JOURNAL OF BACTERIOLOGY, May 1994, p. 2736–2739
0021-9193/94/$04.00+0

Vol. 176, No. 9

NOTES

Chemotaxis in *Bacillus subtilis* Requires Either of Two Functionally Redundant CheW Homologs

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Received 10 December 1993/Accepted 28 February 1994

We have characterized mutants in a novel gene of *Bacillus subtilis*, *cheV*, which encodes a protein homologous to both CheW and CheY. A null mutant in *cheV* is only slightly defective in capillary and tethered cell assays. However, a double mutant lacking both CheV and CheW has a strong tumble bias, does not respond to addition of attractant, and shows essentially no accumulation in capillary assays. Thus, CheV and CheW appear in part to be functionally redundant. A strain lacking CheW and expressing only the CheW domain of CheV is chemotactic, suggesting that the truncated CheV protein retains in vivo function. We speculate that CheV and CheW function together to couple CheA activation to methyl-accepting chemotaxis protein receptor status and that possible CheA-dependent phosphorylation of CheV contributes to adaptation.

Bacterial chemotaxis is one of the best understood biological signaling mechanisms (5, 8, 9, 22, 23). Chemotaxis involves both a phosphorylation-dependent excitation and a methylation-dependent adaptation. Cell surface methyl-accepting chemotaxis proteins (MCPs) act as receptors which regulate the autophosphorylating CheA kinase (13, 20). In *Escherichia coli*, CheW physically bridges CheA to the MCPs to allow regulated phosphotransfer to CheY and CheB (6, 7, 13, 19). Phospho-CheY regulates direction of flagellar rotation (10, 26), while phospho-CheB functions in adaptation by demethylating MCPs (16). Chemotaxis in *Bacillus subtilis* requires many of these same proteins; homologs of CheA (12), CheB (17), CheW (15), CheY (3, 4), and CheR (18) proteins have all been identified.

The accompanying paper describes a new *B. subtilis* chemotaxis gene, *cheV*, encoding a protein homologous to both CheW and CheY (11). In this report, we describe the chemotactic behavior of strains mutant for *cheV*, *cheW*, or *cheY* (Table 1). Our results demonstrate that (i) CheV is required for optimal chemotaxis, (ii) either CheV or CheW is essential for signaling, and (iii) the CheW domain of CheV is sufficient for chemotaxis but not for normal MCP methylation.

Swarm plate analysis. Inactivation of either CheV or CheW leads to a reduction in swarm rate (Table 2), but a discrete ring of chemotactic cells is still observed. In contrast, the double mutant HB4007 (*cheV cheW* mutant) is reduced in swarm diameter (30 to 35% of the wild-type diameter) and fails to form a discrete ring. A *cheY* mutant, which tumbles continuously (3, 5), displays an even smaller disk of growth (<15% of the wild-type size). Thus, the double mutant is more defective in this assay than is either single mutant but not as defective as a mutant lacking CheY. The relationship between swarm rate and chemotactic proficiency is complex; nonchemotactic cells can migrate slowly or rapidly in semisolid agar, depending on the average tumble bias of the cells (27). Therefore, we have assayed chemotaxis directly, using capillary and tethered cell assays.

Capillary assays. We measured the accumulation of bacteria in capillaries containing various concentrations of the nonmetabolizable attractant, azetidine-2-carboxylate (Table 2). A logarithmically growing minimal medium culture was supplemented with 0.005% glucose and 5 mM sodium lactate, diluted to an optical density at 600 nm of 0.001, and allowed to migrate into an attractant-filled capillary (21). In this assay, the absence of either CheW homolog led to a modest reduction in chemotaxis, particularly at low attractant concentrations, while the *cheW cheV* double mutant failed to accumulate in capillaries at all attractant concentrations (<5% of the wild-type level). Strain HB4021 (Table 1), which lacks CheW and expresses a truncated CheV protein, was only as defective as the *cheW* or *cheV* single mutant. These experiments demonstrate that CheV and CheW are partially redundant, and the amino-terminal domain of CheV is sufficient to provide CheW function.

Tethered cell assays. Videomicroscopy of tethered cells (1, 2) allows one to observe both the excitation and adaptation phases of chemotaxis (Fig. 1). Strains lacking either CheV or CheW exhibit essentially wild-type responses to the addition

TABLE 1. *B. subtilis* strains

Strain	Genotype	Relevant characteristic(s)	Reference
OI1085	*trpF7*(Am) *hisB*(Am) *met*	Che^+	25
OI2737	OI1085 *cheW*::*cat*	$CheW^-$	15
OIB055	OI1085 *cheY*::*cat*	$CheY^-$	4
HB4004	OI1085 *cheV*::*kan* (330)[a]	$CheV^-$	11
HB4006	OI1085 *cheV*::*kan* (820)[a]	CheV (truncated)	11
HB4007	OI2737 *cheV*::*kan* (330)	$CheW^-$ $CheV^-$	This work
HB4021	OI2737 *cheV*::*kan* (820)	$CheW^-$ CheV (truncated)	This work

[a] Kanamycin resistance cassettes were inserted in the *BclI* site present at position 330 or position 820 in the *cheV* gene sequence as described in the accompanying paper (11).

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TABLE 2. Phenotypes of various Che$^-$ strains

Strain	Relevant characteristic(s)	Swarm diameter (% wt ± SEM)[a]		Bacterial accumulation in capillary (% wt ± SEM)[b]	
		Mannitol	Tryptone	10^{-2} M	10^{-5} M
OI1085	Wild type	100 ± 2.0	100 ± 3.6	100 ± 13	100 ± 35
OI2737	CheW$^-$	42 ± 0.5	46 ± 1.0	55 ± 16	40 ± 8
HB4004	CheV$^-$	45 ± 1.5	48 ± 2.0	75 ± 10	40 ± 27
HB4007	CheW$^-$ CheV$^-$	32 ± 1.1	28 ± 0.7	0.6 ± 0.1	3.2 ± 0.6
OIB055	CheY$^-$	13 ± 0.5	8 ± 0.4	ND[c]	ND
HB4006	CheV (truncated)	75 ± 2.7	71 ± 2.1	ND	ND
HB4021	CheW$^-$ CheV (truncated)	34 ± 2.3	29 ± 2.3	74 ± 14	25 ± 2.2

[a] Swarm diameter was measured after 8 to 12 h on 0.3% agar plates containing either tryptone or minimal medium with mannitol as described previously (21). Standard error of the mean (SEM) equals $\sigma_{n-1}/\sqrt{n}$, where $n = 5$ in the swarm plate assay.

[b] Capillary assays were performed as described previously (21) with the indicated concentration of azetidine-2-carboxylic acid as an attractant. Background accumulation, measured by the accumulation of bacteria in capillaries without attractant, was less than 2.5% of the wild-type level and was subtracted from values prior to further data analysis. SEM is defined in footnote *a*, except $n = 3$.

[c] ND, not determined.

and removal of an attractant. By contrast, the double mutant lacking both CheV and CheW fails to respond to attractant and has a 90 to 95% tumble bias. This result is similar to that obtained for a *cheA* null mutant (data not shown), consistent with the idea that either CheV or CheW can function to couple CheA to the MCP. Indeed, even a truncated CheV protein is sufficient for a chemotactic response in this assay (Fig. 1) and the capillary assays (Table 2). Slight deviations in the responses of the single mutants compared with that of the wild type, particularly during the adaptation phase, indicate that one CheW homolog is not sufficient to generate an optimal chemotactic response. This is consistent with the reduced migration of these strains in swarm plate assays.

MCP methylation. To determine whether CheV affected methylation of the MCPs, bacterial cells at early stationary phase (180 Klett units) were incubated with [*methyl*-^{3}H]methionine in the presence of chloramphenicol to inhibit protein synthesis. Cells were solubilized, and labeled proteins were visualized by fluorography of sodium dodecyl sulfate-polyacrylamide gels (14, 25). Methylation was near normal in the *cheV* and *cheW* single mutants but was extremely low in the *cheV cheW* double mutant and only somewhat better in the *cheW* mutant expressing a truncated CheV product (Fig. 2). Similar results were obtained when methanol release was assayed (24) from aspartate-stimulated cells (Fig. 3). Either CheV or CheW is sufficient for methanol release, but the double *cheV cheW*

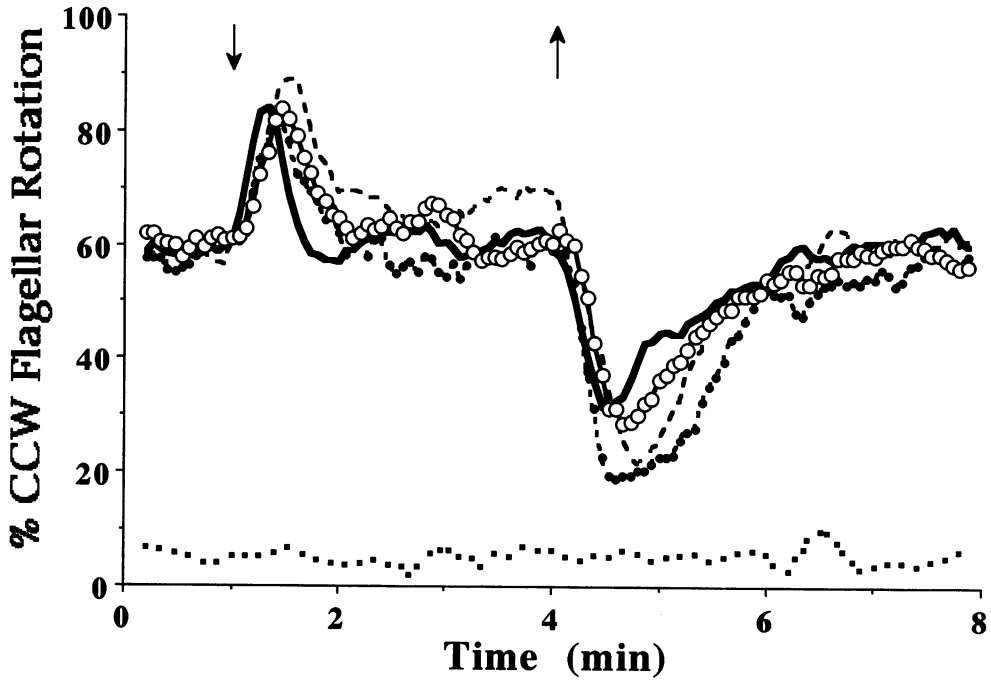

FIG. 1. Behavior of tethered cells of *che* mutant strains. Azetidine-2-carboxylic acid (10^{-5} M) was added at 1 min (downward-pointing arrow) and removed at 4 min (upward-pointing arrow). The average counterclockwise rotation of a population of 20 to 30 cells was determined every 0.25 s by a computer program, and the average values for 4-s intervals were plotted against time. Symbols: ——, wild-type strain OI1085; ○, *cheW* null mutant OI2737; – – – –, *cheV* null mutant HB4004; ■, *cheW cheV* double null mutant HB4007; ●, *cheW* null mutant with truncated CheV, strain HB4021.

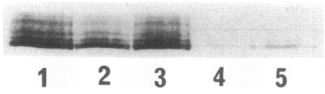

FIG. 2. In vivo MCP methylation. Methylation was done for 5 min with 100 μl of [*methyl*-^{3}H]methionine per sample. Lanes: 1, wild-type strain OI1085; 2, *cheW* null mutant OI2737; 3, *cheV* null mutant HB4004; 4, *cheW cheV* double null mutant HB4007; 5, *cheW* null mutant with truncated CheV, strain HB4021.

mutant, or the *cheW* mutant expressing the truncated CheV protein, allows little methanol release.

Summary. We report the characterization of a novel chemotaxis protein, CheV, with partial functional redundancy to CheW. CheV bears functional resemblance to CheW, rather than to CheY, since CheV can partly substitute for CheW but not for CheY. Mutants lacking either CheV or CheW are somewhat reduced in chemotactic proficiency, as judged by swarm rate and capillary assays (Table 2), but display relatively normal responses in tethered cell assays (Fig. 1) and in MCP methylation and methanol release assays (Fig. 2 and 3). In contrast, a mutant lacking both CheW and CheV is nonchemotactic (Table 1), does not respond to an attractant (Fig. 1), and has greatly reduced methyl turnover on the MCPs (Fig. 2 and 3). Thus, either CheV or CheW is sufficient for modulating CheA activity in response to attractants, but overall chemotactic proficiency is somewhat reduced. We suggest that CheV and CheW may normally function together, perhaps as part of the same receptor-bound multiprotein complex.

The *cheW* mutant can be partially rescued by expression of a truncated CheV protein containing the CheW domain as judged by swarm plate, capillary, and tethered cell assays. Thus, the amino-terminal domain of CheV is sufficient to provide CheW function. Nevertheless, this strain is defective in MCP methylation and methanol release assays, which suggests that CheB activity is significantly reduced. Since *B. subtilis* has an effective methylation-independent adaptation system, low CheB activity does not imply poor chemotaxis (17). Reduced CheB activity could be explained if the MCP complex with truncated CheV and CheA preferentially phosphorylates CheY rather than CheB. Alternatively, the altered MCP complex may be a poorer substrate for interaction with the CheB or CheR enzymes.

Our results suggest that the functional redundancy of CheW and CheV is incomplete. CheV is controlled by the σ^D holoenzyme (11), and CheW is controlled by the σ^A holoenzyme (28). Therefore, CheV is subject to genetic controls different from those for CheW. In addition, phosphorylation on the conserved aspartate in the CheY domain of CheV may play a role in adaptation. Additional experiments will be required to further explore the role of this unique chemotaxis component.

We thank Christopher Kristich for performing the flow assays of methanol production and Michael Kirsch for preliminary tethering experiments.

This investigation was supported by Public Health Service grants (AI20336 to G.W.O. and GM47446 to J.D.H.) and an NIH biotechnology training grant (GM08384 to K.F.).

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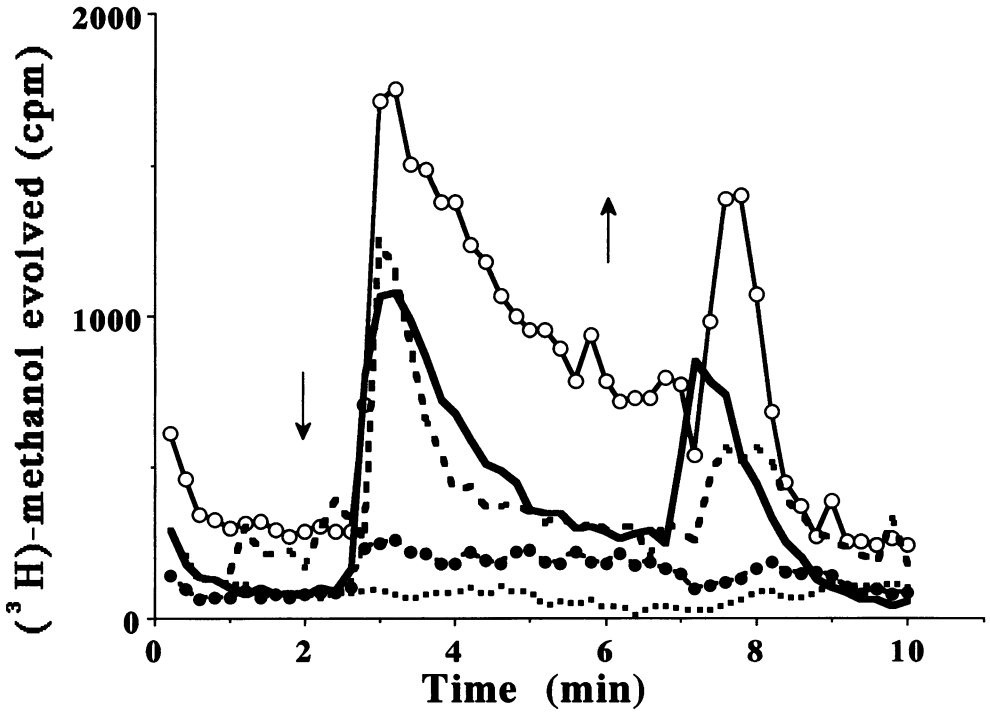

FIG. 3. Methanol production in *che* mutant strains. Cells incubated with [*methyl*-^{3}H]methionine were subjected to the addition (downward-pointing arrow) and removal (upward-pointing arrow) of 0.1 M aspartate in the presence of excess cold methionine. Radioactive methanol release was determined as a function of time. Symbols are described in the legend to Fig. 1.

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