	rod
flaFVII	30	rod
flaFVIII	26	L ring
flaFIX	39	P ring
flaU	31	filament–hook junction
flaV	48, 53	tip of flagellar filament
flaW	59	filament–hook junction
H1	53	flagellar filament
H2	58	flagellar filament

cile purification of relatively large quantities of HBB complex, which could be further fractionated to a partial HBB structure by acid treatment. This group was aided in making protein:gene assignments by generating temperature-sensitive (Ts) mutations of the genes of interest. A reasonable number of these Ts proteins had sufficiently altered electrophoretic mobility that definitive identifications were possible.

Jones et al.[109] recently characterized the HBB–filament complexes assembled by *flaM* and *flaY* mutants of *E. coli*. Under the special conditions caused by hook protein overexpression, these mutants are capable of assembling HBB–filament complexes[110] that can be purified and studied by electron microscopy and biochemical techniques. The results of these studies indicate that *flaM* encodes a 39-kDa protein comprising the L ring.[109] It is interesting that the HBB–filament complexes lacking either the P ring or the L ring are capable of sufficient flagellar rotation (albeit feeble) to impart weak motility to the mutant cells.[109,110]

4. Energy-Transducing Components

Although MotA and MotB are not present in HBB preparations and are not required for flagellar assembly,[111] these proteins are thought to interact with the basal body and to play crucial roles in the functional flagellar motor. Mutations in *motA* or *motB* cause a paralyzed (Mot$^-$) phenotype, although these lesions do not prevent formation of normal flagellar organelles.[111-115] The paralyzed phenotype of *mot* mutants does not result from some impairment of the proton motive force[116] nor does it result from a physical obstruction of the rotor, as these rotors can be driven by an externally supplied rotary force.[117] MotA and MotB are integral membrane proteins,[113,118,119] and it is therefore reasonable to envision their interacting peripherally with the core structural components of the basal body.[26,116,120] Addition of MotA and MotB (e.g., by phage-directed protein synthesis) can cause rotation of flagellar complexes assembled in their absence.[113] With MotB, careful monitoring of the time course and extent of this rescue reveals that the rotation velocity of these cells increases in quantized steps; the final velocity is 16 times the value of an individual increment.[121] Thus, it appears that MotB forms part of a force-generating unit and that each flagellar motor has several such units which can function independently in an additive fashion.

The *motA* and *motB* genes have been isolated from the Clark and Carbon library,[122] sequenced, and placed in expression vectors in attempts to elucidate the roles of these proteins in the flagellar motor.[116,120] Analysis of the amino acid sequence (deduced from the DNA sequence) of MotA indicates a quite hydrophobic protein with four possible hydrophobic membrane-spanning helixes and two highly charged regions (one acidic, one basic) that could conceivably be electrostatically paired in the lipid bilayer,[116] forming a channel for proton translocation to or from the motor. Similar analysis of the deduced MotB amino acid sequence[120] suggests that MotB is an amphipathic protein with only one highly hydrophobic region (at the amino terminus) and one moderately hydrophobic region, which may require stabilizing interactions with other protein components to remain in the membrane. The cytoplasmic membrane appears to have a limited number of sites at which MotB can be accommodated. When MotB is grossly overexpressed (using a *trp* promoter), most of this protein is found in the cytoplasm, whereas normal amounts of MotB are always associated with the cell membrane.[120] Stader et al.[120] propose that other more hydrophobic components are required to achieve membrane insertion of the relatively nonhydrophobic MotB. These stabilizing proteins may include MotA and components of the basal body.

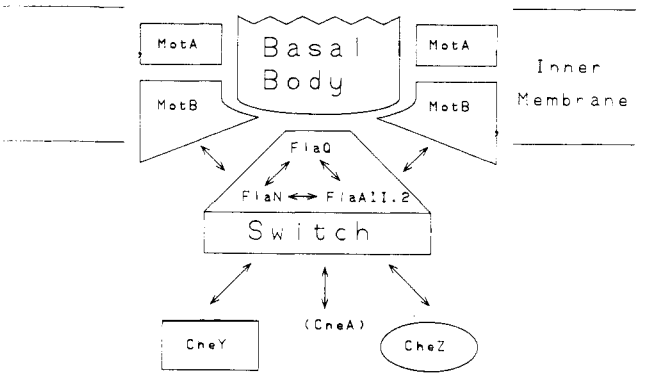


Figure 6. Schematic representation of the switch complex of the *S. typhimurium* flagellar motor and the interactions of the switch with additional chemotaxis components. Protein–protein interactions (denoted by double arrows) among the three components of the switch are suggested by the existence of intergenic suppressors among the corresponding genes as discussed by Yamaguchi et al.[134] As denoted by the double arrows, studies of pseudorevertants indicate that the switch components also interact with CheY, CheZ, MotB, and CheA (shown in parentheses because only one such second-site suppressor has been identified to date).

5. Flagellar Switch Components

The flagellar switch functions at the interface between the flagellar motor and the chemotaxis sensory transduction apparatus. Among its multiple functions are the following: (a) facilitating correct assembly of the basal body components, (b) determining the direction of motor rotation, (c) responding appropriately to intracellular signals that reflect the extracellular environment of the cell. The properties of numerous mutants suggest that at least three different proteins may form a switch complex that carries out these functions (see Figure 6). Point mutations in *flaAII.2*, *flaQ*, or *flaN* in *S. typhimurium* (*flaBII*, *flaAII*, or *motD*, respectively, in *E. coli*) may result in one of four distinct phenotypes,[26,123-130] reflecting the multifunctional nature of the corresponding proteins: (a) nonflagellate (Fla$^-$), which is also the null phenotype; (b)

paralyzed (Mot⁻); (c) generally nonchemotactic (Che⁻) with an abnormally high CW bias; or (d) Che⁻ with an abnormally high CCW bias. Studies performed with *S. typhimurium*[123] indicate that the Che⁻(CW), Che⁻(CCW), and Mot⁻ mutations cluster in distinct regions of these genes. Therefore, specialized regions of the corresponding proteins may participate in different aspects of the multiple functions described above. The multiple phenotypes associated with different mutations of one of the switch genes resulted in multiple names (*mot*, *che*, *fla*) for different alleles of these genes. This can be quite confusing, and we welcome the suggestion by Yamaguchi et al.[123] to use a single symbol for each gene followed by the phenotype in parentheses; e.g., use *flaQ*(Che) instead of *cheC*.

Further evidence that the *flaAII.2* (*flaBII*), *flaQ* (*flaAII*), and *flaN* (*motD*) gene products in *S. typhimurium* (*E. coli*) comprise part or all of the motor switch has been obtained in both E. *coli* and *S. typhimurium*. All three proteins are required for early stages of flagellar assembly.[95,131] However, these three proteins are not part of the basal body complex,[83,125] and at least one (FlaAII) is an integral membrane protein.[124,125] FlaAII (FlaQ) must associate very closely with the flagellar apparatus as evidenced by the maintenance of the extreme CW phenotype in cell envelopes (devoid of cytoplasmic contents) prepared from *flaAII*(Che) mutants.[132] Overproduction of wild-type FlaAII alters cell motility by reducing swimming speed and tumbling frequency.[125] A temperature-sensitive mutant of the *S. typhimurium* equivalent of FlaAII (FlaQ) exhibits a Che⁻ phenotype at permissive temperatures and a Mot⁻ phenotype within 0.5 s of shifting to restrictive temperature,[123] suggesting that this protein plays crucial roles in chemosensory responsiveness and in powering the motor. Interestingly, Ts mutations in several of the flagellar basal body components do not decrease the thermal stability of the HBB's after flagellar assembly has occurred at permissive temperature.[83] This presumably reflects the stabilizing interactions between the Ts protein and other components of the basal body. The absence of such stabilization with the Ts FlaAII.2 protein[123] is consistent with some sort of peripheral association between it and the basal body, perhaps at the cytoplasmic base where it could interact with cytosolic components of the sensory transduction pathway, such as CheY and CheZ (see following section). Such interactions are indicated by the isolation of suppressors of *cheY* and *cheZ* point mutations that map to *flaA* and *flaB* in *E. coli* (ref 130, studies performed before *flaA* and *flaB* loci were known to encode more than single genes). Finally, formation of a complex among FlaAII.2, FlaQ, and FlaN in *S. typhimurium* is suggested by the existence of mutual, allele-specific, intergenic suppressors of mutations in each of these switch components.[133] These interactions are depicted in Figure 6.

The unknown regulator to which the switch responds in generating alternating periods of smooth swimming and tumbling behavior in unstimulated cells does not appear to be regulated in any global manner throughout the cell. Asynchronous motion and switching of flagellar filaments have been observed in filamentous cells of *E. coli*[133] and in normal-sized cells of *S. typhimurium*.[134]

C. Mechanistic Models of the Flagellar Motor

Numerous models have been proposed to explain how the components of the flagellum–motor complex interact to achieve rotation of the filament and, more importantly, how proton motive force is utilized by the motor. Several models are briefly presented below. The interested reader can find extensive discussions of various aspects of these and other models in reviews by Macnab,[14,15] Macnab and Aizawa,[26] Berg,[136] and Khan and Berg.[137,138] At this point it is instructive to review the salient, experimentally determined features of the motor before speculating about possible mechanisms. One caveat the reader should keep in mind when considering these features is the following: for experimental reasons, several important features of the flagellar motor have been revealed by studies utilizing tethered cells. Under these conditions, the motor experiences considerably higher load than that experienced by a motor in free-swimming cells. Future experiments may indicate that some of these features may be slightly different or may not apply at all under the conditions experienced by free-swimming cells (see Note Added in Proof).

Unfortunately (for the motor researcher), *E. coli* and *S. typhimurium* have metabolic properties that obviate or complicate straightforward studies of their flagellar motors.[6] Therefore, much of the available information comes from studies of *Streptococcus*, which lacks an internal energy source and is amenable to experimental manipulations of transmembrane pH gradients and electrochemical potentials;[6] similar studies have been performed with *E. coli*, *S. typhimurium*, *Bacillus subtilis*,[9,11,139,140] and *Rhodospirillum rubrum*.[7] In all cases the flagellar motor is powered by proton motive force (pmf); either a pH gradient or an electrochemical gradient is effective.[6,9,11] Ravid and Eisenbach prepared cell envelopes that lack cytoplasmic contents but have functional flagella.[132] These flagella rotate in simple buffer solutions when a pH gradient is imposed across the envelope membrane, indicating that proton flux itself is sufficient to drive the motor. Investigations of the motor dynamics have indicated that the work output of the motor (or equivalently the angular velocity of the tethered cell) is proportional to the applied pmf, over the entire range of pmf tested in *Streptococcus*[6,137] and at all but very high values in *B. subtilis* and *E. coli*.[9,11] Such studies have also indicated that the torque generated by the motor is independent of the load (viscous drag) experienced by the flagellum under the high load conditions experienced with tethered cells.[6,40] These two observations are of interest because they are most readily explained by the existence of a fixed stoichiometry of proton flux per revolution of the motor (values from 300 to 1000 protons/revolution have been proposed).[2,121] Such a situation is referred to as "tight coupling". A "loose coupling" mechanism (i.e., stoichiometry depends on the load) has also been proposed.[141] Other experimentally determined features of the motor that must be incorporated into models are (1) the reversibility of the motor (this is not achieved by reversing the polarity of Δpmf) and (2) the absence of any temperature or solvent isotope (D_2O) effects on the quantitative or qualitative behavior of the motor[137] under the high load conditions experienced with tethered cells. The latter

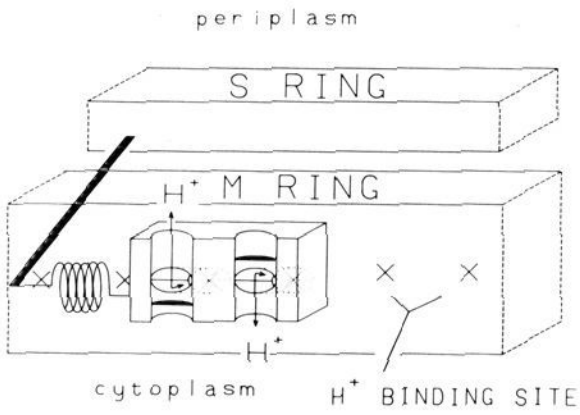


Figure 7. Schematic representation of the bacterial flagellar motor according to the model proposed by Khan and Berg.[137] The mechanism of coupling proton flux to torque generation is discussed in the text.

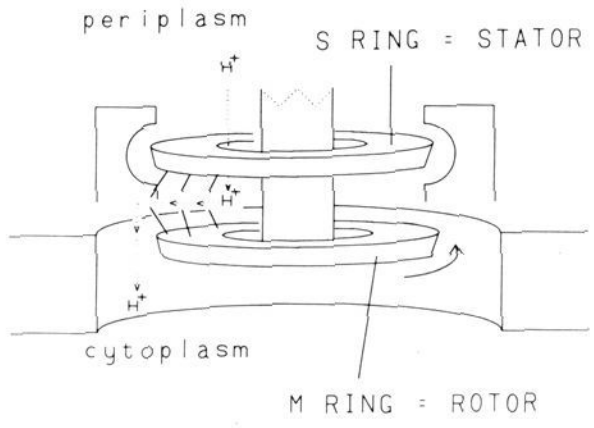


Figure 8. Schematic diagram of the bacterial flagellar motor proposed in Läuger's model.[142] The torque-generating mechanism is discussed in the text.

result suggests that proton transfer events (either entering or exiting the motor), formation of chemical bonds, and generation/relaxation of a "high-energy" chemical intermediate are not limiting steps in the coupling of pmf to rotation of the motor.

With these features of the flagellar motor in mind, we now consider several mechanisms by which this motor might operate. The structural roles proposed for the various observable components of the motor are quite similar in these models. The M ring seen in electron micrographs is assumed to rotate as a unit with the rod within the cell membrane; it serves as the rotor. The S ring is thought to be constrained by the cell wall, and it serves as the stator. Some sequence of events must enable proton flux to generate torque between the M ring and the S ring, and this point is where the various proposed mechanisms diverge.

The most explicitly defined mechanism has been proposed by Khan and Berg[137] and is depicted schematically in Figure 7. In this model, proton flux is mediated by two channels in a membrane-spanning "particle" that serves as the force-generating unit. One channel enables protons from the outside to associate with/dissociate from proton binding sites on the M ring; the other channel makes these proton binding sites accessible to the cytoplasm. The channel complex can move rapidly around the circumference of the M ring, but this movement is tightly coupled to proton translocation as detailed below. When the channel complex is displaced from its equilibrium position, a restoring force is generated in the elastic connection (S ring indicated by spring in diagram) between the channel complex and the cell wall. Movement of the channel around the rotor is constrained by the protonation state of adjacent proton binding sites: the complex can move only when a proton sits in one of the two binding sites exposed by the channels. A negative pH gradient (protons flowing into the cell) results in net movement of the complex to the right in the diagram, and this displacement exerts a force on the outside of the M ring. Note that a positive pH gradient (protons flow out of the cell) is predicted to push the complex to the left of its equilibrium position and would therefore turn the motor in the "backward" direction. Khan and Berg[137] have in fact reported that the *Streptococcus* motor turns CCW when the pH gradient drives protons into the cell and CW when the gradient drives protons out of the cell. Previous experiments had indicated CW rotation for proton flux in either direction.[88] These earlier experiments appear to reflect a chemotactic response by the cells to the decreased pH.[137]

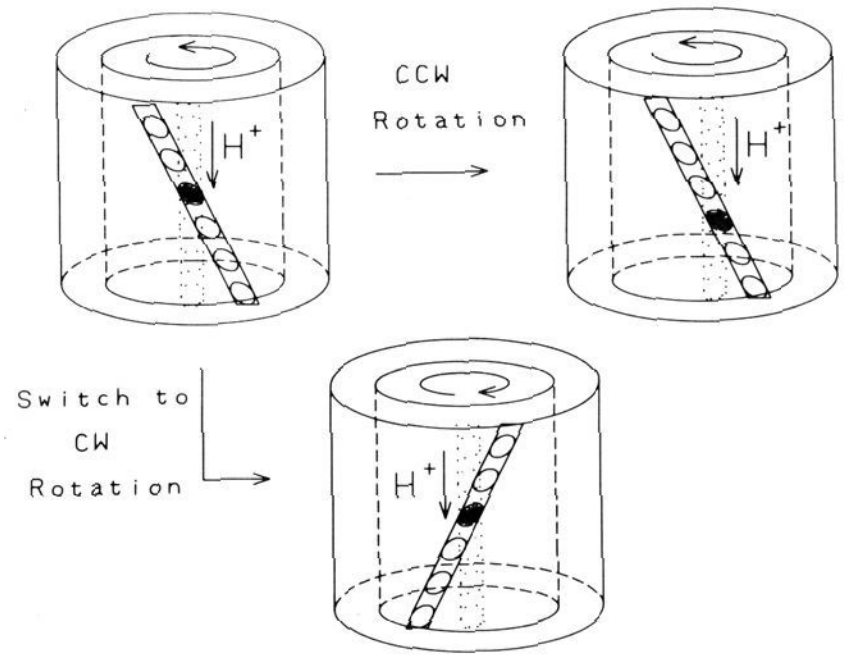


Figure 9. Mechanistic model of the bacterial flagellar motor proposed by Macnab.[15] The mechanism coupling proton flux to torque generation is discussed in the text.

Under high load conditions, Khan and Berg found that the *Streptococcus* motor is not subject to thermal or isotope effects.[137] These results appear to impose restrictions on the models proposed by Läuger[142] and Macnab,[15] which are depicted schematically in Figures 8 and 9. In Läuger's model, a cation (proton) binding site is formed at the intersection of two rows of ligand groups, one on the M ring and the other on the S ring. Multiple rows of such ligands lie on each ring, oriented at a fixed angle so that the proton binding site at each intersection is at a different radial distance on the rings. Migration of a proton from the outside to the inside of the membrane requires rotation of the M ring with respect to the S ring so that the proton is passed outward along the proton binding sites until it has access to the cytosol. Macnab's model[15] is similar to this proposal: protons are passed along arrays of binding sites formed at intersections of channels lining the surfaces of two coaxial cylinders (the M and S rings, for example). Because of the relative arrangement of these channels, a proton can move down the pmf gradient only when the inner cylinder (rotor) rotates with respect to the outer cylinder (stator). In this way proton flux generates torque. Note that a negative pH gradient would reverse the direction of rotation, in agreement with experimental observations.[137] In intact, metabolizing cells, reversal of the direction of rotation does not involve reversal of ΔpH but could result from a conformational change that changes the relative orientation

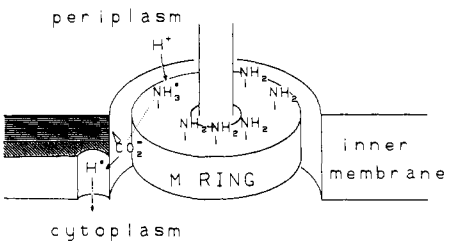


Figure 10. The bacterial flagellar motor according to the model of Glagolev and Skulachev.[7] The proposed operating mechanism is discussed in the text.

of a single set of channels or by different sets of channels that have different relative orientations and that operate in a mutually exclusive manner (see Figure 9). The absence of a solvent deuterium isotope effect[137] requires that the rate-limiting step in the mechanisms of Läuger and Macnab be some kind of elastic deformation that enables proton transfer (which cannot be rate limiting itself).

As shown in Figure 10, Glagolev and Skulachev[7] proposed that protons are passed down the electrochemical gradient by association with amino groups on the motor. Electrostatic attraction between the protonated (positively charged) amino group and a carboxylate moiety in the membrane results in rotation of the motor and enables the proton to dissociate into the cytoplasm. This mechanism has difficulty explaining the backward rotation caused by an outward flow of protons.[137]

III. Detection of Chemotactic Stimuli

A. Roles of the Receptors and Transducers

Four homologous proteins in *E. coli* (Tsr, Tar, Trg, and Tap) have been identified as chemotaxis transducer proteins.[19-22,53-56] The DNA sequences of the genes encoding the transducers[57-60] predict proteins composed of approximately 550 amino acid residues (MW ca. 60 kDa), in agreement with experimental observations.[118,143-146] Changes in receptor ligand occupancy result in some unknown alteration of transducer structure to which cytoplasmic chemotaxis signaling components respond. The transducers also play additional roles in bacterial chemotaxis. Tsr and Tar function directly as chemoreceptors for some amino acids,[56,61,147] and all four transducer proteins are directly involved in the chemotactic adaptation mechanism.[20-22,62-65]

1. Structural and Functional Domains of Transducers

In Tsr and Tar,[58,59,148] and probably in Trg[60] and Tap,[58] the multiple functions of the transducer proteins are accomplished by distinct regions of the proteins. These structural and functional domains have been defined by comparison of the amino acid sequences (deduced from DNA sequences) of the four proteins, by determining the functional effects of specific structural changes, and by determining the probable orientation of these proteins with respect to the cytoplasmic membrane[149] as depicted in Figure 11. Most of the amino-terminal third of each transducer is located on the periplasmic side of the inner membrane, and the carboxy-terminal half is on the cytoplasmic side. Each transducer protein has two markedly hydrophobic

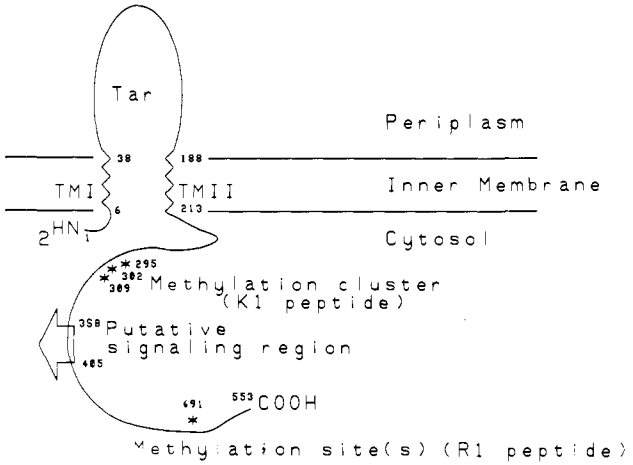


Figure 11. Proposed orientation of transducer proteins across the inner membrane.[58,59,148,149] The numbers refer to amino acid residues of Tar, although very similar orientations have been postulated for Tsr, Trg, and Tap. The transducer protein crosses the inner membrane twice with membrane-spanning regions TMI and TMII. Methylation sites (indicated by asterisks) are clustered in two regions: a lysine-containing tryptic peptide (K1) and an arginine-containing tryptic peptide (R1). Between these methylation regions is a highly conserved stretch of approximately 50 residues (see Figure 12), which may interact with signaling components of the chemotaxis machinery. The periplasmic amino-terminal terminal portion of the protein contains sites for interacting with chemotaxis ligands and various ligand-binding proteins.

stretches (TMI and TMII) of approximately 30 residues, which probably serve as membrane-spanning segments.[57,59,60,150] One or both of these transmembrane segments may be involved in propagating ligand-induced conformational changes from the periplasmic portion of the transducer to its cytoplasmic portion. There is no significant amino acid sequence homology among the TMII segments of the four transducer proteins; however, a strikingly high level of amino acid identity (62%) exists between TMI of Tsr and that of Tar (see Figure 12), suggesting some common function for this region in these two proteins[58,60,151] (see subsection on transducer signaling). TMI also contains the apparent signal sequence that directs insertion of these proteins into the inner membrane.[57,59] These amino-terminal residues do not appear to have been removed by signal peptidase in the mature, functional forms of these proteins.[59] The cleavage of such N-terminal sequences is the exception in prokaryotes. The amino-terminal portion of the transducer proteins appears to contain binding sites for chemoattractants and repellents;[21,60,152] this portion of each protein is appropriately located in the periplasmic space.[57-60] Tsr, Tar, Trg, and Tap respond to distinct sets of stimuli. Therefore, it is not surprising that the ligand-binding domains of these proteins share no regions of significant sequence homology.[58,60] The carboxy-terminal domain of each transducer protein is exposed to the cytoplasm, enabling interactions with signaling components as well as with the components responsible for adaptation. Adaptation involves methylation of specific glutamate residues of the transducer proteins[56,62-65] (see below). The four to six methylation sites of each transducer are clustered in two sequences separated by approximately 200 amino acids. The stretch of amino acids separating the two methylation regions includes a 50 amino acid stretch of extremely high amino acid identity (almost 100%

Figure 12. Comparison of the Tar, Tsr, Trg, and Tap amino acid sequences in regions that are thought to play important functional roles.[58,60,196] Boxes enclose loci where three of the four proteins have identical residues. Asterisks indicate methylation sites of Tsr, Tar, and Trg.

when comparing Tar, Tsr, Trg, and Tap; see Figure 12),[58,60] which may also be involved in adaptation or in the signaling interactions.

Studies of various mutant transducer proteins indicate that the structural domains are in fact the separable functional entities implied by Figure 11. For example, transducer mutants that have defective amino-terminal ligand-binding domains still undergo the methylation reactions associated with adaptation[21,53,60,152] (although not in response to stimuli, of course). Another example of the separability of functional domains was obtained by deleting 35 or 60 amino acids from the C-terminus of Tar[59,146] or Tsr.[153] Such truncated proteins bind ligands normally and direct signal generation in response to stimuli, but they are not readily methylated and are therefore defective in adaptation. Extensive mutagenesis of *tar* and characterization of many *tar* mutants indicate that mutations affecting signaling ability and/or adaptation participation (methylation) fall in the carboxy-terminal portion of the protein and that it is possible to completely incapacitate signaling ability while maintaining normal participation in the adaptation mechanism (differential methylation in response to stimuli).[154]

2. Specificities of Receptors and Transducers

A null mutation of a gene encoding a receptor or one of the four transducer proteins eliminates chemotactic response to a specific subset of stimuli but does not affect cell motility or taxis to the majority of stimuli.[20–22,56] Studies of such mutants[48,52,155–159] have defined the ligand specificity of receptor and transducer proteins in *E. coli* and *S. typhimurium*, as summarized in Figure 13.

For some stimuli, including most amino acids, a single protein (Tsr or Tar) serves as both the chemoreceptor and the transducer. Tsr (*t*axis to *s*erine and from *re*-

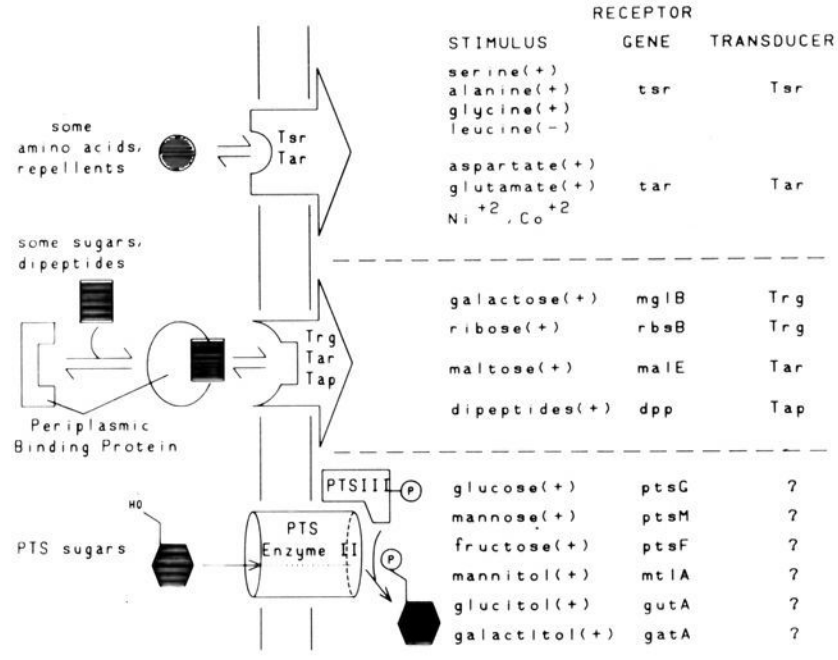


Figure 13. Ligand specificity of chemotactic receptors and transducers of *E. coli*. These assignments are based on genetic[48,52,155–159] and biochemical[56] studies. Each stimulus is denoted as either an attractant (+) or a repellent (–).

pellants) is the receptor–transducer protein for the attractants serine, alanine, glycine, and the nonmetabolizable amino acid analogue α-amino isobutyric acid.[156] Tsr also directly mediates response to repellent stimuli including leucine.[156] Tar (*t*axis to *a*sparatate and from *r*epellents) functions as the receptor–transducer for the attractants aspartate, α-methyl aspartate, and glutamate as well as for the repellents Ni^{2+} and Co^{2+}.[155,156] In vitro studies have yielded K_d's for binding of various chemoattractants and repellents to Tsr and Tar; these values are generally the same as the concentrations required for half-maximal response in in vivo behavioral experiments.[56] Studies using purified Tar indicate that each 60-kDa transducer protein is capable of binding a single molecule of attractant (aspartate).[61] Although some attractants appear to com-

pete for a common binding site on these transducer proteins,[56] it has not been determined whether all of the different attractants and repellents sharing a common transducer compete for a single binding site.

Changes of internal or external pH are also transducer-mediated stimuli:[156,160-163] decreased external pH evokes a repellent (tumbly) response in wild-type cells; decreases of internal pH (arising from weak, membrane-permeant acids such as benzoate, salicylate, and acetate) also elicit a repellent response. There are two pH-sensitive regions of Tsr that appear to mediate these responses.[148,163] The pH-induced change undergone by Tsr may be similar to that observed when other repellents are added (or attractants removed) in that it appropriately signals the excitatory and adaptation[160] components of the chemotaxis machinery. Tar also has an internal and an external pH-sensitive region that evoke chemotactic responses that are the opposite of those associated with Tsr: decreasing pH causes an attractant response by the Tar protein.[148] This inverted response is observable only in *tsr*⁻ cells.[163] For both Tsr and Tar, the intracellular pH detector(s) falls somewhere between residues 258 and 470,[148] a region that may play an important role in signaling (see below). As expected, the extracellular pH detector(s) appears to be found in the periplasmic amino-terminal regions of Tar and Tsr.[148]

Temperature change is another "nonspecific" stimulus to which the chemotactic transducers respond. Rapid temperature increases within the 25–34 °C range cause transient periods of smooth swimming, while temperature decreases cause transient episodes of tumbling.[164] The resulting thermotaxis appears to be predominantly mediated by changes in the signaling mode of Tsr, and adaptation to thermal stimuli involves the normal methylation mechanism.[165,166]

For many chemoattractants, including sugars[42-46,63,188] and dipeptides,[52,167] there are periplasmic receptor proteins that are distinct from the four transducer proteins. As depicted in Figure 13, upon binding an attractant each such receptor protein appears to undergo a conformational change (see below) that enables interaction of the attractant–receptor complex with one of the four transducer proteins. A periplasmic peptide binding protein functions as such a receptor protein for several dipeptides and a few tripeptides.[52] This protein is also a component of the Dpp dipeptide and tripeptide transport system in *S. typhimurium*[167] (and probably in *E. coli* as well).[52] The dipeptide-complexed binding protein interacts with the transducer protein Tap.[52] Similarly, components of various sugar transport systems function as chemoreceptors for specific sugar attractants. The periplasmic maltose binding protein (MBP) interacts with Tar when bound to maltose.[48] When bound to ribose and galactose, respectively, the ribose binding protein (RBP) and the galactose binding protein (GBP) interact with the transducer Trg (*t*axis to *r*ibose and galactose).[49-51] The molar ratio of these binding proteins to chemotactic transducer proteins is quite high (e.g., 40:1 for MBP:Tar),[168] but the affinity of the binding protein–ligand complex for transducer is relatively low, so that changes of ligand concentration in the range of the receptor K_d do result in alterations of the fraction of transducer protein bound by substrate-loaded binding proteins.[168]

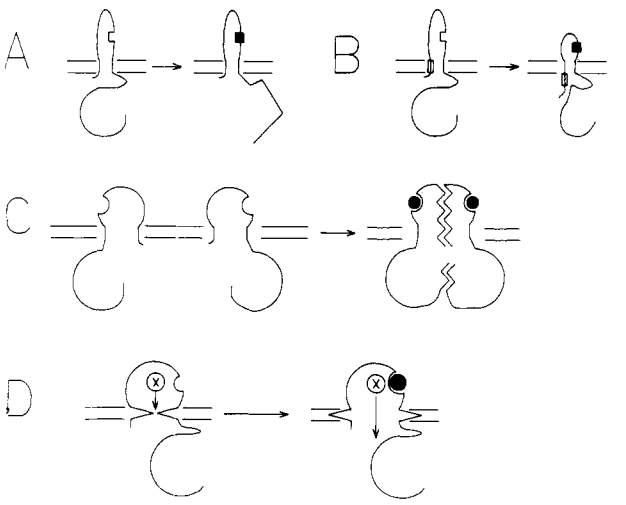


Figure 14. Possible mechanisms of transmembrane communication by chemotaxis transducer proteins. Mechanism A shows the general case of a ligand-induced conformation change that alters the interaction of the transducer protein with cytoplasmic components. In mechanism B, binding the ligand to the transducer "pushes" one of the transmembrane segments, thereby increasing its accessibility to cytosolic chemotaxis components and/or to the cytosolic regions of the transducer protein itself. Mechanism C depicts ligand-induced aggregation of transducers as a signaling mechanism. In possibility D, association of a ligand with the transducer opens a channel through which some "signal molecule" passes.

In *E. coli*, Tsr and Tar are relatively abundant at approximately 1600 and 900 copies/cell, respectively.[22,56] This represents about 1.5% of the total membrane protein. Trg and Tap are significantly less abundant at approximately 150 copies/*E. coli* cell.[22] The transducers from *E. coli* can be exchanged for their counterparts in *S. typhimurium* without diminishing response to any chemotactic stimuli with two known exceptions: (i) Tar from *S. typhimurium* does not support maltose taxis (even when MBP from either host is present);[168] (ii) *S. typhimurium* lacks a functional *tap* gene,[52] but does have an additional receptor or transducer-like gene *tip* (*t*axis *i*nvolved *p*rotein) that encodes a 63-kDa methylatable protein that restores chemotactic ability to mutant strains lacking *tsr*, *tar*, and *trg*.[169]

B. Transmembrane Signaling

The transducer proteins convey information about the ligand occupancy of their periplasmic binding sites to the components of the chemotaxis machinery responsible for generating the signal that determines the bias of flagellar rotation. As will be discussed in a later section, these signaling components are probably cytoplasmic proteins that interact with the carboxy-terminal domains of the transducer proteins. It is likely that the transducers undergo a ligand-induced conformational change[58,147,170] that alters these interactions and thereby enables transmembrane signaling. This general mechanism is depicted in Figure 14. Also presented in Figure 14 are examples of how such a conformational change could alter interactions with other components. For example, ligand binding could alter the accessibility of membrane-associated regions of the transducer as shown in Figure 14B. In such a scheme, adaptation could result from methylation of such an accessible region, thereby enabling its association with the mem-

brane again and eliminating signaling interactions. Another possible mechanism of transmembrane signaling (shown in Figure 14C) involves ligand-induced conformational changes that alter the aggregation state of the transducer proteins.[58] The plausibility of such an aggregation mechanism is supported by studies of receptor systems in eukaryotic cells. For example, signaling mechanisms by the IgE,[171] insulin,[172,173] and EGF[174] receptors appear to involve receptor aggregation. In light of these examples, it is interesting to note that homotetramers of Tsr and Tar appear to exist in vivo in bacterial cells (detected by chemical cross-linking studies)[175] and that purified, detergent-solubilized Tar forms tetramers.[61] However, there appears to be no observable change in the aggregation state of purified Tar when asparatate binds to it.[61] It is possible that the in vitro conditions of this experiment do not allow such a change; for example, an additional component may be required. Although these results are tantalizing, it remains to be seen whether the aggregation of transducer molecules has any functional role in signaling.

Very little is known about the mechanism and extent of the putative ligand-induced conformation changes of the transducer proteins. Physical and spectroscopic investigations of these proteins have been hindered by problems inherent to isolation of membrane proteins and by the susceptibility of these proteins to proteolysis by endogenous proteases.[176] Recent reports of a successful purification protocol yielding milligram quantities of Tar[61] opens the door to detailed investigations of ligand–protein interaction and the resulting structural changes. Conditions that enable in vitro studies of the interaction of purified transducer with other chemotaxis components have also been determined.[177] In contrast to the paucity of information about ligand–transducer interactions, the interactions between the periplasmic sugar-binding chemoreceptors (MBP, GBP, and RBP) and their respective ligands have already been studied extensively. Spectroscopic techniques have been used to demonstrate that, upon binding their respective ligands, MBP,[178,179] GBP,[180,182] and RBP[183] undergo extensive conformational changes that affect protein structure at sites far removed (30–40 Å) from the ligand binding sites. Thus, it may be reasonable to propose that binding of a ligand to the amino-terminal domain of a transducer protein could affect the conformation of the carboxy-terminal domain of the protein located on the other side of the inner membrane some 50–90 Å away.

Studies of mutated transducer proteins have provided some insight into what regions of these proteins may be involved in transmembrane signaling. For example, Parkinson and co-workers[184,185] isolated dominant *tsr* mutations that impart an extremely smooth swimming bias that results in a generally nonchemotactic (*cheD*) phenotype. These mutations apparently lock Tsr into a conformation that constantly signals for smooth swimming.[184–186] Most of these mutations map in the region between amino acid residues 360 and 407,[187] a sequence that is virtually identical in the four transducers.[58,60] One such *trg* mutant (*trg*-21) has been studied in detail; it results from a single amino acid change within the highly conserved region mentioned above.[188] Because of the low relative abundance and/or signaling strength of Trg compared with Tsr, *trg cheD*-like mutations are dominant in an otherwise wild-type background only when present in multiple copies (such as on a high copy number plasmid), whereas *tsr cheD* mutations are dominant in single copy.[184,185] Different mutations in the highly conserved segment of Tar are capable of locking this transducer into one of two signaling modes: one that results in aberrantly smooth swimming behavior or one that causes excessively tumbly behavior.[154] Such studies of mutant transducer proteins suggest that the wild-type transducers have two distinct, interconvertible signaling modes (smooth and tumbly) and that the highly conserved region of these proteins may play some role in signaling.

Another region that probably plays a functional role in signaling is the first membrane-spanning segment (TMI) that includes amino acids 7–37. A significant percentage (~60%) of this sequence is identical in Tar and Tsr[58] (see Figure 12), suggesting some specific function other than anchoring the protein to the membrane.[151] Site-specific mutagenesis of the TMI region of Tar has been used to delete a segment of this region and to place a polar residue (Ala-19 → Lys-19) within the full-length membrane-spanning region.[151] Such mutations affect neither the ability of Tar to associate with the inner membrane nor its ability to bind aspartate. However, these mutant proteins are not modified by the chemotaxis-specific methyltransferase (see below) and result in loss of aspartate chemotaxis in swarm assays. These results suggest that an intact TMI region is required for a functional cytoplasmic region, and they raise the possibility that TMI is involved in transmembrane signaling.

Two major classes of spontaneously arising intragenic suppressors of the Ala-19 → Lys mutation of Tar appear to provide two different ways of compensating for the polar lysine side chain introduced into the hydrophobic membrane-spanning segment. The first class of intragenic suppressors includes mutations that lie within TMII (the putative second membrane-spanning region). The amino acid changes identified in the second group of intragenic suppressors lie between residues 264 and 303 (in the putative cytosolic domain of Tar). The locations of these suppressors in the amino acid sequence of Tar may provide some insight into the orientation of the transducer across the inner membrane. Presumably, Lys-19 Tar can associate with the membrane by distorting its own conformation and/or the orientation of the membrane components so as to compensate for the polar ϵ-amino group of lysine. For example, such changes may enable association of this moiety with polar head groups of membrane phospholipids.[151] Such distortions of the transducer protein and/or the surrounding membrane may be responsible for altering the structure of the carboxy-terminal region of the protein and for adversely affecting its ability to interact with chemotaxis components in the cytosol. The existence of suppressor mutations that lie within TMII raises the possibility that TMI and TMII may be closely associated with one another within the inner membrane.[151] One conceivable mechanism of compensating for the deleterious effects of the Ala-19 → Lys mutation would be to provide a second mutation that provides a compensating charge within the membrane

(e.g., for ion pairing), thereby eliminating the need for a distorted Tar structure and restoring the functional structure of the carboxy-terminal domain. Several of these suppressor mutations do generate potentially negatively charged residues,[151] although there is no direct evidence for such ion pairing. The amino acid changes identified in the second group of suppressors lie within a region that is thought to be part of the cytosolic domain of Tar.[58] Such amino acid changes could alleviate the disrupted conformation of Lys-19 Tar by directly interacting with TMI or (more likely) by interacting with the membrane region that surrounds TMI.[151] The structural implications of these results have been incorporated into Figure 11.

C. Methylation of Transducers

The transducer proteins of *E. coli* and *S. typhimurium* are subject to posttranslational modifications that play a crucial role in the adaptation mechanism. Specific glutamate residues of the transducers are converted to γ-glutamyl methyl esters[189,190] by a chemotaxis-specific methyltransferase (the product of the *cheR* gene).[65] For this reason, the transducers have sometimes been referred to as MCPs (*m*ethyl-accepting *c*hemotaxis *p*roteins). The actual number of identified methylation sites is five for Tsr,[191-193] four for Tar,[194,195] five for Trg,[196] and somewhere within this range for Tap. These methylation sites are clustered in two distinct regions of each transducer protein, separated by approximately 200 amino acid residues. As shown in Figure 12, the sequences surrounding the methylation sites are highly homologous in the four transducers,[58,60,196] and the sites of methylation occur at analogous positions within these regions.[193-196] In Tsr, Tar, and Trg, two of the methyl accepting sites in the first cluster are originally glutamine residues that have been irreversibly deamidated by the product of the *cheB* gene.[192,193,196] The CheB protein is also the chemotaxis-specific methylesterase that catalyzes deesterification of the methylated sites (i.e., hydrolysis of the γ-glutamyl methyl ester bond, forming methanol and regenerating the glutamate γ-carboxyl group).[70] Both the methyltransferase and methylesterase are active in unstimulated cells, resulting in continuous turnover of the methylester groups and generating MCPs with varying levels of methylation. The mobility of the MCPs in SDS polyacrylamide gels is altered by the methylation and deamidation modifications: methylation increases electrophoretic mobility; demethylation and deamidation each decrease electrophoretic mobility.[54,143,196-199] Under appropriate conditions, discrete bands representing the varying levels of methylation are observable, as shown in Figure 15. Such gels are useful for monitoring changes of transducer methylation in response to transducer-mediated chemotactic stimuli. Attractants cause increased levels of transducer methylation (more protein present in the higher mobility bands); repellents have the opposite effect. These changes in methylation level are specific for the transducer class with which a particular stimulus interacts.[54,143,197-199] For example, addition of aspartate or maltose to wild-type cells results in increased methylation of Tar but has no noticeable effect on Tsr methylation. Transducer methylation plays an important role in the mechanism of behavioral adaptation by regulating the ability of ligand-bound transducers to generate the signal(s) that affects the direction of flagellar rotation. Evidence supporting the connection between methylation and adaptation is presented in section V, and the role of methylation in adaptation is discussed in section VI.

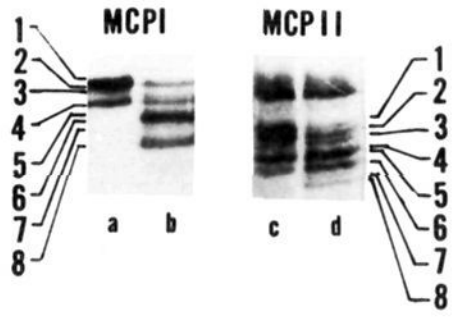


Figure 15. Comparison of the forms of ^{35}S-labeled MCPI (Tsr) and MCPII (Tar) following stimulation with attractants or repellents. Experimental details have been described by Rollins and Dahlquist.[199] Key: lane a, 17 mM leucine plus 17 mM sodium acetate; lane b, 2 mM serine; lane c, 0.5 mM $NiCl_2$; lane d, 28 mM maltose. The protein bands are numbered 1–8, with band 1 having the slowest mobility.

IV. Possible Mechanisms of Communication between Transducers and Flagella

To this point our discussion has focused on the components occupying the two extremes of information flow in bacterial chemotaxis: the membrane-spanning transducers and the membrane-bound complex of flagellar motor and switch. A transducer detects extracellular changes in specific ligands and responds with some appropriate change on its cytosolic side, possibly a conformational change in the cytoplasmic domain.[58,147,170] Such modifications of the MCPs ultimately affect the bias (CW or CCW) of the flagellar motor. The lack of information about the steps that enable communication between the transducers and the flagellar switch is a major obstacle to understanding chemotaxis at a molecular level. In the absence of such information, it has been useful to think of transducers (in response to chemoeffectors) generating or modulating some "signal" that interacts with the flagellar switch and thereby influences the direction of flagellar rotation. There are numerous mechanisms by which this signal might be generated and sent to the switch. For example, modification of some protein or formation of some small molecule could take place at the transducer, and this protein/small molecule could then diffuse through the cytosol to the switch. Alternatively, changes in membrane potential or the intracellular concentration of a specific ion could enable communication between transducers and the flagellar switch. Yet another possibility is that transducer proteins interact directly with the flagellar switch. The limited progress that has been made toward defining the mechanism of communication is covered in this section. CheY and CheZ, their general properties and possible roles in chemotactic signaling, are then discussed.

A. Communication by Electrical Signaling

Some eukaryotic cells utilize changes in membrane potential to transmit receptor-mediated signals rapidly from one part of the cell to another.[200,201] Electrical signaling of this sort appears to be involved in directed motion in several large, nonperitrichous bacteria and

other single-cell organisms. For example, chemotaxis by the ciliated *Paramecium* involves changes in membrane potential[202-204] as does phototaxis in *R. rubrum*[205] and chemotaxis in *Spirochaeta aurantia*.[206]

So it is reasonable to explore the possibility that such a mechanism is utilized by the smaller peritrichous bacteria such as *E. coli*, *S. typhimurium* and *B. subtilis*, although the response latency (200 ms[39]) in *E. coli* is long enough and the cell size small enough (length ~2 μm) to accommodate alternative signaling mechanisms (e.g., chemical transmission).[41,207] Early evidence indicated a correlation between chemotactic behavioral changes and changes in membrane potential. In *E. coli*, *S. typhimurium*,[207-209] and *B. subtilis*[210,211] various chemicals that change the membrane potential also alter the swimming behavior. Addition of chemoeffectors results in altered membrane potential in *E. coli*[207] but not in *B. subtilis*.[210] In a critical test of the role of membrane potential, Margolin and Eisenbach[212] found that clamping it (by means of valinomycin at varying external $[K^+]$) had no effect on the chemotactic behavior of *E. coli* and *B. subtilis*. Similar results were obtained with *Streptococcus*.[6] Thus, modulation of membrane potential does not appear to be necessary for normal chemotactic signaling in these relatively small (short) bacteria.

As Eisenbach pointed out in his insightful review of chemotactic signaling mechanisms,[213] electrical signaling could involve changes in membrane surface charge or intracellular ionic currents instead of membrane potential modulation. The former possibility can be tested by observing the effects of tetraphenylphosphonium ion (TPP^+) on the chemotactic behavior of EDTA-permeabilized cells.[213] By binding to both the inside and outside of the cytoplasmic membrane,[214] the lypophilic TPP^+ changes the net surface charge on both sides of the membrane. However, such treatment does not affect the chemotactic abilities of *E. coli*.[213] Participation of ionic currents within cells can also be ruled out as a likely signaling mechanism because systematically varying the ions present in the suspension medium has no effect on the chemotactic ability of *E. coli*.[213]

B. Signaling by Regulation of Specific Ion Concentrations

Intracellular concentration of a specific ion could change as a result of influx or efflux of the ion across the cytoplasmic membrane or as a result of a change in the ability of some protein to sequester the ion in bound form. In view of the established role of Ca^{2+} in sensory transduction in eukaryotic cells, an interesting possibility is that regulation of intracellular concentration of Ca^{2+} (or some other ion) functions as the chemotactic signaling mechanism. In fact, there is some evidence that Ca^{2+} could play a regulatory role in *B. subtilis*: (a) changing the intracellular Ca^{2+} concentration (using an ionophore and Ca^{2+} buffer) can change the rotation bias of the flagellar motor;[215] (b) Ca^{2+} inhibits MCP methylation, apparently by inhibiting the *B. subtilis* methyltransferase at very low concentrations.[216] In *Halobacterium halobrium*, Ca^{2+} also inhibits MCP methylation, although in this case it appears to result from activation of demethylation.[217] Ordal and Fields[218] postulated that communication between transducers and the flagellar motor is mediated by Ca^{2+} in *B. subtilis*, although a more recent proposal by Ordal[23] abandons Ca^{2+} in favor of a protein serving as the chemotactic signal.

It is unclear whether Ca^{2+} has any role in chemotaxis in *E. coli* and *S. typhimurium*. Depletion of intracellular Ca^{2+} from 33 μM (normal level) to 1.9 μM does not affect the chemotactic responsiveness of *S. typhimurium*.[207] On the other hand, Eisenbach[213] reported that flooding *E. coli* with large extracellular Ca^{2+} concentrations (to prevent normal modulation of intracellular Ca^{2+}) affects the bias of the flagellar rotation and diminishes the ability of the cells to respond to chemotactic stimuli. However, qualitative aspects of these changes and the inability of Ca^{2+} to alter flagellar rotation in cell envelopes[213] suggest that regulation of intracellular Ca^{2+} is not the signal that acts at the flagellar switch. It remains to be seen whether Ca^{2+} affects the activity of any of the chemotaxis-related enzymes or the efficacy of any of the chemotaxis-related proteins.

Unlike Ca^{2+}, which normally has a low intracellular concentration (~30 μM[207]), K^+, Na^+, NH_4^+, and Mg^{2+} have normal intracellular concentrations above 1 mM. Changes in the intracellular concentrations of these ions also do not appear to be a part of the chemotactic signaling mechanism.[212,214]

C. Signaling by Intracellular pH

Utilization of changes of intracellular pH as a mechanism of communication between transducers and the flagellar switch is worth considering because intracellular pH certainly influences bacterial chemotaxis. For example, pH changes can cause transducer-mediated repellent or attractant responses,[156,160-163] as discussed in the preceding section. The importance of proton flux in driving the flagellar motor has been discussed in section II of this review. However, under conditions that buffer intracellular pH sufficiently to prevent pH change, excitatory response to serine stimulus is not affected in *E. coli*.[213] Thus, pH change does not appear to be involved in the events enabling communication between transducers and the flagellar switch except in the special cases of pH taxis and weak acid/base taxis.[160]

D. Signaling by Direct Physical Interaction between Transducers and the Flagellar Switch

It is conceivable that transducer proteins interact directly with the flagellar switch, perhaps by diffusing laterally through the cytoplasmic membrane or by being located directly adjacent to the flagellar complex. However, several observations suggest that such interactions do not occur. First, flagellar rotation in cell envelopes (which lack cytosolic components but which maintain transducer proteins and functional flagella) does not respond to attractants or repellents, although this system apparently has all of the components necessary for the putative direct interactions between the transducers and the flagellar motor.[213] Second, given the range over which the chemotaxis signal is known to operate[41] and the rate at which a transducer–chemoeffector complex could be expected to dissociate, lateral diffusion of such a complex appears to be an unlikely candidate for the chemotactic signaling mechanism.[41] Third, membrane regions surrounding the flagellar

motors are not enriched with transducer proteins,[219] suggesting the absence of physical associations between the two.

E. Signaling by a Chemical Transduction Mechanism

We operationally define a chemical transduction mechanism as one in which a change in the ligand occupancy of a transducer protein results in modification of an existing substance (e.g., a protein) or formation/destruction of some substance (e.g., a nucleotide derivative) that, in turn, interacts directly with the flagellar switch or affects the ability of some other substance to interact with the switch. The embarassing generality and vagueness of this definition serve to underscore the current lack of understanding of the role played by such a mechanism in enabling communication between the chemosensory transducer proteins and the flagellar switch. An imaginative study by Segall et al.[41] provided a glimpse at some of the properties of the elusive "substance", which a priori could be anything ranging from a small molecule to a large protein. By iontophoretically applying attractant to a small, localized region of a large, filamentous cell having multiple marked flagella, Segall et al.[41] were able to monitor the response of flagella separated from the point of stimulation by varying distance and geometry. Their results suggest that *E. coli* has a relatively short-range signaling system in which the signal substance is generated at the transducer (at some rate determined by the ligand occupancy of the receptor); the signal diffuses through the cytoplasm to the flagellar switch. The range over which this diffusing signal acts is limited to ~ 2 μm because it is inactivated as it diffuses through the cytosol. CheZ appears to facilitate this inactivation, as the signal range is considerably longer (6 μm) in a *cheZ* mutant.[41] In principle, such measurements should enable one to calculate the size of the signal molecule; however, due to uncertainties in the experimental measurements and in assumptions necessary to make this calculation, a narrow range of reasonable sizes for the signal cannot be defined. The best estimate yielded by this approach suggests a size of 10–80 kDa for the signal molecule, although the possibility of a considerably smaller molecule (e.g., one the size of a nucleotide) cannot be ruled out because of this calculation.[41]

The properties of the signal defined by these experiments are useful in ruling out several of the possible signaling mechanisms discussed above. Changes in membrane potential or other mechanisms of electrical signaling, lateral diffusion of receptor–ligand complexes, and simple release/binding of a small molecule (e.g., Ca^{2+}) now appear to be unlikely mechanisms for chemotactic signal transduction in *E. coli* and other relatively small (short) bacteria.

1. Proteins Involved in Chemical Transduction Mechanism

There are several chemotaxis-related proteins that could in principle participate in signal generation and inactivation or that could serve as the signal itself. CheR, CheB, CheY, CheZ, CheA, and CheW are all cytosolic proteins with molecular weights that fall within the 10–80-kDa range proposed for the signal by Segall et al.[41] CheR and CheB, however, are unlikely candidates because *cheR*, *cheB*, and *cheRB* deletion mutants still exhibit a normal excitatory response to attractants[19,220] with kinetics approximately the same as those seen in wild-type cells.[40] *E. coli* with null mutations at *cheA*, *cheW*, or *cheY* have extreme CCW (smooth) bias;[12,19,129,222] this swimming phenotype would be expected if the gene product necessary for generating signal (a "tumble generator" by this reasoning) were missing. There is some evidence that CheA and CheW participate in signal generation or modulation (see Note Added in Proof). However, their properties will be discussed in the following section on MCP methylation/demethylation because they may be involved in regulation of the demethylation reaction. There is evidence linking CheY and CheZ to signal generation and/or modulation. The properties of these two proteins and their possible roles in the signaling process are therefore discussed in this subsection.

a. CheY. Point mutations and deletions in *cheY* cause extreme CCW (smooth) bias in the swimming behavior of *E. coli*[19,129,222,223] and *S. typhimurium*.[128] *CheY* mutants can respond to chemotactic stimuli, but only at concentrations well above those eliciting a response from wild-type cells.[19] CheY is a small soluble protein found exclusively in the soluble cytoplasmic fraction of *E. coli*.[118] Sizes ranging from 8 to 14 kDa have been reported for CheY, based on SDS PAGE of labeled protein obtained by phage-directed or plasmid-directed synthesis.[115,224,225] Matsumura et al.[226,228] and Mutoh and Simon[227] reported identical DNA sequences for the *E. coli cheY* gene. The open reading frame corresponding to *cheY* encodes a 129 amino acid protein with a predicted molecular weight of 14.1 kDa.[226,227] The *cheY* gene lies at the end of the *meche* operon, which includes the following genes (transcribed in the indicated order): *tar*, *tap*, *cheR*, *cheB*, *cheY*, *cheZ*.[55,129,222,228] Translational control apparently regulates expression of the proteins encoded by this operon such that the following relative stoichiometries are observed: 4(Tar):1(CheR):1(CheB):18(CheY):3(CheZ).[225] The significance of the elevated relative expression of *CheY* is not clear. The *CheY* gene product is not required for flagellar assembly, but it does appear to interact with the flagellar switch and to partipate in regulation of CCW → CW switching,[19,130,134,213,229,231] as discussed in the following paragraphs.

CheY has been purified to homogeneity in relatively large amounts by Matsumura et al.,[226] making possible experiments that suggest that CheY interacts directly with the flagellar switch to cause CW rotation of the motor.[213,229] Cell envelopes, prepared as described by Eisenbach and Adler,[230] have flagella that rotate exclusively CCW. Addition of purified CheY (from *E. coli*) to such cell envelopes from *S. typhimurium* switches this rotation to CW in a concentration-dependent manner: the more CheY added, the greater the percentage of envelopes with CW-rotating flagella.[229] Two important points to note about these experiments are that (a) the concentrations of CheY used to obtain switching are reasonable approximations of the normal CheY concentration in intact bacteria and (b) the cell envelopes are devoid of cytoplasmic contents. It therefore appears that the CheY–flagellar switch interaction is direct (i.e., not mediated by cytoplasmic

components) and probably occurs in intact cells.

The proposal that CheY is a "tumble factor" is supported by studies examining the behavioral effects of *cheY* overexpression in intact cells. Clegg and Koshland[231] showed that production of greater than normal levels of CheY in *E. coli* imparts an extreme CW bias to flagellar rotation. In addition, this effect can be obtained by overexpressing *cheY* in host cells that lack transducer proteins as well as in host cells lacking most of the other chemotaxis-related proteins (a *cheA* → *Z* deletion strain). These results agree with those of Ravid and Eisenbach[229] in suggesting that CheY interacts directly with the flagellar switch and does not require additional chemosensory components to mediate this interaction.[231]

Parkinson and co-workers[19,130] presented genetic evidence that in *E. coli* CheY interacts directly with component(s) of the flagellar switch: 95% of second-site suppressors of *cheY* point mutations map to the *flaA* or *flaB* locus. Moreover, these compensatory mutations (*scy*'s) are allele-specific, suggesting that CheY interacts directly with the *flaA* and *flaB* gene products. It should be noted that these studies were performed before the demonstrations that the *E. coli flaA* locus contains two genes, *flaAI* and *flaAII*[124] and that the *E. coli flaB* locus encompasses three genes: *flaBI*, *flaBII*, and *flaBIII*.[232] It remains to be seen whether *scy* mutations map in *flaBII* and *flaAII*, which probably encode switch components.[26,134] Work performed by Yamaguchi et al.[134] provided strong evidence for interactions between CheY and the three components (FlaAII.2, FlaQ, and FlaN) of the flagellar switch in *S. typhimurium*. The *cheY* mutations isolated by these workers suppress the effects of mutations in the switch component genes in an allele-specific manner.

The ability of CheY to directly function as a tumble factor raises the possibility that CheY itself is the chemotactic signal that enables communication between the transducers and the flagellar switch. In such a model, CheY bound to a transducer would be released into the cytoplasm or "activated" at some rate determined in part by the ligand occupancy of the transducer. Once released (or activated), CheY could diffuse to the flagellar switch and shift the bias toward CW. However, Clegg and Koshland[231] reported that transducer-mediated stimuli cannot influence flagellar rotation in cells that have Tsr, CheY, and functional flagella but lack the putative sensory transduction components: CheA, CheW, CheZ, CheR, and CheB. Any or all of these additional components may be required to enable CheY to interact with the switch in a transducer-directed manner. Another possibility is that while CheY is not itself the signal, its interaction with the flagellar switch is influenced by the signal. Alternatively, CheY may affect the ability of the signal to interact with the switch.

Other properties of CheY, including its interactions with CheR[19,233] and AdoMet,[226] are discussed in the following section on transducer methylation reactions.

b. CheZ. CheZ is also a soluble, cytoplasmic protein.[118] Its size, based on SDS PAGE of labeled protein obtained by phage-directed or plasmid-directed synthesis, is approximately 24 kDa in *E. coli*[115,224] and approximately 28 kDa in *S. typhimurium*.[225] No significance is attributed to this slight difference. The DNA sequence of *E. coli cheZ* reported by Mutoh and Simon[227] predicts a 24-kDa polypeptide composed of 213 amino acids. In *S. typhimurium*, allele-specific suppressors of mutations in *flaAII*, *flaQ*, and *flaN* (the genes encoding the flagellar switch components) have been mapped to *cheZ* by Yamaguchi et al.[134] These results corroborate and extend those of Parkinson et al.[130] who isolated allele-specific second-site suppressors of *cheZ* point mutants and mapped them to the *flaA* or *flaB* locus of *E. coli*. These compensating second-site mutations (*scz*'s) probably indicate direct interactions between CheZ and components of the flagellar switch. Evidence indicating interaction between CheZ and CheB[19,233] is discussed in the following section on transducer methylation reactions.

Point mutations and deletions in *cheZ* are generally associated with a tumbly swimming phenotype that reflects an extreme CW bias of flagellar rotation,[19,128,129,222,234] although a *cheZ* mutation that imparts an extreme smooth bias has recently been reported.[134] *CheZ* mutants have an abnormally high threshold for response to chemotactic stimuli.[19,40,220] Detailed kinetic studies[40,220] of the response of wild-type chemotactic *E. coli* and *cheZ* point mutants to pulses of chemoattractant indicate that *cheZ* mutants are defective in some aspect of excitatory signaling. Specifically, the response latency of *cheZ* mutants (~2 s) is approximately 10 times longer than the typical response latency of wild-type cells (~0.2 s).[40,220]

Earlier in this section, evidence was presented indicating that CheZ facilitates inactivation of the diffusable signal generated at the transducers.[41] A reasonable model has been proposed to integrate this finding with the increased response latency observed with *cheZ* mutants.[41] An additional piece of evidence necessary for building this model is the following: there is a pronounced CCW bias of flagellar motors in intact cells that lack transducer proteins[235] and in cell envelopes.[230] Apparently, this is observed because unstimulated transducers are involved in generating a signal that enables CW flagellar rotation (a tumble factor). Under resting conditions in wild-type cells, the steady state level of tumble factor is relatively constant at some level determined by its rate of formation by transducer and its rate of inactivation by CheZ. When attractant binds to a transducer protein, the rate of tumble factor formation decreases; because CheZ is present to facilitate rapid inactivation of the existing tumble factor, the concentration of tumble factor decreases. The net result is an increase in the CCW bias of the flagellar motor. However, when CheZ is defective or absent, the rate of tumble factor inactivation is slowed considerably. Consequently, when attractant binds to transducer and slows the rate of tumble factor formation, the rate at which the concentration of tumble factor declines is much slower than normal; a longer than normal time interval elapses before this concentration is low enough to noticeably affect the flagellar bias. In this model, *cheZ* mutants have a higher than normal steady-state level of tumble factor because they are defective in inactivating this signal, and this overabundance causes the tumbly phenotype associated with *cheZ* mutants.

c. Homologies of Putative Chemotaxis Signaling Components with Components of Other Signal Transduction Mechanisms That Respond to En-

vironmental Changes. Nixon et al.[236] noted that CheA and CheY share significant amino acid sequence homology with a number of other proteins that are involved in enabling responses to environmental changes in a variety of bacterial species. The roles played by some of these proteins in their respective sensory transduction mechanisms have been characterized in some detail and so may provide some insight into the interactions that are involved in the chemotaxis signal transduction mechanism. In this regard, it is interesting that CheA shares regions of homology with the gene products of *envZ*[227] (from *E. coli*), *phoR* (from *E. coli*), *virA* (from *Agrobacterium tumefaciens*), and *ntrB* (from *Klebsiella pneumoniae* and *Bradyrhizobium sp.*),[236] which are thought to directly modify their respective partners, the *ompR*, *phoB*, *virG*, and *ntrC* gene products,[237-240] to direct appropriate responses (e.g., altered transcription of specific genes) to environmental changes. For the *ntrB–ntrC* couple, this modification appears to be the altered level of phosphorylation of the *ntrC* product in response to signals from the *ntrB* product, reflecting ammonia availability.[240] The observation that the CheY proteins from *E. coli* and *S. typhimurium* share significant amino acid sequence homology with the proteins of the "*ntrC* group" raises the possibility that CheA may serve as the interacting (regulating) partner of CheY[236] and may hint at a role for phosphorylation or some other chemical modification in regulating CheY tumble-inducing activity. The N-terminal region of CheB (the MCP-specific methylesterase) is also homologous to proteins of the *ntrC* group,[236] again suggesting that interaction with CheA and/or modification of this enzyme could be involved in regulating its activity in response to chemotactic stimuli (as discussed in section V).

2. Role of ATP or a Related Nucleotide

Over 10 years ago it was established that arsenate inhibits chemotaxis in *E. coli* and *S. typhimurium*[5] under conditions in which motility is normal. Arsenate-treated cells lose the ability to tumble. Originally, it was assumed that the ATP depletion caused by arsenate[241] was responsible for this effect. It should be noted that arsenate depletes the bacterial cell of not only ATP but also many other high-energy phosphate compounds, any of which might be responsible for the observed effect on chemotaxis. However, in more recent studies in which ATP depletion has been achieved by alternative means (adenine starvation of *hisF* mutants),[242] results very similar to those reported with arsenate have been obtained. This suggests that ATP or a closely related derivative is required for tumbling.

Because ATP is one of the substrates required for AdoMet synthesis by AdoMet synthase (the other substrate is methionine), the effect of ATP depletion on chemotaxis could readily be explained by the following scenario: ATP depletion results in AdoMet depletion, which in turn disables transducer methylation by CheR; MCP methylation is necessary for adaptation and for maintenance of a normal CCW to CW ratio (*cheR* mutants cannot adapt normally and have high CCW bias, i.e., are smooth swimmers; see following section). So ATP depletion ultimately could inhibit chemotaxis by generating an abnormally high CCW bias and by incapacitating the adaptation system. An increasing body of evidence suggests that the situation may actually be more complicated than this and that ATP or some closely related derivative plays some role in chemotaxis in addition to enabling transducer methylation.[242] Results from Parkinson's lab provide a clear demonstration of this additional role: following arsenate treatment, *cheRB* deletion mutants lose their ability to tumble, although motility is maintained.[22] Prior to arsenate treatment, these mutants exhibit alternating smooth swimming and tumbling behavior. These cells can respond to chemotactic stimuli, but of course they cannot adapt normally because they lack the ability to reversibly methylate the MCPs.[243] Stock et al.[244] also reported that arsenate abolishes the tumbling ability of mutants that cannot methylate MCPs; these mutants appear to be *cheRB* double mutants also.[245] The nature of the additional role played by ATP and the identity of the participant (ATP or otherwise) are not clear. In spite of the in vivo results, ATP does not appear to be essential for CW flagellar rotation in vitro. In Eisenbach's cell envelopes, flagella rotate exclusively CCW and cannot be made to rotate CW by addition of ATP or AdoMet.[230] These flagella can be made to rotate CW by adding purified CheY,[213,229] however, and this switch does not require ATP. These results indicate that ATP or an ATP derivative is an unlikely candidate for the signal that communicates transducer changes to the flagellar switch. The role of ATP in bacterial chemotaxis remains unclear.

V. Proteins Involved in Adaptation: MCP Methylation and Demethylation

As discussed in detail earlier in this review, the four known transducer proteins in *E. coli* (Tsr, Tar, Trg, and Tap) and *S. typhimurium* are also referred to as methyl-accepting chemotaxis proteins (MCPs) because they are reversibly methylated[170,189,190,246] by a chemotaxis-specific methyltransferase (the *cheR* gene product). This enzyme catalyzes transfer of the activated methyl group from AdoMet to specific MCP glutamic acid γ-carboxylate groups in *E. coli*[65] and *S. typhimurium*.[233] The *cheB* gene product is the methylesterase that catalyzes hydrolysis of these methylesters,[70] producing methanol and regenerating the γ-carboxylates of the glutamic acid residues.[247]

In *E. coli*, *S. typhimurium*,[17,20,248] and *B. subtilis*,[23,249] MCP methylation is thought to play an important role in behavioral adaptation, i.e., the return of swimming behavior to the prestimulus pattern of alternately running and tumbling.[32] Mutants deficient in CheR and/or CheB are defective with respect to adaptation and have extremely abnormal bias of flagellar rotation in their unstimulated swimming behaviors.[64,127,129,223,250] In *E. coli* and *S. typhimurium*, increased levels of MCP methylation are associated with adaptation to positive stimuli (addition of attractant or removal of repellent), while adaptation to negative stimuli is accompanied by decreased methylation.[54,55,62,143,197-198,248] The time course of the change in MCP methylation level approximates the time course for behavioral adaptation following such stimuli.[62,248] The four known MCPs serve as transducers for four different sets of stimuli. A given stimulus appears to alter the methylation level of only the MCP class that is responsible for detecting its concentration.[54,55,62] Therefore, it appears that the

activity of the methyltransferase and/or that of the methylesterase is regulated in response to concentrations of chemoeffectors. This regulation causes increased methylation of a specific subset of the total MCP pool. This section of the review covers what is known about CheR and CheB and the regulation of their activities. CheA and CheW are also discussed in this section because they may be involved in this regulation. Although our focus is primarily on the enzymes from *E. coli* and *S. typhimurium*, some mention is made of the considerable progress that has been made in characterizing the methyltransferase and esterase from *B. subtilis* (see ref 23 review). *B. subtilis* has MCPs that are methylated and demethylated in response to chemoeffectors. However, in this bacterium, attractant stimuli cause decreased MCP methylation levels, and repellents cause increased levels of methylation.[249]

A. CheR

On the basis of its DNA sequence,[227] the methyltransferase (CheR) from *E. coli* is a 286 amino acid, 32.7-kDa polypeptide encoded by the third gene in the Meche operon. Subcellular fractionation studies indicate that CheR is located in both the cytoplasm and the inner cytoplasmic membrane.[118] This distribution apparently reflects the fact that CheR is a soluble globular protein, but it binds quite tightly to its substrate, the membrane-bound MCPs ($K_d \sim 1\ \mu M$[251]). The molecular weight of this protein has been estimated to be 28–31 kDa based on SDS PAGE.[115,118,224,225] Stock et al. reported a procedure for partial purification of CheR and reviewed the in vivo and in vitro assays for this enzyme.[251] CheR does not appear to methylate proteins other than the MCPs,[65,251,252] and it methylates only specific glutamate residues on these.[189,190] Other than MCP methylation, the only known reaction involving CheR is in vivo synthesis of *S*-methylglutathione in *E. coli*.[253] This compound does not appear to have any role in chemotaxis, however, and it may simply arise from the "parasitic" reaction of glutathione with the enzyme-bound AdoMet.[253]

In vivo, CheR methylates each of the four sites on Tar with quite different intrinsic rates:[254] two sites are methylated quite readily (relative rates 1.0 and 0.63); the other two sites are methylated much more slowly (relative rates 0.09 and 0.02). Terwilliger et al.[254] attributed these rate differences to the primary amino acid sequences in the vicinity of the methylation sites, and they determined a consensus sequence of Glu-Glu*-X-X-Ala-Thr/Ser, where the asterisk designates the site of methylation. The two faster methylation sites of Tar have amino acid sequences corresponding to this consensus sequence; the two slower sites differ from this sequence by one residue. Incorporating data for Trg as well as for Tar and Tsr, Nowlin et al.[196] suggested that the consensus sequence for methyl-accepting sites is Ala/Ser-X-X-Glu-Glu*-X-Ala/aa-OH-Ala-Ala/aa-OH, where the asterisk designates the site of methylation, X denotes any amino acid, and aa-OH represents serine or threonine. In contrast with the Tar results,[254] methylation of the one Trg site that deviates from this consensus sequence does not appear to be considerably slower than methylation of "nondeviating" sites.[196]

When cells are exposed to saturating concentrations of attractants, the overall level of MCP methylation increases two- to fourfold above the prestimulus level;[62,170,254] repellents cause methylation levels to fall to approximately half of the prestimulus level.[170,252,255] In Tar, each of the possible methylation sites becomes more methylated in response to attractant,[254] suggesting that all four sites participate in adaptation. However, in Tar (and probably in the other MCPs) this increase occurs to varying extents at the four esterification sites. Under unstimulated conditions Terwilliger et al.[254] found approximately 0.5 methyl ester/Tar protein (in an *E. coli* strain lacking Tsr). The distribution of methyl groups among the four esterification sites reflects the different intrinsic rates of methylation and demethylation of each site. Following a saturating aspartate stimulus, the level of methylation of each site increases with a characteristic time course that could in principle result from both an increased rate of methylation and a decreased rate of demethylation. Approximately 2.0 methyl groups/MCP molecule are found after adaptation. The relative distribution of these methyl groups among the four sites is now quite different from that in the unstimulated MCP because it results from the intrinsic reactivities of the sites in the aspartate-bound form of Tar. The rate of methylation increases at all four sites in the presence of aspartate, but to varying extents ranging from 3- to 30-fold enhancement of the calculated rate constant of methylation. At three of these sites the enhanced rate of methylation accounts largely for the increased methylation level,[254] while at the fourth site the transiently decreased rate of demethylation[255-257] makes a significant contribution as well.[254]

Previous observations of increased methylation in response to attractants in reconstituted in vitro systems[147,258] suggest that his response may not require additional regulatory components; increased methylation may result directly from an attractant-induced conformational change in the MCP that increases accessibility of the esterification sites to the methyltransferase.[147,170,258] Such a scenario could account for the specificity of the MCP methylation response.

CheR mutants are smooth swimming[12,19,129,222] due to an extreme CCW bias of flagellar rotation.[64] Such mutants will respond to repellent stimuli by tumbling, although with a high response threshold. In addition, the duration of the tumbly response is considerably longer (up to 50 times longer) than that observed with wild-type cells, indicating a defect in adaptation.[63,64] This defect appears to result from the inability of *cheR* mutants to methylate MCPs;[63,259] unmethylated or undermethylated MCPs are thought to generate a CCW signal,[17,20,199,248] which would account for the smooth swimming phenotype of *cheR* mutants.

In spite of their inability to methylate MCPs, some *cheR* mutants do exhibit a gradual return toward the prestimulus CCW bias following their initial excitatory (CW) response to repellents.[12,64,244,260] This may result from a methylation-independent adaptation mechanism,[244,260] which is somewhat slower and considerably less effective than the methylation-dependent mechanism. This possibility is discussed in a later subsection.

There is genetic evidence that CheR interacts with CheY: both *cheR* and *cheY* from *E. coli* must be sup-

plied to functionally complement an *S. typhimurium* mutant lacking either of these.[233] This implies that the CheR protein from *E. coli* cannot interact properly with the CheY protein from *S. typhimurium*. This is one of the few instances in which individual chemotaxis components are not interchangeable between *E. coli* and *S. typhimurium*,[233] although the putative CheR–CheY complex is apparently interchangeable. The influence of CheY on the methyltransferase is not clear. Because CheY binds AdoMet quite tightly,[226] it is conceivable that CheY could regulate the concentration of AdoMet accessible to the methyltransferase. However, CheY is not required for normal methyltransferase activity in vivo or in vitro,[65,246,254] and *cheY* overexpression does not noticeably affect the level of transducer methylation.[261]

CheR mutants respond to attractant and repellent stimuli with normal (wild-type) response latencies.[40] So although it is intimately involved in the normal adaptation mechanism, CheR does not appear to play a role in generating the excitatory signal that enables communication between the MCPs and the flagellar switch.[40] Rather, CheR indirectly affects the signal level by modifying the ability of the MCPs to generate signal.

B. subtilis has two distinct MCP methyltransferases, both of which utilize AdoMet as methyl donor.[216,262] Methyltransferase II is a 30-kDa protein primarily responsible for methylation of all of the *B. subtilis* MCPs in vivo.[262] In vitro studies indicate that this enzyme can methylate the MCPs from *E. coli* as well.[262] Methyltransferase I, on the other hand, appears to utilize only one of the *B. subtilis* MCPs as a substrate. This 19-kDa enzyme has been purified to homogeneity and characterized.[216,262] Micromolar concentrations of Ca^{2+} inhibit this enzyme, leading to speculation about the possible regulatory role of Ca^{2+} in chemotaxis of *B. subtilis*.

B. CheB

The *cheB* gene product is thought to be a soluble cytoplasmic enzyme.[70,118,263,264] Subcellular fractionation of *E. coli* indicates that CheB is located in both the cytoplasm and the inner cytoplasmic membrane,[118] presumably reflecting the affinity of this cytosolic enzyme for the membrane-bound MCPs.[263] The DNA sequence of *cheB* from *E. coli*[227] predicts a corresponding amino acid sequence of 349 residues comprising a 37.5-kDa protein. In agreement with these predictions are the observed molecular weights of the *E. coli* (38-kDa[115,118,224]) and *S. typhimurium* (37-kDa[225,264]) proteins and the amino acid content of the purified enzyme from *S. typhimurium*.[264] CheB catalyzes hydrolysis of the γ-glutamyl methyl esters formed by CheR; the products of the hydrolysis are methanol and the free γ-glutamyl carboxylates.[70,247] CheB is also responsible for the irreversible deamidation of two MCP glutamines, converting them to glutamates, which are among the methyl esterification sites in wild-type cells.[192,193,195,243] In vitro assays of the methylesterase activity of CheB (reviewed in ref 263; see also ref 264) enabled development of purification procedures for this enzyme.[263,265] A more straightforward and reproducible purification protocol was recently reported by Simms et al.[264] In addition to the intact 37-kDa esterase, this procedure also yields a 21-kDa proteolytic fragment, which represents the carboxy-terminal three-fifths of the uncut esterase and which has remarkably high activity (15-fold higher than that of the 37-kDa parent protein).[264] The possible significance of this finding with respect to regulation of methylesterase activity is discussed below. Some of the kinetic and physical properties of the purified esterase have been determined.[263,264] The enzyme is readily inhibited by several thiol reagents,[263] perhaps indicating an essential role for one of the two cysteine residues[227,264] of CheB. Simms et al.[266] further explored this possibility by using oligonucleotide-directed mutagenesis to convert these cysteine residues to alanines (individually). Cys^{207} → Ala CheB has normal esterase activity, while the Cys^{309} → Ala protein is completely inactive. In the sequence of CheB, the catalytically essential Cys^{309} residue follows what may be a nucleotide-binding fold formed by residues 277–307.[266] One exciting possibility is that this region is involved in regulating the activity of CheB (see below). Oligonucleotide-directed mutagenesis of a key element of the putative nucleotide-binding fold does result in inactive enzyme,[266] but no evidence supporting a regulatory role for this region of the protein has been reported yet.

The unstimulated swimming phenotype of *cheB* mutants is invariably tumbly.[70,129,222,267] This extreme CW bias of flagellar rotation can be altered by attractant stimuli, resulting in periods of smooth swimming.[40,129,222,260,267] The response thresholds for attractant stimuli are higher in *cheB* mutants than in wild-type cells.[267] However, CheB does not appear to be involved in generating the excitatory signal that links the transducers to the flagellar switch, because *cheB* mutants exhibit normal response latencies to attractants.[40] By virtue of its role in determining methylation states of MCPs, CheB appears to be a part of the adaptation machinery. The MCPs in esterase-deficient mutants have only about half of the methylation sites available in $cheB^+$ hosts (due to the loss of the sites generated by the CheB-catalyzed deamidation reaction[192,193,195,243]). Methyl groups can be added to, but not removed from, the residual esterification sites. The resulting "overmethylation" apparently causes the nondeamidated MCPs to generate "tumble signal" constantly.[17,19,20,248] Stock et al.[260] demonstrated that "tight" *cheB* mutants (no detectable esterase activity) are capable of some form of behavioral adaptation to stimuli of limited intensity. These findings are discussed in one of the following subsections.

Several research groups demonstrated that the activity of CheB is regulated in response to MCP-mediated stimuli.[255–257,268] By monitoring the disappearance of MCP methyl groups or the accumulation of methanol, Toews et al.[255] first demonstrated that the methylesterase is transiently inhibited by attractant stimuli; repellent stimuli (or removal of attractant) result in a transient increase in methylesterase activity.[255] Kehry et al.[256,257] extended these studies by utilizing a flow assay that greatly facilitates monitoring of the in vivo methylesterase activity of CheB. This system (depicted in Figure 16) basically involves pumping media over cells maintained on a filter; stimuli are presented to the cells simply by switching the inlet tubing from one source of media to another. The methyl groups on the MCPs in these cells have been prelabeled (using [^{3}H]-methionine) prior to placing the cells on the filter, and

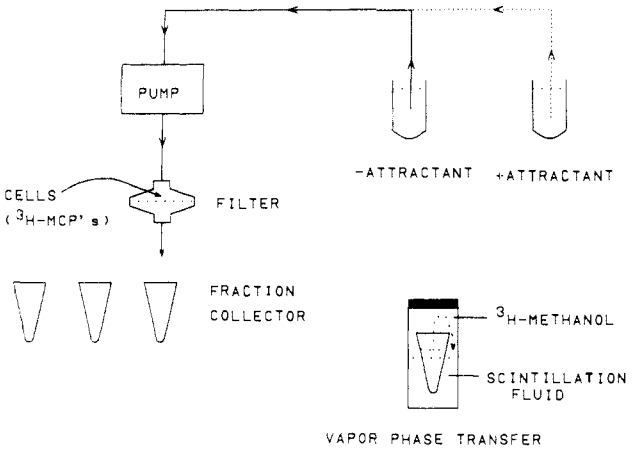


Figure 16. Schematic diagram of the procedure for measuring methanol evolution by intact cells in the continuous flow apparatus developed by Kehry et al.[256] Media is pumped over prelabeled cells maintained on a 0.2 μm filter, and fractions are collected at constant intervals. Stimuli are presented as single-step concentration increases or decreases by switching the source of media pumped over the cells.

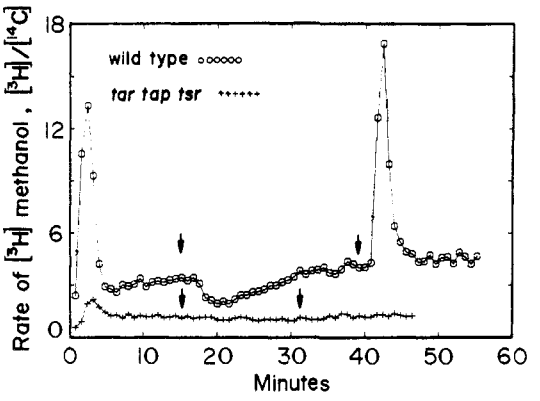


Figure 17. Response of steady-state [^{3}H]methanol production to chemotaxis stimuli (serine addition and removal). Experimental details have been described by Kehry et al.[256] Serine (75 μM) was added at the first arrow (t = 15 min) and removed at the second arrow (t = 40 min). Data are shown for chemotactic wild-type *E. coli* (0, RP487) and for a *tar tap tsr* deletion strain (+, MS5228). Note the transient, moderate decrease in [^{3}H]-methanol evolution in response to attractant addition and the dramatic increase in response to attractant removal (a repellent stimulus).

esterase activity is monitored by collecting fractions of media flowing over the cells and determining the [^{3}H]methanol content of each. This is easily achieved by placing each fraction tube into a partially filled scintillation vial, capping the vial, and allowing vapor-phase transfer to equilibrate the [^{3}H]methanol between the aqueous flow fraction and the scintillation cocktail in the larger vial. In "steady-state flow" experiments, [^{3}H]methionine is present in the flow medium at all times; typical results for wild-type and control cells are shown in Figure 17 for an experiment using serine addition and removal as stimuli. The transient decrease in methanol evolution (CheB activity) in response to serine addition is readily apparent, as is the dramatic increase in CheB activity when attractant is removed (a repellent stimulus). "Flow chase" experiments are performed in the same manner, except that only cold methionine is available to the cells in the flow medium following the initial prelabeling. Results from a typical flow chase experiment are shown in Figure 18. Again, the esterase activity appears to de-

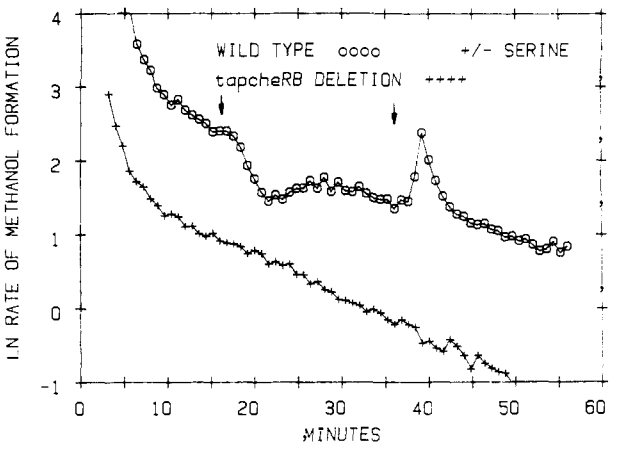


Figure 18. Response of [^{3}H]methanol production to chemotaxis stimuli in flow chase mode. Experimental details have been described by Kehry et al.[256] Serine (35 μM) was added at the first arrow (t = 15 min), then removed at the second arrow (t = 40 min). Data are shown for chemotactic wild-type *E. coli* (0, RP487) and for a *tap cheR cheR* deletion strain (+, RP2867). In the absence of stimuli, the rate of methanol production follows an approximately logarithmic decay as [^{3}H]methyl groups are chased with cold methyl groups. Addition of attractant causes the rate to fall transiently below the unstimulated rate; repellent stimuli cause the rate to increase transiently to levels well above the unstimulated rate.

crease transiently following attractant addition and to increase transiently in response to attractant removal. It is gratifying to note that $t_{1/2}$ for an unstimulated flow chase (reflecting the net, weighted-average rate of deesterification of multiple methyl sites on each MCP; see ref 256) agrees reasonably with the $t_{1/2}$ values for demethylation of individual sites as determined by Terwilliger et al.[254]

There is some evidence that the methylesterase activity may be regulated by the same signal generated by the transducers to communicate with the flagellar switch (or by the same mechanism responsible for signal generation). First, the response of the methylesterase activity to addition or removal of chemoeffectors always exhibits a characteristic biphasic time course: a very rapid change (faster than the mixing time of the flow apparatus) in one direction followed by a slower change (requiring 5–6 min) in the opposite direction that gradually restores the methylesterase activity to its prestimulus level. Although the mixing time of the flow apparatus ($t_{1/2} \sim 3.6$ min) limits detailed determination of the kinetics of these changes, the time course of CheB activity change appears to mimic the sequence of behavioral events following stimulation of wild-type cells: a rapid excitatory response (latency ~0.2 s[39,40]) followed by adaptation to the prestimulus behavior (requires several minutes[38]). Second, input from different classes of receptors is integrated in some manner prior to the event(s) that results in regulation of CheB activity,[257] a situation reminiscent of the integration that takes place in determining the swimming behavior of the cell.[32] Third, in *cheZ* mutants (which are known to be defective in excitatory signaling[39,40]) the methylesterase activity responds to stimuli somewhat slower than is observed in *cheZ*$^+$ cells (Kehry, M. R.; Doak, T. G.; Dahlquist, F. W., manuscript in preparation).

Cells lacking most or all of the known flagellar and chemotaxis-associated proteins (*flaI* or *flaA* → *flbB* deletions[92]) of course show no methylesterase activity.

When such cells are provided with CheR, CheB, and MCPs by expressing genes carried on plasmids, methylesterase activity is observable, but flow experiments show no regulation of this activity in response to chemotactic stimuli (Russell, C. B.; Stewart, R. C.; Dahlquist, F. W., unpublished results). Therefore, additional components of the chemotaxis sensory transduction machinery may be required for CheB regulation. Some progress has been made toward identifying those components. CheZ was a reasonable candidate because interspecific complementation studies suggest that CheZ interacts with CheB to form a complex that has some essential role in chemotaxis.[233] Furthermore, some *cheZ* mutants have low in vitro methylesterase activity.[265] However, CheZ is not required for methylesterase activity.[264,265] Flow experiments indicate that CheZ is not required for regulation of CheB per se, but rather for normal kinetics of regulation (Kehry, M. R.; Doak, T. G.; Dahlquist, F. W., manuscript in preparation). CheY also does not appear to be involved in methylesterase regulation, as *cheY* mutants exhibit wild-type regulation patterns in flow experiments (Kehry, M. R.; Doak, T. G.; Dahlquist, F. W., manuscript in preparation). *cheA* and *cheW* mutants, on the other hand, do not appear to be capable of regulating methylesterase activity in response to chemoeffectors, suggesting that the corresponding proteins may be involved in this regulation. The currently available information about the roles of CheA and CheW in chemotaxis is very limited and is discussed below.

In view of the demonstrated regulation of methylesterase in response to chemotactic stimuli, the following question arises: can this regulation account for the stimulus-specific changes in methylation levels of corresponding MCPs? If inhibition of demethylation were primarily responsible for these changes, then one might expect interactions between the stimulated MCP and the methylesterase (e.g., accessibility) to directly determine the activity of the enzyme in response to stimuli (i.e., "local regulation"[147,170,258]). However, CheB activity appears to be "globally regulated" in response to some parameter that reflects the integrated input from different classes of transducers.[257] This suggests that regulation of methylesterase activity is not primarily responsible for the stimulus-specific MCP methylation changes and predicts that this specificity results primarily from local regulation of the methyltransferase. In support of this prediction, Terwilliger et al.[254] presented evidence that the increased level of Tar methylation following an aspartate stimulus is due primarily to increased rates of methylation at all four esterification sites, not to decreased rates of demethylation. The observation of stimulus-induced methylation changes in in vitro systems[147,258] suggests that this regulation of CheR may be at the local level.

Studies of CheB proteolytic fragments[264] and of the *cheB* DNA sequence[269] may provide some insight into the molecular aspects of CheB regulation. When the 37-kDa methylesterase is proteolyzed to a limited extent, a highly active 21-kDa fragment is obtained.[264] This fragment is relatively resistant to further proteolysis and appears to be the carboxy-terminal three-fifths of the intact 37-kDa protein. The specific activity of the proteolytic fragment is fifteen times that of the parent protein, perhaps suggesting that the amino-terminal portion of CheB plays some role in reversibly regulating the activity of the intact (37-kDa) enzyme. It remains to be seen whether the existence of at least two proteolyzed forms of CheB in extracts from rapidly lysed wild-type *S. typhimurium* suggests that there is an in vivo role for proteolytic activation of the relatively low-activity 37-kDa enzyme.[264] Stock et al.[269] determined that significant amino acid sequence homology exists between the entire CheY protein and the amino-terminal region of CheB. Such homology raises the possibility that CheY and CheB interact with a common or similar substrate (e.g., MCPs) or perhaps with a common regulatory element[269] (see subsection IVE1c).

In *B. subtilis*, in vitro studies[270] demonstrated that the activity of the purified methylesterase increases in response to MCP-mediated attractants; this result parallels in vitro results, which have demonstrated that the decreased levels of MCP methylation observed in response to attractants is due to increased methylesterase activity.[249] The *B. subtilis* methylesterase has been purified and characterized;[271] it is a 41-kDa protein that requires a divalent cation for activity. This enzyme will utilize methylated MCPs from *E. coli* as substrates;[271] however, attractant stimuli have no effect on the methylesterase activity in this hybrid system.

C. CheA and CheW

Mutants in *cheA* or *cheW* have extreme CCW bias of flagellar rotation; they are smooth swimmers and seldom tumble.[65,128,129,222,243b] Such mutants do respond transiently[162,243b] to a strong pH repellent stimulus (benzoate) but do not appear to be capable of responding to other less potent CW stimuli.[128,243b] Therefore, CheA and CheW do not appear to be required for CW flagellar rotation per se, although they seem to be essential for communicating MCP–chemoeffector binding events to the flagellar switch (see Note Added in Proof). The mapping of suppressors of *cheA* point mutants to genes encoding flagellar components[19] (and vice versa[134]) further supports such a signaling role for CheA.

CheA also appear to be involved in regulation of methylesterase activity in response to MCP-mediated stimuli. Springer and Zanolari[268] found that several *cheA* point mutants and a *cheA* deletion mutant are defective in regulation of methylesterase activity in response to negative (repellent) stimuli in vivo, although regulation in response to positive stimuli is essentially normal. Work in our laboratory indicated that some *cheA* mutants exhibit no regulation of CheB in response to positive or negative stimuli in flow chase experiments (Kehry, M. R.; Doak, T. G.; Dahlquist, F. W., manuscript in preparation). These biochemical results, in conjunction with the previously described behavioral and genetic studies, suggest that CheA may have some role in signaling and that this signal (or some species affected by it) is also involved in regulation of CheB activity.

A partial *cheA* sequence and the entire *cheW* DNA sequence were reported by Mutoh and Simon.[227] Two polypeptides are encoded by the *cheA* locus of *E. coli*;[224,272,273] they are designated p[*cheA*]$_S$ (66–69 kDa) and p[*cheA*]$_L$ (76–78 kDa). The same coding sequence and reading frame are utilized for both CheA proteins, but the translation start site of p[*cheA*]$_L$ precedes that

of p[*cheA*]$_S$ such that the larger protein has an additional 90 or so amino acids on its amino-terminal end.[272] The two proteins have identical amino acid sequences beyond the first 90 residues of p[*cheA*]$_L$. Results of complementation studies suggest that the amino-terminal portion of p[*cheA*]$_L$ has a function distinct from that shared by p[*cheA*]$_S$ and the remainder of p[*cheA*]$_L$.[272] The short form of CheA is found only in the cytoplasm of *E. coli*, while the longer form of CheA is found in both the cytoplasmic and inner membrane fractions.[118] So it is conceivable that the amino-terminal region of p[*cheA*]$_L$ enables association of the longer protein with a different set of chemotaxis components from those that interact with the cytoplasmic pool of CheA.[19,272]

The function of CheW remains unknown. This 167 amino acid, 18-kDa protein[118,224,225,227,273,274] is found exclusively in the cytoplasm of *E. coli*.[118] The *S. typhimurium* protein was purified to homogeneity by Stock and co-workers,[274] who found that CheW exists as a homodimer under nondenaturing conditions (confirming the results of cross-linking studies[175]). Analysis of the CheW amino acid sequence (predicted from the determined DNA sequences) by Stock et al.[274] indicates that residues 128–160 may form a purine nucleotide binding site, an interesting possibility in view of the demonstrated requirement of nucleotides in chemotaxis (see section IV). Whether CheW actually binds nucleotides and the role of such an activity in chemotaxis has yet to be established. It seems likely that CheW has a function related to those of the other components of the *mocha* operon (*motA*, *motB*, and *cheA*), i.e., enabling or regulating motility. Some *cheW* mutants have overmethylated MCPs,[12] perhaps suggesting that the CheW protein is involved in regulation of CheB and/or CheR activity as well. Some role for CheW in generating the signal to which methylesterase responds is supported by the results of flow chase experiments that indicate that methylesterase activity is not regulated in response to chemotactic stimuli in *cheW* mutants (Kehry, M. R.; Doak, T. G.; Dahlquist, F. W., manuscript in preparation).

D. Adaptation without MCP Methylation

1. MCP-Mediated Stimuli

In previous sections evidence has been presented indicating that methylation of the transducer proteins is intimately involved in behavioral adaptation to MCP-mediated chemoeffectors. Binding of attractant to an MCP causes it to generate a "CCW signal"; methylation of the MCP counteracts or turns off this signaling[17,20,199,248] so that the original signal level is reestablished and the bias of the flagellar motors returns to the prestimulus condition. According to this scheme, mutants lacking or defective in CheR or CheB should not be capable of adapting to MCP-mediated stimuli. However, although such mutants certainly are defective in adaptation, they do appear to have some ability to adapt.[244,260] The following differences between adaptation in the absence of methylation and that in the presence of a functional methylation system should be emphasized: (a) wild-type cells can adapt to a wide range of stimuli, resulting in exact restoration of the prestimulus behavior pattern; in *cheR*$^-$ and/or *cheB*$^-$ mutants, the extent of this restoration depends on the stimulus strength, and the adaptation to prestimulus bias is observed only at very low concentrations of stimuli; (b) the time required for adaptation of wild-type cells is considerably shorter than that required for *cheR*$^-$ and/or *cheB*$^-$ cells to reach their "adapted" state.[260] In spite of these adaptation defects, Stock et al.[260] reported that the "limited adaptation" available to *cheR*$^-$*cheB*$^-$ double mutants is sufficient to enable reasonable chemotaxis as indicated by swarm plate assays (mutant swarm rate ~25% of wild-type rate). Compared with the double mutant, single mutants in either *cheR* or *cheB* perform markedly worse in swarm assays.[260] This difference appears to result from the considerable aberrations in motor bias (CCW in *cheR*$^-$; CW in *cheB*$^-$) imposed by the single mutations since *cheR*$^-$ and *cheB*$^-$ cells do have the same limited adaptation capacity as is observed in *cheR*$^-$*cheB*$^-$ cells.[244,260] The observation of limited adaptation in *cheB*$^-$ and *cheR*$^-$*cheB*$^-$ mutants demonstrates that this adaptation cannot be mediated by CheB-catalyzed deamidation of MCPs.[192,193,243a]

Stock et al.[260] suggested that the excitatory response of chemotaxis (which is ulaltered by *cheR* and *cheB* mutations[39,40]) results from rapid release of an activated form of signal from the MCP upon binding of chemoeffector; the limited, methylation-independent adaptation then results from two slower steps: inactivation of the signal and reassociation of the inactivated signal with the MCP. In this scheme, the first round of activated signal production would be fast, limited only by the rate of activation and release from the MCP. However, subsequent rounds of active signal production would be limited by the slower inactivation and reassociation steps. The net result would be a burst of activated signal followed by its decay to a new steady-state level.

An alternative explanation may be the dissipation of signal by some buffering mechanism of the cell. For example, some metabolic cycles may share a component with the MCP signal generation pathway. Alternatively, one of the MCP-independent chemotaxis systems (e.g., the PTS sugar system) could have a signaling step in common with the MCP-dependent system. Processing of the activated signal by any such system could lead to the observed adapatation.

Another group of double mutants that are capable of chemotaxis in the absence of MCP methylation was reported by Parkinson et al.[275] These are *cheR*$^-$ mutants that have a second compensatory mutation in *tsr*. The suppressor mutations are designated as *scrS* (suppressor of *cheR*$^-$ in *tsr*). These *tsr* mutations are not allele-specific and will in fact compensate for *cheR* deletions. The *scrS* mutations impart a CW flagellar bias to *cheR*$^+$ hosts. It is conceivable that these mutations function in *cheR*$^-$ cells by restoring a more normal flagellar rotation bias to the extent that the limited methylation-independent adaptation system proposed by Stock et al.[260] can function adequately. Such a bias shift could be accomplished by a *tsr* mutation that increases the basal level of CW signaling by the Tsr protein.[275] Alternatively, these mutations could enhance the sensitivity of Tsr to the feedback mechanism responsible for methylation-independent adaptation.

2. Non-MCP-Mediated Stimuli

Chemotactic response to some stimuli does not require any of the four known transducer proteins;[69,277,278] furthermore, methylation of the transducers is not required for adaptation to such stimuli, which include oxygen and sugars whose transport is mediated by the phosphotransferase system (PTS sugars).[66-68,277] Some progress has been made in identifying the receptors for these stimuli and in defining the signaling systems that link changes in stimulus intensity to regulation of flagellar bias.

With the PTS sugars, components of the phosphotransferase system (sugar-specific forms of Enzyme II) appear to function as the receptors.[66-68,278] Each sugar-specific form of Enzyme II is responsible for phosphorylating its particular sugar. This phosphorylation requires the phosphorylated form of another protein (HPr) to serve as phosphate donor. Phosphorylation of HPr requires Enzyme I and phosphenolpyruvate. Each of these proteins is required for chemotactic response to the PTS sugars. It seems reasonable to propose that changes in the concentration of some phosphorylated compound (caused by changes in sugar concentration) could play some signaling role in PTS sugar chemotaxis.[66] This proposal appeared to be supported by the loss of chemotaxis to PTS sugars (but not to amino acids) in some adenylate cyclase and phosphodiesterase mutants[279] even though transport and phosphorylation of PTS carbohydrates was normal. However, recent results demonstrate that in both *S. typhimurium* and *E. coli*[281] cyclic nucleotides are not involved in the chemotaxis signal transduction pathway and that cAMP can affect the chemotactic ability of cells only because it is required for synthesis of chemotaxis proteins that are subject to catabolite repression. For example, Tribhuwan et al.[280] found that removal of cAMP from *S. typhimurium cya* mutants following growth under inducing conditions (cAMP present) had no effect on phosphotransferase chemotaxis. Normal chemotaxis was also observed with *S. typhimurium cpd* mutants having elevated cAMP and cGMP levels. Vogler and Lengeler[281] demonstrated that *E. coli cya* mutants have normal chemotaxis toward PTS carbohydrates and that the *cya-854* mutant (on which most of the previous studies were performed) has altered expression of an as yet unidentified component of the Enzyme II mediated signal transduction pathway.[281] This unknown protein does not appear to be a *pts* protein or a cAMP binding protein. Thus, adenylate cyclase, cAMP, and cGMP do not appear to have any direct role in mediating chemotaxis to PTS carbohydrates. It remains to be seen whether the signal for PTS sugar chemotaxis is related to the signal for MCP-mediated chemotaxis and whether the respective signaling pathways converge at some point before reaching the flagellar motor.

Chemotactic response to oxygen does not appear to involve a classic receptor–ligand interaction that directs the behavior-determining components. Rather, oxygen generates changes in the flux of electrons through the electron transport system.[282,284] It is thought that the resulting changes in proton motive force are detected by a "protometer"[277,283] that can appropriately direct flagellar bias. Whether the protometer achieves this goal by regulating the same signal as is utilized in response to amino acid stimuli is unknown.

VI. A Model for Information Processing during Bacterial Chemotaxis

A. Limitations on Possible Models

There are several features of bacterial chemotaxis that appear to restrict the sorts of molecular models that one can propose for the comparator that allows the current state of receptor occupancy to be compared with the level of methylation of the transducers to generate excitatory signals.

1. Binding of Chemoattractants Is Rapid Compared with Methylation

Binding events at the receptor sites appear to be rapid. From the impulse response studies from Berg's laboratory[39,40] it is clear that responses to both positive (addition of attractant) and negative (removal of attractant) stimuli occur on the fraction of a second time scale. Thus, the forward and reverse rate constants for the binding reactions must be on the order of 1/s or faster. However, the methylation/demethylation reactions appear to be slower.[54,62,246,248,255] For stimuli of the size typically encountered by the cell, adaptation times are on the seconds time scale.[285] Adaptation times of several minutes are seen for large stimuli.[36-38] Similarly, the turnover times for methyl groups are about 15 min.[254-258,268] These observations have two important implications. First, it is clear that ligands will exchange off receptors much faster than methyl groups turn over. Second, large stimuli appear to saturate the adaptation system, suggesting that some of the kinetic events associated with the adaptation processes are not likely to be first order.

2. Integration of Signals from the Transducers Is Required

The extreme sensitivity of the chemotaxis apparatus to small changes in attractant concentration with time is well documented. Using defined spatial gradients, we demonstrated that changes in attractant concentration as small as 1 part in 10^4/bacterial length produced a half-maximal response.[35,286] Bacteria swim about 10 body lengths/s, so temporal gradients of about 1 part in 10^3 s^{-1} produce a half-saturating response. Using defined temporal gradients, Segall and Berg showed that stimulation of about one receptor in 1000 resulted in a demonstrable response in the cell.[40,41] While estimates vary slightly, there appear to be a few thousand receptor transducer proteins per cell.[22,56] Thus, stimulation of a few receptors above the adapted level results in response. This sensitivity presents several issues to consider in attempting to model the system. The intrinsic statistical noise that results from a few thousand molecules of receptor must be overcome. If 2000 receptors were half-saturated, statistical fluctuation of $1000^{1/2}$ or 30 receptors would be expected. Yet the system appears to have nearly single-receptor sensitivity. One way to overcome the statistical fluctuations would be to integrate the information from all receptors for a period of time that is long compared with the lifetime of the attractant–receptor complex, thus giving each receptor the opportunity to contribute many

times to the integrated signal from both its bound and unbound states. To reduce the statistical noise by a factor of 30, it is necessary to integrate the receptor occupancy information for a period of about 10^3 bound lifetimes. For ligands with affinities in the range of 10^5–10^6 M^{-1}, bound lifetimes can be estimated to be about a millisecond, assuming a nearly diffusion controlled bimolecular rate constant (10^8–10^9 M^{-1} s^{-1}) for the encounter of receptor and ligand. This suggests integration times on the second time scale, which compares favorably with the time constant for excitation of a fraction of a second mentioned above.

Berg and Tedesco[32] directly demonstrated another form of signal integration. They observed that the application of a positive stimulus through Tsr and a negative stimulus through Tar resulted in no behavioral response if the sizes of the stimuli were appropriately adjusted. This is not the expected result if the response to the combined stimuli were the simple sum of the individual responses, since adaptation to large negative stimuli is several times faster than that to large positive stimuli. Thus, a simple sum of the responses would predict a rapid negative and slower positive response followed by return to the unstimulated pattern. Since no transient responses were observed, they concluded that the signals were integrated *before* adaptation. Further, they concluded that information from each receptor is converted into a common signal by each transducer system.

Finally, the high sensitivity to small temporal changes suggests that signals from distant receptors must be integrated. This suggests a highly efficient communication system between the receptor/transducers and the integration system. This may rule out models in which an association of transducers is required to produce signal. Such a model would predict a highly nonlinear dependence of signaling on receptor occupancy in disagreement with the linear dependence observed by Berg and Tedesco.[32]

3. Nature of the Adapted State

Unlike some sensory systems, bacterial cells in the adapted state are able to respond to both positive and negative stimuli. For example, cells adapted to 50 μM serine respond both to the addition of more serine and to the removal of serine from the environment. Experiments from our laboratory suggest that negative and positive stimuli are detected with the same sensitivity.[257] In these experiments, cells were allowed to adapt to a moderate aspartate concentration. The aspartate was removed, and an appropriate amount of serine was added such that no behavioral response was observed. Several minutes were allowed for adaptation. Then the serine was removed, and the original aspartate concentration was added back. Again, no behavioral response was observed.

Let us suppose that addition of serine or aspartate is detected with unit sensitivity while their removal is detected with sensitivity X. Since no net response is observed in either order of addition/removal, we can write two equations for the integration of the serine and aspartate signals:

If ΔS and ΔA are the changes in serine and aspartate concentration then

$$0 = (1)\Delta S - X\Delta A \qquad (1a)$$

for the addition of serine and removal of aspartate and

$$0 = (1)\Delta A - X\Delta S \qquad (1b)$$

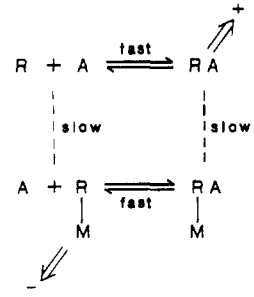
for the addition of aspartate and removal of serine. These relationships require the sensitivity of the detection of "downjumps", X, to be equal to unity, and equal to that of concentration increases.

As indicated by the redistribution of methyl groups on Tar and Tsr, adaptation had occurred during the period between the reversal of stimuli in this experiment. The implication of the absence of a behavioral response to these compensating stimuli is that adapted transducers (or at least methylated transducers) signal downjumps in attractant concentration with equal sensitivity but opposite sign as unmethylated transducers signal attractant concentration increases. Another interesting result of such a "compensating jump" experiment is that no modulation of methylesterase activity is observed immediately following application of the compensating stimuli, implying that the opposing signals are integrated *before* the point at which they influence methylesterase activity.

This conclusion is not consistent with "downregulation" of the transducer during adaptation. Potential mechanisms of downregulation could include sequestering of the transducer or lowering its affinity for chemoattractant as a result of methylation. Clarke and Koshland[56] reported no change in aspartate affinity when the methylation state of Tar was changed (comparing proteins from wild-type and *cheR*$^-$ hosts), while Yonekawa and Hayashi[287] reported a dramatic decrease in the serine affinity of Tsr with increased levels of methylation (comparing proteins from *cheR*$^-$ and *cheRB*$^-$ hosts). More independent data are necessary to establish if any form of downregulation occurs in the bacterial chemotaxis system. For our purposes, we shall assume no change in receptor affinity with methylation state.

B. A Speculative Model for Chemotaxis

The model shown below is based on ideas originally proposed by Rollins,[199] which have recently been independently developed by Knox, Devreotes, Goldbeter, and Segal.[288] The model presented here is a special case of their "adapting box" with some modifications.



For the sake of simplicity, we consider a single methylation event denoted by an M on each receptor/transducer denoted by R. Attractant molecules, A, bind to form the complex RA. Both the receptor and the receptor–attractant complex are subject to slow, reversible methylation reactions to form R–M and RAM. The methylated form of the receptor binds the attractant equally as well as the unmethylated form. The dashed lines indicate that the methylated and un-

methylated forms interconvert slowly, but not necessarily by a simple first-order process. The bound but unmethylated form RA generates a positive signal, while the methylated unbound receptor R–M generates a negative signal. We shall assume here that the unbound, unmethylated and the bound, methylated forms of the transducers do not signal. We propose that the positive and negative signals are the result of discrete action by the appropriate form of the receptor. This is consistent with the discovery of dominant transducer mutants that appear to lock the signaling mode in either the positive or negative sense. This simplified version of the adapting box allows some insight into how these transducers could function. Imagine we begin with unbound receptor R in the absence of A. Under these conditions there is very little RA and R–M. Sudden addition of attractant results in binding and an increase in RA, which signals in a positive sense. This is slowly converted into the methylated form RAM as the cells adapt, and RAM rapidly equilibrates to form R–M, which then signals in the negative sense. Adaptation occurs when the concentrations of the positive and negative signaling forms RA and RM are equal. Removal of attractant results in conversion of all the methylated forms into RM and the unmethylated forms into R. As a result, there is an excess of negative signal, and the cells tumble. Adaptation to this repellent stimulus occurs when R–M is demethylated, converting the transducer into its nonsignaling form R. Knox et al.[288] demonstrated that reasonably good simulations of signaling time course in response to large stimuli can be obtained with some combinations of the first-order rate constants for the methylation/demethylation reactions, signaling properties of the four species, and ligand affinity constants. The model does not appear to fit the data for small stimuli nearly so well. There may also be quantitative features of their model that are not consistent with experimental observations.

We believe that the integrating device then integrates the positive and negative signals from all receptors. As discussed in previous sections, it appears that this integrated signal is used to modulate the activity of the methylesterase serving as a feedback control mechanism in regulating the level of methylation of the total transducer population. This modulation of esterase, combined with the linear dependence of adaptation time on receptor occupancy, suggests that the methylation/demethylation events cannot be viewed as first-order processes. This deviates from the elegant mathematical form of the adapting box model proposed by Knox et al.[288] and may change some quantitative aspects of their treatment. Although the qualitative aspects of the model continue to provide insight into how such a system may function, we may now enlarge our view to include all the information we have in hand concerning information flow during chemotaxis. This is shown in Figure 19. Here the integration device generates a signal that controls the sense of flagellar rotation via the action of CheY and CheZ proteins. This requires the presence of functional CheA and CheW proteins and may involve components not yet identified at the genetic level.

The integrated signal is also used to modulate the activity of the methylesterase. It is not yet clear if the methyltransferase activity is modulated by the integrated signal. It is clear from both in vitro and in vivo studies that attractant-bound transducers are methylated at about a twofold higher rate than the free transducer in the absence of signal.[147,254,258]

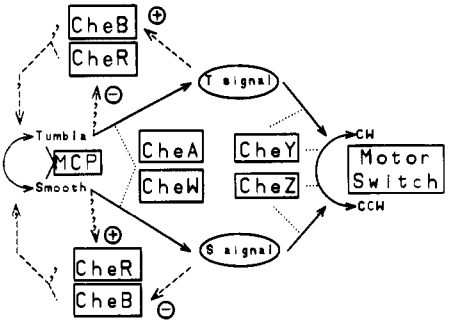


Figure 19. Information flow in bacterial chemotaxis. The transducer protein can alternate between smooth (S) and tumbly (T) signaling modes. This equilibrium is influenced by the binding of ligands (attractants, repellents, periplasmic binding proteins) to the transducer and by the transducer methylation state as determined by CheR and CheB. The signaling inputs from all transducers are integrated over time by some unidentified component that directs formation/destruction/modification of a key signal molecule: T (tumble) signal and S (smooth) signal. As indicated by the dotted lines, integration of transducer input and/or generation of these signals require CheA and CheW, and interaction of the signal molecule with the flagellar motor switch requires CheY and CheZ. Alternatively, modified forms of CheY and/or CheZ may serve as the signal(s) themselves. Feedback processes that enable adaptation are indicated by dashed lines: some form of the integrated signal regulates the activity of CheB, and CheR activity is directly influenced by the ligation status (bound vs. unbound) of the transducers. Whether the components of the flagellum or switch also participate in the adaptation mechanism has not been established.

How might the integrating device function? We find this to be one of the most perplexing aspects of bacterial chemotaxis, since there is virtually no experimental information available. We imagine distinct positive and negative signals. These could also correspond to high and low levels of a single entity. How can these be compared and an output produced that reflects the integrated sum of all receptors in the positive and negative signaling states? The simplest mechanisms appear to involve the production of a pool of effector molecules that serve as the integrating device. This pool could be modulated by chemoeffector binding events at the transducers. Alternatively, the transducers could serve as enzymes that generate and/or degrade the pool of effector molecules in analogy with the action of G protein systems in higher organisms.[289–291] It is difficult to see how direct binding events can help to give integration over time, although it does provide a means to integrate over all receptors. This follows because we would again expect statistical fluctuations in the bound complexes to drastically increase the noise of the system. A potential means to reduce the noise is to make the binding events nonindependent, i.e., cooperative. Since we have little information suggesting cooperative interactions of this sort, we shall not consider direct binding events as the signal. However, if the transducers act as enzymes on some effector pool, integration over both time and all transducers follows directly.

For example, suppose RA and RM of the adapting box correspond to two forms of the transducer with catalytic activities or that regulate the activities of other enzymes that generate and degrade some effector E from some buffered precursor P_0. This idea was pres-

ented by Macnab and Koshland[36] to account for the rapid and slow steps observed during excitation and adaptation. We assume that each transducer species has the potential for such activities associated with its conserved cytoplasmic signaling domain.

The following scheme is suggested:

$$P_0 \xrightarrow{[RA]} E \xrightarrow{[RM]} \text{products} \quad (2)$$

If RA is saturated with P_0 and if the amount of E is such that RM is always saturated and we assume that the turnover number is the same to form and degrade E, the following rate equation describes the situation:

$$\frac{dE}{dt} = k[RA] - k[RM] = k([RA] - [RM]) \quad (3)$$

by integration to establish the time course of E

$$E(t) = k\int_0^t (RA - RM)dt \quad (4)$$

In our model, the effector E will go on to control the sense of flagellar rotation, perhaps via CheY, and will feed back on the methylation/demethylation system to control the activity of at least the methylesterase. This will make the detailed solution of eq 3 complicated and of little value. However, we see that such a scheme integrates the difference between the positive and negative signaling forms of the receptor and stores this information in the concentration of the effector molecule E. As mentioned in an earlier section, Segall, Ishihara, and Berg showed that tumbly signal was produced at a longer distance in *cheZ* mutant cells.[41] They suggested that CheZ may function to break down the tumbly signal. This view is consistent with the model presented here.

Clearly, the model we propose here speculates about events for which little or no experimental data yet exist. However, it does have some of the general features we believe are required for the chemotaxis system to function and may be a useful framework to use when thinking about chemotaxis.

These ideas are not unique to us. They have been discussed by many members of the chemotaxis scientific community—usually late at night after several libations. While they should not be blamed if these ideas prove worthless, any credit for useful ideas should be spread over the entire chemotaxis community.

Note Added in Proof. We draw the attention of the reader to two important papers published while this article was in press. The publication (Meister, M.; Lowe, G.; Berg, H. C. *Cell (Cambridge, Mass)* **1987**, *41*, 643) demonstrates that some properties of the flagellar motor observed under high load conditions (tethered cells) are quite different from those determined under low load conditions (free swimming cells). A publication (Wolfe, A. J.; Conley, P.; Kramer, T.; Berg, H. C. *J. Bacteriol.* **1987**, *169*, 1878) demonstrates a clear role for CheA and CheW in the chemotaxis signaling pathway and further elucidates the antagonistic roles of CheY and CheZ.

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