Both CheA and CheW Are Required for Reconstitution of Chemotactic Signaling in *Escherichia coli*

M. PATRICIA CONLEY, ALAN J. WOLFE, DAVID F. BLAIR, AND HOWARD C. BERG*

Department of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts 02138

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If cells of *Escherichia coli* deleted for genes that specify transducers and all known cytoplasmic chemotaxis proteins are reconstituted with CheA, CheW, and CheY, they spin their flagella alternately clockwise and counterclockwise. If the aspartate receptor also is present, clockwise rotation is suppressed upon addition of aspartate. If either CheA or CheW is absent, the fraction of time that the flagella spin clockwise is reduced and responses to aspartate do not occur.

Amino acids and certain sugars are sensed in *Escherichia coli* by membrane-spanning proteins called transducers, receptors, or methyl-accepting chemotaxis proteins. The cells are propelled by flagella that spin alternately clockwise (CW) and counterclockwise (CCW). The cytoplasmic domains of the transducers transmit information about receptor occupancy to the flagellar motors, changing the probabilities of CW and CCW rotation. Addition of a chemical attractant enhances CCW rotation. Four chemotaxis gene products have been implicated in this excitation pathway: CheA, CheW, CheY, and CheZ. A feedback loop allows for adaptation to a chemotactic stimulus: a methyltransferase (CheR) methylates the cytoplasmic domain of a transducer in response to an increase in the level of attractant, and a methyl-erase (CheB) demethylates the transducer in response to a decrease in the level of attractant (for recent reviews on bacterial chemotaxis, see references 8, 10, and 13).

Our interest has focused on the excitation pathway that involves CheA, CheW, CheY, and CheZ. In work described earlier (16), we constructed null (guttled) strains deleted for genes coding for all the known transducers (Tsr, Tar, Tap, and Trg) and all the known cytoplasmic chemotaxis proteins (CheA, CheW, CheY, CheZ, CheR, and CheB). If the flagellar motors of these cells were wild type, they spun exclusively CCW. When the cells were reconstituted with CheY, their motors spun alternately CW and CCW. CheZ behaved as a CheY antagonist. When the gutted cells were reconstituted with CheA and CheW, their motors continued to spin CCW. However, when they also contained the receptor for aspartate (Tar) and CheY, they spun CW and responded to aspartate. CheA and CheW were not tested separately. We now report that responses to aspartate require both CheA and CheW. Our earlier work also suggested that CheA or CheW or both act synergistically with CheY to increase the fraction of time that the flagella spin CW. We now report that this effect requires both CheA and CheW.

For the work described here, three parental gutted strains were prepared with two deletions of the *che* gene region (Fig. 1). Strain HCB437 is $\Delta(tsr)7021 \Delta(trg)100$ *zbd::Tn5* $\Delta(cheA-cheZ)2209$, strain HCB440 is $\Delta(tsr)7021 \Delta(cheA-cheY)::XhoI(Tn5)$, and strain HCB721 is $\Delta(tsr)7021 \Delta(cheA-cheY)::XhoI(Tn5) \text{ } trg::Tn10$. The second and third strains differ only by the disruption of *trg*. Strain HCB437 was used for our earlier work (16). The deletion $\Delta(cheA-cheZ)2209$

(11) gives rise to the expression of two CheA::CheZ fusion proteins (7) that have been found to enhance CW rotation in the presence of CheY (15). The deletion $\Delta(cheA-cheY)::XhoI(Tn5)$ was used to construct parental strains free of these fusion proteins. $\Delta(cheA-cheY)::XhoI(Tn5)$ has the *XhoI* fragment of *Tn5*, conferring kanamycin resistance, inserted in place of the chemotaxis genes that link the two *Sall* sites (Fig. 1). This new deletion was transduced from strain TH1594-r10 (from S. Harayama via G. L. Hazelbauer, Washington State University, Pullman) into a strain (HCB317) that carried $\Delta(tsr)7021$ to yield HCB440. This leaves *cheZ* intact but without its native promoter. The transposon *trg::Tn10* from RP1131 (J. S. Parkinson, University of Utah, Salt Lake City) was inserted in strain HCB440 (which is *trg*⁺) to disrupt *trg*; the resultant strain is HCB721 (15). Complementation of a *cheZ* mutant was not observed when the deletion $\Delta(cheA-cheY)::XhoI(Tn5)$ was present in single copy (15), but weak complementation was observed when this deletion was carried on a multicopy plasmid (data not shown). The expression of CheZ was measured in strain HCB721 by S. C. Kuo (Washington University, St. Louis, Mo., personal communication), who found a level of less than 5% that of the wild type.

Three plasmids and two λ bacteriophage were constructed so that subsets of the *che* genes could be added back (Fig. 1). The reconstituted strains are listed in Table 1. *cheA*, *cheW*, and *cheA cheW* are carried on the plasmids pDFB41, pDFB32, and pDFB38, respectively (Fig. 1). These genes are under the control of the *E. coli* B arabinose promoter, *para*, linked to *araC*, which encodes the arabinose activator-repressor. This allowed variable expression by addition of the inducer arabinose. *para* and *araC* were derived from pJH120 (from J. Haack via F. W. Dahlquist, University of Oregon, Eugene). The *cheA* and *cheA cheW* plasmids include sequences from *motA* and *motB* required for translation of the long and short forms of CheA at their native ratio (Parkinson, personal communication). *cheY* is carried on lysogenic phage λ (λ DFB19; Fig. 1) and is under the control of *plac*, which allowed variable expression by addition of the inducer isopropyl- β -D-thiogalactopyranoside (IPTG). *cheY*, from pJH123 (Haack via Dahlquist), was placed under the control of the *E. coli* lactose promoter on pUC9 (14) to yield plasmid pDFB13 (not shown). Synthetic linkers (New England BioLabs, Inc.) were used to change the restriction sites flanking *plac-cheY* to *EcoRI* sites. The resulting *EcoRI* cassette was inserted into the single *EcoRI* site in λ gt4, yielding the hybrid phage λ DFB19. Phage λ AJW3, which

* Corresponding author.

FIG. 1. Portions of the *mocha* and *meche* operons deleted in different backgrounds and encoded by the plasmids and phage constructed for this work. Endpoints are indicated by the restriction sites, which were either present in the native sequence or prepared with synthetic linkers. A_L and A_S , Translational start sites for the long and short proteins Che A_L and Che A_S , respectively; P, codon for the phosphorylation site in Che A_L . Double lines between genes indicate the presence of noncoding sequences.

also carries *tar* under control of its native promoter, was made in a similar fashion. The *BalI* fragment of the *tar* plasmid pAK101, in which the *AvaI* site in *tap* is converted to an *EcoRI* site (6), was fused to *plac-cheY* (from pDFB13 partially digested with *PvuII*).

Cells were grown in T broth in the presence of the requisite inducers and antibiotics, harvested, washed, and then tethered. Their rotational behavior was recorded on videotape and analyzed by computer (1). Computations of bias (the fraction of time that cells spun CCW) were based on measurements spanning 3 min for each of 10 to 20 cells. Most responses to attractants and repellents were scored by eye. Other materials and methods have been described previously (15, 16).

TABLE 1. Strains reconstituted in two deletion backgrounds by addition of the plasmids and phages depicted in Fig. 1

Reconstituted strain	Parental strain	Chemotaxis genes expressed ^a
$\Delta(cheA-cheZ)2209$ background		
HCB681	HCB437	<i>cheA cheY tar</i>
HCB690	HCB437	<i>cheW cheY tar</i>
HCB660	HCB437	<i>cheA cheW cheY tar</i>
$\Delta(cheA-cheY)::XhoI(Tn5)$ background		
HCB699	HCB440	<i>cheA cheY tar trg</i>
HCB697	HCB440	<i>cheW cheY tar trg</i>
HCB698	HCB440	<i>cheA cheW cheY tar trg</i>
HCB728	HCB721	<i>cheA cheY</i>
HCB725	HCB721	<i>cheW cheY</i>
HCB726	HCB721	<i>cheA cheW cheY</i>

^a *cheA*, *cheW*, and *cheA cheW* expressed from *para*, inducible by arabinose; *cheY* expressed from *plac*, inducible by IPTG; *tar* and *trg* expressed from their native promoters.

The effects of varying expression of *cheA*, *cheW*, and *cheY* on the response to aspartate were measured in cells with transducers grown in the presence of different concentrations of the inducers arabinose and IPTG. Responses to the addition of aspartate, and subsequently to its removal, were observed only when *cheA* and *cheW* were coexpressed (Table 2). Responses to aspartate were stronger in cells containing the $\Delta(cheA-cheY)::XhoI(Tn5)$ deletion than in those containing the $\Delta(cheA-cheZ)2209$ deletion (Table 2). However, if cells were induced with high concentrations of either arabinose or IPTG, the responses disappeared in both deletion backgrounds. The absolute induction levels, rather than their ratio, appeared to determine aspartate responses. Arabinose or IPTG at 25 μ M led to the synthesis of approximately wild-type levels of CheA and CheW or of CheY, respectively, as judged by optimum complementation of *cheA cheW* or *cheY* mutants in swarm plate and capillary assays (15, 16). In general, cells that responded well to the attractant aspartate also responded to the repellent Ni^{2+} , which is sensed by the same transducer (0.1 mM; data not shown). Responses to aspartate in cells expressing *cheA* and *cheY* or *cheW* and *cheY* could be judged readily only in the $\Delta(cheA-cheZ)2209$ background (in strains HCB681 or HCB690), because biases in the $\Delta(cheA-cheY)::XhoI(Tn5)$ background (in strains HCB699 or HCB697) were effectively 1. However, cells of the latter strains also failed to respond to Ni^{2+} . No responses to aspartate or Ni^{2+} were observed in strains HCB681 or HCB690, even in cells that spent a sizable fraction of their time spinning CW.

Within a given data set, the bias of individual cells varied widely (Fig. 2). Responses to aspartate, however, did not appear to be correlated to the unstimulated bias: within a given data set, a cell with a small bias was not more likely to respond than one with a large bias, nor did the mean bias indicate how well the cells might respond. Note for strain HCB698 (Fig. 2 and Table 2) the similar distributions and

TABLE 2. Bias and response of strains with transducers reconstituted in two deletion backgrounds

Genes under variable control	Strain	Concn (μM) of:		Mean bias ^a ± SEM (n)	Response to 0 to 2.5 μM aspartate ^b
		Arabinose ^c	IPTG ^d		
Δ(<i>cheA-cheZ</i>)2209 background					
<i>cheA cheY</i>	HCB681	10	10	0.99 ± 0.004 (20)	0
		25	10	0.80 ± 0.06 (20)	0
		25	25	0.91 ± 0.04 (16)	0
<i>cheW cheY</i>	HCB690	25	10	0.94 ± 0.04 (17)	0
		25	25	0.97 ± 0.02 (20)	0
		50	25	0.99 ± 0.01 (19)	0
<i>cheA cheW cheY</i>	HCB660	10	10	0.89 ± 0.05 (19)	0
		25	10	0.89 ± 0.06 (15)	+/-0
		10	20	0.52 ± 0.14 (10)	+
		20	20	0.45 ± 0.09 (17)	+
		25	20	0.41 ± 0.10 (10)	+
		50	20	0.73 ± 0.13 (12)	0
Δ(<i>cheA-cheY</i>::<i>XhoI</i>(Tn5) background					
<i>cheA cheY</i>	HCB699	25	10	1 (11)	0
		25	25	1 (14)	0
		100	25	1 (11)	0
<i>cheW cheY</i>	HCB697	25	10	0.99 ± 0.01 (15)	0
		25	25	1.00 ± 0.001 (20)	0
		25	50	1 (20)	0
<i>cheA cheW cheY</i>	HCB698	25	10	0.81 ± 0.07 (20)	++
		100	10	1.00 ± 0.001 (13)	0
		10	25	0.51 ± 0.15 (10)	++
		25	25	0.43 ± 0.10 (17)	++
		50	25	0.53 ± 0.08 (19)	+/-0
		25	50	0.42 ± 0.15 (11)	++
		50	50	0.92 ± 0.04 (15)	-/-0
	25	100	0.53 ± 0.10 (16)	0	

^a Fraction of time that tethered cells spin CCW.^b ++, All cells CCW only; +, most cells increased bias; +/-0, some cells increased bias; 0, no response; -/-0, some cells decreased bias.^c Induction of *cheA*, *cheW*, or *cheA cheW*.^d Induction of *cheY*.

mean biases of cells induced with 25 μ M arabinose–25 μ M IPTG or 50 μ M arabinose–25 μ M IPTG. In the former case, the cells responded strongly to aspartate; in the latter case, they did not (Table 2). In general, responses were not enhanced significantly by a further step to 100 μ M aspartate.

FIG. 2. Distributions of bias (fraction of time that tethered cells spin CCW) of individual cells of strain HCB698 expressing *cheA cheW* and *cheY*. The concentrations of arabinose (A) used to induce *cheA cheW* and of IPTG (I) used to induce *cheY* are listed along the abscissa. The means and standard errors of the means for each datum set are given in Table 2.

When *cheA* and *cheW* were coexpressed, the unstimulated bias was lower than when they were expressed separately (Table 2). This was true in both the presence and absence of transducers (Tables 2 and 3). However, in the latter case, the effect was less pronounced: compare the entries for strains HCB726 (Table 3) and HCB698 (Table 2) at equivalent concentrations of inducers.

In summary, we have found that CheA, CheW, and CheY together increase the fraction of time that cells spin their

TABLE 3. Bias of strains without transducers reconstituted in the second deletion background^a

Genes under variable control	Strain	Concn (μ M) of:		Mean bias ^b $\pm$ SEM (n)
		Arabi-nose ^c	IPTG ^d	
<i>cheA cheY</i>	HCB728	25	25	1 (12)
		25	50	1 (10)
<i>cheW cheY</i>	HCB725	25	25	1 (17)
		25	50	1 (16)
<i>cheA cheW cheY</i>	HCB726	25	10	0.94 $\pm$ 0.06 (10)
		25	25	0.80 $\pm$ 0.09 (12)
		50	25	0.98 $\pm$ 0.02 (20)
		100	25	0.96 $\pm$ 0.04 (18)
		25	100	0.79 $\pm$ 0.08 (19)

^a $\Delta(\text{cheA-cheY})::XhoI(\text{Tn5}) \text{ } \text{trg}::\text{Tn10}$.^b Fraction of time that tethered cells spin CCW.^c Induction of *cheA*, *cheW*, or *cheA cheW*.^d Induction of *cheY*.

flagella CW. This activation is enhanced by the presence of transducers (Tar or Tar and Trg) but suppressed when transducers are bound to ligand. These results support and extend our earlier findings on the activation of CheY (16). They complement the work of M. I. Simon and co-workers, who have shown that CheA is autophosphorylated in vitro in the presence of ATP and that phosphorylated CheA transfers phosphate to CheY (3-5; also see reference 17). In particular, they complement their recent work, showing that the rate of phosphorylation of CheY is enhanced manyfold by CheA, CheW, and a membrane fraction containing Tar and that this phosphorylation is blocked by the addition of aspartate (2). However, in the in vitro system, the effects of the addition of aspartate to preparations lacking only CheA or CheW were not tested.

If CheA and CheW enhance CW rotation via a phosphorylation cascade that activates CheY, how do they suppress this activation when attractant is bound to the receptor? *cheA* encodes two proteins, CheA_L and CheA_S, that are produced in approximately equal amounts in wild-type cells (9, 12; Fig. 1). The N-terminal portion of CheA_L, which includes the phosphorylation site (3), is missing in CheA_S. One possibility is that complexes of CheW with CheA_L are excitatory, while complexes of CheW with CheA_S are inhibitory. The interactions of these complexes with transducers might differ depending on whether or not an attractant is bound. We plan to test this hypothesis by reconstituting strains which allow variable expression of *cheA_L* and *cheA_S*.

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