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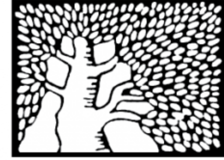

Signal transduction in bacterial chemotaxis

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Signal Transduction in Bacterial Chemotaxis

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I. INTRODUCTION

The first systematic studies of bacterial chemotaxis were by Engelmann [1882] and by Pfeffer [1881–1885, 1888]. Interest in the field was strong until Metzner [1931] published his studies, after which it was all but forgotten. In 1965, Julius Adler renewed interest in this behavior [Adler, 1965], since when the studies of

this topic can be divided into several relatively short research periods. The first period was devoted mainly to identification of attractants and repellents. The subsequent periods dealt with isolation of receptors for the stimulants, with identification of genes and gene products involved in chemotaxis, with the function and structure of the receptors, and with the biochemical events involved in adaptation to chemotactic stimuli. Studies of the mechanism of bacterial movement, without which chemotaxis cannot occur, were carried out in parallel. Throughout these periods, the mechanism of sensory transduction in bacterial chemotaxis was a major subject of scientific curiosity, but it remained a mystery until very recently. The current period of research, which is just at its outset, may perhaps be defined as the period of revealing the mechanism of sensory transduction.

The focus of this review is on transduction of sensory signals in bacterial chemotaxis. Other aspects of chemotaxis that are directly related to signal transduction will not be dealt with here. Adler [1987], Stewart and Dahlquist [1987], Macnab [1987b], Ordal [1985] and Simon et al. [1985] reviewed in recent years the field of bacterial motility and chemotaxis, in general. Parkinson [1981] and Parkinson and Hazelbauer [1983] reviewed the genetics of bacterial chemotaxis. Berg et al. [1982], Eisenbach [1990], Iino [1985], Macnab [1987a], Macnab and Aizawa [1984], and Macnab and DeRosier reviewed the structure and mechanism of function of the bacterial flagellar motor.

This review was completed in May 1989. Subsequent references had been submitted to me as preprints prior to this date.

II. BACKGROUND

A. Bacterial Behavior

Chemotaxis is an oriented movement toward favorable substances (attractants) and away from unfavorable ones (repellents). Bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, and *Streptococcus* sp. chemotax by modulation of two main swimming patterns: a run, which is a smooth swimming in rather straight lines, and a tumble, which is a chaotic angular motion with no net translational movement [Berg and Brown, 1972; Macnab and Koshland, 1972]. In the absence of a stimulus (an attractant or a repellent), the tumbles are short but frequent. (The tumbling frequency varies from strain to strain, being about one tumble per1–5 sec.) When the bacteria sense an increase in the concentration of an attractant, the runs are prolonged and the tumbles are depressed. When they sense a large increase in the concentration of a repellent or a large decrease in the concentration of an attractant the tumbles are enhanced. (Other modulatable swimming patterns in other bacteria are forward and backward swimming, as in *Halobacterium halobium* [Hildebrand and Dencher, 1975; Spudich and Stoeckenius, 1979; Alam and Oesterhelt, 1984], or a run and a stop, as in *Rhodobacter sphaeroides* [Armitage and Macnab, 1987] or *Rhizobium meliloti* [Gotz and Schmitt, 1987].)

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decrease in an attractant c
and pause more frequently
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The swimming of the bacteria results from rotation of flagella attached to the cells [Berg and Anderson, 1973; Silverman and Simon, 1974]. Peritrichously flagellated bacteria (bacteria whose flagella are randomly distributed around the cell) usually have 5 to 12 flagella per cell. The rotation of the flagella, unlike eukaryotic flagella [Adler, 1985], is carried out by a motor located at the base of each flagellum and embedded in the cytoplasmic membrane [DePamphilis and Adler, 1971; for reviews, see Berg et al., 1982, and Macnab and Aizawa, 1984]. The primary driving force of the motor is the proton-motive force ($\Delta\tilde{\mu}_{H^+}$) [Larsen et al., 1974a; Thipayathasana and Valentine, 1974; Manson et al., 1977; Matsuura et al., 1977; Glagolev and Skulachev, 1978; Shioi et al., 1978; Khan and Macnab, 1980b; Manson et al., 1980; Berg et al., 1982; Ravid and Eisenbach, 1984b], defined as $\Delta\tilde{\mu}_{H^+} = \Delta\psi - Z\Delta pH$ (where $Z = 2.3RT/F = 59$ mV at 25°C); and $\Delta\psi$ is membrane potential). In alkalophilic *Bacillus*, sodium ions rather than protons drive the motor [Hirota et al., 1981].

By tethering bacterial cells of wild-type *E. coli* to glass by their flagella, it became clear that the motor alternates between counterclockwise rotation, clockwise rotation, and brief pauses [Silverman and Simon, 1974; Larsen et al., 1974b; Lapidus et al.,1988]. When wild-type bacteria detect an increase in the concentration of an attractant (which is equivalent to a decrease in the concentration of a repellent), their flagella rotate counterclockwise and do not pause; when they detect an increase in the concentration of a repellent (or a decrease in an attractant concentration), their flagella gain a clockwise bias and pause more frequently [Larsen et al., 1974b; Lapidus et al., 1988]. The result of counterclockwise rotation in bacteria like *E. coli* and *S. typhimurium* is a run [Anderson, 1975; Macnab, 1977], and the result of clockwise rotation [Macnab and Ornston, 1977] and of pausing [Eisenbach et al., 1990b] is a tumble. It was recently shown that a separate signal for pausing is unlikely [Eisenbach et al., 1990b]. Therefore, only signals for switching the direction of rotation will be dealt with in this review.

The attractants for *E. coli* and *S. typhimurium* are primarily amino acids, sugars, and dipeptides [see Adler, 1978, Koshland, 1980, and Macnab, 1987b, for elaborated reviews]. The receptors for most of these attractants have been identified and thoroughly investigated. The repellents for these species are weak organic acids, certain cations, certain amino acids, and other compounds such as indole and glycerol. Table I shows selected attractants and repellents of *E. coli*. Most of the *E. coli* stimulants are effective also for *S. typhimurium* [see Macnab, 1987b, for a comparison]. In this context it should be noted, however, that repellents for a given species may act as attractants for another species. A few examples are shown in Table II. Other stimuli are oxygen (acting as either an attractant or a repellent, depending on its concentration; see

TABLE I. Selected Attractants and Repellents of *E. coli*

Stimulant	Att/rep	Receptor			MCP[†]	References
		Identity	Location	K_d or K_m*		
L-Serine	Att	MCP I	Membrane	5 μM	I	Mesibov and Adler [1972], Clarke and Koshland [1979], Hedblom and Adler [1980, 1983]
L-Alanine	Att	MCP I	Membrane	?	I	Mesibov and Adler [1972], Hedblom and Adler [1983]
L-Cysteine	Att	MCP I	Membrane	?	I	Mesibov and Adler [1972], Hedblom and Adler [1983]
Glycine	Att	MCP I	Membrane	?	I	Mesibov and Adler [1972], Hedblom and Adler [1983]
L-Aspartate	Att	MCP II	Membrane	5 μM	II	Mesibov and Adler [1972], Springer et al. [1977], Clarke and Koshland [1979], Hedblom and Adler [1983]
L-Glutamate	Att	MCP II	Membrane	?	II	Mesibov and Adler [1972], Hedblom and Adler [1983]
Maltose	Att	MBP	Periplasm	2 μM	II	Adler et al. [1973], Springer et al. [1977], Manson et al. [1985]
D-Galactose	Att	GBP	Periplasm	0.5 μM	III	Adler et al. [1973], Zukin et al. [1977b], Kondoh et al. [1979], Hazelbauer and Harayama [1979]
D-Ribose	Att	RBP	Periplasm	0.1 μM	III	Adler et al. [1973], Kondoh et al. [1979], Hazelbauer and Harayama [1979]
D-Glucose	Att	GBP	Periplasm	0.2 μM	III	Zukin et al. [1977b]
D-Glucose	Att	E_{II}	Membrane	20 μM	None	Adler and Epstein [1974], Postma and Lengeler [1985]
D-Mannose	Att	E_{II}	Membrane	30 μM	None	Adler et al. [1973], Postma and Lengeler [1985]
Dipeptides	Att	MCP IV	Membrane	?	IV	Manson et al. [1986]
Oxygen	Att	cyt *o* and *d*	Membrane	0.7 μM	None	Laszlo et al [1984], Shioi et al. [1987]
Oxygen	Rep	?	?	1 mM	None	Shioi et al. [1987]
Acetate	Rep	MCP I	Membrane	pH dependent	I	Repaske and Adler [1981], Kihara and Macnab [1981]
Benzoate	Rep	MCP I	Membrane	pH dependent	I	Repaske and Adler [1981], Kihara and Macnab [1981]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

D-Glucose	Att	E_{II}	Membrane	20 μM	None	Adler and Epstein [1974], Postma and Lengeler [1985]
D-Mannose	Att	E_{II}	Membrane	30 μM	None	Adler et al. [1973], Postma and Lengeler [1985]
Dipeptides	Att	MCP IV	Membrane	?	IV	Manson et al. [1986]
Oxygen	Att	cyt *o* and *d*	Membrane	0.7 μM	None	Laszlo et al [1984], Shioi et al. [1987]
Oxygen	Rep	?	?	1 mM	None	Shioi et al. [1987]
Acetate	Rep	MCP I	Membrane	pH dependent	I	Repaske and Adler [1981], Kihara and Macnab [1981]
Benzoate	Rep	MCP I	Membrane	pH dependent	I	Repaske and Adler [1981], Kihara and Macnab [1981]
Indole	Rep	MCP I	Membrane	?	I	Tso and Adler [1974], Springer et al. [1977], Eisenbach et al. [1990a]
Leucine	Rep	MCP I	Membrane	?	I	Tso and Adler [1974], Springer et al. [1977], Eisenbach et al. [1990a]
Ni^{2+}	Rep	MCP II	Membrane	?	II	Tso and Adler [1974], Springer et al. [1977], Eisenbach et al. [1990a]
Co^{2+}	Rep	MCP II	Membrane	?	II	Tso and Adler [1974], Springer et al. [1977], Eisenbach et al. [1990a]
Glycerol	Rep	?	?	0.2 M	Anyone	Oosawa and Imae [1983]
Isobutanol	Rep	MCPs?	?	~20 mM	Anyone	Tso and Adler [1974], Eisenbach et al. [1990a]

*K_d is the dissociation constant. K_m is the "behavioral Michaelis constant" [Macnab, 1987b], i.e., the stimulant concentration yielding half-maximal response. K_m is especially important in the case of repellents for which receptors have not been identified. The values of K_d and K_m are similar.

†Abbreviations: cyt, cytochrome; GBP, galactose-binding protein; MBP, maltose-binding protein; MCP, methyl-accepting chemotaxis protein; RBP, ribose-binding protein; E_{II}, enzyme II of the phosphotransferase system (PTS); att, attractant; rep, repellent.

TABLE II. Stimuli With Different Functions in Different Species

Stimulus	Attractant for	Repellent for	References
Phenol	*E. coli*	*. typhimurium*	Lederberg [1956], Tsang et al [1973] Koshland [1980], Imae et al. [1987] Tsang et al. [1973]
Leucine	*B. subtilis*	*E. coli, S. typhimurium*	Tsang et al [1973], Tso and Adler [1974], Ordal and Gibson [1977]
Valine	*B. subtilis*	*E. coli, S. typhimurium*	Tsang et al [1973], Tso and Adler [1974], Ordal and Gibson [1977]
Tryptophan	*B. subtilis, Chromatium vinosum*	*E. coli, S. typhimurium, R. sphaeroides*	Tsang et al [1973], Tso and Adler [1974], Ordal and Gibson [1977], Armitage et al. [1979]
Acetate	*C. vinosum*	*E. coli, S. typhimurium, R. sphaeroides*	Tsang et al [1973], Tso and Adler [1974], Armitage et al. [1979]
Benzoate	*Pseudomonas putida*	*E. coli*	Tso and Adler [1974], Harwood et al. [1984]

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In the process of identify
chemotaxis machinery, gen
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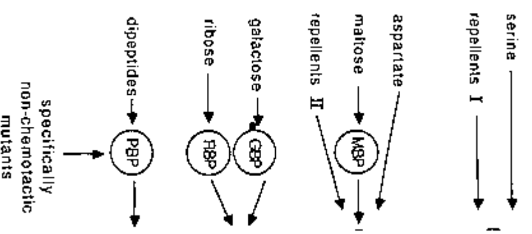

Fig. 1. Schematic representatio
mediated stimuli are drawn. The
maltose-, galactose-, ribose-, and
the genes that encode for MCPs I
marked at the bottom.

section IV.C.),pH (see section IV.D.), light (see the chapter by Bogomolni and Spudich, this volume), and temperature (see section VI.).

In the process of identifying the receptors and the other components of the chemotaxis machinery, genetics played a major role. By isolation of mutants with defects in chemotaxis toward specific or multiple chemicals, it became possible to identify genes and gene products involved in reception and in transduction of the sensory signals [see Adler, 1978, and Parkinson, 1981, for reviews]. Based on the phenomenological behavior of the so-called specifically, multiply, and generally nonchemotactic mutants, it became clear that the sensory transduction system in bacteria is a convergent system of the type shown in Figure 1. Thus the defective genes in the specifically nonchemotactic mutants code for specific receptors; the defective genes in the multiply nonchemotactic mutants code for methylatable, transmembrane proteins called methyl-accepting chemotaxis proteins (MCPs), sensory transducers, or—for some attractants (Table I)—receptors; and the generally nonchemotactic mutants have defects in the *che* genes (*che* for chemotaxis; Table III). The products of the latter genes are located predominantly in the cytoplasm [Ridgway et al., 1977].

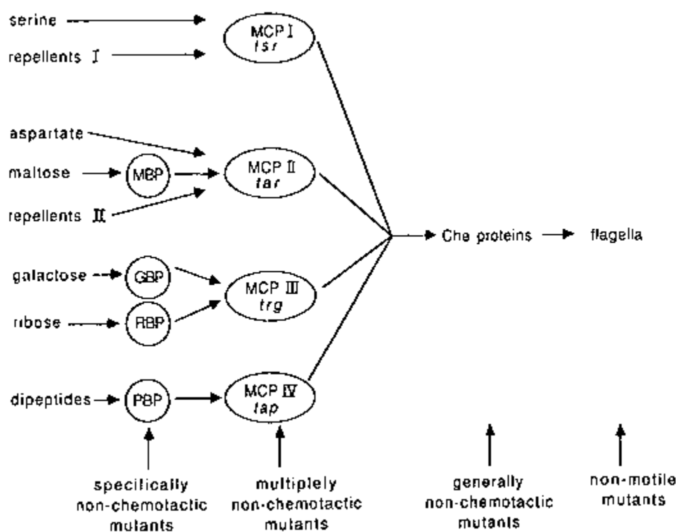

Fig. 1. Schematic representation of the information flow in chemotaxis of *E. coli*. Only MCP-mediated stimuli are drawn. The repellents are listed in Table I. MBP, GBP, RBP, and PBP are maltose-, galactose-, ribose-, and peptide-binding proteins, respectively. *tsr, tar, trg* and *tap* are the genes that encode for MCPs I–IV, respectively. The types of mutants found at each stage are marked at the bottom.

TABLE III. Cytoplasmic Che Proteins

Protein	Role in	Polymeric form	Monomeric M_r*	No. of molecules per cell†	References‡	Comments
CheA	Excitation	Dimer	71,000 60,000	?	1–4, *5*, 6–*8*, 25	There are long and short polypeptides of CheA. The protein is a kinase
CheB	Adaptation	?	37,500	~200	1–3, *5*, 9–*11*, 12	N-terminal of CheB is homologous to the entire length of CheY. CheB is methylesterase
CheR	Adaptation	Monomer	33,000	~200	2, 3, *5*, 14–*16*	CheR is methyltransferase. It is a very slow enzyme
CheW	Excitation	Monomer	18,000	?	2, 3, *5*, *17*, 25	Has a concensus nucleotide-binding site
CheY	Clockwise rotation	Monomer	14,000	~4,000	2, 3, *5*, *11*, 18, *19*, 20, 21	CheY is homologous to the N-terminal of CheB
CheZ	Counterclockwise rotation	Polymer (≥20 monomers)	24,000	~700	2, 3, *5*, 22–24	Very acidic, methylatable protein. Has phosphatase activity

*The values given are for *E. coli*.

†Unless otherwise specified, the calculation is based on the molecular stoichiometry between *tar* and *che* gene products [DeFranco and Koshland, 1981] and the number of Tar (MCP II) molecules in a cell of *E. coli* [Hazelbauer et al., 1982].

‡Reference numbers in italics include the seqeunce analysis of the protein. The references are: 1, Parkinson [1976]; 2, Ridgway et al. [1977]; 3, Silverman and Simon [1977b]; 4, Smith and Parkinson [1980]; 5, Mutoh and Simon [1986]; 6, Hess et al. [1987]; 7, Macnab [1987b]; 8, Stock et al., [1988a]; 9, Stock and Koshland [1978]; 10, Yonekawa et al. [1983]; 11, A. Stock et al. [1985]; 12, Simms et al. [1985]; 13, Goy et al. [1978]; 14, Goy et al. [1977]; 15, Springer and Koshland [1977]; 16, Simms et al. [1987b]; 17, Stock et al. [1987a]; 18, Clegg and Koshland [1984]; 19, Matsumura et al. [1984]; 20, Ravid et al. [1986]; 21, Wolfe et al. [1987]; 22, A. Stock and Stock [1987]; 23, Stock et al. [1987b]; 24, Stock [1988]; 25, Gegner and Dahlquist [1991] (this information was added after completion of the manuscript).

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B. Sensory Transduction

The chemotaxis machin
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The final target of the sensory signals is presumably the "switch" of the motor, i.e., the *fliM (cheC)*, the *fliG (cheV)*, and the *fliN* gene products [Parkinson et al., 1983; Yamaguchi et al., 1986], located at, or associated with, the cytoplasmic membrane [Ravid and Eisenbach, 1984a]. (The gene names are according to the universal nomenclature for chemotaxis [DeFranco and Koshland, 1981] and flagellar [Iino et al., 1988] genes.) These proteins were assigned as components of the presumed switch, because a mutation in any given gene of this group yielded both counterclockwise-biased and clockwise-biased mutants. This is not the case with other *che* mutants, where any defect in a given gene leads almost always to a mutant with the same bias: exclusive counterclockwise rotation (*cheA, cheR, cheW, cheY* mutants) or a predominant clockwise rotation *(cheB, cheZ)* [Parkinson, 1981]. (One exception was the isolation of a *cheZ* mutant with a counterclockwise bias [Yamaguchi et al., 1986].)

It should be noted that there may be basic differences in the chemotaxis machinery, in general, and in the mechanism of sensory transduction, in particular, among different bacterial species. This is primarily so between small (a few microns in length) and large bacterial species. In the large species, the sensory signal is expected to travel much longer distances. Because *E. coli* and *S. typhimurium* (classified as small bacteria) are the best-studied bacterial species, the information provided will be for these two species, unless otherwise mentioned. (See Ordal and Nettleton [1985] for a review of the chemotaxis machinery in *B. subtilis*.) When relevant information on large bacterial species is available, as in the case of excitatory signaling, a separate section will be devoted to those species.

B. Sensory Transduction

The chemotaxis machinery probably involves various sensory signals, and each signal may undergo several steps of transduction or be a composite of several signals. Thus, when dealing with sensory transduction, one should define the sensory signal in terms of space (from where to where in the bacterial cell the signal is transduced), cellular components (the origin and target of the signal), and function. In this review, I shall try to distinguish between the various signals spatially and functionally and then try to examine how these signals are integrated into a functional, harmonic machinery.

As shown in Figure 2, there are at least two, sometimes three, spatial regions between the receptor and the switch of the motor. For those stimuli that interact directly with the MCP or with other membrane receptors (Table I), sensory signaling involves two steps, a signal across the cytoplasmic membrane and a signal within the cytoplasm to the switch–motor complex. For those stimuli whose receptors are periplasmic binding proteins, an additional transduction step within the periplasm precedes the other steps. I shall not deal in

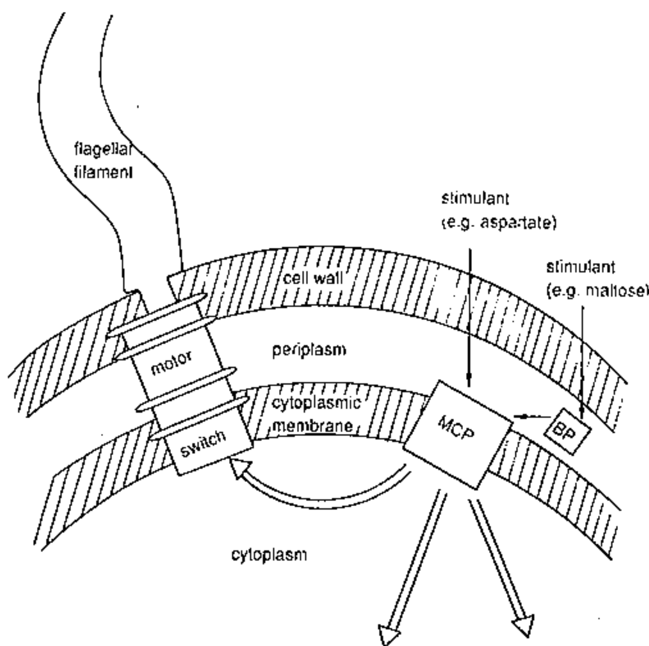

Fig. 2. Spatial regions of information flow. Single arrows represent direct interaction; double arrows, indirect interaction. BP, binding protein. The scheme is not drawn to scale.

this review with other theoretical modes of interaction between the receptors or the MCPs and the motor, such as direct interactions or signaling via lateral diffusion in the periplasm or cytoplasmic membrane. These possibilities have been reviewed elsewhere and shown to be improbable [Eisenbach et al., 1985].

Two distinct processes are known to be involved in sensory transduction in bacteria: excitation and adaptation [see Springer et al., 1979, for review]. The excitation process involves the first or the more rapid signaling process between the receptor and the switch–motor complex. In wild-type strains the excitation is observed within 0.2 sec in response to a large step change in the concentration of a stimulant [Segall et al., 1982] or within about a second in response to brief and very small changes in the concentration [Block et al., 1982; Segall et al., 1986]. The adaptation process prompts a behavior similar to the unstimulated behavior of the bacteria. The bacteria can then respond again to stimuli, even though the original stimulant is still present. In comparison with the excitation process, adaptation is a much slower process in response to a large step stimulus, but a comparable one in response to a brief and small stimulus [Block et al., 1982]. The excitation and adaptation processes might,

in principle, be initiated by
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III. SENSORY SIGNALI

This section deals with
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The analysis revealed the
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ten van der Waals contacts

in principle, be initiated by a single, common sensory signal, or each of them may be triggered by separate signals. These alternatives will be dealt with in detail in the following sections. Eventually, other types of taxis in bacteria will be compared with bacterial chemotaxis from the point of view of sensory transduction.

III. SENSORY SIGNALING THROUGH THE PERIPLASM

This section deals with those stimuli whose receptors are periplasmic (cf. section II and Table I). The issue of signal transduction via the periplasm is intriguing in light of evidence that the periplasmic space is rather a gel-like material [Hobot et al., 1984] and that the rate of lateral diffusion of proteins such as the maltose-binding protein (the maltose receptor) or the galactose-binding protein (the galactose receptor) in the periplasm is very low (the lateral diffusion coefficient is 100-fold lower than would be expected for such proteins in the cytoplasm and 1,000-fold lower than in aqueous medium [Brass et al., 1986]).

It seems quite established that two steps are involved in sensory transduction within the periplasm: 1) conformational changes of the periplasmic receptor (the sugar- or peptide-binding protein) as a result of attractant binding and 2) binding of the formed complex to the appropriate MCP. (This is sketched in Fig. 3 for the attractant galactose.) Evidence in support of the first step was given by Boos et al. [1972] and by Zukin et al. [1977a] for the galactose receptor and by Szmelcman et al. [1976] and by Zukin [1979] for the maltose receptor. The evidence is based on attractant- stimulated changes in the electrophoretic mobility of the binding protein, changes in the apparent dissociation constants, and changes in the fluorescence of the tryptophan residues of the receptor and in the fluorescence of an extrinsic fluorophore, 5-(iodoacetamido)fluorescein, attached at a single methionine residue of the receptor. Evidence in favor of the second step came from genetic analysis of mutants in the maltose-binding protein. Kossmann et al. [1988] found two *malE* mutants that are specifically nonchemotactic toward maltose in which residue 53 or 55 of the maltose-binding protein was replaced by another amino acid. These chemotactic defects were suppressed by point mutations in *tar*, the gene that codes for MCP II. These results indicate a physical interaction between the maltose-binding protein and MCP II. X-ray crystallography was carried out for the galactose-binding proteins from a wild-type strain and from a mutant whose receptor binds galactose normally, but the strain is nevertheless specifically nonchemotactic to galactose, presumably because of a defect in the site for binding MCP III. The analysis revealed the possible site in the galactose-binding protein for interaction with MCP III [Vyas et al., 1988]. This study also indicated that ten van der Waals contacts, a salt link, and a positive entropic effect (but no

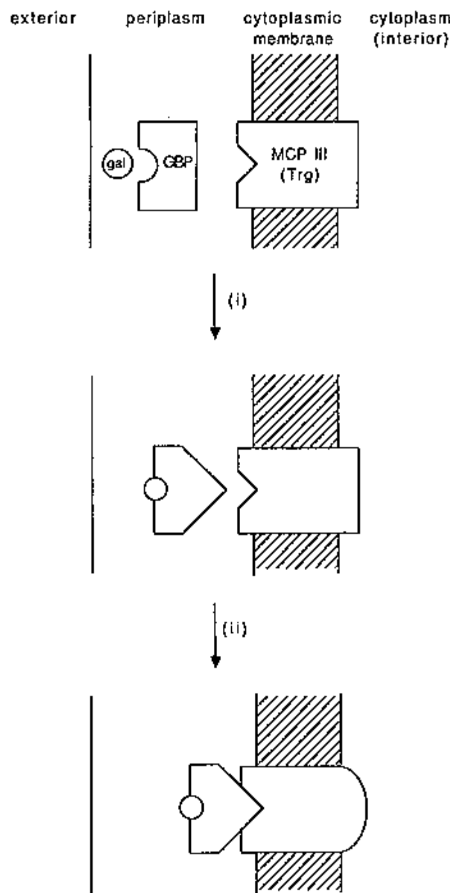

Fig. 3. Schematic representation of the first steps involved in galactose-stimulated signal transduction. Gal, galactose; GBP, galactose-binding protein. The scheme is not drawn to scale.

hydrogen bonds) comprised the interaction. Supportive evidence for both the first and second steps, i.e., conformational changes and binding, was supplied for the maltose receptor by Koiwai and Hayashi [1979]. These investigators made an affinity column containing purified maltose receptor and then used this column to chromatograph the solubilized membrane fraction of *E. coli*. They found that the receptor interacted with MCP II only in the presence of maltose. Such an interaction was not found with MCP I (cf. Fig. 1). In a similar approach, Richarme [1982a] studied the binding of the purified maltose receptor to membrane vesicles of *E. coli*. He found that the binding was dependent on the presence of maltose in the medium and on the presence of

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MCP II in the membrane; membrane vesicles from mutants defective in the *tar* (but not *tsr*) gene product did not bind the maltose receptor even in the presence of maltose. Another finding was that imposition of membrane potential, positive outside, across the vesicles membrane by a valinomycin-induced potassium efflux significantly increased the binding of the receptor to the vesicles [Richarme, 1982a]. This requirement of membrane potential for binding is, almost always, naturally maintained in vivo by the proton-motive force [Padan et al., 1976].

Interestingly, but not necessarily correlated, the inverse relationship between binding and membrane potential has also been found: Binding of an attractant to the receptor causes a transient increase in the membrane potential of intact *E. coli* cells [see Eisenbach, 1982, 1983, for summaries of previous reports and for resolving apparently conflicting data]. By using different probes and techniques, it was shown that the measured change is a true increase in membrane potential rather than a change in the surface charge of the membrane [Eisenbach et al., 1984]. This increase in membrane potential, or hyperpolarization, was found to occur in response to each of the nine different attractants tested [Eisenbach, 1982]. The hyperpolarization was thoroughly investigated for the attractant D-galactose and its nonmetabolizable analog D-fucose. It was found to be correlated with chemotaxis to galactose rather than with transport of galactose or its metabolism [Eisenbach et al., 1983a] and possibly to be the result of an efflux of the organic cation decarboxylated *S*-adenosylmethionine (M. Eisenbach, unpublished data). The significance of this hyperpolarization or cation efflux is still a mystery. It is puzzling that the hyperpolarization depends only on binding of a substrate to the receptor [Eisenbach et al., 1983a] and not on the binding of the receptor to the MCP, as *trg* mutants do become hyperpolarized by galactose or fucose (M. Eisenbach, unpublished data). Currently there is no obvious explanation for this puzzle.

How can sensory transduction in the periplasm, as described above, occur in view of the low mobility of the receptors within the periplasm? Calculations of Brass et al. [1986], based on the number of protein molecules in the periplasm and on the dimensions of the periplasmic space, have revealed that there may be little or no aqueous space in the periplasm. Brass et al. [1986] therefore suggested that the periplasm may contain semiordered chains of associated binding protein molecules and that substrate molecules may move along these chains of receptor molecules toward the cytoplasmic membrane (three to four protein molecules are sufficient to fill in the distance between the outer and cytoplasmic membranes). Evidence for such association has been already reported [Rasched et al., 1976; Richarme, 1982b; Mowbray and Petsko, 1983]. However, the idea of receptor chains is not obligatory. It appears that even if the diffusion of ligand is buffered, the ligand will cross the periplasm and reach the receptor near the cytoplasmic membrane in a relatively short time.

In either case, when the signal eventually reaches the membrane, the complex galactose–receptor may either interact with the appropriate MCP, Trg (MCP III) in this case, or interact with the membrane components of the transport system, MglA [Harayama et al., 1983; Muller et al., 1985], depending on the location of the receptor.

IV. SENSORY TRANSDUCTION ACROSS THE CYTOPLASMIC MEMBRANE

The versatility of systems that transduce the sensory signal across the cytoplasmic membrane may imply a diversity of transduction mechanisms. I shall therefore deal initially with each system separately.

A. Sensory Transduction by Methyl-Accepting Chemotaxis Protein (MCP)

The MCP serves three functions in bacterial chemotaxis: It binds the ligand (either the attractant or the attractant–receptor complex; see Table I), it sends an excitatory signal into the cytoplasm, and it is involved in adaptation to attractants or repellents by being methylated or demethylated, respectively (see section V.B.). Sequencing of the genes that code for the MCPs allowed the determination of the regions that are responsible for these functions. Each MCP molecule contains two stretches of hydrophobic residues, one near the N terminus and the other further down the sequence at a distance equivalent to 40% of the polypeptide length [Boyd et al., 1983; Krikos et al., 1983; Russo and Koshland, 1983; Bollinger et al., 1984]. Assuming that these stretches are the transmembrane segments of the protein, Krikos et al. [1983] and independently Russo and Koshland [1983] suggested a model for the MCPs (Fig. 4), according to which the domain between these two segments is on the periplasmic side of the membrane (residues 33–190 of Tsr), and the domain between the second hydrophobic stretch and the C terminus (residues 216–end) is on the cytoplasmic side of the membrane. The periplasmic segment is considered to be the ligand-binding domain of the MCP. This domain is the segment with the largest divergence among the four known MCPs (only 26% homology), which fits well with the requirement for specificity of the binding sites. In line with this model are 1) studies with chimeric MCPs exhibiting ligand specificities [Krikos et al., 1985], 2) the finding that a proteolytic fragment of the Tar protein from residues 1 to 259 retains the aspartate-binding function [Mowbray et al., 1985], and 3) the discovery that all MCP mutants with defects in chemotaxis to specific attractants have single amino acid substitutions in this segment only [Park and Hazelbauer, 1986a; Lee et al., 1988; Wolff and Parkinson, 1988]. The cytoplasmic portion of the MCP contains the methylation sites and the binding sites for the enzymes methyltransferase and methylesterase (see section V.B.1.). The stretches that contain the pre-

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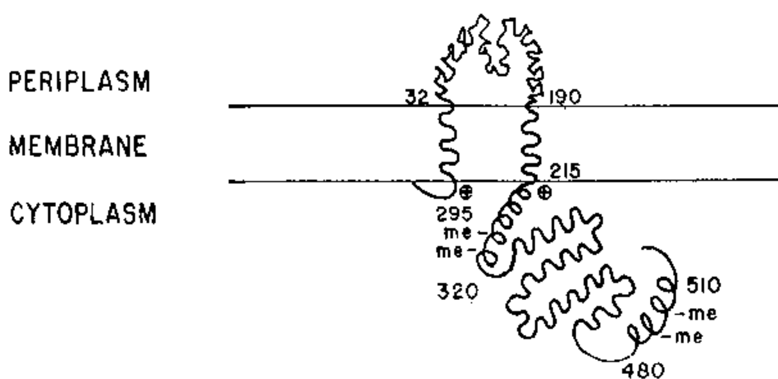

Fig. 4. A model for MCP. Me, Sites of methylation. See text for details. [Reproduced from Krikos et al., 1983, with permission of the publisher.]

sumed binding sites are highly conserved (91–100% identity among the four MCPs), as would be expected for stretches on different proteins that interact with a common enzyme. This part of the model is supported by 1) site-directed mutagenesis affecting specific residues in the cytoplasmic portion of the MCP [Terwilliger et al., 1986b; Nowlin et al., 1988], 2) amplification of a mutational change of a single amino acid in this portion [Park and Hazelbauer, 1986b], 3) studies of proteolytic fragments of this region [Mowbray et al., 1985; Nowlin et al., 1987], 4) studies with hybrid MCPs [Slocum et al., 1987], and 5) the phenotypic behavior and the molecular activities retained in bacteria containing a truncated *tar* gene, encoding only the C-terminal subdomain of the MCP [Oosawa et al., 1988b]. This portion of the MCP should be responsible for signaling to the subsequent components of the chemotaxis machinery. And indeed, nearly all MCP mutations with defects in signaling were located in the cytoplasmic portion of the MCP [Mutoh et al., 1986; Kaplan and Simon, 1988; Ames and Parkinson, 1988].

In a very elegant study involving site-directed cross-linking, Milligan and Koshland [1988] have shown that MCP II is a dimer. This conclusion probably holds also for other MCPs. What are the possible ways by which an MCP dimer can transduce from its periplasmic to its cytoplasmic face the information that a ligand is bound there? By analogy to other systems, several modes may be possible in principle: 1) opening of an ion channel, as in the acetylcholine receptor [Changeux and Revah, 1987]; 2) association of monomers (or dissociation of dimers), as suggested for the insulin receptor [Heffetz and Zick, 1986] and the epidermal growth factor (EGF) receptor [Yarden and Schlessinger, 1987]; 3) major conformational change of the protein or vertical displacement

of the protein within the membrane [Borochov and Shinitzky, 1976]. What is known about these modes of function?

1. Specific ion channels have not been found in *E. coli* or in any other small bacterium. The only channels found in *E. coli* were of two types: pressure-activated ion channels [Martinac et al., 1987] that most likely reside in the outer membrane, not in the cytoplasmic membrane [Saimi et al., 1988] and therefore cannot participate in signal transduction across the cytoplasmic membrane; and voltage-activated channels having no selective permeability [Saimi et al., 1988]. Also the latter channels appear to be irrelevant for chemotaxis, because they open more frequently upon membrane depolarization [Saimi et al., 1988], whereas such depolarization or any other consistent change in membrane potential does not occur in *E. coli* in response to attractants or to repellents (other than those that lower intracellular pH) [Snyder et al., 1981], unless respiratory inhibitors are present [Eisenbach, 1982]. Furthermore, indirect studies of whether ion channels play a role in chemotaxis yielded negative results. Thus, if ion channels were involved in sensory transduction, systematic variations of the ionic composition of the medium in which the bacteria are suspended should have affected chemotaxis [Jaffe, 1979]. This was not the case: Neither elimination of specific ions from the suspending medium [Adler, 1973] nor addition of specific ions to perturb passive efflux through ion channels [Eisenbach et al., 1985] affected the chemotaxis process.

2. Milligan and Koshland [1988] have shown that the signaling is not by a change in the aggregation state of the MCP. MCP II was found to retain its dimeric state even after the addition of saturating concentrations of its ligand, the attractant aspartate. On the contrary, the observed effect of aspartate was stabilization of the dimer: It prevented the rapid exchange between the subunits of the MCP.

3. Unlike the first two possibilities, conformational changes of the MCP do appear to play a major role in signaling. Overproduction of the C-terminal half of MCP II of wild-type *E. coli* and of two *tar* mutants, one locked in "smooth swimming signal" and the other in "tumbling signal," revealed major differences in the oligomeric sizes, the susceptibility to degradation, and the ion-exchange behavior of the three fragments, indicative of different conformations for each of the signaling modes [Kaplan and Simon, 1988]. In a different approach, Falke and Koshland [1987] constructed seven different mutants by site-directed mutagenesis, each containing a new cysteine residue at a different position in the primary structure of MCP II. They measured the rate of crosslinking two solubilized monomers of MCP II into a dimer via disulfide bonds between the cysteine residues and found that addition of aspartate had a large effect on the rate of formation of the disulfide bonds. This again indicates that binding of ligand, in this case the attractant aspartate, brings about

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B. Sensory Transductioı

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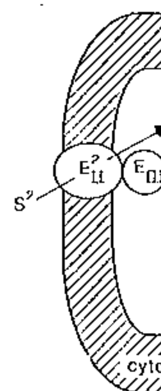

Fig. 5. A model for the mech
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[Postma and Lengeler, 1985].
S, sugar; HPr, histidine-contai
adenylate cyclase. Based on Pe
communication with J. Lengele

major changes in the conformation of the protein. It remains to be seen whether these conformational changes, stimulated at the periplasmic side of the membrane and expressed throughout the protein molecule, are sufficient to transmit the sensory signal to the next target or whether vertical displacement of the protein within the membrane also occurs as a result of the conformational changes.

B. Sensory Transduction by the Phosphotransferase System

The phosphoenolpyruvate-dependent carbohydrate:phosphotransferase system (PTS) enzymes II are membrane proteins that transport certain hexoses, hexosamines, polyhydric alcohols, and disaccharides [Postma and Lengeler, 1985]. They were identified as the chemoreceptors for these sugars by Adler and Epstein [1974]. Twelve such systems are known. The mechanism of transport is summarized in Figure 5. The following is a description of the main steps [Kaback, 1968; Kundig and Roseman, 1971a,b; Pecher et al., 1983]. Enzyme II is activated by phosphorylation, carried out by two cytoplasmic protein kinases, enzyme I and histidine-containing protein (HPr), with phosphoenolpyruvate as the phosphate donor. In the case of the D-glucose and a plasmid-

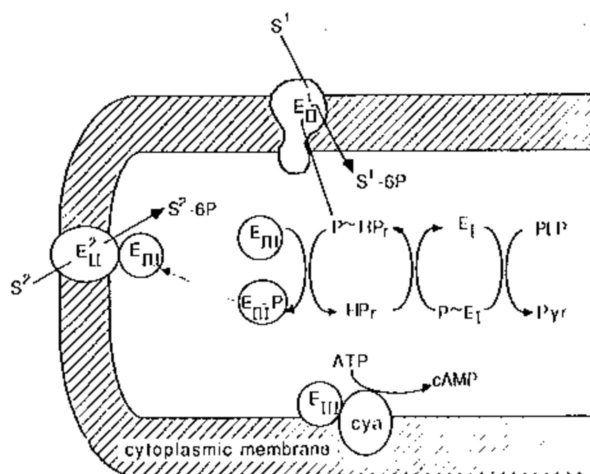

Fig. 5. A model for the mechanism of sugar transport by the PTS system. See text for details. S^1 and E^1_{II} are the sugars and enzymes II, respectively, involved in the transport of *N*-acetylglucosamine, trehalose, mannose, galacticol, fructose, glucitol, mannitol, β-glucosides, L-sorbose, dihydroxyacetone, and lactose. S^2 and E^2_{II} are involved in the transport of glucose and sucrose [Postma and Lengeler, 1985]. E_I, E_{II}, and E_{III} represent enzymes I, II, and III, respectively; S, sugar; HPr, histidine-containing protein; PEP, phosphoenolpyruvate, Pyr, pyruvate; cya, adenylate cyclase. Based on Pecher et al. [1983], Postma and Lengeler [1985], and on personal communication with J. Lengeler.

encoded sucrose transport system, a third kinase, enzyme III, is required for the phosphorylation. The carbohydrate binds to the phosphorylated enzyme II and then is translocated across the membrane. At the same time the carbohydrate is phosphorylated by the enzyme. Enzyme II is rephosphorylated by the cytoplasmic proteins as before, and a new cycle of transport starts. As shown in Figure 5, there are two classes of enzyme II: enzyme III–dependent enzyme, e.g., enzymes II of glucose and sucrose; and enzyme III–independent enzyme, e.g., enzymes II of manitol and *N*-acetylglucosamine [Postma and Lengeler, 1985]. During the transport of the carbohydrate, a phosphate group is transferred from phosphate–HPr to enzyme III, in the first class, or to the enzyme III-like domain at the C terminus of enzyme II, in the second class. In a second step, the phosphate group is transferred from enzyme III to enzyme II or from the enzyme III-like domain to the enzyme II domain. In each case a histidine–phosphate is involved. In the presence of a substrate, the phosphate group is transferred from the phosphorylhistidine to form substrate- 6-phosphate [Ebner and Lengeler, 1988; Saier et al., 1988; Vogler and Lengeler, 1988]. Like MCP, enzyme II contains hydrophobic (probably membrane-spanning) domains, a substrate-binding site toward the periplasm, and a hydrophilic domain in the cytoplasm [Ebner and Lengeler, 1988; J. Lengeler, personal communication].

The signal transduction system of the PTS across the cytoplasmic membrane is independent of the MCP transduction system. This was shown by studying deletion mutants having no MCPs and no CheR and CheB proteins. Even though these mutants could not respond chemotactically to MCP-recognized stimuli, they did respond to PTS sugars [Niwano and Taylor, 1982; Pecher et al., 1983; J. Lengeler, personal communication]. The functions of enzyme II as a transporter and as a sensory transducer are tightly linked, probably unified. The evidence for this statement can be summarized as follows.

1. All enzyme II–negative mutants for a single carbohydrate that have been isolated were invariably defective in both chemotaxis and transport/phosphorylation [Lengeler et al., 1981]. Several thousand mutants in 10 different systems (D-mannitol, D-glucitol, galactitol, D-glucose, D-mannose, *N*-acetyl-D-glucosamine, D-fructose, β-glucopyranosides, cellobiose, and D-sorbose) have been tested (J. Lengeler, personal communication). Despite intensive searches, no mutant has been found having an enzyme II–catalyzed transport without chemotactic activity or showing a positive chemotaxis while lacking transport activity [Lengeler, 1975; Lengeler et al., 1981].
2. Substrate specificity and affinity are similar in the transport/phosphorylation and chemotaxis processes [Lengeler et al., 1981].

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3. Enzyme II mutants with altered affinity for the substrate have altered K_m values for transport, phosphorylation, and chemotaxis [Lengeler et al., 1981].

A major functional difference between enzyme II and MCP is that in the former it is probably the phosphorylation/dephosphorylation of enzyme II/ enzyme III, not the binding of a substrate, that triggers the chemotactic signal [Lengeler et al., 1981]. This conclusion is based on the observations that no metabolism of the attractant is needed beyond phosphorylation to elicit chemotactic response [Adler and Epstein, 1974; Lengeler et al., 1981], on the one hand, and that binding of the sugar attractant to enzyme II is not always sufficient to bring about chemotaxis (e.g., *pts* mutants cannot respond chemotactically to PTS sugars in spite of the presence of enzyme II in their membrane [Lengeler et al., 1981]), on the other hand. The signal is not the phosphorylated attractant, as is evident from the observation that nonmetabolizable analogs of sugar attractants are chemotactically active, whereas phosphorylated sugars, e.g., glucose-6-phosphate or fructose-6- phosphate, are not active. Thus, in the case of the PTS, it appears that the chemotactic signal originates at the cytoplasmic side of the membrane; the attractant, not the signal, crosses the membrane. A somewhat similar phenomenon was recently found in *Rhod. sphaeroides*, in which uptake [Ingham and Armitage, 1987] and metabolism [Poole and Armitage, 1989] of the attractant are required in order to elicit a chemotactic response.

C. Sensory Transduction via Other Membrane Components

Other membrane components that recognize chemotactic stimuli are the system(s) specific for oxygen and the system that senses changes in proton-motive force. Oxygen is, for the time being, the only substance known to be either an attractant or a repellent for *E. coli* and *S. typhimurium*, depending on its concentration [Shioi et al., 1987]. When the concentration of dissolved oxygen is equal to or lower than the concentration in equilibrium with air ($\leq$0.25 mM), oxygen is an attractant. When the concentration is higher (e.g., when a bacterial suspension is exposed to atmosphere of pure oxygen), oxygen acts as a repellent. The difference between the opposing sensitivities is more than three orders of magnitude: the half-maximal attractant response is at 0.7 μM dissolved oxygen, whereas that of the repellent response is at 1 mM [Shioi et al., 1987]. Based on the different affinities for oxygen, Shioi et al. [1987] concluded that there are distinct receptors for oxygen, one for attraction and one for repulsion.

The receptors for attractant oxygen were identified as cytochromes *o* [Laszlo et al., 1984] and *d* [Shioi et al., 1988]. However, these cytochromes are not receptors in the usual sense of the word, because strict binding of oxygen to

them does not bring about a chemotactic response. It is the metabolism of oxygen or the effect of oxygen on the redox potential of a signaling component [Glagolev and Sherman, 1983] or on the proton-motive force of the cell that initiates the response.

It is possible that the attraction to oxygen is a particular case of a more general one, a protometer, i.e., a hypothetical device that senses changes in proton-motive force [Glagolev, 1980; Taylor, 1983]. It should be noted, however, that there is no direct proof for the existence of such a device. It is also worth mentioning that an increase in the proton-motive force is not the only change in $\Delta\tilde{\mu}_{H^+}$ that causes smooth swimming. A change to the other extreme, i.e., a reduction in the proton-motive force, also causes smooth swimming [Khan and Macnab, 1980a], albeit not an attractant response: There is no adaptation to low proton-motive force; Che^- mutants are affected too [Khan and Macnab, 1980a]; and uncouplers, which reduce the proton-motive force, are repellents, not attractants [Ordal and Goldman, 1975; Shioi et al., 1988]. The reader is referred to Taylor (this volume) for an elaboration of oxygen taxis and proton-motive force-sensing.

The receptor for oxygen that brings about a repellent response has not been identified. This is not surprising in view of the fact that any specific receptor for repellents in the usual sense of the word, i.e., a receptor that initiates a repellent behavior in response to binding of the repellent, has not been identified.

D. Repellent Sensing

Discussing signal transduction via the membrane that leads to a repellent response is not simple because of the lack of knowledge on repellent receptors. As discussed above, such receptors have not been identified, excluding perhaps the sensors for acidification and for weak organic acids, such as formate, acetate, benzoate, and salicylate, which act by lowering the internal pH of the cell [Repaske and Adler, 1981; Kihara and Macnab, 1981].

For both the acidification and the organic acids the sensor was identified as MCP I [Slonczewski et al., 1982; Krikos et al., 1985]. In the case of a change in the external pH, a site in the periplasmic domain of MCP I responds to the pH change [Krikos et al., 1985]. The result is possibly a major conformational change that can be read by the cytoplasmic components of the chemotaxis machinery, as in the case of the attractants serine and aspartate (see section IV.A.). In the case of weak organic acids, the signal is transduced by passive diffusion of the nonionized form of the acid across the membrane. The acid is ionized within the cell, the intracellular pH is lowered, and the change in pH is detected by MCP I. Thus, even for the case of external acidification or weak organic acids, MCP I is not a receptor in the usual or narrow sense of the word. These are pH-sensitive sites, not attractant-binding sites, that bring about the presumed conformational changes of MCP I. In the

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broader sense of the word, however, these sites may be considered as proton (or hydroxyl)-binding sites. By constructing mutants with chimeric MCPs, Kirkos et al. [1985] found that such sites reside on both the periplasmic and cytoplasmic domains of MCP I. MCP II has analogous sites, but these are attractant sites: A decrease in pH (internally or externally) causes an attractant response. The MCP I response is dominant. Therefore, when both MCPs are functional, the observed response is that of a repellent. In a mutant in which MCP I is missing or not functional, the MCP II–mediated attractant response is observed.

What about sensory transduction in the case of other repellents? A definitive answer to this question cannot be given. I shall try, however, to consider possible modes of signaling in view of the available data. A variety of different substances are included in this group of repellents: amino acids such as leucine and valine, indole and its derivatives, alcohols such as isopropanol, ions such as Co^{2+} and Ni^{2+}, and others [Tsang et al., 1973; Tso and Adler, 1974]. A common denominator for all these repellents is not obvious.

On the basis of competition experiments and additivity experiments, both Tsang et al. [1973] and Tso and Adler [1974] concluded that repellent receptors should exist. This conclusion was further supported by the isolation of mutants defective in their response to L-leucine but not to indole [Tso and Adler, 1974]. (Both repellents are detected by MCP I [Table I].) However, because no receptor for the above-mentioned repellents has been identified, we should consider the repellent receptors as putative ones. The lack of positive identification of repellent receptors should not be considered as evidence against their existence. The repellent concentrations required to elicit a chemotactic response are in the millimolar range or even higher. Thus, if repellent receptors exist, their affinity for the ligands (=repellents) is low. Receptors with low affinity for their ligands are difficult to identify. A similar situation exists in the olfactory system, where a large repertoire of odorants can be detected, albeit with a very low affinity. For this reason the issue of whether there are receptors in the olfactory system is still controversial [Lancet and Pace, 1987]. In a different system, in at least one case in which the existence of receptors is unquestionable, the affinity of the yet-unidentified receptors to their ligands is very low. These are the taste receptors and especially the sugar receptors. The concentration of sugar needed to elicit a half-maximal response is in a range as high as 0.5 M(!) [Jakinovich, 1981; Teeter and Brand, 1987]. The chemotactic signal triggered by most of the known repellents is mediated by a specific MCP [Springer et al., 1977]. (An exception is the group of aliphatic alcohols and polyalcohols, the signal to which is mediated by any MCP [Oosawa and Imae, 1983, 1984; Eisenbach et al., 1990a].) Therefore, if indeed there are receptors in the bacterial cell for most of the repellents, these receptors should be either periplasmic or the MCPs themselves (cf. Fig. 2). Ravid

and Eisenbach [1984a] and Eisenbach et al. [1990a] found that spheroplasts lacking periplasm retain their responsiveness to repellents but lose their responsiveness to maltose, the receptor for which is periplasmic. In view of these findings, the probability of periplasmic receptors for repellents appears to be low. Therefore the most likely possibility in this category is that the MCPs are also receptors for repellents, albeit with a low affinity. In such a case the sensory signal is presumably transduced across the membrane by major conformational changes of the MCP, as was concluded for the case of attractants (see section IV.A.). Perhaps we have here a unique combination of a versatile receptor with a large variety of low-affinity ligands. The MCP appears to combine the versatility of cytochrome P-450 (which exhibits nonselectivity and high versatility [Black, 1987]) with the low affinity of the sugar receptors of the taste system.

A different possibility that has been raised is that there are no receptors for repellents and the repellents have a nonspecific effect on one or more of the membrane properties [Seymour and Doetsch, 1973; Ordal and Goldman, 1976], e.g., the membrane fluidity [Macnab, 1985]. The high concentrations of repellents needed to elicit a chemotactic response and the lack of stereospecificity of the repellent response (e.g., the potency of D- and L-leucine or D- and L-phenylalanine as repellents is comparable [Tso and Adler, 1974]) are in line with this possibility. A change in the membrane fluidity may, e.g., cause a vertical displacement of the MCP and thus transduce the signal across the membrane or it may affect the turnover of the protein activity. As a matter of fact, many of the substances classified as unsaturated fatty acids, short aliphatic alcohols, hydrophobic amino acids, and aromatic compounds in the extensive repellent screening of Tso and Adler [1974] for *E. coli* may induce a disorder in the lipid domain expressed as an increase in the membrane fluidity [Shinitzky, 1984; Yuli et al.,1982; M. Shinitzki, personal communication], the more effective substances being more potent repellents. However, two main lines of evidence argue against the possibility that the classic repellents work by fluidizing the membrane: 1) Direct measurements of the changes in membrane fluidity of *E. coli* in response to repellent stimulation do not reveal a consistent value that elicits a behavioral response [Eisenbach et al., 1990a], and 2) there is no effect of the membrane fluidity on the tumbling frequency of nonstimulated cells [Miller and Koshland, 1977]. Another general membrane property that could, in principle, mediate a repellent response is the membrane potential, but, again, consistent changes in the potential in response to repellents have not been found [Snyder et al., 1981]. It thus appears that the possibility that repellents have nonspecific effects on a membrane property is not likely.

It was recently found that some repellents may cause an additional response at the level of the flagellar motor. One of the effects of repellents is an increase in the frequency of pauses. A high repellent concentration may bring the motor

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E. Conclusion

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V. SIGNAL TRANSDUC

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A. Excitation in Small B

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to a complete stop. Removal of the repellent restores the rotation [Lapidus et al., 1988]. This complete stop occurs also in energized, cytoplasm-free flagellated envelopes of *S. typhimurium* [Eisenbach et al., 1990b]. Because the envelopes lack the chemotaxis machinery [Eisenbach and Adler, 1981; Ravid and Eisenbach, 1984a], it may be concluded that under certain conditions some repellents may affect the motor directly. Alternatively, under these conditions the repellents may be sensed by the cytoplasmic membrane and the information transduced at the membrane level directly to the motor. In view of the findings described in the preceding paragraph, the likelihood of this alternative is low.

E. Conclusion

Based on the survey in this section, it seems that there are versatile mechanisms for transducing the signal across the membrane: major conformational changes, as in the case of MCP-mediated recognition of attractants and repellents; active transport of the attractant across the membrane, as in the case of PTS; stimulant diffusion through the membrane followed by MCP modulation from the inside, as in the case of weak organic acids and bases; and general modulation of a membrane property such as the proton-motive force.

V. SIGNAL TRANSDUCTION WITHIN THE CYTOPLASM

The points of origin of sensory signaling within the cytoplasm are from the MCPs, the PTS, and the other membrane receptors. The final target is the switch at the base of the flagellar motor. I shall first deal with the excitatory signal from a single point of origin. Then the available data will be examined to determine whether they indicate one common excitatory signal from all the points of origin or separate signals. A separate discussion will be devoted to the adaptation signal and to the question of whether both the excitation and adaptation processes are triggered by a common signal or whether the adaptation process is triggered by a feedback mechanism and follows the excitation. In view of the distinct differences between large and small bacterial species with respect to excitatory signaling, each of these species will be dealt with separately.

A. Excitation in Small Bacterial Species

In principle, the excitatory signal between the points of origin at the membrane and the membrane-associated switch could be a change in the membrane potential, a change in the concentration of a specific ion within the cytoplasm, or chemical reactions of diffusible substances in the cytoplasm. Cytoskeleton-like interactions will not be considered here because, to the best of my knowledge, no solid or confirmed data have been published to show the existence of cytoskeleton filaments in the bacterial cell.

Historically, changes in the membrane potential and changes in the concentrations of specific ions were the first signaling candidates to be investigated, presumably because of the analogy to sensory transduction in the nervous system. As discussed in section III above, changes in membrane potential, stimulated by attractants, were indeed found. Additional support for electrical signaling was the finding that an externally applied, electromagnetically induced electric field inhibits the chemotaxis process by 70% and at the same time enhances the motility of the cells [Eisenbach et al., 1983b]. However, both the inhibitory effect of the electric field and the attractant-stimulated changes in the membrane potential could well be indirect. Two subsequent lines of evidence argued against the involvement of membrane potential in signaling. 1) *E. coli* cells were found to be excitable, adaptable, and fully chemotactic under conditions of clamped membrane potential, i.e., conditions under which signaling by fluctuations in the membrane potential is impossible [Margolin and Eisenbach, 1984]. Similar but less extensive observations were reported also for *Streptococcus* [Manson et al., 1977] and *B. subtilis* [Margolin and Eisenbach, 1984]. 2) Segall et al. [1985] produced long filamentous cells of *E. coli* and showed that only flagella near the stimulation site responded to an attractant, added by iontophoretic pulse. This indicated that the range of the excitatory signal (only a few micrometers) is shorter than the range calculated for a change in the membrane potential. As a matter of fact, this evidence argues against any long-range mode of signaling, and, indeed, surface charge changes were also eliminated as a factor in chemotactic signaling [Eisenbach et al., 1985]. It therefore seems justified to conclude with a high degree of confidence that the excitatory signal is not electrical in nature.

The same experiments with filamentous cells of *E. coli* were used to eliminate signaling by diffusion of ions in the cytoplasm. Segall et al. [1985] calculated that simple release and binding of a small molecule or an ion is improbable, because this requires a diffusion coefficient several hundred times smaller than the actual measured coefficients in cytoplasm-like media. This negative conclusion came on a background of nonconclusive evidence for some kind of involvement of ions, predominantly Ca^{2+}, in the chemotaxis machinery [see, e.g., Ordal, 1977, but also section I in Ordal, 1985]. A reasonable statement about the state of the art with regard to ions in chemotaxis is that changes in the concentration of a specific ion are not the excitatory signal [see Eisenbach et al., 1985, for summary of data on Ca^{2+} as well as on other ions and intracellular pH]. All the supportive phenomena that have been observed could be explained on the basis of indirect effects on enzymes within the cell. For example, in a recent study Matsushita et al. [1988] demonstrated partial inhibition of chemotaxis by Ca^{2+} channel blockers, as measured by swarm and capillary assays. However, rings were formed in the swarm assays, and accumulation was observed in the capillary even with the most potent blockers. It therefore

seems to me that, unlike the conclusion of Matsushita et al., Ca^{2+} is not likely to play a major role in bacterial chemotaxis in general, and in the excitation process in particular. The involvement of H^+, K^+, Na^+, $NH_4{}^+$, and Mg^{2+} fluxes in the chemotaxis machinery was eliminated by direct measurements [Eisenbach et al., 1985]. The possibility of indirect participation of Ca^{2+} in the signaling mechanism still calls for further experimentation. The success of Gangola and Rosen [1987] to measure the intracellular concentration of Ca^{2+} in *E. coli* cells opens perhaps the way for a direct determination of the involvement of Ca^{2+} in the signaling process.

One of the most important conclusions from the study of the range of signaling in filamentous cells is that the excitatory signal is inactivated as it moves away from its point of origin [Segall et al., 1985]. This is in favor of the possibility that the excitatory signal is a combination of chemical reactions among diffusible substances within the cytoplasm. Recent data, summarized below in a semichronological order, support this notion.

The "natural" rotation of the bacterial motor is counterclockwise. At least part of the chemotaxis machinery is needed for clockwise rotation. This has been shown by both genetic and in vitro approaches. Flagella of mutants, that lack large parts of the cytoplasmic chemotaxis machinery because of deletions of *che* genes, always rotate counterclockwise [Parkinson and Houts, 1982; Wolfe et al.,1987; Eisenbach et al., 1990b]. Accordingly, cytoplasm-free cell envelopes [Eisenbach and Adler, 1981], isolated from wild-type bacteria by relatively short penicillin treatment and subsequent osmotic lysis (Fig. 6); rotate their flagella exclusively counterclockwise [Eisenbach and Adler, 1981; Ravid and Eisenbach, 1984a]. The counterclockwise bias of the envelopes is due to the loss of cytoplasm. This was proven by preparing envelopes from clockwise mutants (Fig. 7) and showing that envelopes prepared from mutants whose defective, clockwise-causing gene product was cytoplasmic, acquired a counterclockwise bias, just like wild-type envelopes, whereas mutants whose defective gene product was associated with the membrane retained their clockwise bias (Table IV; see Fig. 7 for a more detailed explanation) [Ravid and Eisenbach, 1984a; see Eisenbach and Matsumura, 1988, for review]. Neither mutants with the cytoplasmic *che* genes deleted [Parkinson and Houts, 1982] nor wild-type envelopes [Ravid and Eisenbach, 1984a] respond to chemotactic stimuli.

The next stage was the identification of the cytoplasmic constituent that causes clockwise rotation, followed by the identification of the constituents that participate in regulating the direction of rotation and in transducing the excitatory signal. Searching for a protein candidate rather than low-molecular-weight candidates was a logical first step, because the nonresponsive, counterclockwise-biased intact *che* mutants presumably contained close to normal levels of low-molecular-weight substances. Six of the *che* gene products are cytoplasmic proteins (Table III). Two of them, the CheY and CheZ pro-

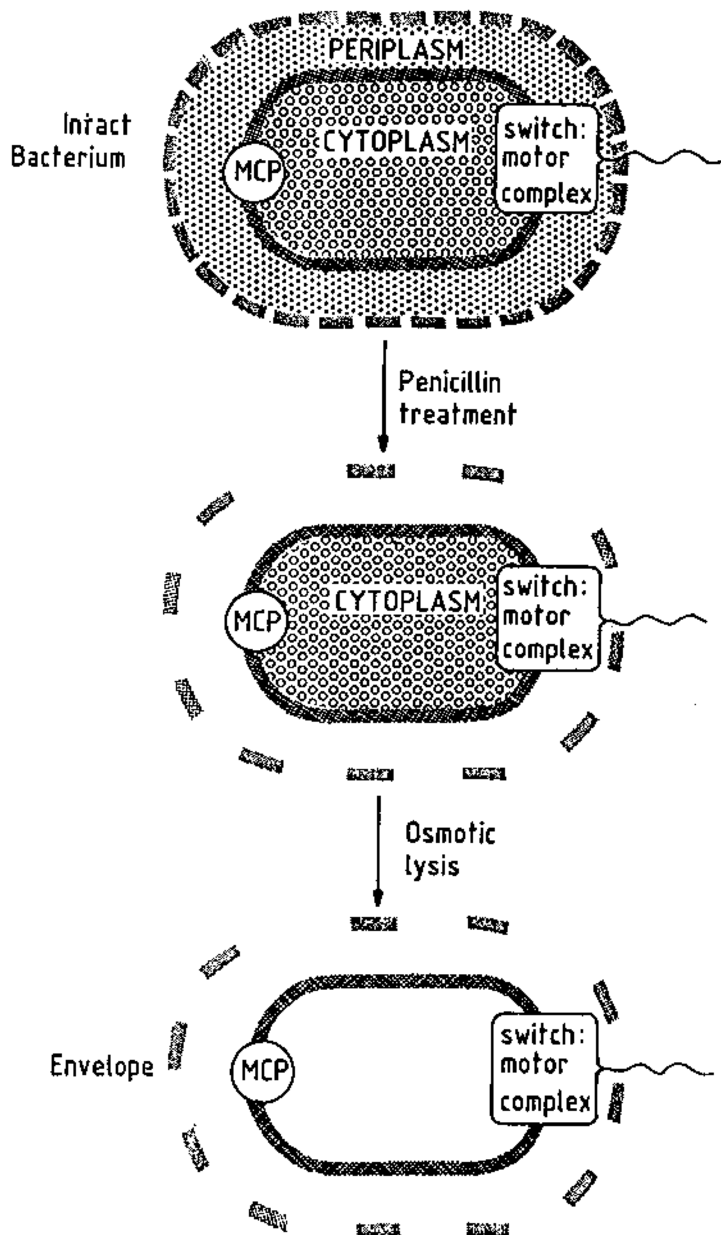

Fig. 6. The main steps in the preparation of cell envelopes. The scheme is not drawn to scale. [Reproduced from Eisenbach et al., 1985, with permission of the publisher.]

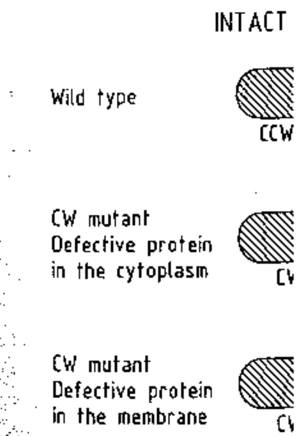

Fig. 7. The rationale of experim
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TABLE IV. Flagellar Rotation i

Strain	Relevant phenotype	Loca of g proc
ST1	Wild type	
ST171	CheA,B‡	Cytop
ST450	CheB	Cytop
ST120	CheC	Mem
MY1	CheV	Mem

*Reproduced from Eisenbach and
†CCW-cw, counterclockwise rotati
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‡Strain ST171 [Aswad and Koshla
and Iino [1985] showed it to be a

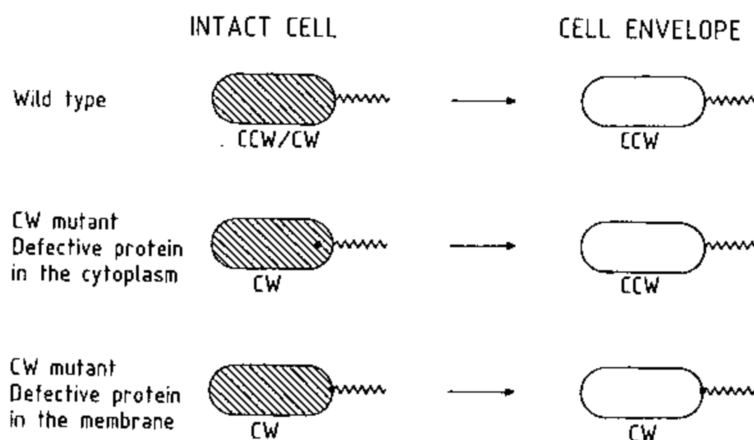

Fig. 7. The rationale of experiments aimed at determining whether the cytoplasm release is the cause of the counterclockwise bias of tethered envelopes. The scheme demonstrates that envelopes, prepared from mutants whose defective gene product is in the cytoplasm, should be similar to those prepared from the wild-type parent and have therefore counterclockwise (CCW) bias (the defective gene product presumably being released with the rest of the cytoplasm). Envelopes prepared from mutants whose defective gene product resides in the cytoplasmic membrane should contain the defective gene product and might retain the clockwise (CW) bias [Ravid and Eisenbach, 1984a].

teins, have been shown by genetic reversion analysis (second-site suppression analysis) to interact with the switch [Parkinson et al., 1983; Yamaguchi et al., 1986] and thus became primary candidates to be tested. Mutation in the *cheZ* gene leads to clockwise bias [Parkinson, 1978, 1981], indicating that its gene product may be involved in counterclockwise rotation. Using the same logic, the CheY protein may be involved in clockwise rotation, because *cheY* mutants

TABLE IV. Flagellar Rotation in Behavioral Mutants and Their Derived Cell Envelopes*

Strain	Relevant phenotype	Location of gene product	Cell proportion in: Intact bacteria	Cell proportion in: Cell envelopes
ST1	Wild type		20% CCW, 80% CCW-cw†	100% CCW
ST171	CheA,B‡	Cytoplasm	17% CW-ccw, 83% CW	100% CCW
ST450	CheB	Cytoplasm	16% CW-ccw, 84% CW	100% CCW
ST120	CheC	Membrane	100% CW	100% CW
MY1	CheV	Membrane	84% vibrating, 16% CW	86% CCW, 14% CW

*Reproduced from Eisenbach and Matsumura [1988], with permission of the publisher.
†CCW-cw, counterclockwise rotation with occasional, brief periods of clockwise rotation; CW-ccw, clockwise rotation with occasional, brief periods of counterclockwise rotation.
‡Strain ST171 [Aswad and Koshland, 1975b] was considered to be a *cheZ* mutant, until Kutsukake and Iino [1985] showed it to be a *cheAcheB* double mutant.

rotate exclusively counterclockwise [Parkinson, 1978, 1981]. These data made the CheY protein the preferred candidate for being tested as the primary cytoplasmic constituent that causes clockwise rotation [Parkinson, 1981; Lengeler, 1982; Ravid and Eisenbach, 1984a; Segall et al., 1985].

Two approaches have been used for testing CheY. In one approach a plasmid containing the *cheY* gene and a regulatable promoter was constructed, introduced into wild-type and mutant strains, and used to express the *cheY* gene at different levels [Clegg and Koshland, 1984; Wolfe et al., 1987]. Overproduction of CheY caused clockwise bias in both the wild-type and the mutant strains lacking all other cytoplasmic *che* gene products. The ability of CheY to cause clockwise rotation in a strain that lacks any other cytoplasmic *che* gene products indicated that CheY causes clockwise rotation by interaction with the switch. This approach could not, however, distinguish between a direct interaction of CheY with the switch and an indirect interaction, e.g., via some small molecule [Clegg and Koshland, 1984]. The other approach, carried out in parallel, involved insertion of purified CheY into cytoplasm-free envelopes. The CheY protein was overexpressed and purified [Matsumura et al., 1984]. Wild-type envelopes containing purified CheY were prepared by inclusion of the protein in the lysis medium during the preparation of the envelopes (Fig. 6) [Ravid et al., 1986]. As a result, some of the tethered envelopes rotated exclusively clockwise (Fig. 8). The fraction of envelopes that did so increased

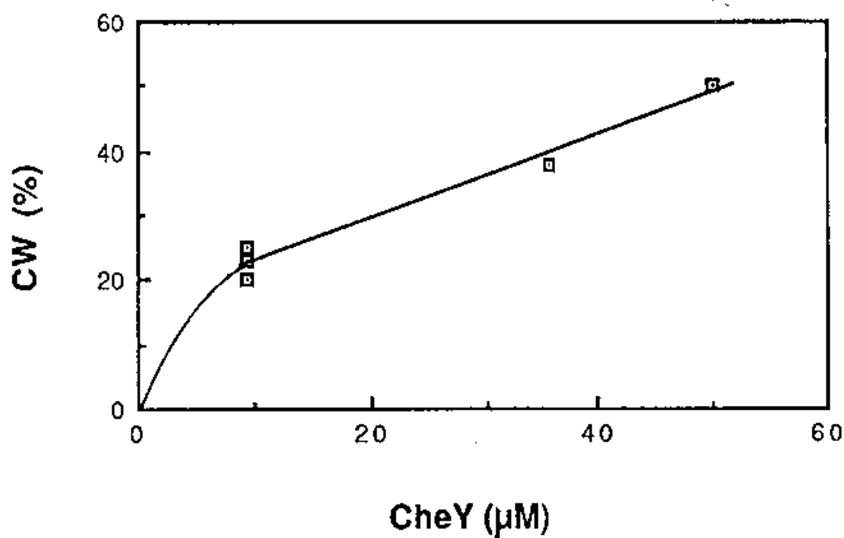

Fig. 8. The fraction of clockwise (CW)-rotating envelopes as a function of the presumed internal concentration of CheY. The rotation was driven by DL-lactate.

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with the concentration of CheY. When the internal concentration of purified CheY was 40–45 μM (around 44,000 molecules per cell), about one-half of the envelopes rotated clockwise [Eisenbach and Matsumura, 1988]. Because of the absence of any of the original cytoplasmic constituents in the envelopes, including small molecules, it was concluded that the interaction between CheY and the switch is direct.

On the basis of the above observations it is possible to conclude that repellents cause the MCP to transmit a clockwise signal to the flagellar motor, i.e., to trigger a cascade of events that eventually leads to direct interaction of CheY with the switch and to clockwise rotation. What about a counterclockwise signal? Because in the absence of a clockwise signal the motor rotates counterclockwise, one could argue that there is no need for a counterclockwise signal. However, to respond quickly to attractants, the bacteria must have means to deactivate or destroy the clockwise signal. Indeed, recent studies of Ames and Parkinson [1988] with MCP mutants locked in counterclockwise rotation indicated that the MCP can actively signal the motor to rotate counterclockwise. (Destruction of a clockwise signal is also considered here as an active counterclockwise signal.) Thus, while a Δ*tsr* mutant alternates between both directions of flagellar rotation, there are mutants with point mutations in *tsr* that rotate in only one direction, either clockwise or counterclockwise, indicative of active clockwise and counterclockwise signaling. Furthermore, this active signaling is independent of the presence of other MCPs, because a strain deleted of all MCPs that alternates between both directions of rotation because of overproduction of CheY can be made to rotate predominantly counterclockwise or clockwise by introducing a second plasmid with an appropriately biased *tsr* signaling defect (P. Ames and J.S. Parkinson, personal communication).

The estimated number of switch molecules per bacterial cell is in the order of 100 or less [Ravid et al., 1986; R.M. Macnab, personal communication]. Why was there a need of about 400-fold larger number of CheY molecules to make one-half of the envelopes rotate clockwise? The same question applies also to intact bacteria, where the concentration of monomeric CheY in the cytoplasm is estimated to be about 20 μM [A. Stock et al., 1985] or in the order of 20,000 molecules per cell. One possibility is that the CheY protein is sequestered by being bound, e.g., to the MCPs, but thus far no evidence for an interaction between CheY and the MCPs has been obtained from second-site suppression analyses. Furthermore, CheY is homologous to the N terminus of CheB (see below), and as such one could argue that CheY is likely to interact with the MCP just as CheB does. However, because a proteolytic fragment of CheB that does not contain the CheY-homologous domain has even higher esterase activity (see section V.B.1.) than that of intact CheB [Simms et al., 1985], the MCP-binding site in CheB is probably not in the CheY-homologous domain. In other words, CheY apparently does not contain this

MCP-binding site. Another possibility, perhaps more likely, is that only a small fraction of the CheY molecules within the envelopes were active. Possibly related observations in the envelopes are that all the so-called active batches of CheY lost their activity with time and that the majority of the preparations were nonactive from the start [Eisenbach and Matsumura, 1988]. Apparently other substances are required to confer activity to CheY. These may be other Che proteins or low-molecular-weight substances in the cytoplasm.

1. Proteins. Among the cytoplasmic *che* gene products, the CheA, CheW, and CheZ proteins are considered to be involved in the excitation process. This is based on studies of the behavior of *che* mutants [Parkinson, 1981] and on overproduction of the Che proteins in bacteria deleted for genes that code for other cytoplasmic *che* gene products and MCPs [Kuo and Koshland, 1987; Wolfe et al., 1987]. What is the current knowledge about these three proteins?

a. CheZ. CheZ is a hydrophilic and very acidic protein [A. Stock and Stock, 1987]. It appears to be a homopolymer, constituted from at least 20 monomeric subunits [see Stock, 1988, for a review of CheZ]. Based on second-site suppression analysis it was concluded that CheZ interacts with the flagellar switch [Parkinson and Parker, 1979; Parkinson et al., 1983]. Because CheZ counteracts the action of CheY [Kuo and Koshland, 1987], one of the suggestions raised with regard to the role of CheZ was that it is a counterclockwise signal [e.g., Lengeler, 1982; Kuo and Koshland, 1987], in analogy to CheY, which was found to be a clockwise signal (see above). However, CheZ-free envelopes rotate counterclockwise [Ravid and Eisenbach, 1984a], and intact *cheZ* mutants respond to attractants with counterclockwise rotation [Parkinson, 1981; Wolfe et al., 1987; Eisenbach et al., 1990b]. It is therefore more reasonable to assume that CheZ terminates the interaction of CheY with the switch rather than being a signal itself [Ravid et al., 1986]. Evidence that apparently supports this suggestion was supplied by Wolfe et al. [1987], who found that intracellular production of CheZ increased the counterclockwise bias only when the cell also contained CheY and that cells containing CheA, CheW, CheY, and the MCPs did respond to attractants and repellents even though CheZ was absent. In spite of not being a counterclockwise signal, CheZ presumably does participate in the excitation process, because the response delay time of *cheZ* mutants is abnormally long [Segall et al., 1982; Block et al., 1982]. (The response delay time is the period of time lapsed between the addition of a stimulant and the response of the flagella. It therefore stands for the excitation time.)

b. CheA and CheW. In vitro studies indicate that CheA and CheW form a complex [P.I. Matsumura, personal communication; McNally et al., 1985] with a stoichiometry of two CheW monomers per CheA dimer ($K_d \sim 17\ \mu M$) [Gegner and Dahlquist, 1991[1]]. It appears that both of them participate in the trans-

[1]This information was added after the completion of the manuscript.

mission of the signal rather than in controlling the direction of flagellar rotation, because signal transduction in mutant cells that lack CheA and CheW is not functional; nevertheless, the direction of rotation in such a mutant is still modulatable by CheY and CheZ [Clegg and Koshland, 1984; Wolfe et al., 1987]. CheA and CheW together are required for the transduction of the signal; production of both of them (in addition to CheY) is required for the purpose in a strain lacking all other *che* gene products [Conley et al., 1989]. Although initially CheA was considered to be a trimer or tetramer [Hess et al., 1987; Stock et al., 1988a] and CheW to be a homodimer [Stock et al., 1987a], recent data suggest that CheA is a dimer and CheW is a monomer [Gegner and Dahlquist, 1991].[2] Based on a consensus sequence, CheW may have a nucleotide-binding site [Stock et al., 1987a] but none has thus far been demonstrated. Unlike CheA (see below), little is known of the molecular function of CheW. These proteins are further discussed in section V.A.2.c.

2. Low-molecular-weight substances. Low-molecular-weight substances, examined for their involvement in signal transduction, are primarily *S*-adenosylmethionine (AdoMet), cGMP or cAMP, and ATP. The evidence accumulated in the literature for the possible involvement of these and other substances in the excitation process is summarized below.

a. AdoMet. Armstrong [1972a,b] and Aswad and Koshland [1975a] found that AdoMet is essential for chemotaxis. AdoMet was then found to be the precursor for MCP [Springer and Koshland, 1977] and CheZ [Stock et al., 1987b] methylation. MCP methylation is not involved in the excitation process of *E. coli* and *S. typhimurium*, but it may be involved in that of *B. subtilis*. Only in this species changes in the methylation levels of the MCPs occurred within less than 5 sec, much before adaptation is observed [Thoelke et al., 1988]. The role of this methylation in *B. subtilis* is, however, still obscure. Similarly, no role has been assigned to the methylation of CheZ [Silverman and Simon, 1977a], which is unaffected by chemotactic stimuli and is independent of any other component of the chemotaxis system [Stock et al., 1987b; Stock, 1988]. It does not seem that AdoMet may have any other role in chemotaxis besides these methylations [Borczuk et al., 1987] and being the precursor of decarboxylated AdoMet (dSAM) (see below).

b. cGMP and related compounds. Several reports proposed the possible involvement of cGMP [Black et al., 1980; Omirbekova et al., 1985] and cAMP or the corresponding cyclases [Black et al., 1983] in chemotaxis. However, subsequent studies called these propositions into question [Taylor et al., 1985; Tribhuwan et al., 1986; Vogler and Lengeler, 1987]. A reproducible experimental result obtained in several laboratories was that addition of a high concentration of cGMP (about 30 mM) caused a prolonged smooth swimming

[2]See footnote 1.

(about 30 min) [Black et al., 1980]. Although there was no attraction to cGMP-containing capillaries [Black et al., 1980], it is still possible that cGMP or a related compound is an attractant for *E. coli*. One way to discriminate between cGMP being an attractant or a regulator of chemotaxis is to carry out temporal assays for cGMP with specific MCP mutants to determine whether only one specific MCP senses the cGMP (as would be anticipated in the case of an attractant) or whether all the MCPs sense it (as would be expected in the case of a chemotactic regulator). Recent temporal assays carried out by K. Lewis (personal communication) indeed showed that a smooth swimming response is absent in mutants with deleted *tsr* or with point mutation in this gene but present in any other MCP mutants. It therefore seems reasonable to conclude that cGMP (or cAMP, guanylate cyclase, or adenylate cyclase) is not directly involved in transducing the chemotactic signal but rather is an attractant for *E. coli*. This conclusion appears to be justified in view of the extremely low number of cGMP molecules in a bacterial cell, e.g., less than one to three molecules per *E. coli* cell [Vogler and Lengeler, 1987].

c. ATP and phosphorylation. ATP seems to be the only low-molecular-weight substance for which there is consistent strong evidence for being directly involved in the chemotaxis machinery. The involvement is beyond the ATP requirement for AdoMet synthesis. Bacteria depleted of ATP fail to tumble [Aswad and Koshland, 1975a; Springer et al., 1975; Galloway and Taylor, 1980; Khan and Macnab, 1980a; Kondoh, 1980; Arai, 1981; Shioi et al., 1982; Taylor et al., 1985], and the rotation of their flagella is counterclockwise biased. The minimal ATP level needed for tumbling is about 0.2 mM [Shioi et al., 1982]. The need of clockwise rotation for ATP is in the cytoplasm; envelopes prepared from a clockwise-biased switch mutant that is sensitive to ATP depletion rotated their flagella clockwise in spite of being devoid of ATP [Ravid and Eisenbach, 1984a; Eisenbach and Matsumura, 1988]. Based on these observations, it was logical to look for phosphorylation of Che proteins within the cell. Several laboratories have looked for such phosphorylation in vivo but were unable to detect any. A breakthrough was made when Hess et al. [1987], followed by Wylie et al. [1988] succeeded in isolating and purifying CheA and found that it is autophosphorylated in vitro by ATP.

The *cheA* gene encodes two polypeptides, long and short, the long one having additional amino acids in the N-terminal domain [Smith and Parkinson, 1980]. Because the phosphorylation site is His 48 [Hess et al., 1988b], only the long polypeptide is phosphorylated [Hess et al., 1987]. Phosphorylated CheA can rapidly phosphorylate CheY in vitro (Fig. 9) [Hess et al., 1988c; Wylie et al., 1988]. This phosphotransferase activity of CheA is fully retained in the N-terminal domain of the protein. By using ATP analogs, Wong et al. [1988] found an ATP-binding site on CheY in addition to the site found by Hess et al. [1987]. Only pure CheY could be labeled by the analogs; no label-

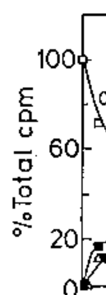

Fig. 9. Phosphorylation of Che
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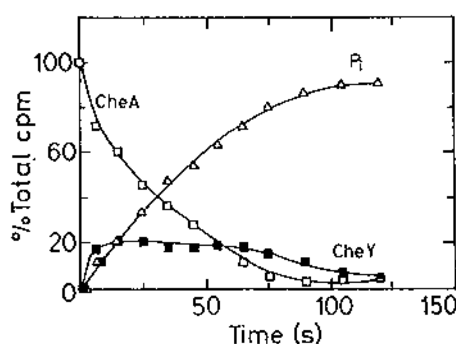

Fig. 9. Phosphorylation of CheY by CheA and its dephosphorylation. The counts on the ordinate are ^{32}P counts obtained from radiolabeled bands of an autoradiogram of the time courses of CheY- and CheB-mediated dephosphorylation of CheA. [Reproduced from Hess et al., 1988c, with permission of the publisher.]

ing or phosphorylation of CheY was found in a cell extract. The pure CheY, however, was neither autophosphorylated nor adenylated by ATP [Wong et al., 1988]. Based on this finding, Smith et al. [1988] suggested that there are two ATP-binding sites in the chemotaxis pathway of CheY. By EPR measurements of a spin-labeled analog of ATP and by NMR measurements of ATP and AMP, Kar et al. [1988, 1991] concluded that the binding of ATP to CheY is via the phosphate groups and that the adenosine plays little or no role in the binding. This conclusion is in line with the observation of Stock et al. [1987a] that the CheY sequence (as well as that of CheA) does not contain discernible nucleotide-binding fingerprints. Because the CheY protein as a whole is homologous to the N-terminal portion of the CheB protein [A. Stock et al., 1985], it was not surprising to find out that also CheB, like CheY, was phosphorylated in vitro by CheA [Hess et al., 1988c]. Although the rate of CheA dephosphorylation by CheY was comparable with the dephosphorylation rate by CheB, the degree of phosphorylation of CheY was one order of magnitude higher than that of CheB: one to two phosphate groups per one CheY molecule and 0.1 to 0.2 groups per one CheB molecule [Hess et al., 1988c]. On the basis of the amino acid residues that are completely conserved between all proteins belonging to the group of response regulators (see end of section V.A.3.), Stock et al. [1988b, 1989[3]] have suggested that aspartyl groups are the sites of phosphorylation (residues 13 and 57 in the case of CheY). Subsequent studies of Sanders et al. [1989] and Bourret et al. [1990] con-

[3]See footnote 1.

firmed that Asp-57 of CheY is the phosphorylation site and that Asp-13 has an important role in the activity of the protein.[4]

Unlike phosphorylated CheA, which is very stable, phosphorylated CheY is quickly and spontaneously dephosphorylated in vitro [Hess et al., 1988c; Wylie et al., 1988]. Both the phosphorylation and dephosphorylation processes of CheY are enhanced by other proteins. Thus CheW enhances (in rate and extent) the phosphorylation of CheY in vitro [Borkovich et al., 1989], whereas CheZ enhances its dephosphorylation [Hess et al., 1988c]. The effect of CheW is much more pronounced in the presence of MCP. CheZ enhances the dephosphorylation without becoming phosphorylated itself. When both CheW and CheZ are present, CheZ dominates, i.e., the phosphorylation of CheY by CheA is slower than in the absence of these proteins [Borkovich et al., 1989]. CheZ is also able to dephosphorylate CheA (but not CheB), albeit at a very slow rate. Being so slow in comparison with the dephosphorylation effect of CheY on CheA or CheZ on CheY [Hess et al., 1987, 1988c], this CheZ-dependent dephosphorylation of CheA is presumably secondary and has no significant role in vivo. The observations that the excitory signal is unstable [Segall et al., 1985], that CheY interacts with the switch (see above), and that CheZ enhances the dephosphorylation of CheY, taken together with the suggestion that CheZ terminates the interaction of CheY with the switch, argue for CheY–phosphate being a signal for clockwise rotation. The supporting evidence in favor of this proposition can be summarized as follows. 1) Production of CheY by a plasmid within a MCP-less mutant that also lacks all the cytoplasmic *che* gene products caused clockwise rotation that was dependent on the presence of ATP internally [Smith et al., 1988]; 2) site-specific mutagenesis that changed the phosphorylation site, His 48, to 13 different amino acids caused total loss of the chemotactic activity of the mutants [Hess et al., 1988b]; and 3) defective CheA proteins were isolated from *cheA* mutants and tested for phosphorylation activity in vitro [Oosawa et al., 1988a]. The large majority of the proteins were found to be defective in at least one aspect of phosphorylation: They either had abnormal levels or rates of autophosphorylation or were unable to phosphorylate CheY and/or CheB. Mutations with similar phenotypes were found to be grouped together in the same region of the *cheA* gene. Mutations with different phenotypes were found to be in different locations in the gene. Based on the classification of the phenotypes and the location of the mutations, Oosawa et al. [1988a] concluded that there are three functional domains in CheA: one domain (the N terminus) for the interaction with CheY and CheB, one domain for regulating the phosphorylation and the stability of the protein, and one domain (the C terminus) for receiving input signals regulating CheA activity.

[4]See footnote 1.

In a very elegant study, Borkovich et al. [1989] looked for the minimal system needed for affecting CheY phosphorylation by MCP in vitro. This system involves, in addition to MCP II–containing membrane vesicles and CheY, the proteins CheA and CheW. Thus the phosphorylation of CheA and CheY in the presence of CheW was much enhanced by the presence of MCP. Addition of aspartate (an attractant for MCP II) to this mixture caused a drastic inhibition of CheY phosphorylation. Hence the modulation of CheY phosphorylation involves CheA and CheW. Another illuminating observation of Borkovich et al. [1989] was that the MCP-mediated enhancement of the rate of CheA autophosphorylation was dependent on the presence of CheY, indicative of the existence of a complex between CheA and CheY.

If indeed phosphorylated CheY is the active form of the protein, as the above observations suggest, an intriguing question that comes up is how could CheY bring about clockwise rotation in a mutant that lacks CheA and the other cytoplasmic Che proteins [Clegg and Koshland, 1984; Kuo and Koshland, 1987; Wolfe et al., 1987; Smith et al., 1988] or in wild-type, cytoplasm-free cell envelopes [Ravid et al., 1986]? The question is even more intriguing in view of the finding that CheY is not autophosphorylated in vitro. The data available to date cannot supply an answer to this question. Possible explanations, all without any supporting evidence, are as follows.

1. Nonphosphorylated CheY may also be active, i.e., it may also interact with the switch and bring about clockwise rotation, but its affinity to the switch may be much lower than that of CheY–phosphate [Hess et al., 1988c]. Thus overproduction of CheY in intact bacteria or inclusion of high CheY concentrations in envelopes may compensate for the low affinity or activity of nonphosphorylated CheY.

2. Assuming the existence of "cross-talk" among sensory transduction systems [Ronson et al., 1987], it is possible that a small fraction of the CheY molecules in the cells becomes phosphorylated by homologous systems. The CheY–phosphate thus produced may be sufficient to cause clockwise rotation [Hess et al., 1988c]. A cross-talk between proteins involved in nitrogen assimilation (Ntr) and chemotaxis was indeed demonstrated recently: CheY could be phosphorylated by NR_{II} (NtrB), and the latter could even suppress the smooth swimming phenotype of a *cheA* mutant [Ninfa et al., 1988]. How can cross-talk be responsible for CheY phosphorylation in the case of CheY-containing envelopes, which apparently do not contain sufficient residual ATP in the cell? (Assuming at least 10^4 dilution of the original internal cell content during the lysis of the cells to form envelopes, the average ATP content in the envelopes should be less than 0.5 μM.) Furthermore, inclusion of a high ATP concentration together with CheY in the envelopes was not sufficient to activate

apparent nonactive CheY (M. Eisenbach, unpublished data). Perhaps membrane-bound, autophosphorylated kinases (e.g., EnvZ, PhoR, and CpxA [Igo and Silhavy, 1988; Stock et al., 1988b; Bourret et al., 1989]) can transfer their phosphate groups to the inserted CheY in the absence of their original substrate proteins. This possibility can be put to the test by comparing the activity of CheY in wild- type envelopes with that of CheY in envelopes of mutants lacking membrane-bound kinases.

3. It is possible that some preparations of envelopes contained remnants of CheA and ATP, sufficient to make the inserted CheY active. This may explain also the large variability in the activity of different CheY batches. However, the reservations just raised with regard to the very large (at least 10^4) dilution of the cytoplasmic content holds also here. It is possible, perhaps, that there are variations in the degree of cytoplasm release from one envelope to another. Though variations are likely, they should be rather small because each envelope studied is first examined for lack of endogenous energy source, indicative of lack of cytoplasm [Ravid and Eisenbach, 1984a,b]. Therefore the remnants of CheA and ATP in the envelopes are probably at very low concentrations, much below substrate quantities. Thus it seems that this explanation may hold only if extremely low concentrations of CheY–phosphate are sufficient to cause clockwise rotation in envelopes.

4. An explanation analogous to the one in 3 above, but for intact bacteria, is that the strains used in the experiments with CheY overproduction carried the deletion Δ*(cheA–cheZ)*2209, which causes the formation of two CheA::CheZ fusion proteins [Kuo and Koshland, 1987; Wolfe et al., 1988]. One of these proteins still contains the long polypeptide of CheA, which may be sufficient for CheY phosphorylation [Hess et al.,1988b].

In principle, any of the above explanations or a combination thereof may hold. Further experiments are required to determine which, if any, of the explanations is correct.

One direct way to test the hypothesis that phosphorylated CheY is the clockwise signal is to include CheY together with CheA and ATP in flagellated envelopes and to look for restoration of clockwise rotation in comparison to the same but CheA-free envelopes. Such studies are in progress (R. Barak and M. Eisenbach, unpublished data). Until there is direct evidence for the role of CheY–phosphate, one may not conclude that the phosphorylation of CheY is the whole picture. The overall mechanism of the transduction of the excitatory signal within the cytoplasm is probably more complicated.

d. Acetyladenylate. Another low-molecular-weight substance that may be required for the activity of CheY is acetyladenylate. Wolfe et al. [1988] found that acetate, but not the more potent repellent benzoate, enhanced clockwise

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rotation in strains having normal levels of CheY but lacking other cytoplasmic Che proteins and MCPs. The effect was not observed in the absence of CheY. By using metabolic inhibitors and mutants deficient in acetate metabolism and by modifying the growth conditions, Wolfe and colleagues showed that acetyladenylate is the substance needed, together with CheY, for generating clockwise rotation and for normal chemotaxis. It is not clear at this stage whether the activation of CheY by acetyladenylate is correlated with the hypothesized activation of CheY by CheA-mediated phosphorylation and, if it is, how. Because CheY phosphorylation by CheA was observed in the absence of acetyladenylate [Borkovich et al., 1989], it seems reasonable to assume that acetyladenylate is somehow involved in the interaction of CheY–phosphate with the switch rather than in the phosphorylation itself. This involvement should be in trace amounts, to comply with the observation that CheY causes clockwise rotation in cytoplasm-free envelopes [Ravid et al., 1986]. Other possibilities are that acetyladenylate acts on the switch rather than on CheY or that its action is to counteract the function of CheZ and thus to avoid dephosphorylation of CheY or its removal from the switch. Again, experiments with cell envelopes containing CheY, acetyladenylate, and perhaps also other *che* gene products may clarify the picture. Because it is reasonable to assume that the metabolic state of bacteria may affect the chemotaxis process directly and because acetyladenylate is related (via acetyl-CoA) to the citric acid (Krebs) cycle, it is possible that the effect of acetyladenylate on the apparent activity of CheY is the link between the metabolic state of the bacteria and the chemotaxis process.

3. Model. To suggest a model for the transduction of the excitatory signal in the cytoplasm, let us summarize the observations discussed above that have been made about the excitation process.

i. The direction of flagellar rotation in the absence of cytoplasmic Che proteins is exclusively counterclockwise.

ii. A mutant deleted of the *cheA, cheW,* or *cheY* gene is always counterclockwise biased. An unstimulated mutant deleted of the *cheZ* gene is always clockwise biased.

iii. The MCP can trigger clockwise and counterclockwise signals. The counterclockwise signal may be one that actively terminates the clockwise signal.

iv. The excitatory signal is quickly deactivated in vivo.

v. CheY interacts with the flagellar switch both in vivo and in vitro and causes clockwise rotation.

vi. Tethered, cytoplasm-free cell envelopes can rotate only in one direction, either counterclockwise (wild-type envelopes) or clockwise (envelopes from switch mutants or CheY-containing envelopes from wild type). They seldom reverse.

vii. Acetyladenylate might be required in vivo intracellularly for expressing the activity of CheY.

viii. There is an ATP requirement for clockwise rotation. The requirement is at the level of the cytoplasm.

ix. CheA is autophosphorylated in vitro by ATP.

x. Phosphorylated CheA phosphorylates CheY in vitro and, to a lesser degree, CheB.

xi. Many *cheA* mutants have a phosphorylation-related defect.

xii. CheY-phosphate is unstable and quickly releases inorganic phosphate in vitro.

xiii. CheZ acts on the interaction of CheY with the switch in vivo and terminates it. CheZ has no effect in the absence of CheY.

xiv. CheZ enhances the dephosphorylation of CheY in vitro.

xv. CheW and MCP together enhance the CheA-mediated phosphorylation of CheY in vitro.

xvi. The effect of CheZ on CheY (observation xiv) is dominant over the effect of CheW on CheY (observation xv).

xvii. CheY interacts with CheA in vitro in stoichiometric, not catalytic, ratios.

xviii. CheA and CheW are required for transduction of the signal from MCP in vivo.

xix. MCP-mediated enhancement of the rate of CheA phosphorylation is dependent on the presence of CheY.

xx. CheA and CheW form a complex in vitro.

xxi. Based on a consensus sequence, CheW appears to have a nucleotide-binding site.

Because no experimental data are available with regard to the function of CheW and the data for the functions of other Che proteins are, at best, incomplete, every model suggested at this stage will be deficient and merely a speculation. Nevertheless, such a model warrants publication because—to quote a proverb attributed to Picasso—"a model is a lie that helps you see the truth," and it may serve as a working hypothesis for future experiments. A speculative model for repellent-stimulated excitation process, in which I try to comply with most of the observations above, is proposed below. In addition to the listed observations, the model is based on two ideas that were discussed above, for which the evidence is indirect and incomplete: a) Only CheY–phosphate

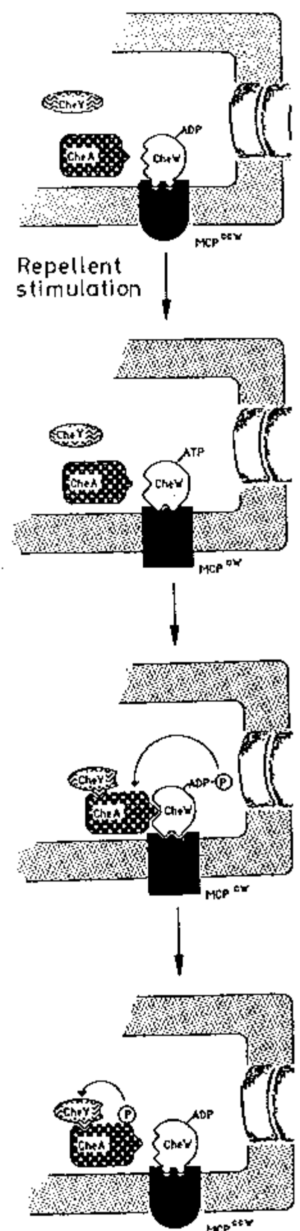
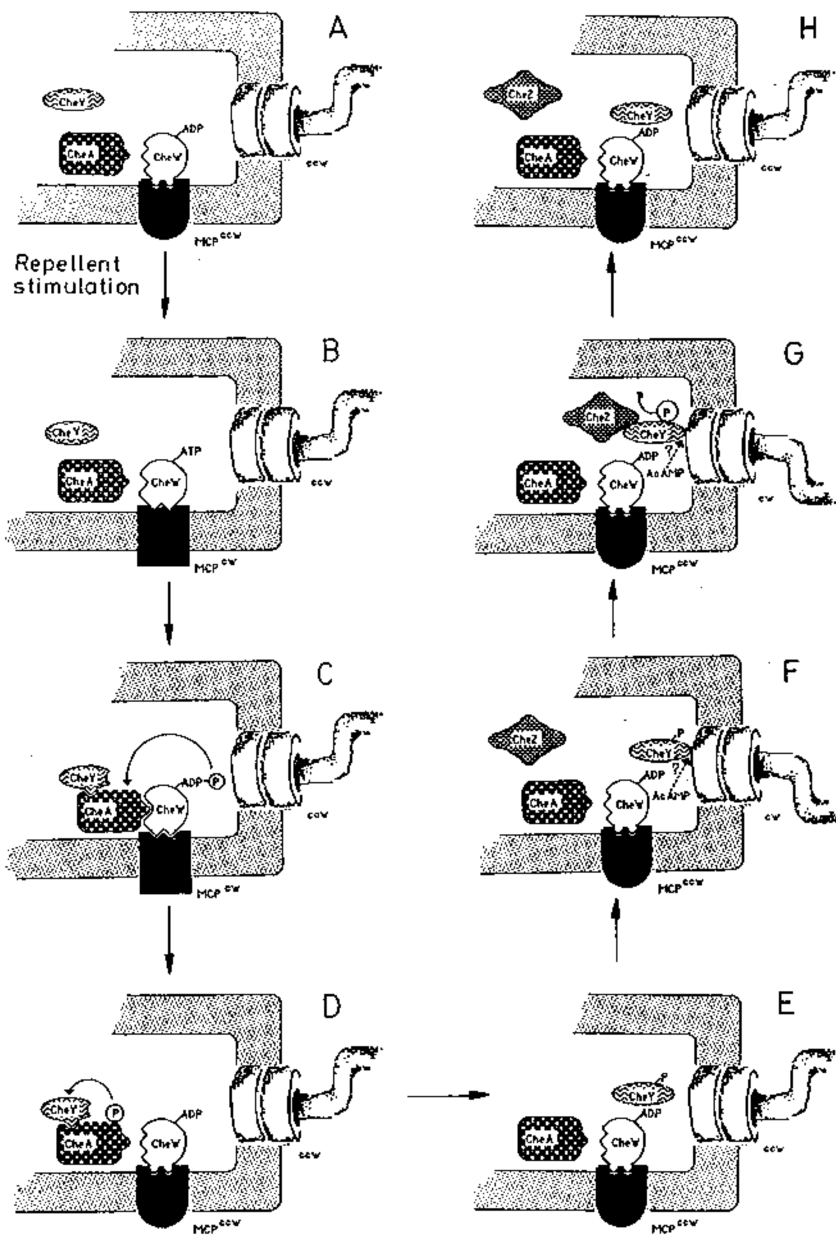


Fig. 10. Schematic model for excitatory signal transduction triggered by repellents. The drawing is for a single stimulatory event. Under continuous stimulation, the MCP conformation in panels D–H should be clockwise (MCP^{cw}), and the cycle shown in Figure 12 should function. See text for details.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MCP ccw
MCP cw

can interact with the switch and bring about clockwise rotation, and b) MCP can be in distinct clockwise and counterclockwise conformations (MCP^{cw} and MCP^{ccw}, respectively). The model is schematically drawn in Figure 10 for repellent stimulation, and it consists of the following stages and steps.

1. Repellent stimulation (either repellent addition or attractant removal) shifts the conformation of MCP from the counterclockwise (Fig. 10A) to the clockwise (Fig. 10B) conformation. (Based on observation iii and idea b.)

2. CheW, reminiscent of G proteins in eukaryotic systems [Dunlap et al., 1987; Gilman, 1987], binds either ATP or ADP. MCP^{cw} has high affinity for CheW(ATP), whereas MCP^{ccw} has high affinity for CheW(ADP). Thus the change from MCP^{ccw} to MCP^{cw} in response to repellent stimulation results in a conformational change of CheW that facilitates exchange of ATP for ADP and, at the same time, increases the affinity of CheW for CheA (Fig. 10B). (Based on observations viii, xviii, xx, xxi, and idea b. The basis for the proposed direct interaction between the MCP and CheW is the recent finding of Liu and Parkinson [personal communication] that there is allele-specific suppression between *tsr* and *cheW* mutations. Furthermore, the existence of MCP–CheW and CheW–CheA complexes is indicated by the observation of Parkinson [personal communication] that overexpression of CheW has a very inhibitory effect on chemotaxis in wild-type cells, inhibition that can be removed by expressing either MCP or CheA at high levels. This suggests that these three proteins are in mutual interaction. There is no information in the literature for the number of CheW molecules per cell; therefore the stoichiometry between MCP [at least 3,500 MCP molecules per cell; Clarke and Koshland, 1979; Hazelbauer and Harayama, 1983] and CheW is unknown.)

3. CheW(ATP) binds CheA, which in turn binds CheY. As a result of CheY binding, the phosphorylation of CheA is enhanced (Fig. 10C). (Based on observations xv and xvii–xx. The complex between CheA and CheY is proposed on the basis of observation xix [Borkovich et al., 1989]. The requirement of CheW to be bound to the MCP while phosphorylating CheA provides the reason for the observation that a mutant deleted for all the MCPs can rotate its flagella counterclockwise only. This requirement, however, is not absolute, because CheW in the absence of MCP [but in the presence of CheA] can augment clockwise rotation. Yet MCP^{cw} can further enhance the clockwise bias provided that CheW is present [Liu and Parkinson, personal communication].)

4. As a consequence of CheA phosphorylation, the complex CheA–CheY is detached from CheW and CheY is phosphorylated by CheA (Fig. 10D). (Observations x and xi.)

5. CheY–phosphate, just formed, is detached from CheA (Fig. 10E), interacts with the switch, and causes it to change into the clockwise conformation (Fig. 10F). (Observations i, ii, and v and idea a.)

6. As long as CheY–phosphate is bound to the switch, the switch is in the clockwise conformation. Only when CheY is dephosphorylated by CheZ (Fig. 10G) can it detach from the switch and the latter can relax to the energetically favored counterclockwise conformation (Fig. 10H). (Observations i, ii, iv, vi, xii–xiv and xvi and idea a. See Khan and Macnab [1980a] and Eisenbach et al. [1990b] for discussions about the energy levels of the switch. It is assumed here that spontaneous dephosphorylation of CheY [Hess et al., 1988c; Wylie et al., 1988] can occur only when the protein is free; when it is bound to the switch [Fig. 10F], CheZ is needed for the task. This assumption, however, is not obligatory for the model.)

Figure 10 was drawn for a single event of stimulation. It should be noted that as long as the repellent stimulation persists, the clockwise conformation of MCP will prevail (unlike Fig. 10D). MCP^{cw} can return to MCP^{ccw} only when the repellent stimulation ceases (as in Fig. 10) or adaptation commences (see section V.B.1.). If these conditions are not fulfilled, another cycle, starting with step 2, can operate in parallel to steps 4–6.

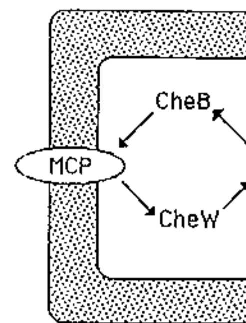
In the case of attractant stimulation, the mechanism of excitation appears to be different than just the reverse mechanism, because neither CheW nor CheZ are obligatory for counterclockwise signaling [Stewart et al., 1988; Liu and Parkinson, personal communication]. It is possible that counterclockwise signaling involves CheA binding to MCP^{ccw} with a resultant inhibition of CheA phosphorylation, thus accounting for observation iii, i.e., that the counterclockwise signal is active. Furthermore, CheW(ADP) perhaps stabilizes the counterclockwise conformation of MCP, as indicated by the finding of Liu and Parkinson (personal communication) that excessive levels of CheW within the cell cause counterclockwise rotation. It is also possible that the activity of CheZ is regulatable, thus that attractant stimulation either activates CheZ or makes it available. P. Matsumura (personal communication) recently found evidence for a complex between the short polypeptide of CheA and CheZ. Matsumura precipitated either of these proteins by specific antibodies and then probed with antibodies against the other protein. On the basis of this finding it is possible to postulate that CheA regulates the activity or the availability of CheZ as well.

What is the case under unstimulated conditions? What does determine the conformation of the switch then? Because the flagella reverse stochastically and there is no reversal synchrony among flagellar motors on a given cell in the absence of a stimulus [Macnab and Han, 1983; Ishihara et al., 1983], the effect on the switch should be local. It is not likely that the MCP fluctuates between clockwise and counterclockwise conformations and thus affects the switch. If this were the case, all the flagella on a given cell should have reversed synchronously. Thus the switch either fluctuates between its clockwise and

counterclockwise conformations without any stimulation, spending more time in the counterclockwise conformation because of being energetically favored [Khan and Macnab, 1980a; Eisenbach et al., 1990b], or responds to occasional encounters with CheY–phosphate. The former alternative of uncontrolled switching is not likely, because in such a case the phenotypic behavior of a CheA$^-$, CheW$^-$, or CheY$^-$ mutant should have been the same as that of the wild type. This is not the case. Furthermore, low levels of CheY–phosphate are maintained even in the presence of CheZ [Borkovich et al., 1989].

A legitimate question that may result from the model is why is there a need for a mediator (CheW) between CheA and the MCP. In principle, if CheA could interact directly with the MCP, the excitation should have been as efficient as in the current model. Two types of answers may be given here, one factual and the other argumentative. Factually, CheW enhances the CheA-mediated phosphorylation. This is so even in vitro [Borkovich et al., 1989]. But what is the logic behind such an arrangement? 1) It may provide initial amplification. The gain of the processing system for a positive signal (attractant stimulation) in *E. coli* is very high [Berg, 1988]. Although the gain may be lower in processing a negative signal [Koshland et al., 1988], any model for signal transduction should contain a step or steps of amplification. According to the model, one molecule of repellent can stimulate several cycles of CheW(ADP)–CheW(ATP) exchange, during which time several CheY–phosphate molecules are formed [cf. steps b–e in Fig. 12, below). Thus one molecule of CheW can activate, in principle, many CheA and CheY molecules. (There appears to be a large excess of nonactive CheY molecules in the cell; Table III.) 2) Such an arrangement fits the central role of CheA in regulating the signal transduction process. CheW presumably fullfils in the excitation process a role similar to the one that CheB fullfils in the adaptation process (see section V.B.1.). In other words, this design allows CheA to sense the MCP conformation via CheW and, at the same time, to affect this conformation via CheB. As shown in Figure 11, such an arrangement together with the interaction of CheA with CheY indeed confers CheA a central regulatory role.

The model can provide immediate explanations for all the phenotypes observed in chemotaxis mutants. 1) CheA$^-$ and CheY$^-$ mutants are counterclockwise biased because of being unable to make CheY–phosphate. 2) An unstimulated CheZ$^-$ mutant is clockwise biased because of being unable to dephosphorylate CheY. The response delay time of this mutant for attractants is abnormally long [Segall et al., 1982; Block et al., 1982] because the level of CheY–phosphate in the cell may be abnormally high and its life time is prolonged in the absence of functional CheZ. Note that the response-delay time for attractants depends, according to the model, on the rate of CheY dephosphorylation. 3) CheW$^-$ mutants as well as mutants deleted of all their MCPs rotate only counterclockwise because of being unable to make CheY–



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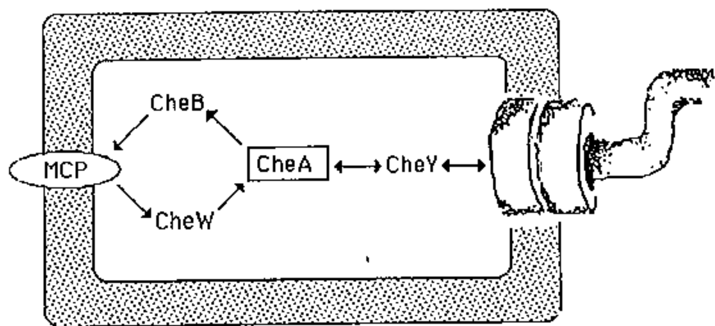

Fig. 11. A simplified scheme for emphasizing the central role of CheA in regulating signal transduction in bacterial chemotaxis.

phosphate. The phosphorylation of CheY by free CheA is much slower than the CheW-mediated phosphorylation and cannot compete with the rapid dephosphorylation of CheY by CheZ [Borkovich et al., 1989]. 4) *cheW cheZ* double mutants rotate clockwise (J.S. Parkinson, personal communication). Here, again, the fast phosphorylation pathway of CheY cannot occur because of the absence of CheW, but this time CheY can be slowly phosphorylated by CheA because the phosphatase (CheZ) is absent.

One point that has not been considered yet is the function of acetyladenylate in the excitation process (observation vii). In the absence of any experimental results to indicate the mode of function of acetyladenylate, the possibilities discussed above (see section V.A.2.d.) may all be valid and they all fit the model.

It was shown in section IV that there are several independent systems that transduce the sensory signal across the membrane. Do the signals from all these systems converge in the cytoplasm to one common pathway of signal transduction, or do the pathways remain separated? The little information that has been published indicates convergence. It appears that all the systems require the cytoplasmic transduction system that involves CheA, CheW, CheY, and CheZ. Thus, an *E. coli* mutant lacking the MCPs, CheA, and CheW is unable to respond to oxygen or to the PTS sugars fructose or mannose [Taylor et al., 1988]. A CheAWYZ$^+$ strain responds to these stimuli. How these systems integrate with the major transduction system is still obscure. For the PTS it is possible, e.g., that, enzyme II, like MCP, also binds CheW and thus triggers the chain of events discussed above. Another possibility is that one or more of the phosphate-intermediates in the cytoplasm (Fig. 5) functions like CheW or even CheA. For example, it is quite conceivable that enzyme III functions like CheW. The involvement of CheA or CheW alone in PTS chemotaxis has not been examined yet. To distinguish between these possibilities, *cheA* and *cheW* mutants should be tested

for their chemotactic responsiveness to PTS sugars. Recent complementation assays indicate that the phosphorylation state of HPr is sensed by the chemotaxis system [Lengeler and Vogler, 1989]. It is possible, for instance, that HPr phosphorylates CheY or CheA. Another interesting possibility raised by J. Lengeler (personal communication) is that the phosphoryl-chemotaxis protein (PCP) of the PTS system dephosphorylates CheY just like CheZ does. (PCP is a putative protein that was postulated to react to changes in the phosphorylation/dephosphorylation equilibrium of HPr/enzyme I [Lengler, 1982].) This may explain the somewhat-intriguing observation of Taylor et al. [1988] that CheZ is not obligatory for chemotaxis to a PTS sugar (but is obligatory for chemotaxis to oxygen).

Nucleotide sequence analysis of pairs of genes, whose products are involved in mutual regulation in bacteria, indicated that it is possible to divide the proteins into two groups: sensors that identify specific stimuli (not chemotactic) in the environment and regulators that control the expression of specific genes in response to the stimuli [Ronson et al., 1987]. The sensors have about 200 amino acid residues in the C terminus that are conserved. The regulators have a conserved region of about 120 residues in the N terminus. Based on this observation, Kofoid and Parkinson [1988] examined additional bacterial proteins involved in other signaling systems, including the chemotaxis system, for the presence of the consensus domain of the sensor ("transmitter") and that of the regulator ("receiver"). And indeed they found that the MCPs as well as enzymes II of the PTS had a transmitter consensus sequence, whereas CheB and CheY had the receiver sequence. CheW (J.S. Parkinson, personal communication) and CheZ [Kofoid and Parkinson, 1988] had partial receiver sequences. Interestingly, CheA had both the receiver and transmitter sequences: three domains (of which two are partial, one at each end of the molecule) with receiver sequence and one domain with transmitter sequence. The suggested model (Figs. 10, 11) is in line with these findings of Kofoid and Parkinson, which emphasize the central role of CheA in regulating signal transduction [see also Parkinson, 1988].

There is no doubt that a major breakthrough in the understanding of excitatory signaling in small bacterial species has been made in recent years. It is conceivable that the near future will further clarify the picture and will provide the missing segments of the puzzle.

B. Adaptation

The adaptation process is an obligatory part of every behavioral system. For being able to detect small changes in the concentration of a stimulant, the bacteria must have a means of adjusting to constant levels of the stimulant. Today at least two systems of adaptation are known in small bacteria, but only one of them has been sufficiently investigated to supply the molecular basis for its mechanism. Each of these systems will be dealt with separately.

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1. MCP-dependent adaptation. The MCP-dependent adaptation was the first system to be discovered and intensively investigated. The main actor in this system is the MCP. The secondary actors—but not the least important—are the methyltransferase (CheR) and the methylesterase (CheB). The story began when it was found that L-methionine is essential for chemotaxis: methionine-depleted cells lost their ability to tumble and became generally nonchemotactic [Adler and Dahl, 1967; Aswad and Koshland, 1974; Springer et al., 1975]. The need for a continuous supply of methionine was for AdoMet [Armstrong, 1972a,b; Aswad and Koshland, 1975a]. The fact that AdoMet is a methyl donor initiated a search for methylation reactions involved in chemotaxis. Soon after, Kort et al. [1975] identified attractant-stimulated methylation of membrane proteins and named them MCPs. The kinetics of the attractant-stimulated methylation of MCP was comparable to the kinetics of the behavioral adaptation of the bacteria to the attractant, and, vice versa, the rate of the repellent-stimulated demethylation was comparable to that of the adaptation to the repellent. It was therefore assumed that the methylation system is responsible for the adaptation [Goy et al., 1977; Springer et al., 1979]. Strong supportive evidence for this assumption came from the findings that a *cheR* mutant [Goy et al., 1978; Parkinson and Revello, 1978], in which the MCP cannot be methylated because of a defective methyltransferase, and a *cheB* mutant [Yonekawa et al., 1983], in which the MCP cannot be methylated because of a defective methylesterase, were severely impaired in adaptation.

What is currently known about the main actors in this adaptation system? I shall restrict myself to information that appears to be relevant to signal transduction. Other details can be found in the excellent review of Stewart and Dahlquist [1987] and in other reviews.

a. MCP. Much has been said about the MCPs in section IV.A., so only the aspects related to methylation and adaptation will be discussed here. In general, methylation of proteins is found either as *N*-methylation of basic amino acids, lysine or histidine, or as carboxymethylation of acidic residues, aspartate or glutamate. The common carboxymethylation in eukaryotes is of the aspartate residue [Clarke, 1985; van Waarde, 1987]. This is not the case in prokaryotes, where the methylation of MCP occurs only on glutamate residues [Kleene et al., 1977; Van der Werf and Koshland, 1977]. There are four to five methylation sites per molecule of MCP, of which two are glutamine residues converted to glutamate by CheB-catalyzed deamidation [Kehry et al., 1983; Terwilliger and Koshland, 1984; Nowlin et al., 1987, 1988]. Interestingly, no single methylation site is absolutely required for the function of the MCP, but loss of even a single methylatable residue prolongs the time required for adaptation [Nowlin et al., 1988]. This observation, taken together with the well-known phenomenon that methylation of MCP increases the clockwise bias of flagellar rotation whereas demethylation increases the counter-

clockwise bias, indicates that the MCP is modulatable to various extents by the methylation reaction and that these modulations are efficiently translated to signals affecting the flagellar switch. The level of MCP methylation is determined by the relative rates of its methylation and demethylation by the *cheR* and *cheB* gene products.

b. CheR. Like most other *che* gene products (Table III), pure CheR is a monomer [Simms et al., 1987b]. There are only about 200 CheR molecules per wild-type *S. typhimurium* cell. Most of them are bound to the MCP [Simms et al., 1987b], in accordance with the finding of Ridgway et al. [1977] that CheR is located in both the cytoplasm and the cytoplasmic membrane. The affinity of CheR to the MCP is in the range of $K_d = 1\ \mu M$ (Stock et al., 1984], about one order of magnitude higher than to AdoMet, the other substrate of CheR [Simms et al., 1987b]. No other proteins besides the MCPs were found to be methylated by CheR [Springer and Koshland, 1977; Clarke et al., 1980]. The only other reaction in which CheR was found to participate in vivo is the synthesis of *S*-methylglutathione in *E. coli*, a substance that does not appear to have any role in chemotaxis [Terwilliger et al., 1986a]. Indirect evidence for an interaction of CheR with CheY was supplied by DeFranco et al. [1979], who studied the functional homology of chemotaxis genes in *E. coli* and *S. typhimurium*. They found that both CheR and CheY from *E. coli* must be supplied to an *S. typhimurium* mutant lacking either of these proteins. Unlike most other Che proteins that are interspecies specific, this interaction between CheR and CheY is species specific. The CheR protein is a very slow enzyme (10 [mol methyl] $\cdot$ [mol enzyme]$^{-1}$ $\cdot$ min^{-1} at 30°C), so slow that it takes about 6 sec to accomplish a single methylation event [Simms et al., 1987b; Stock and Simms, 1987]. This sluggishness fits well the observed kinetics of the adaptation process. The methylation rate of MCP by CheR is not constant for each glutamate residue. For example, two sites on MCP II are readily methylated, and the other two sites are methylated 10–30-fold slower [Terwilliger et al., 1986c]. The relatively slow rate of the methylation, on the one hand, and the observation that *cheR* mutants are excitable and have normal excitation latencies [Block et al., 1982], on the other hand, strongly support the idea, mentioned above, that CheR and the methylation reaction are involved only in the adaptation process. The activity of CheR in vitro is enhanced by attractants and reduced by repellents [Kleene et al., 1979].

c. CheB. Like CheR, CheB is also found during subcellular fractionation to be located in both the cytoplasm and the cytoplasmic membrane [Ridgway et al., 1977], in spite of being a soluble cytoplasmic protein [Snyder et al., 1984; Simms et al., 1985]. CheB is a thiol enzyme [Snyder et al., 1984]. It contains two cysteine residues, of which one is crucial for the activity of the enzyme and is adjacent to a putative nucleotide-binding fold [Simms et al.,

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1987a]. There is a remarkable sequence homology between the entire length of the CheY protein and the N terminus domain of CheB [A. Stock et al., 1985], which fits well with the phosphorylatability of both CheB and CheY by CheA (see section V.A.2.). CheB has two functions in chemotaxis: It demethylates the γ-glutamyl methyl esters of the MCP, forming methanol and γ-glutamyl carboxylate as products [Stock and Koshland, 1978; Toews and Adler, 1979); and it catalyzes the deamidation reaction discussed above, in which two glutamines on each MCP are transformed to methylatable glutamates. The deamidation reaction is irreversible. The methylesterase activity is specific for MCP [Snyder et al., 1984] and is fully maintained in the C-terminal half of CheB [Simms et al., 1985; Stewart and Dahlquist, 1988]. This activity in vitro is 5–10-fold higher when CheB is phosphorylated [Hess et al., 1988a; Stock et al., 1988b]. The function of the N-terminal region in vivo appears to be regulation of the activity of the protein [Stewart and Dahlquist, 1988]. This was not observed in vitro [Borczuk et al., 1986]. Like CheR, CheB is also not involved in the excitation but only in the adaptation process [Block et al., 1982; Yonekawa et al., 1983]. Its activity is affected by stimuli, albeit differently than CheR: attractants inhibit the demethylation activity, and repellents enhance it [Toews et al., 1979; Kehry et al., 1984]. This response of CheB to chemotactic stimuli requires the presence of CheA [Springer and Zanolari, 1984; Stewart and Dahlquist, 1988]. The response to repellents requires also CheW [Stewart and Dahlquist, 1988]. As in the case of CheR and CheY, there is circumstantial evidence for functional interaction between CheB and CheZ [DeFranco et al., 1979].

Based on the observations summarized above, it is apparent that methylation of MCP is the basis of adaptation of *E. coli* or *S. typhimurium* to attractants, whereas demethylation is the basis of adaptation to repulsive stimuli. Hazelbauer et al. [1989] have nicely established that methylation is crucial for MCP-dependent chemotaxis. They substituted alanyl residues for the methyl-accepting sites on MCP III, thus knocking out completely the methylatability of the protein without altering significantly its structure and stability. A mutant carrying this substituted MCP III and lacking other MCPs was normally excitable by ribose, an MCP III attractant, but it was not adaptable.

Of great interest is the recent observation of cross-talk among different MCPs. Hazelbauer et al. [1989] constructed a mutant carrying the substituted alanine-for-glutamate MCP III and normal MCPs I, II, and IV. They found that the chemotaxis defect caused by the lack of methyl-accepting sites on MCP III could be suppressed by the presence of the other MCPs, indicating that adaptation can commence by methylation of an MCP other than the one that was excited. Accordingly, Sanders and Koshland [1988] found that stimulation of MCP II by aspartate resulted in methylation of MCP I. Thus, while the excitation process is strictly MCP specific, the adaptation process is not. This

finding endorses the idea discussed in section V.A. that the clockwise and counterclockwise signals transmitted by the MCPs are two active signals. The finding also suggests that methylation of MCP does not simply restore its prestimulus conformation, i.e., adaptation does not necessarily mean resetting the whole system to the starting point. The same conclusion emerges from studies of the mutual effects of two attractants interacting with the same MCP. MCP II has distinct binding sites for its ligand attractants maltose (being in complex with the maltose-binding protein) and aspartate [Mowbray and Koshland, 1987; Wolff and Parkinson, 1988]. Although saturating concentrations of either aspartate or maltose do not block the chemotactic response to the other attractant, thus confirming the distinct site for each attractant, the magnitude of the response is reduced [Wolff and Parkinson, 1988]. This reduced response implies not only that conformational changes at one site affect the signaling efficiency at the other site, but also that the adaptation process does not simply reverse the attractant-stimulated conformational change of the MCP [Wolff and Parkinson, 1988].

Are the MCPs, CheR, CheB, and AdoMet the only actors in the MCP- dependent adaptation? Probably not. Based on studies with ATP-depleted cells of *S. typhimurium*, Smith et al. [1988] have shown that there is no need in ATP for methylation (other than for the synthesis of AdoMet). However, as discussed above, the CheB protein is phosphorylated by CheA, and this phosphorylation enhances its activity and hence lowers the methylation level of MCP. Thus the methylation–demethylation process itself may not need ATP, but the signal for adaptation seems to involve ATP. Other actors may be the CheA and CheW proteins, because the aspartate-induced methylation of MCP I (but not of MCP II) requires the presence of these proteins [Sanders and Koshland, 1988] and because CheW overproduction causes a significant increase in the level of MCP methylation [Stewart et al., 1988]. Obviously, a clear distinction is made here between the methylation or the actual adaptation processes, on the one hand, and the signal for adaptation, on the other hand.

What is the signal for adaptation? Does stimulation of MCP trigger both the excitation and adaptation processes simultaneously or is there a kind of feedback mechanism for adaptation? Measurements carried out by Berg and Tedesco [1975] and endorsed by Kehry et al. [1985] apparently negate the former possibility, i.e., that the excitation and adaptation processes commence in parallel. In these measurements it was demonstrated that it is possible to adjust the magnitude of a positive stimulus (an attractant) through one MCP so as to balance exactly a negative stimulus (a repellent) through another MCP so that no behavioral response is observed. The adaptation time of a positive stimulus is longer than that of an equivalent negative stimulus, e.g., cells will adapt to addition of an attractant after a longer period of time than to the removal of the attractant. Therefore, if both the excitation and adaptation processes

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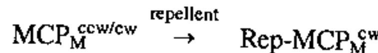
commenced simultaneously, the expected behavior of a mixture of positive and negative stimuli would be a transient negative response, i.e., a fast shift to clockwise bias and a gradual return to the unstimulated behavior. This has not been observed [cf. Stewart and Dahlquist, 1987]. Thus it appears that the signal for methylation and adaptation is triggered only subsequent to the initiation of the excitation process. This idea of a feedback mechanism is further supported by the findings (discussed above) that the demethylation activity is regulated by CheA [Springer and Zanolari, 1984] and also requires CheW [Stewart et al., 1988], that CheA phosphorylates CheB in vitro [Hess et al., 1988c] and increases its activity [Hess et al., 1988a; Stock et al., 1988b], and that methylation of MCP I, stimulated by an attractant for MCP II, requires CheA and CheW [Sanders and Koshland, 1988].

How does this feedback mechanism of MCP-dependent adaptation integrate within the general mechanism of signal transduction? Let us summarize the experimental observations that should be now incorporated into the model of signaling.

1. Methylation of MCP adapts the cell to attractants. This methylation is catalyzed by the methyltransferase CheR.
2. Demethylation of MCP adapts the cell to repulsive stimuli. The process is catalyzed by the methylesterase CheB.
3. Methylation of MCP increases the clockwise bias of flagellar rotation. Demethylation increases the counterclockwise bias.
4. The methylation process, assayed in vitro, is enhanced by attractants and inhibited by repellents. The demethylation process is enhanced by repellents and inhibited by attractants.
5. CheA is involved in the modulation of CheB activity by attractants, and both CheA and CheW are involved in its modulation by repellents.
6. Adaptation does not reset the conformation of the MCP to the prestimulus conformation.
7. Stimulation of one MCP can lead to methylation of another MCP with consequent adaptation. This cross-talk requires both CheA and CheW.
8. CheR and CheY may interact with each other and so may CheB and CheZ.
9. The turnover of CheR is very low and so is the number of CheR molecules per cell.
10. The degree of CheB phosphorylation in vitro by CheA is one order of magnitude lower than that of CheY.
11. Phosphorylation of CheB increases its esterase activity.

Considering these observations, the model may be expanded to include the adaptation process as follows. On the basis of observation 6, nine major con-

formational degrees of MCP should be considered: nonstimulated, attractant-stimulated, and repellent-stimulated conformations (designated as MCP, Att-MCP, and Rep-MCP, respectively), and in each of them the MCP may be highly methylated, or it may have medium or low levels of methylation (MCP_H, MCP_M, and MCP_L, respectively). Some of these conformations are clockwise conformations (MCP^{cw}), i.e., they transmit a clockwise signal, and the others are counterclockwise conformations (MCP^{ccw}). As long as the repellent stimulation persists, the cycle described for the excitation process (steps 1–5 on p. 176) can continue, subjected to the limitation of step 6 (p. 177). Under these conditions, the MCP conformation in Figure 10D–G should be Rep-MCP_M^{cw}, i.e., a repulsive stimulus (either repellent addition or attractant removal) causes the following reaction:

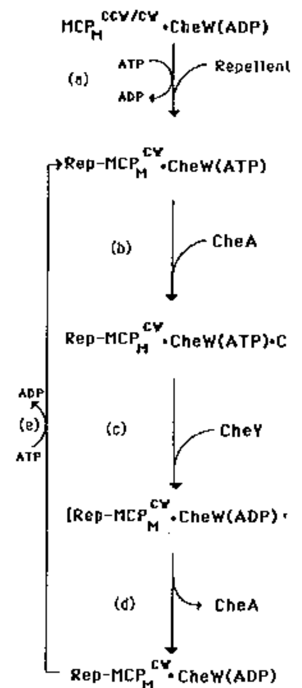


$$MCP_M^{ccw/cw} \xrightarrow{\text{repellent}} \text{Rep-}MCP_M^{cw}$$

Adaptation to repellents is caused by CheB-dependent demethylation of MCP. As a result of demethylation, the MCP conformation is changed to the counterclockwise conformation and CheY–phosphate is no longer formed. This is probably the reason for observation 3, that demethylation increases the counterclockwise bias.

How is the demethylation process regulated according to the model? As discussed in section V.A.3. and as suggested by observation 8, CheB and CheZ may form a complex in the cytoplasm. Because of the analogy of the N-domain of CheB to the entire length of CheY, it is reasonable to assume that CheB can form a complex with CheA similar to the CheA–CheY complex, though with a smaller prevalence and lower affinity. Thus interaction of CheB–CheZ with CheA may result in formation of CheA–CheB, during which CheA phosphorylates CheB and activates it to demethylate the MCP. CheZ is now free to dephosphorylate CheY–phosphate and thus to terminate the clockwise rotation. In this manner both the switch and MCP conformations are simultaneously modulated by CheZ and CheB, respectively.

According to the model, the sequence of events (for both the excitation and adaptation processes) that follow a repellent stimulation may be written as in Figure 12. As long as the repellent stimulation persists and adaptation is not yet effective, a cycle consisting of steps b–e can operate many times and produce much CheY–phosphate. Step f completes the excitation process. Some CheZ is always present and therefore the cycle b–e should continuously operate to produce sufficient amounts of CheY(P) and to compete with the CheZ-catalyzed dephosphorylation of CheY (step g). During adaptation, however, significant amounts of CheZ are produced in step h. Steps h–j are involved in the adaptation of the MCP, and step g in the adaptation of the switch.



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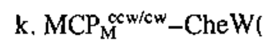

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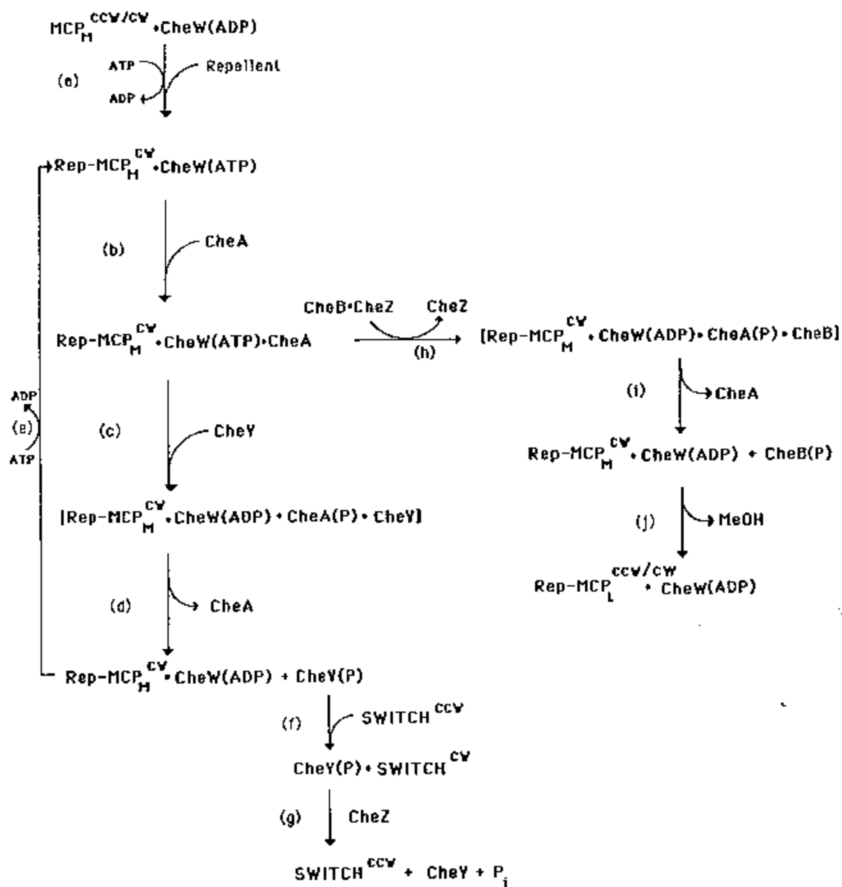
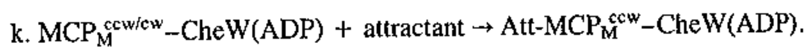


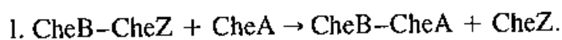
Fig. 12. Sequence of excitation and adaptation events that follow a repellent stimulation.

In analogy, the sequence of events that follow an attractant stimulation may be described as follows:



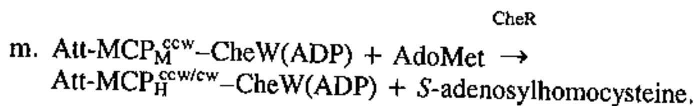
k. $MCP_M^{ccw/cw}$–CheW(ADP) + attractant → Att-MCP_M^{ccw}–CheW(ADP).

To account for observation 5, CheZ may become available or active and CheB inactive by:



l. CheB–CheZ + CheA → CheB–CheA + CheZ.

Whatever the mechanism is for CheZ activation, the switch acquires or remains in the counterclockwise conformation according to step g in Figure 12 or according to any of the mechanisms discussed on p. 177, and the adaptation affects the MCP according to step m:

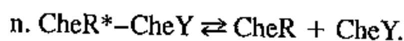


$$\text{m. Att-MCP}_{\text{M}}^{\text{ccw}}\text{–CheW(ADP)} + \text{AdoMet} \xrightarrow{\text{CheR}} \text{Att-MCP}_{\text{H}}^{\text{ccw/cw}}\text{–CheW(ADP)} + S\text{-adenosylhomocysteine.}$$

Now CheY(P) can be regenerated by a cycle similar to that in steps b–e in Figure 12.

How, according to the model, is the adaptation in response to a large-step stimulation so much slower than the excitation? For the case of attractant stimulation, the presumed cause of the difference is the sluggishness of CheR and the low number of CheR molecules per cell (observation 9). For the case of repellent stimulation, the proposed cause of the difference is the low degree of CheB phosphorylatability (observation 10).

All the observations but one were taken into account in the adaptation model. The observation that was not considered is the inhibition of the methylation reaction by repellents. This can be done by assuming that CheR is active (CheR*) only when it is in a complex with CheY (observation 8) and that the two forms are in equilibrium:



$$\text{n. CheR*–CheY} \rightleftarrows \text{CheR} + \text{CheY.}$$

The dissociation constant of CheR*–CheY is presumably very low. Excitation by repellents temporarily consumes CheY (eq. c in Fig. 12), shifting the equilibrium in eq. n to dissociation of the complex and resulting in a temporary decrease in CheR activity. As the adaptation proceeds, free CheY is formed (eq. g) and the equilibrium in eq. n is shifted back to association, thus restoring activity to CheR. The extremely small number of CheR molecules per cell enables regulation of CheR activity by CheY without perturbing other processes. Thus, during CheR activation, the formation of the complex CheR*–CheY has no significant effect on the concentration of CheY that is available to other functions of CheY. This is because of the small number of CheR molecules per cell [Simms et al., 1987b], on the one hand, and the relatively large number of CheY molecules per cell [Ravid et al., 1986], on the other hand. It would be of great interest to put to test the validity of eq. n as follows: 1) Examine whether addition of purified CheY to in vitro assays of CheR activity will increase the turnover of CheR, as the equation predicts. Thus the very low activity of purified CheR found in vitro by Simms et al.

[1987b] may be the consequence of CheY absence in those in vitro assays. On the other hand, in the cell-free extract used by Kleene et al. [1979] in their assays for examining the effects of attractants and repellents on the methylation activity in vitro, CheY was presumably present. 2) Examine whether the level of MCP methylation in a *cheY* mutant is as low as in a *cheR* mutant, as the equation predicts.

It is interesting to note that, according to the model, addition of a repellent to unstimulated bacteria is not completely equivalent to removal of an attractant from a suspension of adapted bacteria. Although both cases are considered as repellent stimulation, the initial conformation of the MCP is different in each case, $MCP_M^{ccw/cw}$ and Att-$MCP_L^{ccw/cw}$, respectively. The different conformations may be the cause of the difference in the behavioral responses to stimulus addition and removal observed, for example, in *che* mutants [Eisenbach et al., 1990b].

Although this review is devoted primarily to *E. coli* and *S. typhimurium*, the adaptation in *B. subtilis* is remarkably different, and the comparison between these species deserves special attention. Similarities between the adaptation machineries of *E. coli* and *B. subtilis* include: structural resemblance of the MCPs [Nowlin et al., 1985]; similar sites of methylation [Burger-Cassler and Ordal, 1982]; functional homologies between *E. coli's* CheR and CheB and *B. subtilis*'s methyltransferase and methylesterase, respectively [Burgess-Cassler and Ordal, 1982; Nettleton and Ordal, 1989]; and attractant-stimulated cross-talk between MCPs [Bedale et al., 1988; Sanders and Koshland, 1988]. In spite of these similarities, the methylation and demethylation processes appear to fulfill in *B. subtilis* the opposite roles that they do in *E. coli:* Adaptation to attractants was found to be correlated with demethylation, whereas MCP methylation is apparently correlated with the excitation process [Goldman and Ordal, 1981; Goldman et al., 1982; Thoelke et al., 1988]. Accordingly, attractants inhibit methylation and stimulate demethylation of MCP in vitro [Goldman and Ordal, 1984]. Repellents have no effect on MCP methylation in vivo (M. Thoelke, W. Bedale, and G. Ordal, unpublished observations cited in Thoelke et al. [1987]). Another difference is that in *B. subtilis* there appears to exist an intermediate methyl acceptor, to which methyl groups are transferred from the MCPs. It was suggested by Tholke et al. [1987] that demethylation of this intermediate is the function that affords adaptation to attractants and formation of methanol. More studies are required for proposing a model for the excitation and adaptation processes in *B. subtilis* and for relating them to the corresponding mechanisms in *E. coli* and *S. typhimurium*.

2. MCP-independent adaptation. This type of adaptation covers two classes of stimuli: those that work by MCP-dependent excitation and those that work by MCP-independent excitation. Each of these classes will be dealt with separately.

The issue of MCP-independent adaptation (sometimes called methylation-independent adaptation) to MCP-mediated stimuli was controversial for a number of years. Recent careful studies appear, however, to remove at least some of the controversy. Chemotactic responses to MCP-mediated attractants or repellents were observed in the absence of CheR and/or CheB [J. Stock et al., 1981, 1985a,b; Block et al., 1982]. The responses were observed in swarm plates, capillary assays, and with tethered cells. The responses, however, were not complete and were only to small or moderate concentrations of stimulants. In light of these observations, J. Stock and Stock [1987] proposed that MCP methylation does not provide the main adaptation mechanism but rather provides a back-up mechanism. They suggested that MCP deamidation could be a way for adaptation. On the other hand, Hazelbauer et al. [1989] have demonstrated that MCP III is unable to mediate net chemotactic response in the absence of methylation. Weis and Koshland [1988] found no chemotactic response (i.e., no migration) to the attractant aspartate in a CheR$^-$CheB$^-$ mutant by the light-scattering technique of Dahlquist et al. [1972] unless excessive concentrations of aspartate were used. Under similar conditions but in tethering experiments, the mutant did respond and adapt. By careful studies of various mutants and using both the tethering and light-scattering techniques, Weis and Koshland [1988] were able to eliminate the possibilities that the MCP-independent adaptation was the result of 1) leakiness of the mutants used, 2) response to gradients of MCP-independent stimuli formed during the experiment (e.g., an oxygen gradient), or 3) cross-talk with another MCP-independent system. They showed that the methylation system is required for adaptation, but that CheR$^-$CheB$^-$ mutants can respond to steep gradients of stimuli though with an efficiency that is far below wild-type cells. The mechanism of this MCP-independent adaptation is not known. To examine what components of the chemotaxis machinery are involved in this adaptation, mutants having excitable but nonadaptable MCPs and lacking one or more of the Che proteins should be studied in temporal assays or tethering experiments.

Niwano and Taylor [1982] found that an *E. coli* mutant lacking MCPs I–III adapts to PTS sugars or oxygen stimulation. In retrospect, the finding that adaptation to these stimuli is MCP independent, is not surprising. It is quite reasonable that the entity that is involved in excitation is the one on which adaptation should act, thus restoring its ability to sense further changes in the stimulant concentration. In other words, if MCP is not involved in the excitation process, there is no apparent reason for being involved in the adaptation process. The mechanism of this MCP-independent adaptation is not known. As discussed in section V.A.3., enzyme II may have a function similar to that of MCP in excitation. If this is so, enzyme II is a likely candidate to be involved in the adaptation process to PTS sugars. As a matter of fact, rephosphorylation of any of the components involved in the excitation (Fig. 5) may initiate the

adaptation process. Furth
adaptation to PTS sugars
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C. Signal Transduction i

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adaptation process. Further studies are required to reveal the mechanism of adaptation to PTS sugars. (See Taylor, this volume, for a discussion of the adaptation process to oxygen.)

C. Signal Transduction in Large Bacterial Species

In large bacterial species (longer than 20 μm), a signaling system of the type discussed in section V.A. is not suitable, because it is a short-range signal [Segall et al., 1985]. Furthermore, even if the signal were not inactivated during traveling along this large distance, the delay response time would be longer than 4 sec for a 20 μm-long bacterium [Lee and Fitzsimons, 1976]. Because the delay response time in such species is very short, e.g., less than 20 msec in *Spirillum volutans* [Krieg et al., 1967], a signal based on diffusion is improbable. The signal should be electrical in nature [Berg, 1975; Snyder et al., 1981]. Direct electrophysiological measurements in large bacterial species have not been reported, but there is circumstantial evidence to support the idea of electrical signaling. Neurotoxins that block the activity of ion channels were found to inhibit the motility of *Rhodospirillum rubrum* and *Thiospirillum jenense* [Faust and Doetsch, 1971] and the chemotactic activity of *Spirochaeta aurantia* [Goulbourne and Greenberg, 1983b]. The act of reversing polarity, or of breaking or closing the electrical circuit, caused *Spirill. volutans* in an electric field instantly to reverse [Caraway and Krieg, 1972]. This observation is at best suggestive, because electric field was found to be effective also in *E. coli* [Eisenbach et al., 1983b], in which signaling is not electrical in nature. Perhaps the most convincing evidence was obtained in *Spiroch. aurantia*, in which a voltage clamp was found to inhibit chemotaxis [Goulbourne and Greenberg, 1983a], unlike in *E. coli* and *B. subtilis* [Margolin and Eisenbach, 1984]. It should be pointed out that although electrical signaling was suggested also for gliding cyanobacteria [Murvanidze and Glagolev, 1982], direct electrical measurements with vibrating electrodes did not reveal extracellular electrical currents [Jaffe and Walsby, 1985].

Although the mode of signaling appears to be known in large bacterial species, nothing is known of the identity of the transmitter and the receiver in them. These species may contain MCPs analogous to those of *E. coli*, *S. typhimurium*, or *B. subtilis* [Nowlin et al.,1985], but their involvement in signaling is at best speculative. The situation is similar with regard to adaptation. Attractant-stimulated carboxymethylation was found in *Spiroch. aurantia* [Kathariou and Greenberg, 1983], but the mechanism of adaptation and the role of methylation in this mechanism are totally unknown. Ironically, the electrical nature of the excitatory signal in large bacterial species had been known many years before anything was learned about the nature of the excitation signal in small bacterial species. Nowadays, the wheel has turned. Very little progress has been made in large species, probably because their genetic sys-

tems are less tractable. Much progress has been made in small bacteria because of the availability of genetic and molecular biology approaches that could be combined with other biochemical and physiological approaches.

VI. SIGNAL TRANSDUCTION IN OTHER TAXES

Bacteria are known to perform other types of taxis, in addition to chemotaxis, depending on the species and their habitat. Taxes that have been reported are thermotaxis, phototaxis, magnetotaxis, osmotaxis, and galvanotaxis. Recently also rheotaxis was identified in gliding mycoplasma [Rosengarten et al., 1988], i.e., gliding of cells upstream in a moving fluid, but nothing is known on the mechanism of this phenomenon. The most recently discovered phenomena in *E. coli* are the last ones in the list, osmotaxis and galvanotaxis. I shall deal with them first.

Li et al. [1988] found by using the chemical-in-plug method that most chemicals at concentrations as high as 1 OsM repel *E. coli* cells. Even substances that are attractants at lower concentrations, e.g., D-galactose, D-ribose, D-glucose, and D-mannose (cf. Table I), were found to be repulsive at concentrations of 1 M and above. This was not the case with the most potent attractants, L-serine, L-aspartate, and maltose, which were attractive at any concentration. In temporal assays, lower osmolarities than 1 OsM were sufficient to freeze the swimming of the bacteria for 1–2 min followed by incessant tumbling for up to hours [Li et al., 1988]. In analogy to repellents, which cause an attractant response (smooth swimming) upon their removal from the medium, it is interesting to know whether a change from high to normal osmolarity causes an attractant response. This has not been determined. However, the finding that *E. coli* are attracted to optimal osmolarities [Adler et al., 1988] is suggestive that this should indeed be the case. It appears that the chemotaxis machinery is involved in osmotaxis: Che^- mutants as well as mutants lacking MCPs I and II fail to carry out osmotaxis in the chemical-in-plug assay. It seems, however, that a large step increase in osmolarity may affect the flagellar motor directly, because even chemotaxis mutants, including switch mutants, respond in temporal assays to such an increase by tumbling that, surprisingly, results from counterclockwise rotation [Adler et al., 1988]. The sensing mechanism of either of these phenomena is not known.

Galvanotaxis in *E. coli* and *S. typhimurium* was discovered by Adler and Shi [1988] (see references cited there for earlier work in other bacterial species). Wild-type *E. coli* migrates toward the anode, whereas wild-type *S. typhimurium* migrates toward the cathode. Alignment of nonmotile bacteria in an electric field indicated that bacteria have a fixed dipole [Adler and Shi, 1988]. It is thus possible that galvanotaxis is composed of passive alignment and active swimming. Accordingly, tumbly bacteria show poor galvanotaxis,

and none of the known gene products for chemotaxis is necessary for this type of taxis [Adler and Shi, 1988].

Similarly, magnetotaxis does not appear to have an active mechanism. Magnetosomes, composed of magnetite (Fe_3O_4) and located intracellularly, orient the cells in the geomagnetic field and thus direct them to swim along the lines of the magnetic field [see Blakemore, 1982, for a review]. A separate signaling system for magnetotaxis is therefore highly unlikely.

Although thermotaxis and especially phototaxis have been extensively investigated during the last decade, most of the research was at the level of the receptors and less, if any, at the level of signal transduction. From the little that is known, it appears that both the phototaxis and thermotaxis systems share the transduction system of the chemotaxis machinery. The difference between these systems seems to be at the receptor level rather than in the signal transduction. Recently, Spudich et al. [1988] and Alam et al. [1989] presented data that suggest the involvement of MCP-like membrane proteins in phototaxis of *H. halobium*. Phototaxis will not be dealt with here, and the reader is referred to Bogomolni and Spudich in this volume. *E. coli* has two thermoreceptors, MCPs I and II [Maeda and Imae, 1979; Mizuno and Imae, 1984; Imae, 1985]. MCP I mutants, defective in chemotaxis to serine, exhibit normal thermotaxis, indicating that there are distinct sites for serine binding and for thermosensing [Lee et al., 1988]. Adaptation to chemoattractants alter the thermosensing ability of the MCPs: MCP I, when adapted to L-serine, loses its ability to detect thermal stimuli; MCP II, when adapted to L-aspartate, generates an inverted sensory signal in response to thermal stimuli. It was suggested that the MCPs transduce the thermosensed signal across the membrane by conformational changes, and these changes are affected by the methylation levels of the MCPs [Mizuno and Imae, 1984; Imae, 1985]. Because the MCPs respond to alcohols that change the membrane fluidity [Eisenbach et al., 1990a] and the membrane fluidity is temperature dependent, it would be of interest to examine whether changing the fluidity of the membrane by rigidifying or fluidizing substances affects the thermoresponse of the bacteria. The transduction of the signal from the MCPs to the switch appears to be the same mechanism as in chemotaxis, because *cheA*, *cheW*, and *cheY* mutants do not respond to thermal stimuli [Imae, 1985].

Figure 13 shows the three major segments of the chemotaxis machinery: the membrane MCP, the cytoplasmic Che proteins, and the membrane switch–motor complex. The MCP is apparently a unique sensory transduction protein, having specific sensitivities to attractants, repellents, temperature changes, pH changes, and osmolarity changes. Some of the other stimuli do not use the MCPs but use other receptors that transduce the sensory signal to the chemotaxis machinery. Other extreme conditions may affect the motor directly.

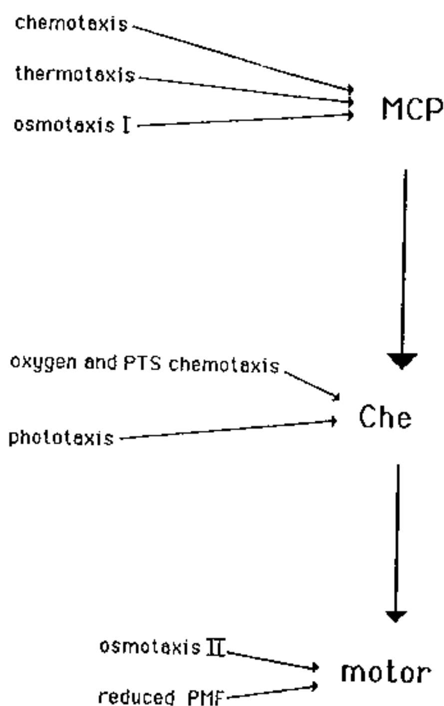

Fig. 13. A simplified scheme showing the involvement of the chemotaxis machinery in other taxes.

To conclude, the more that is known about the signal transduction in chemotaxis of bacteria, the more similarities to eukaryotic sensory systems (described elsewhere in this volume) emerge. It will not be a surprise if the molecular principles of signal transduction turn out to be universal, as is the case with genetics and metabolism.

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VII. NOTE ADDED IN F

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118:640–645.

VII. NOTE ADDED IN PROOF

Within the period of time between the date of the completion of this review and the date of its publication, several new observations, which support the model shown in Figures 10 and 12 and discussed on pp. 173–180 and 185–189, were published. For example, the existence of ternary complexes involving CheA, CheW, and MCP was indicated by the studies of both Borkovich and Simon [Cell (1990) 63:1339–1348] and Gegner and Dahlquist [Proc Natl Acad Sci USA (1991) 88:750–754].

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