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# Response Regulation in Bacterial Chemotaxis

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**Abstract** The signal transduction system that mediates bacterial chemotaxis allows cells to moduate their swimming behavior in response to fluctuations in chemical stimuli. Receptors at the cell surface receive information from the surroundings. Signals are then passed from the receptors to cytoplasmic chemotaxis components: CheA, CheW, CheZ, CheR, and CheB. These proteins function to regulate the level of phosphorylation of a response regulator designated CheY that interacts with the flagellar motor switch complex to control swimming behavior. The structure of CheY has been determined. Magnesium ion is essential for activity. The active site contains highly conserved Asp residues that are required for divalent metal ion binding and CheY phosphorylation. Another residue at the active site, Lys109, is important in the phosphorylation-induced conformational change that facilitates communication with the switch complex and another chemotaxis component, CheZ. CheZ faciliates the dephosphorylation of phospho-CheY. Defects in CheY and CheZ can be suppressed by mutations in the flagellar switch complex. CheZ is thought to modulate the switch bias by varying the level of phospho-CheY. 

**Key words:** CheY, CheZ, response regulators, histidine kinase, switch complex

Bacteria move via a series of runs and tumbles that result in a three-dimensional random walk [1]. The runs are caused by counterclockwise rotation of the flagella, and tumbles occur when rotation is reversed to clockwise [2,3]. Chemotaxis or movement in a favorable direction along a chemical gradient is caused by a bias in the random walk [1,4]; this bias is set by a series of interactions of cytoplasmic proteins with transmembrane chemoreceptors [5–7].

Transient protein phosphorylation is a key feature in the mechanism by which the cytoplasmic components control the tumbling frequency. This type of protein modification has been noted in studies of sensory transduction systems involved in chemotaxis, nitrogen regulation, osmoregulation, regulation of mucoidy, endospore formation, and virulence [8–10]. Signal transduction proteins that mediate these processes can be classified into two families on the basis of sequence homology and the identity of the phosphorylated amino acid residues. One family is defined by a homologous domain that is generally associated with the intracellular signalling domain of a membrane receptor protein. Proteins in this family are kinases that use ATP to phosphorylate themselves at histidine residues. The second family is comprised of cytoplasmic response regulators that transfer phosphoryl groups in the kinases to one of their own aspartic acid residues.

A general scheme illustrating this chain of events in bacterial chemotaxis is shown in Figure 1. The process begins with stimulatory ligands interacting with the transmembrane receptors. The receptors either bind ligands directly (e.g., aspartate, serine) or interact with auxilary periplasmic binding proteins that bind ligands (e.g., maltose, ribose, galactose, oligopeptides). The receptors communicate with chemotaxis components in the cytoplasm. An auxiliary protein, CheW, acts together with the receptors to regulate, at least in part, the rate of autophosphorylation of a histidine protein kinase, CheA [11,12]. Phospho-CheA is central to the mechanisms involved in excitation and adaptation. Excitation is the almost immediate response to attractant or repellent stimuli. This is followed by an adaptation phase in which behavior is restored to its prestimulus state despite the continued presence of stimulatory ligands [4]. In the excitation process, phospho-CheA is a substrate for CheY, a phospho-activiated response regulator. Phospho-CheY interacts with the flagellar motor switching apparatus (FliG,

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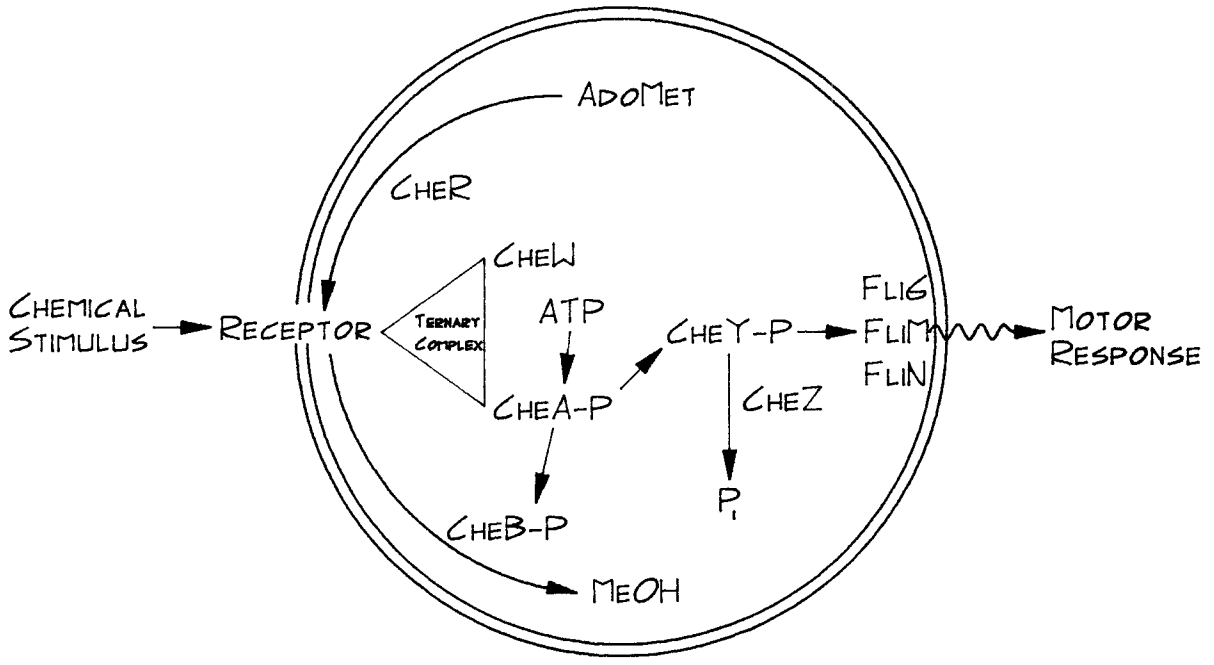


**Fig. 1.** Signal transduction in bacterial chemotaxis Six cytoplasmic components, the Che proteins, mediate chemotaxis CheA, a histidine protein kinase, is regulated by interactions with receptors and CheW Phospho-CheA is a source of phosphoryl groups for CheY and CheB Phospho-CheY interacts with the switch complex to cause tumbly swimming responses CheZ facilitates the dephosphorylation of phospho-CheY Phospho-CheB catalyzes the demethylation of methylglutamyl residues in the receptor CheR catalyzes the methylesterification of receptor glutamyl residues

FliM, FliN) to produce a tumbly response [13–17]. A protein designated CheZ acts directly to facilitate the dephosphorylation of phospho-CheY [14]. Another component, the methylesterase, CheB, acts in parallel to CheY, using phospho-CheA as a source of phosphoryl groups [11,18]. Phospho-CheB together with a methyltransferase (CheR) [19] functions to control the methylation state of the receptors, and fluctuations in methylation provide a feedback mechanism to control function of the active receptor–CheW–kinase complex [11,12].

## STRUCTURE OF APO-CheY

In order to understand the molecular mechanisms involved in chemotaxis signaling, a detailed knowledge of the protein structures involved is ultimately required. The process of defining structural characteristics of components central to signal transduction in bacteria has progressed with the reported crystal structures of CheY from *Salmonella typhimurium* [20] and *Escherichia coli* [21]. These structures open the way for determination of how CheY phosphorylation results in CheY activation and interaction with the flagellar switch. Furthermore, the CheY structures together with the homologies within the family of response regulators support the idea that these response regulators contain a regulatory domain that has a common structural motif and active site [22–24].

CheY is a single-domain protein comprised of a doubly wound five-stranded parallel β-sheet surrounded by five α-helices (Fig. 2). Three highly conserved aspartate residues (Asp12, Asp13, and Asp57) form an acidic pocket between the C-terminal ends of two adjacent β-strands (β1 and β3) [23]. The site of phosphorylation (Asp57) [25] and a divalent metal binding site [26] are located in this acidic pocket. Another highly conserved residue, Lys109, is located at the C-terminal end of β5 with its side chain pointing inward toward the acidic pocket. In the *E. coli* structure, the ε-amino group of the Lys109 side chain is bound to one of the carboxyl oxygens of Asp57 and hydrogen bonded to a $H_2O$ molecule bridging to the carboxyl group of Asp12 [21].

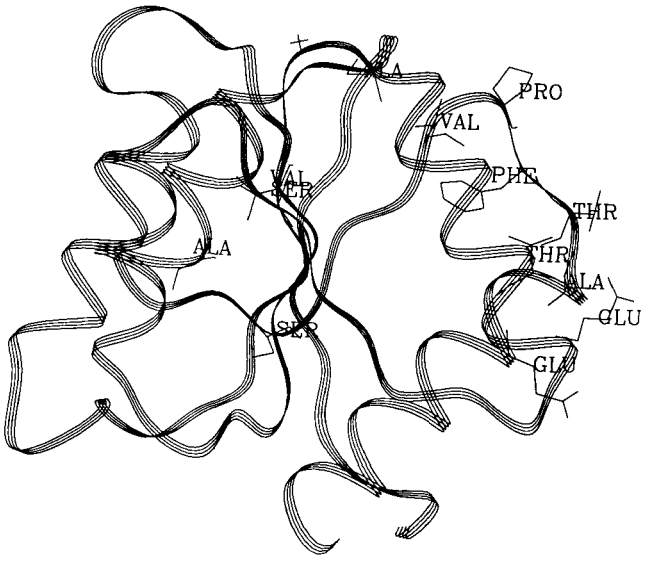


**Fig. 2.** Three-dimensional structure of CheY with $Mg^{2+}$ bound. The $Mg^{2+}$ ion, shown as an X at the top of the figure, is coordinated at the active site of the CheY protein (from X-ray crystallographic data at 1.7 Å resolution [A. Stock: unpublished results]. The amino acid side chains of suppressors of *fliM* and suppressors of *fliG* are shown; they generally fall on one face of the molecule [17,44]. This view looks down the edge of the β-sheets.

## CONTRIBUTIONS OF MAGNESIUM ION AND Lys109 TO THE ACTIVATED CheY STRUCTURE

A divalent metal ion is required for both phosphorylation and dephosphorylation of CheY [26]. Metal binding causes a dramatic quenching of the intrinsic fluorescence of the only tryptophan residue (Trp58) in CheY [26]; this tryptophan is immediately adjacent to the acidic pocket and the site of phosphorylation. The change in intrinsic fluorescence of the tryptophan upon metal binding, and the lack of metal binding to mutant CheY proteins with aspartates in the acidic pocket changed to asparagines, indicates that the acidic pocket provides a site for a divalent metal ion to bind to CheY [26]. The mutant CheY protein, Lys109Arg, binds $Mg^{2+}$ in a manner similar to wild-type protein (1 $Mg^{2+}$/protein), indicating that the side chain of the highly conserved Lys is not a site of metal coordination [26]. In addition to causing a conformational change affecting the region of the tryptophan side chain, introduction of a divalent $Mg^{2+}$ into the CheY active site has the potential to alter the electronic nature of the active site significantly, priming it to take a phosphoryl group from phospho-CheA.

Asp to Asn mutations in the acidic pocket abolish CheY function in vivo [25,27]. This observation is easily explained by the lack of CheY phosphorylation, which is a direct result of the inability of these mutants to bind $Mg^{2+}$. The Lys109Arg CheY protein is also unable to produce tumbly behavior in vivo despite the fact that it is readily phosphorylated [28]. Since the metal binding to the Lys109Arg protein is similar to that of wild-type CheY [26], binding of a divalent metal ion to the side chain of Asp13 and Asp57 probably pushes the Lys109 side chain out of hydrogen-bonding range with Asp57. This is consistent with preliminary crystal structure data on metallated CheY. These data, obtained by soaking $Mg^{2+}$ into CheY crystals, show that the metal ion is ligated to the protein by side chain oxygens of Asp13 and Asp57, backbone carbonyl oxygen from residue 59, and a water molecule hydrogen bonded to Asp12 [A. Stock: unpublished results]. The introduction of a phosphate at Asp57 requires the displacement or alteration in the coordination of $Mg^{2+}$ in the acidic pocket because of further "crowding" of the pocket. The result of these rearrangements is an activated conformation of CheY that probably has the side chain of Lys109 in a new orientation. It is likely that Lys109 plays a central role in an event subsequent to phosphorylation that is necessary for further propagation of the signal.

The Asp13Lys CheY mutant protein cannot be phosphorylated with phospho-CheA yet causes cells to tumble frequently [27]. Two unusual CheY homologs, FlbD and FrzG, for which no cognate kinases are known, also have lysines at positions corresponding to Asp13 in CheY

[29,30]. It has been proposed that the Asp13Lys CheY mutant, and by extension FlbD and FrzG, are locked in an activated conformation normally induced by phosphorylation.

The scheme presented for chemotaxis in Figure 1 shows CheY and another response regulator, CheB, using phospho-CheA as a phosphodonor. CheB is composed of two distinct domains, a N-terminal regulatory domain homologous to CheY and a C-terminal catalytic domain [22,31]. Phosphorylation of the regulatory domain causes an increase in methylesterase activity, which functions in adaptation [18]. Mutations in the regulatory domain of CheB at amino acid residues that are highly conserved within the response regulator family block CheB phosphorylation [32]. By analogy to the CheY case, the inability to generate phospho-CheB in these mutants can be explained in terms of the deficiency of the D10N, D11N, and D11E mutant CheB proteins in their ability to bind divalent metal ions. The D56N and D56E mutants no longer contain the amino acid residue required at the site of phosphorylation. A conformational change in the regulator domain of CheB upon phosphorylation similar to the one proposed for phospho-CheY could be responsible for the enhanced methylesterase activity.

## SMALL MOLECULES AS RESPONSE REGULATOR PHOSPHODONORS

Recent studies with low-molecular-weight phosphodonors have shown that both CheY and CheB can use phosphoramidate as a substrate to produce phosphorylated response regulators [33]. This clearly demonstrates that CheY and CheB play active roles in their own phosphorylations. The fact that phospho-CheA provides a substrate for CheY is supported by the observation that the reaction of CheY and a phospho-CheA fragment, which lacks the kinase domain, still produces phospho-CheY at a rate comparable to the rate of reaction of intact phospho-CheA and CheY [34].

The precise nature of the response regulator active sites provides a degree of specificity for phosphodonors. CheY is able to use acetylphosphate and carbamoylphosphate as phosphate sources; by contrast, CheB is not capable of using these two small molecule donors as a phosphate source [33]. The response regulator phosphorylation with low-molecular-weight phosphodonors, acetylphosphate, carbamylphosphate, and/or phosphoramidate, has been demonstrated for CheY, CheB, OmpR, NrI, PhoB, ArcA, and AlgR [33,35] [B. McCleary and J. Stock: unpublished results]. The contribution of small-molecule phosphodonors to CheY phosphorylation in vivo has not been delinated. However, depending on the stage of growth and carbon source, acetylphosphate levels in the cell can reach levels that could provide significant steady state rates of CheY phosphorylation [B. McCleary and J. Stock: unpublished results].

The introduction of CheY in a gutted strain (deleted of all the *che* genes and the receptors) is sufficient to induce tumbling [36,37]. As CheY concentration increases, the percentage of tumbling cells increases [36,37]. The level of phospho-CheY necessary for tumbling is unknown. To obtain half-maximal levels of tumbling with CheY expressed in a gutted strain, higher concentrations of CheY (30 μM) [36] relative to that found in wild-type cells (8–20 μM) [22,36] are required. This observation can be explained in terms of CheY activation due to phosphorylation by small-molecule phosphodonors (i.e., acetylphosphate), whereby a level of phospho-CheY can be maintained in the gutted strain. At higher levels of CheY in the absence of CheZ, phospho-CheY generated with acetylphosphate and possibly by "crosstalk" from other histidine kinases [38] would be sufficient to explain the observed tumbles.

## SWITCH COMPLEX AND POSSIBLE INTERACTIONS WITH CheY

The final step in signal transduction is regulation of flagellar motor rotation. A switch complex, composed of *fliG, fliM,* and *fliN* gene products [17,39,40], is believed to be necessary for energization and switching of the motor, and it has been coisolated with basal flagellar structures [41]. The switch proteins form a bell-like complex that is found in strains lacking the genes for motility-associated Mot proteins or for the Che proteins, but not in *mot* mutants produced by lesions in *fli* genes [41]. FliG has been localized to the cytoplasmic face of the M ring of the flagellar basal body [42]. The initial evidence suggesting physical interactions between CheY and the flagellar motor was that most intergenic cheY pseudorevertants contained compensatory mutations in the *fliG* or *fliM* locus and that suppression between the *che* and *fli* genes was at least partially allele specific [13,43]. Amino acid changes in FliM and FliG that result in suppression and the CheY mutations that compensate for these variants have been determined. Suppressible CheY mutations appear to be clustered

on one face of the CheY structure (Fig. 2) (suppressors of *fliM:* A99T, S104R, P110T, A113V, T115A [17]; suppressors of *fliG;* V11, S56, E27, A90, V108, F111, T112, E117 [44]), suggesting that this face may be important in CheY switch interaction.

## PHOSPHATASE ACTIVITY OF CheY AND CheZ

CheY has an inherent phosphatase activity that is responsible for the short half-life of phospho-CheY. The first order rate constant for the decay of phospho-CheY to CheY is approximately 2 $min^{-1}$ [14,28]. The hydrolysis rate constant of denatured phospho-CheY in the absence of $Mg^{2+}$ (0.02 $min^{-1}$) corresponds to the rate constant observed for acetylphosphate hydrolysis under similar conditions of temperature and pH [45,46]. Magnesium ion appears to be involved mechanistically in the autodephosphorylation process, since its removal via EDTA chelation stablizes the phosphorylated protein [26].

While phospho-CheY induces tumbly behavior, smooth swimming is observed upon expression of CheZ in cells producing large amounts of CheY [36,37]. Cells that lack CheZ tumble incessently but still run in response to attractant stimulation [47]. The response latency is a measure of the time needed for signals generated by external stimuli to be processed through the complete signal transduction pathway. While the response latency of wild-type cells is ~0.2 s; latencies of ~2 s are observed for strains containing *cheZ* mutations [48,49]. The long response latency time in the absence of CheZ presumably correlates with the time required for autophosphatase activity of phospho-CheY to reduce phospho-CheY levels sufficiently to prevent tumbles. At the molecular level, these observations are consistent with phospho-CheY interaction with the switch complex and significant enhancement of the rate of phospho-CheY dephosphorylation by CheZ.

At substoichiometric levels of CheZ relative to CheY, CheZ acts in a catalytic manner to facilitate phospho-CheY dephosphorylation [14]. Retention of CheZ on a CheY affinity column suggests involvement of a CheZ/CheY complex in the catalytic phosphatase activity [44]. Consistent with the existence of a CheZ/CheY complex is the fact that a mutant CheY protein (Lys109Arg) is phosphorylated and dephosphorylated normally but is totally resistent to dephosphorylation rate enhancement by CheZ [28]. Thus the Lys109 of CheY is important in recognition of phospho-CheY by CheZ as well as by the flagellar switch complex. Lys109 lies on the face of CheY defined by CheY mutations that are suppressors of *fliM* and *fliG*.

The *cheZ* coding sequence predicts an extremely acidic, hydrophilic protein with a molecular weight of 23,900 [50]. N-Terminal analysis of pure CheZ yields an amino acid sequence in agreement with that predicted and shows that the amino-terminal methionine residue is N-methylated [51]. Analysis of purified CheZ by molecular sieve chromatography reveals that the protein is a homopolymeric structure with apparent native molecular weights ranging from 115,000, to >500,000 [50]. How polymerization is related to CheZ activity and its regulation is unclear. Each subunit of the homopolymer has a protease-sensitive site that allows cleavage of a segment of the C-terminus; this cleavage, however, does not effect the polymerization state of CheZ [50].

The common occurance of *fliG, fliM,* and *fliN* alleles that suppress mutations in *cheZ* was initially interpreted as an indication of an interaction between CheZ and the flagellar motor [13,43]. However, suppression of nonsense and frameshift mutations in cheZ by alterations in *fliM* strongly suggest that adjustment of the switch bias (the level of phospho-CheY required to interact with the switch complex in order to generate a tumble) is the only factor required for suppression [17]. Genetic studies comparing *cheZ/fliM* pseudorevertants with *cheB/fliM* double mutants show that the differential ability of *cheB* and *cheZ* mutations to suppress was relatively unaffected by the particular *fliM* allele being suppressed. Since there is no evidence for CheB–motor interactions, these data support the idea that the suppression data do not indicate a specific CheZ–FliM interaction [17].

Control of CheZ activity may be regulated by the receptor and CheW in a manner similar to regulation of CheA autophosphorylation. A short form of CheA, $CheA_s$, is produced from an alternate translational initiation site in approximately equal amounts to full-length CheA [52]. Although the function of $CheA_s$, which lacks the site of autophosphorylation [14], is unknown, complexation between $CheA_s$ and CheZ has been reported.

With the discovery of a phosphorylation cascade in chemotaxis signal transduction, a mechanism for transducing information from the cell surface to the flagellar motor has been defined. The regulation and fine tuning of various steps in this cascade are currently being studied. Sig-

nificant responses can be elicited from cells when attractant ligands bind only a few receptors. Ligand binding to a small number of receptors would be expected to cause a small percentage decrease in kinase activity. This, in turn, would result in a correspondingly small decrease in phospho-CheY levels. Hence, the current state of understanding of the biochemistry of signal transduction would require that the motor sense very small fluctuations in phospho-CheY. This would require very tight control over the steady-state level of phospho-CheY. Alternatively, there may be a mechanism for signal amplification from the receptors. The solution of this issue will require a greater understanding of the mode of regulation of CheY dephosphorylation by the CheZ protein.

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