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Biochemistry

Role of CheW protein in coupling membrane receptors to the intracellular signaling system of bacterial chemotaxis

(chemoreceptors/protein phosphorylation/sensory transduction)

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ABSTRACT **Chemotactic behavior in *Escherichia coli* is mediated by membrane-associated chemoreceptors that transmit sensory signals to the flagellar motors through an intracellular signaling system, which appears to involve a protein phosphorylation cascade. This study concerns the role of CheW, a cytoplasmic protein, in coupling methyl-accepting chemotaxis proteins (MCPs), the major class of membrane receptors, to the intracellular signaling system. Steady-state flagellar rotation behavior was examined in a series of strains with different combinations and relative amounts of CheW, MCPs, and other signaling components. At normal expression levels, CheW stimulated clockwise rotation, and receptors appeared to enhance this stimulatory effect. At high expression levels, MCPs inhibited clockwise rotation, and CheW appeared to augment this inhibitory effect. Since overexpression of CheW or MCP molecules had the same behavioral effect as their absence, chemoreceptors probably use CheW to modulate two distinct signals, one that stimulates and one that inhibits the intracellular phosphorylation cascade.**

Chemotactic movements in *Escherichia coli* are carried out by modulating the pattern of flagellar rotation in response to chemical stimuli: counterclockwise (CCW) rotation produces forward swimming and clockwise (CW) rotation causes directional changes or tumbles (reviewed in refs. 1 and 2). Many of these responses are mediated by transmembrane receptors known as methyl-accepting chemotaxis proteins (MCPs), which monitor chemoeffector concentrations through ligand-binding sites arrayed on the periplasmic side of the membrane and modulate intracellular signals that alter the rotational behavior of the flagellar motors. The manner in which these chemoreceptors are coupled to the locomotor control system remains poorly understood.

Six cytoplasmic proteins (CheA, CheB, CheR, CheW, CheY, and CheZ) comprise the intracellular signaling system that processes receptor information (Fig. 1). CheR and CheB are enzymes that, respectively, add or remove methyl groups on MCP molecules. These functions are not needed to initiate flagellar responses but rather serve to bring about sensory adaptation and a subsequent cessation of responses by altering MCP methylation state. CheY and CheZ appear to interact with flagellar components of the rotational switching mechanism (3) and are probably directly involved in eliciting stimulus responses. Several lines of evidence show that CheY produces CW rotation, whereas CheZ serves as an antagonist of CheY function. Mutants with *cheY* defects exhibit exclusively CCW rotation; *cheZ* mutants are CW-biased (4). Overproduction of CheY causes a dramatic increase in CW bias, both in wild-type cells (5, 6) and in "gutted" cells missing other chemotaxis components (7). Moreover, CheY imparts CW rotational bias to flagellar motors in cell envelopes that are devoid of cytoplasm (8). Although overproduction of CheZ causes CCW bias, CheZ alone has no effect on rotational behavior in the absence of CheY, suggesting that it acts by inhibiting the CW-promoting effects of CheY (7). Consistent with this idea, the duration and range of flagellar signals are greatly extended in mutants lacking CheZ (9).

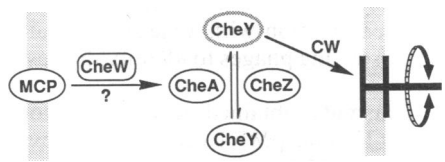

FIG. 1. A working model of the excitatory signaling pathway in *E. coli* chemotaxis. Sensory information is relayed from the membrane receptors or MCPs through cytoplasmic proteins to the membrane-associated flagellar motors. (The sensory adaptation pathway involving feedback control of MCP signal output by the cytoplasmic CheR and CheB proteins is not shown.) Chemoreceptors may modulate rotational behavior by controlling the functional activity of CheA (or CheZ). CheW is somehow involved in mediating this control.

CheA and CheW are needed not only for the feedforward transmission of receptor signals to the flagellar motors through CheY/CheZ (7, 10) but also for the feedback control of receptor methylation through CheB/CheR (11). Recent studies indicate that protein phosphorylation events may be involved in these signaling activities (12–16). CheA possesses autophosphorylation activity *in vitro* and is able to transfer its phosphoryl groups to both CheY and CheB, which may serve to regulate their functional activities. Since CheZ accelerates the dephosphorylation of CheY *in vitro*, phospho-CheY may represent the CW signal *in vivo*, and CheZ may antagonize CW rotation by removing phosphoryl groups from CheY. Moreover, mutants lacking either CheA or CheW are CCW-biased, implying that both functions are involved in controlling the flow of phosphate into CheY to regulate flagellar behavior. The role of CheW in this phosphorylation cascade has been unclear. Recent *in vitro* work has shown, however, that CheW together with MCP receptors can stimulate the ability of CheA to transfer phosphate to CheY (17), suggesting that CheW may serve to couple membrane receptors to the CheA-dependent phosphorylation cascade.

To test the idea that CheW functions as an intermediary between the membrane receptors and CheA (see Fig. 1), we measured the rotational behavior of strains in which the normal complement and stoichiometric proportions of CheW and the other signaling components were altered. Our findings indicate that CheW participates in two different signaling

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Abbreviations: MCP, methyl-accepting chemotaxis protein; CW, clockwise; CCW, counterclockwise; IPTG, isopropyl β-D-thiogalactopyranoside.

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events, both of which may involve direct interactions between CheW and MCP molecules.

MATERIALS AND METHODS

Bacterial Strains. Strains with nonpolar deletions of various chemotaxis genes (Fig. 2) are listed in Table 1. All are derivatives of RP437, an *E. coli* K-12 strain wild-type for chemotaxis (18). CP362 (19) was obtained from G. Hazelbauer (Washington State University); all of the others were either from our laboratory collection or constructed in the course of this work. Deletions were combined by cotransduction with linked Tn*10* insertions; individual tetracycline-resistant transductants were tested for Che and MCP functions by complementation with λche22 (18) and λtsr70 (20) specialized transducing phages to identify the desired recombinants.

Plasmids. Plasmids obtained from other sources were pPA144 (*tsr*$^+$) (21) and pMK1 (*tar*$^+$) (22), provided by M. Manson (Texas A&M University); and pCS20 (*tsr*$^+$ under P_{tac} control), provided by F. Dahlquist (University of Oregon). Plasmids containing the *cheA* or *cheW* genes were constructed as follows (see Fig. 2). pJL60 (*cheA*$^+$) contains a *Sau*3A1–*Eco*RV fragment from λche22 (18) inserted at the *Hin*dIII site of pKK177-3, a pBR322 derivative carrying the P_{tac} promoter (23). The *motA* and *motB* functions were subsequently inactivated by removal of a *Bgl* II–*Nsi* I fragment. pJL31 (*cheW*$^+$) contains a partial *Sau*3A1 fragment from λche22 inserted at the *Bam*HI site of pACYC184 (24). The *motA*, *motB*, and *cheA* functions were subsequently inactivated by removal of an *Mlu* I fragment. pJL63 (*cheW*$^+$ under P_{tac} control) carries the *cheW* locus of pJL31 subcloned in pKK177-3. To provide a source of *lac* repressor, the *lacI*^{q} gene was subsequently introduced from plasmid pMC7 (25), provided by D. E. Koshland (University of California, Berkeley). pJL54, a derivative of pJL63 containing a nonfunctional *cheW* gene (not shown in Fig. 2) was constructed by opening pJL63 at a unique *Sty* I site in the *cheW* gene, treating with S1 nuclease, and religating the linear DNA to create a small deletion.

Behavioral Assays. Chemotactic ability was assessed on semisolid agar swarm plates (26) and flagellar rotation patterns were measured by tethered cell assays (27).

Measurement of Intracellular CheW Concentration. CheW expression from plasmid pJL63 at various concentrations of isopropyl β-D-thiogalactopyranoside (IPTG) was measured by antibody titration, using anti-CheW antiserum kindly provided by P. Matsumura (University of Illinois, Chicago). First, a concentrated, labeled sample of CheW was prepared by growth of RP1078 carrying pJL63 in minimal medium

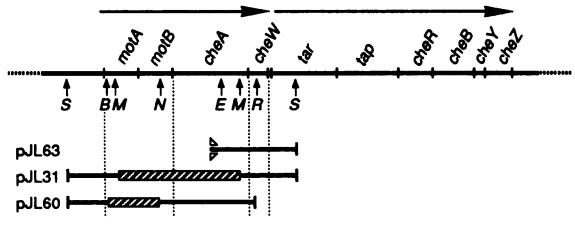

FIG. 2. Plasmid clones used in this study. The relative sizes and transcriptional organization of chemotaxis genes in the *cheW* region are shown, with the positions of restriction-site landmarks indicated by vertical arrows as follows: *S*, *Sau*3A1; *B*, *Bgl* II; *M*, *Mlu* I; *N*, *Nsi* I; *E*, *Eco*RI; *R*, *Eco*RV. Genetic material present in plasmid clones is indicated by thick solid lines, and deleted segments are represented by hatched boxes. The *cheW* gene in pJL63 is expressed from the regulatable P_{tac} promoter (denoted by paired triangles at the upstream end of its insert); genes in pJL31 and pJL60 are expressed from the native promoter of the *cheW* operon.

Table 1. Bacterial strains

Strain	Relevant genotype
RP437	Wild type for chemotaxis
RP1078	Δ *(cheW–tap)2217*
RP1788	Δ*(cheA)10211*
RP7009	Δ*(cheY)6021*
RP7010	Δ*(cheZ)6725*
RP7011	Δ*(cheY–cheZ)4313*
RP9322	Δ*(cheA–tap)2260* Δ*(cheZ)6725*
RP9351	Δ*(cheW–tap)2217* Δ*(cheZ)6725*
RP9352	Δ*(tsr)7028* Δ*(tar–tap)5201* Δ*(trg)100* Δ*(cheZ)6725*
RP9411	Δ*(tsr)7028* Δ*(trg)100* Δ*(cheW–tap)2217* Δ*(cheZ)6725*
RP9447	Δ*(cheA)10211* Δ*(cheZ)6725*
CP362	Δ*(tsr)7028* Δ*(tar–tap)5201* Δ*(trg)100*

containing [^{35}S]methionine (50 μCi/ml; 1 μCi = 37 kBq) and 1 mM IPTG, followed by immunoprecipitation essentially as described (28). A calibration curve was derived by mixing equal volumes of labeled CheW with unlabeled samples prepared by serial dilution of proteins extracted from RP1078/pJL63 cells grown in 100 μM IPTG. The mixtures were precipitated with a limiting amount of anti-CheW antiserum and proteins were separated by polyacrylamide gel electrophoresis. The labeled CheW was visualized by autoradiography and quantified by measuring the band intensity with a Zeineh soft-laser scanning densitometer (model SCR-504-XL, Biomed Instruments, Fullerton, CA). The calibration curve was then used to determine the relative amounts of CheW in other unlabeled samples treated and analyzed in the same manner.

RESULTS

To investigate the role of CheW and the membrane receptors in modulating the CheY activation process, we measured steady-state (i.e., unstimulated) flagellar rotational bias in strains with different combinations and quantities of CheW, MCP receptors, and other signaling components. Nonpolar deletions were used to eliminate various functions, and plasmid clones were used to overexpress selected gene products. The rotation patterns (Fig. 3) are presented as histograms showing the relative proportion of cells in a culture that were assigned to each of five rotation categories, from exclusively CCW at one extreme to exclusively CW at the other. The results are described in the context of the provisional signaling pathway shown in Fig. 1.

Rotation Patterns at Normal Stoichiometries. Strains lacking MCP receptors, CheW, CheA, or CheY rotated their flagella exclusively in the CCW direction (Fig. 3, lines 2–5), whereas a strain lacking CheZ rotated its flagella almost entirely in the CW direction (line 6). In contrast, wild-type cells exhibited frequent reversals (line 1). These findings are consistent with the results of previous studies and the signaling pathway of Fig. 1: MCPs, CheW, CheA, and CheY augment CW rotation; CheZ opposes their action and enhances CCW rotation. In the absence of CheZ, defects in the different CW functions produced distinctive rotational phenotypes (Fig. 3, lines 7–11). CheY is the most critical for CW rotation since the Y$^-$Z$^-$ strain (line 7) was exclusively CCW, whereas the others exhibited at least some CW capability. It appears that CheY is essential for CW rotation of wild-type flagellar motors and that CCW rotation is the default or ground state of the motors when CheY is missing.

The A$^-$Z$^-$ strain (line 8) was predominantly CCW-biased, but with a few reversing individuals, indicating that CheA function plays a major role in promoting CW behavior but is not absolutely essential for CW rotation. These properties are consistent with the working model of Fig. 1, in which CheA function is viewed as activating CheY for CW rotation. The

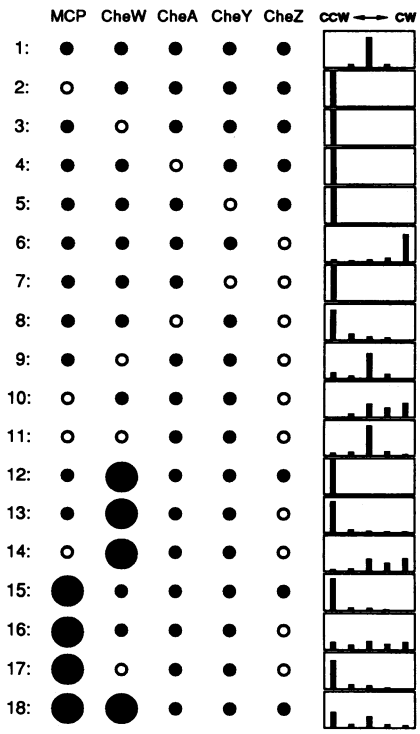

FIG. 3. Flagellar rotation patterns of chemotaxis mutants. Cells were tethered to microscope slides with flagellar antiserum and examined in the absence of chemotactic stimuli. For each strain measured at least 100 rotating cells were observed for 15 sec apiece and classified into one of five categories: exclusively CCW, CCW-biased with an occasional reversal, frequent reversals with no apparent directional bias, CW-biased with an occasional reversal, and exclusively CW. The heights of the bars in the histograms on the right of the figure indicate the percentage of rotating cells in each category. (These rotation profiles were quite reproducible. The variability between strains with similar genotypes or for repeated measurements on the same strain was at most a few percentage points in each category. Where several measurements were made, the averages are plotted.) Each line represents the rotation profile of one or more strains with the indicated combination of gene products: open circles denote missing products; large filled circles denote products present in large excess. The strains were as follows: 1, RP437; 2, CP362; 3, RP1078; 4, RP1788; 5, RP7009; 6, RP7010; 7, RP7011; 8, RP9447, RP9322/pJL63 (no IPTG); 9, RP9351; 10, RP9352, RP9411/pJL63 (no IPTG); 11, RP9411; 12, RP437/pJL63 (100 μM IPTG); 13, RP7010/pJL63 (100 μM IPTG); 14, RP9352/pJL63 (100 μM IPTG), RP9411/pJL63 (100 μM IPTG); 15, RP437/pPA144; 16, RP7010/pPA144; 17, RP9411/pPA144; 18, RP437/pJL63+pPA144 (100 μM IPTG).

few CW episodes seen in the A^-Z^- double mutant might be due to activation of CheY through low-level crosstalk from a different signaling pathway.

The W^-Z^- and MCP^-Z^- strains (lines 9 and 10, respectively) displayed substantial CW behavior, indicating that neither CheW nor MCP function is essential for CW rotation. (Some of the W^- strains contained different combinations of the four MCP species known in *E. coli*, with no discernable difference in behavior, so for simplicity we refer to the MCP receptors collectively rather than individually.) Since the CheZ defect was not fully epistatic in either case (compare lines 9 and 10 with line 6), CheW and the MCP receptors evidently do not promote CW rotation simply by antagonizing CheZ action but rather, like CheA, must play an active role in generating or stimulating CW rotation. Moreover, the roles played by CheW and the MCPs are different. When compared to the MCP^-W^- strain (line 11), CheW enhanced CW rotation in the absence of receptors (line 10), whereas MCPs had no effect on rotational behavior in the absence of CheW (line 9) but did augment CW rotation in the presence of CheW (line 6). We conclude that CheA and CheY are directly responsible for generating CW rotation, whereas the MCPs and CheW are involved in modulating the activity of this CW-generating system (Fig. 1).

Inhibition of Chemotaxis by Excess CheW. The ability of CheW at normal levels to augment CW rotation, both in the presence and in the absence of receptors, suggested that overexpression of CheW might further enhance CW rotation. To explore this possibility, we constructed two multicopy *cheW* plasmids (Fig. 2): pJL31, in which *cheW* is expressed from its native promoter (29), and pJL63, in which *cheW* is expressed from the IPTG-regulatable P_{tac} promoter. Under conditions leading to high-level expression of CheW, both plasmids inhibited the chemotactic ability of wild-type cells. Results with pJL63 are shown in Fig. 4. Swarm size was essentially normal at internal CheW concentrations ranging from about wild type (5–10 μM IPTG) to several times greater than wild type (20–25 μM IPTG), but larger amounts of CheW caused a proportionate reduction in swarm size. This inhibitory effect appeared to be due to a functional activity of the overexpressed CheW protein, since a derivative of pJL63 with a small deletion in *cheW* (pJL54) had no effect on wild-type behavior, even at very high inducer concentrations (data not shown).

Stoichiometric Compensation Between CheW and MCPs. The inhibition of chemotaxis by high levels of CheW might be caused by titration or inactivation of another component of the signaling machinery with which CheW normally interacts. Consequently, we attempted to alleviate the CheW effect by expressing other chemotaxis proteins at correspondingly high levels, using compatible plasmids to introduce additional copies of selected genes into cells containing a *cheW* plasmid. We found that overexpression of Tsr or Tar, the major MCP species in *E. coli*, partially compensated for high CheW levels, whereas overexpression of CheA (from pJL60; see Fig. 2) did not. Results with plasmid pPA144, which expresses *tsr* at high levels (21), are shown in Fig. 4. Similar results were obtained with pCS20, which expresses *tsr* from the IPTG-regulatable P_{tac} promoter, and with pMK1, a *tar* plasmid (data not shown). In all three cases, chemotactic ability was inhibited by high levels of either CheW or MCPs alone, but improved when the proteins were jointly overex-

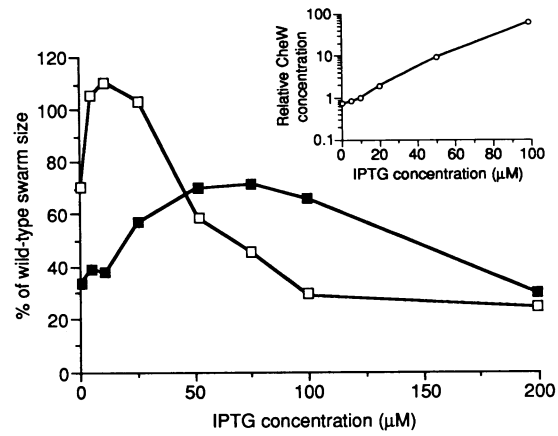

FIG. 4. Effect of CheW and MCP levels on chemotactic ability. RP1078 carrying pJL63 (*cheW*$^+$ under P_{tac} control) alone [□] or in combination with pPA144 (*tsr*$^+$) [■] was inoculated on tryptone semisolid agar plates containing various concentrations of IPTG to manipulate CheW levels in the cells. Chemotactic ability was assessed by comparing colony size to that of a wild-type control on the same plate (RP437 carrying plasmid pBR322) after incubation for 8 hr at 35°C. Each data point is the average of two measurements. (*Inset*) In parallel experiments, the relative amount of CheW made at each inducer concentration was determined by immunoprecipitation.

pressed. We conclude that these compensation effects are due to restoration of a more balanced stoichiometry between CheW and MCP molecules, which may interact directly with one another during sensory signaling.

Inhibition of CW Rotation by Elevated Levels of CheW or MCPs. The inhibition of chemotactic ability at high CheW or MCP levels was accompanied by a drastic reduction in CW rotation (Fig. 3, lines 12 and 15 vs. line 1). When both CheW and MCPs were expressed at elevated levels, which partially alleviated the chemotaxis defect (Fig. 4), CW rotational episodes were also restored (Fig. 3, line 18). These results suggest that a stoichiometric excess of CheW or MCP molecules impairs chemotactic ability by inhibiting CW rotational behavior, presumably by blocking the activation of CheY by CheA or by accelerating the destruction of activated CheY.

Neither CheW- nor MCP-mediated inhibition of CW rotation was greatly dependent on CheZ, the only function clearly implicated in CheY inactivation (Fig. 3, line 12 vs. line 13 and line 15 vs. line 16). However, CheZ removal partially reversed both effects, particularly the MCP effect, suggesting that CheZ function contributes to the reduction of CW rotation produced by overexpression of CheW or MCP molecules. We conclude that excess CheW and MCP probably inhibit CW rotation by slowing the rate of CheY activation and that CheZ augments these effects by acting at the CheY inactivation step (see Fig. 1).

Both the CheW and MCP overproduction effects were sufficiently attentuated by CheZ removal to assess interactions between CheW and MCP functions in the inhibition of CW rotation. [Although the two effects were reversed to different extents (Fig. 3, line 13 vs. line 16), this could reflect relatively small differences in steady-state levels of activated CheY.] Under CheZ-less conditions, the CheW effect was dependent on MCP receptors (Fig. 3, line 14 vs. lines 10 and 13), whereas the MCP effect was not dependent on CheW and was actually enhanced by CheW removal (Fig. 3, line 17 vs. lines 9 and 16). We conclude that MCP molecules alone, when present in stoichiometric excess, are capable of inhibiting CW rotation, whereas high levels of CheW probably inhibit CW rotation by augmenting this MCP-dependent effect.

DISCUSSION

The production of CW flagellar rotation and the ability to modulate rotation pattern are essential for chemotactic behavior by *E. coli*. This study reports the unusual finding that high intracellular levels of either CheW or MCP molecules have the same behavioral effect as the absence of either of these signaling components—both conditions prevent CW rotation and, consequently, chemotaxis. These results indicate that CheW and the MCP receptors have at least two distinct signaling roles, and suggest molecular mechanisms that could account for dual signaling activity. Because a great deal of circumstantial evidence suggests that CW rotation is activated by CheY phosphorylation, we will discuss our findings in terms of the phosphorylation hypothesis, even though alternative signaling mechanisms cannot be ruled out.

CheA and CheY Comprise the CW Generator. CheY and CheA were essential for CW rotation, whereas MCPs and CheW were not. In cells lacking MCPs, CheW, and CheZ, CheA and CheY were sufficient to generate a wild-type level of CW rotation (Fig. 3, line 11 vs. line 1). In contrast, Conley *et al.* (10) observed essentially no CW rotation in gutted cells containing various levels of CheA and CheY. The difference in behavior may be due to the CheR and CheB proteins, which were present in our system but absent in the gutted cells. Alternatively, the gutted cells may have contained enough residual CheZ activity to obscure a slow rate of CheY activation (10). In any case our findings are consistent with *in vitro* work which indicates that CheA is able to transfer phosphate groups to CheY in the absence of other chemotaxis proteins (13, 16). We conclude that *in vivo*, CheA, possibly assisted by CheR or CheB, can activate CheY for CW rotation, whereas MCP and CheW molecules modulate that activation process to elicit stimulus responses.

The CW Generator Is Modulated in Two Ways. At normal stoichiometries, CheW alone or in combination with MCP molecules produced an increase in CW bias, whereas MCPs alone did not. Thus, CheW plays a direct role in *stimulating* the rate of CheY activation, and MCPs can augment this effect. Overproduction of MCP molecules, alone or in combination with normal levels of CheW, resulted in a reduction in CW rotation. Overproduction of CheW produced similar effects, but only in the presence of normal levels of MCP molecules. Thus, MCPs play a direct role in *inhibiting* the rate of CheY activation, and CheW can augment this effect.

Mechanisms of Receptor Coupling. An explicit model that accounts for these two opposing modes of rotational control, which we presume to reflect signaling activities involved in stimulus responses, is shown in Fig. 5. We propose that flagellar rotation is modulated by two control systems: one actively enhances CW rotation, the other actively enhances CCW rotation. In terms of the phosphorylation cascade, CW signals would stimulate the rate of CheY phosphorylation, whereas CCW signals would inhibit the rate of CheY phosphorylation. These two signaling modes are thought to correspond to different conformational states of the MCP receptors, whose relative proportions are modulated by chemoeffectors and methylation changes to control rotational behavior (21, 30, 31).

Receptors in the CW mode might stimulate CheY phosphorylation by enabling CheW to enhance CheA activity, possibly through the formation of CheW–CheA complexes. Since CheW can enhance CW rotation in the absence of MCPs, the receptors do not seem to play an essential role in this process but rather may function by accelerating the formation of CheW–CheA complexes, perhaps by serving as a scaffold on which to bring the components together in proper orientation. The nature of the activated form of CheA is unknown, and a variety of plausible alternatives can be envisioned. For example, CheW might serve as a conformational effector to keep CheA in the catalytically active form, or it might serve to funnel ATP or CheY substrates to CheA,

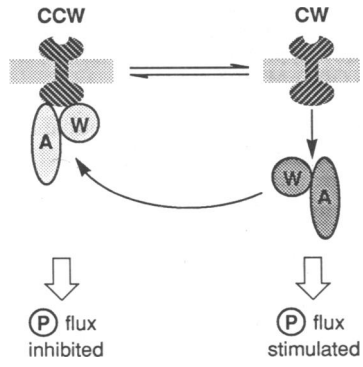

FIG. 5. Model of dual control of flagellar rotation by MCP and CheW molecules. Stimuli that elicit CCW rotational responses might inhibit the rate of CheY phosphorylation by shifting MCP molecules into a conformation that binds CheA (or CheY) in inactive form. CheW facilitates, but is not essential for, this effect. Stimuli that elicit CW rotational responses might stimulate the rate of CheY phosphorylation by shifting MCP molecules into a conformation that facilitates the formation of enzymatically active complexes between CheA and CheW. CheW is essential for this effect, whereas MCP molecules merely augment it. Ⓟ, phosphate.

enhancing turnover rate. It is also unclear whether the active form of CheA functions catalytically or must be regenerated after each autophosphorylation/phosphotransfer cycle.

Receptors in the CCW mode might inhibit CW rotation by binding CheA or CheY molecules, thereby reducing the rate of CheY phosphorylation. (In Fig. 5 we pictured CheA as the target molecule because this mechanism could also explain CheB control; see below.) Since CheW is not essential for this MCP-mediated inhibition, it appears that the receptor can bind the target molecules without the assistance of CheW. Thus, MCP overproduction would lead to a stoichiometric excess of inhibitor over target molecules and a consequent reduction in CW rotation. However, overproduction of CheW also reduces CW rotation, suggesting that CheW can influence either the equilibrium proportions or the relative affinities of the two receptor conformations. For example, CheW–CheA complexes may have higher affinity for the CCW form of MCP than does CheA alone. Alternatively, CheW may bind preferentially to the CCW form of MCP, effectively trapping receptors in the inhibitory state when it is present in stoichiometric excess.

The dual nature of the receptor coupling mechanism in this model may also apply to the feedback control of CheB by receptor signals. CheB appears to be activated by phosphorylation *in vitro* (32), and, like CheY, its *in vivo* activity is subject to both stimulation and inhibition in response to chemoeffectors (33, 34). Stewart *et al.* (11) have demonstrated that CheA is required for both control effects, but CheW is required only for CheB activation, not for its inhibition, consistent with the notion that there are two sorts of CheA-mediated receptor signals, only one of which (the CheA-stimulating signal) requires CheW.

Biochemical Support for the Dual Coupling Model. Evidence exists for several of the protein–protein interactions implicit in the model. The stoichiometric compensation between MCP and CheW molecules observed in the present study (Fig. 4) provides strong circumstantial support for interactions between these components, and recent demonstrations of conformational suppression between *tsr* and *cheW* mutations lend additional weight to this conclusion (unpublished data). CheW–CheA complexes have been detected by immunoprecipitation (P. Matsumura, personal communication), but it is not known whether their catalytic properties are different from those of uncomplexed CheA. Finally, in cell fractionation experiments, a substantial proportion of CheA molecules appeared to be loosely associated with the cell membrane (35), consistent with the possibility that CheA interacts with membrane-bound receptors.

Some aspects of the model are also consistent with recent *in vitro* work by Borkovitch *et al.* (17), who showed that CheA-mediated phosphorylation of CheY was greatly stimulated by a combination of CheW and solubilized membranes containing MCP molecules. Moreover, that stimulation was reversed by attractant stimuli, which would be expected to shift the receptors into the CCW conformation. However, some effects predicted by our model have not yet been seen *in vitro*. For example, CheW alone should enhance the rate of CheA autophosphorylation or the subsequent phosphorylation of CheY, whereas MCPs alone and in sufficient excess should inhibit those reactions. Since the relative stoichiometry of the interacting components is undoubtedly a critical factor, further *in vitro* study of the protein phosphorylation cascade is needed to properly test these predictions.

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