Effect of loss of CheC and other adaptational proteins on chemotactic behaviour in *Bacillus subtilis*

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*Bacillus subtilis* has a more complex mechanism of chemotaxis than does the paradigm organism, *Escherichia coli*. In order to understand better the role of the novel chemotaxis proteins – CheC, CheD and CheV – mutants in which increasing numbers of the corresponding genes had been deleted were studied as tethered cells and their biases and sometimes durations of counterclockwise (CCW) and clockwise (CW) flagellar rotations in response to addition and removal of the attractant asparagine were observed. The *cheC* mutant was found to have considerably reduced switching frequency (that is, prolonged CCW and CW rotations) without a significantly different prestimulus CCW bias, compared with wild-type. This result may indicate that in absence of CheC the switch might be in a conformation less resembling the transition state than in presence of CheC. Conversely, the *cheB* (methyltransferase) mutant showed considerably increased switching frequency without affecting CCW bias, compared with wild-type. Removal of all known adaptation systems – the methylation, CheC and CheV systems – resulted in a mutant (*cheRBCDV*) that still retained some adaptation following the addition of attractant.

### INTRODUCTION

Chemotaxis is the process by which bacteria travel to high concentrations of attractant or low concentrations of repellent and it occurs by biasing an otherwise random walk (Berg & Brown, 1972). The basic mechanism in *Bacillus subtilis* (reviewed by Aizawa et al., 2001) and in *Escherichia coli* (reviewed by Bourret & Stock, 2002; Bren & Eisenbach, 2000; Falke & Kim, 2000; Falke & Hazelbauer, 2001; Stock & Levit, 2000; Taylor et al., 1999) involves complexes of transmembrane receptors (called methyl-accepting chemotaxis proteins or MCPs), CheA autokinase and CheW coupling protein, which allows the receptors to activate the kinase (Morrison & Parkinson, 1997). The CheA kinase autophosphorylates at the expense of ATP with production of ADP (Garrity & Ordal, 1997; Hess et al., 1988a, b). The response regulator, CheY, phosphorylates itself using CheA-P as the phosphoryl donor (Hess et al., 1988b) and is thought to bind to the switch protein FliM (Bren & Eisenbach, 1998; Szurmant et al., 2003; Toker & Macnab, 1997; Toker et al., 1996). Since the default direction of flagellar rotation in *E. coli* is counterclockwise (CCW), CheY-P binding causes an increase in the probability of CW rotation (Alon et al., 1998; Sourjik & Berg, 2002; Bren & Eisenbach, 2001; Scharf et al., 1998), to produce tumbling, which is uncoordinated motion that permits the next smooth swim to be in a different direction (Berg & Brown, 1972). Since the default direction of flagellar rotation in *B. subtilis* is clockwise (CW) (Bischoff et al., 1993), binding of CheY-P to FliM causes smooth swimming in *B. subtilis* (Bischoff et al., 1993; Szurmant et al., 2003). In the unstimulated, prestimulus state, the flagella in *B. subtilis* rotate CCW approximately 55% of the time. Stimulation by attractants results in an upregulation of CheA kinase and leads to increased CheY-P levels. This results in an increase in the CCW flagellar rotation.

Adaptation, which brings the levels of CheA-P and CheY-P back to prestimulus levels following addition of attractant, involves a coordinated methylation and demethylation of different sites on the receptors in *B. subtilis* (Zimmer et al., 2000) and involves only methylation of several possible sites in *E. coli*/*Salmonella enterica* (Terwilliger et al., 1986). CheR methyltransferase carries out the methylation in these organisms (Burgess-Cassler et al., 1982; Springer & Koshland, 1977) and the CheB methylesterase, the demethylation (Goldman et al., 1984; Stock & Koshland, 1978). The *B. subtilis* chemotaxis mechanism is more elaborate than that of the paradigm organism *E. coli* and involves several proteins not found in *E. coli* – CheC, CheD and CheV (Aizawa et al., 2001; Fredrick & Helmann, 1994; Karatan et al., 2001; Kirby et al., 2001; Kristich & Ordal, 2002; Rosario & Ordal, 1996; Rosario et al., 1995). However, *B. subtilis* lacks CheZ, which in *E. coli* dephosphorylates CheY-P (Blat & Eisenbach, 1994; Lukat & Stock, 1993;
Zhao et al., 2002). CheD deamidates certain selected glutamine residues in the receptors, changing them to glutamate residues (Kristich & Ordal, 2002), a reaction that makes the receptors more amenable to stimulation by attractant, possibly due to an effect on higher-order complexes within receptor clusters (Kirby et al., 2001). The other two proteins – CheC and CheV – are involved in mechanisms of adaptation. CheV has two domains, a CheW-type coupling domain and a CheY-type response-regulator domain (Fredrick & Helmann, 1994), which undergoes phosphorylation to help bring about adaptation (Karatan et al., 2001). CheC works by an unknown mechanism. There is evidence that it can bind to the receptor complex, including the receptors, the CheA kinase and CheD, and it is homologous to a region within the switch proteins FliM and FliY (Kirby et al., 2001). It has been suggested that CheC binds to one or both of these switch proteins (Kirby et al., 2001). Interestingly, CheC and CheD interact (Rosario & Ordal, 1996) but the purpose is unknown, for there are many bacteria that have cheD but lack cheC (Kirby et al., 2001). A summary of the properties of the chemotactic proteins of B. subtilis is presented in Table 1 and a diagram of their proposed locations is given in Fig. 1.

In order to gain further insight into the roles of the various chemotaxis proteins, we created strains lacking combinations of them, subjected them to addition and removal of attractant, and observed the effect on the time-course of direction of flagellar rotation using tethered cells. Tethered cells are cells that are sheared of most of their flagella and tethered using anti-flagellar antibody by the remaining flagellum to a glass cover slip that forms the ceiling of a laminar-flow chamber. The stimuli of addition and removal of attractant are administered by flowing buffer with or without attractant past the bacteria and the resulting changes in rotational behaviour of the bacteria are monitored using a microscope fitted with a CCD camera and suitable recording devices (Kirby et al., 1999).

**METHODS**

**Growth of bacteria.** Bacteria were grown and prepared for the tethered-cell assay as described by Kirby et al. (2001) for capillary assays except that the bacteria were grown overnight on tryptose blood agar (TBAB) plates at 30°C, suspended at O.D.550 0.014, and grown in minimal medium supplemented with 0.02% tryptone. The methods of Ordal et al. (1983) were followed for transformation and transduction.

**Construction of strains.** The strains studied in this article are given in Table 2. Strain OI3627, the cheRBCD mutant, was constructed by the same procedure as described by Kirby et al. (1999). The linkage of cheCD, as determined by Cm’, and unkU29::spc (due to integration of a Spc’ cassette in an unknown gene upstream of the major fla/che operon), was 70% by PBSI transduction. Strain OI3642, the cheRBCDV mutant, was created by transducing OI3627 to Kn’ using PBSI grown on HB4004, which contains a null mutation in cheV (Rosario et al., 1994). Strain OI3511, the cheRBCD mutant, was created in two stages. First, plasmid pC11 (Hanlon et al., 1992), which contains part of cheA as well as cheW, cheC, cheD and part of sigD, was gutted of cheW, cheC and cheD, which was replaced with a terminatorless cat cassette (Bischoff & Ordal, 1991) to make plasmid pK37. This plasmid was linearized and introduced into the cheB mutant OI2715 by transformation, with selection for Cm’, to make strain OI3512. Finally, strain OI2680 was made His+ by selection for histidine prototrophy, and the cheR allele was introduced into OI3512 by PBSI transduction with selection for His+. Presence of the cheR allele was confirmed by growing PBSI on candidates, transducing OI1085 to His+ and scoring for Cm’.

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**Table 1.** Roles of proteins in B. subtilis chemotaxis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Role</th>
<th>Bias of null mutant</th>
<th>Comments</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CheA</td>
<td>Autokinase</td>
<td>Tumbly</td>
<td>For excitation</td>
<td>Fuhrer &amp; Ordal (1991); Garrity &amp; Ordal (1997); Zimmer et al. (2002)</td>
</tr>
<tr>
<td>CheY</td>
<td>Response regulator</td>
<td>Completely tumbly, no switching</td>
<td>Binds to flagellar switch to cause CCW rotation</td>
<td>Bischoff &amp; Ordal (1991); Bischoff et al. (1993)</td>
</tr>
<tr>
<td>CheW</td>
<td>Coupling</td>
<td>Random</td>
<td>Couples receptor to CheA</td>
<td>Fredrick &amp; Helmann (1994); Hanlon et al. (1992); Karatan et al. (2001); Rosario et al. (1994)</td>
</tr>
<tr>
<td>CheV</td>
<td>Coupling, adaptation (cheWcheV is tumbly)</td>
<td>Random</td>
<td>Couples receptor to CheA and plays role in adaptation</td>
<td>Fredrick &amp; Helmann (1994); Karatan et al. (2001); Rosario et al. (1994)</td>
</tr>
<tr>
<td>CheB</td>
<td>Methylesterase</td>
<td>Random</td>
<td>Demethylates receptors upon being phosphorylated</td>
<td>Goldman et al. (1984); Kirby et al. (1997, 2000); Kirsch et al. (1993b); Zimmer et al. (2000, 2002)</td>
</tr>
<tr>
<td>CheR</td>
<td>Methyltransferase</td>
<td>Tumbly</td>
<td>Methylates receptors using S-adenosylmethionine</td>
<td>Burgess-Cassler &amp; Ordal (1982); Burgess-Cassler et al. (1982); Kirsch et al. (1993b); Rosario et al. (1995); Zimmer et al. (2000, 2002)</td>
</tr>
<tr>
<td>CheC</td>
<td>Adaptation</td>
<td>Random</td>
<td>Plays role in adaptation</td>
<td>Kirby et al., 2001; Rosario &amp; Ordal (1996); Rosario et al. (1995)</td>
</tr>
<tr>
<td>CheD</td>
<td>Deamidation of receptors</td>
<td>Tumbly</td>
<td>Deamidates receptors to activate them</td>
<td>Kirby et al. (2001); Kristich &amp; Ordal (2002); Rosario &amp; Ordal (1996); Rosario et al. (1995)</td>
</tr>
<tr>
<td>FliY</td>
<td>CheY-P hydrolysis</td>
<td>Non-motile (fliY56.1.15 is smooth swimming)</td>
<td>Dephosphorylates CheY-P</td>
<td>Szurmant et al. (2003)</td>
</tr>
</tbody>
</table>
The tethered-cell assay and calculation of the averaged behaviour
Assay of behaviour of tethered cells and analysis of data.

The tethered-cell assay and calculation of the averaged behaviour
have been previously described by Kirby et al. (1999). A CCW bias
for an individual behavioural period of a single cell was calculated
by averaging all 4 s data points within that period. The CCW biases
for all cells of a given strain were then averaged to obtain the mean
by averaging all 4 s data points within that period. The CCW biases
for an individual behavioural period of a single cell was calculated

RESULTS
Mean CCW and CW event durations in cheD, cheC and cheB mutants in the prestimulus state
Both cheC and cheB mutants have wild-type prestimulus biases (Table 3). However, analysis of mean CCW and CW durations of flagellar rotations in these mutants showed gross differences. When compared to the wild-type, the mean CCW and CW event durations in the cheC mutant were increased by 70 % and 67 %, respectively, both of which were statistically significant values (Table 3). Because both values increased by a similar magnitude, the cheC prestimulus bias was very close to the wild-type value. In contrast, in the cheB mutant, mean CCW and CW values were lower than that of the wild-type by 37 % and 27 %, respectively (Table 3). Again, because both parameters decreased equally, the cheB strain exhibited a wild-type prestimulus bias.

Interestingly, although the wild-type adapted completely (Fig. 2, Table 4), the mean postaddition CCW and CW event durations were significantly longer than those of the prestimulus period (Table 4).

Comparison of the effect of the cheD, cheC and cheB null alleles on bias
CheD is known to interact with CheC and the MCP receptor complex (Kirby et al., 2001; Rosario & Ordal, 1996). It was therefore of interest to understand the effect of deleting cheD on the event duration parameters described above. It has long been known that the cheD strain has a very low prestimulus CCW bias (Kirby et al., 2001; Rosario & Ordal, 1996); analysis of the mean event duration parameters showed that this low bias was due to a large decrease in mean CCW event duration and a small increase in the mean CW event duration (Table 3). This phenotype was very similar to that of the cheCD strain, indicating that the cheD null allele is much more important than the cheC null allele in determining bias. However, even in these strains, the cheC allele had a qualitatively similar effect as in wild-type: the mean CCW and CW event durations were longer in the strain lacking cheC, the $p_{\text{type I}}$ values being 1.43E–02 and 3.51E–17, respectively. Additionally, the cheD null allele was also much more important than the cheB null allele, at least when the cheC null allele is also present, in that the bias was also very low (Table 3). These results underscore the importance of CheD in setting the prestimulus bias.

Chemotactic behaviour of the cheBcheCcheD mutant
Behavioural responses of cheB and cheC mutants to addition
and removal of asparagine have been reported (Kirby et al., 2000, 2001). The cheB mutant could respond to high concentrations of asparagine (504 μM, corresponding to 90 % receptor occupancy; Ordal et al., 1977). However, it was impaired in adaptation to this stimulus as well as its

http://mic.sgmjournals.org 583
response to the removal (Kirby et al., 2000; Zimmer et al., 2002). The cheC mutant showed impaired adaptation to the addition of 504 μM asparagine, but normal response to its removal (Kirby et al., 2001; Rosario et al., 1995). To determine the behavioural responses of the cheBCD mutant, tethered-cell assays were performed with this concentration of asparagine. Previous analysis of rotational behaviour of the cheD and cheCD strains had indicated that only 50% of the cells were capable of responding to the addition of asparagine in either of the strains (Kirby et al., 2001). However, in the cheBCD mutant every cell analysed showed a response to the addition of asparagine (Fig. 2). This strain was also able to partially adapt to the addition of asparagine.

## Chemotactic behaviour generated by minimal systems

In order to determine the minimal chemotaxis system still capable of response and adaptation to a positive stimulus,
three strains were constructed: cheRBCD (Kirby et al., 1999), cheRBCDW and cheRBCDV. The latter two strains each lack one of the two proteins that couple the CheA autokinase to the MCP receptor complex. A cheWV strain has a very tumbly bias and is unresponsive to all stimuli (Karatan et al., 2001; Rosario et al., 1994). The cheRBCD strain had a very low prestimulus CCW bias and was unresponsive to the addition and removal of asparagine (Fig. 3), and the mean prestimulus CCW and CW event durations (Table 5) were not significantly different from those of the other strains lacking CheD (Table 5). Interestingly, further deletion of cheW restored the responsive nature of the chemotaxis system (Fig. 3) and significantly increased the prestimulus CCW bias (Table 5). This increase in bias was entirely due to an increase in the mean CCW event duration.

The cheRBCDV strain did not show a statistically significant increase in bias; however, the response to addition and removal of asparagine was restored in this strain as well.

Furthermore, the ability of this strain to partially adapt to the addition of asparagine is significant because it suggests that there still exists some measure of post-additional adaptation, despite the lack of all three known adaptation mechanisms: CheR/CheB methylation/demethylation (Hanlon et al., 1993; Kirby et al., 1997; Zimmer et al., 2000, 2002), the CheC mechanism (Kirby et al., 2001; Rosario et al., 1995), and CheV phosphorylation (Karatan et al., 2001). It is possible that a fourth (as-yet-unidentified) adaptation mechanism is also present in the chemotaxis system of B. subtilis. In this connection, FliY has recently been found to have CheY-P phosphatase activity (Szurmant et al., 2003).

**DISCUSSION**

Over the past 20 years much insight has been gained into the roles of chemotaxis proteins in both B. subtilis and other bacteria by analysing the rotational behaviour of cells that are subjected to addition and removal of chemoeffectors. The response profile of mutant strains reveals which phase of the chemotactic response – prestimulus, addition, post-addition, removal or post-removal – the strain is defective in. The parameter used has been the CCW bias, that is, the fraction of time the flagella rotate CCW. Here we have shown that while the CCW bias is an important parameter for analysis of chemotactic behaviour of cells, it can sometimes be incomplete. A complementary parameter to use is the mean durations of CCW and CW events. By using this parameter, we were able to show that cheB and cheC mutants were altered in their prestimulus behaviour even though they had normal prestimulus biases and that adaptation in wild-type was complete for bias but not for these parameters. The cheC mutant had increased CCW and CW event durations and the cheB mutant had decreased CCW and CW event durations. In other words, the cheC and cheB mutants exhibited a significant decrease and increase in the flagellar switching frequencies, respectively. These defects suggest that the switch complexes in these mutants are in a different

**Table 5. CCW bias and mean event durations for cheRBCD, cheRBCDW, cheRBCDV**

<table>
<thead>
<tr>
<th>Strain</th>
<th>CCW bias</th>
<th>CCW event duration</th>
<th>CW event duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.55</td>
<td>1.02</td>
<td>0.75</td>
</tr>
<tr>
<td>cheRBCD</td>
<td>0.10</td>
<td>0.31</td>
<td>1.30</td>
</tr>
<tr>
<td>cheRBCDW</td>
<td>0.27*</td>
<td>0.56*</td>
<td>1.31</td>
</tr>
<tr>
<td>cheRBCDV</td>
<td>0.15</td>
<td>0.45*</td>
<td>1.19*</td>
</tr>
</tbody>
</table>

*Statistically different from corresponding cheRBCD value. Values of $p_{type}$ were as follows: CCW bias for cheRBCD compared with cheRBCDW, 4.91E–03; CCW event duration for cheRBCD compared with cheRBCDW, 2.91E–26; CCW event duration for cheRBCD compared with cheRBCDV, 2.28E–08; CW event duration for cheRBCD compared with cheRBCDV, 4.87E–02.
conformational state compared to the wild-type strain as well as each other.

The observation that the cheC mutant has reduced flagellar switching frequency suggests that CheC reduces the durations of CCW and CW events. This result is consistent with a reduction in the energy of activation for the transition between CCW and CW directions of flagellar rotation. One way this might occur would be by binding of CheC to FliY or FliM (see Kirby et al., 2001) and stabilizing a conformation of the switch proteins that is closer to the transition state. Kirby et al. (2001) suggested that since CheC has similarity to switch proteins, it might transiently associate with the switch complex and since it binds to the receptors, CheD and CheA, it might go from the switch to the receptors as part of a mechanism of adaptation (cheC mutants adapt poorly: Kirby et al., 2001; Rosario et al., 1995). Our results support this hypothesis by showing that CheC does have an effect on switch properties and it is hard to imagine a way that such an effect can occur other than by direct binding.

Not only is it likely that CheC binds to the switch, but also two lines of evidence are consistent with the hypothesis that CheC is at least transiently associated with the ternary receptor complex. (i) CheC was shown to interact with the asparagine receptor McpB as well as CheA and CheD in a yeast two-hybrid assay (Kirby et al., 2001). (Both CheA and CheD interact with receptors directly: see Introduction.) (ii) It has also been shown that receptor methylation is affected by CheC because cheC mutants have overmethylated receptors (Rosario & Ordal, 1996). Thus, not only is there evidence that CheC can interact at both the receptors and at the switch – and hence might travel between them as part of a mechanism – but also it is possible that the affinity of CheC for the receptors could be determined by the methylation state of the receptors.

In order to account for the effect of a null mutation in cheB on switching frequency (much shorter durations of CCW and CW rotations; see Table 3), two hypotheses are apparent. One is that CheB binds directly to the switch to affect switching frequency and the other is that in the cheB mutant, there is more CheC available to bind to the switch. We favour the latter hypothesis since there is no evidence that CheB, an enzyme that demethylates receptors, binds to the switch. By contrast, the effect of the cheB null mutation on switching frequency can be accounted for by assuming that overmethylated receptors have minimal affinity for CheC (see preceding paragraph). In this hypothesis, a surplus of CheC could result in increased binding at the switch and further lowering of the energy of activation of transition beyond that in wild-type, leading to the increased switching frequency observed in the cheB mutant.

This hypothesis that effects of different conditions (such as lack of CheB methylesterase) on switching frequency are mediated via CheC levels also may provide understanding of the incomplete adaptation for durations of CW and CCW rotation following addition of attractant (Table 4). It is generally accepted that bacteria fully adapt to stimuli (Alon et al., 1999; Barkai & Leibler, 1997; Macnab & Koshland, 1972), and it is true that B. subtilis adapts fully in terms of bias (Table 4). However, it does not adapt in terms of durations of CCW and CW rotations; in fact, both are longer after ‘adaptation’ (Table 4). No current model of chemotaxis accounts for this failure of adaptation. However, if we assume that the ‘adapted’ receptor complex has increased affinity for CheC, then there would be less CheC binding at the switch and the switching frequency would be longer.

This line of thinking, where CheC binding to the switch affects switching frequency, might provide insight into a longstanding puzzle. Many archaea and some bacteria have CheC or the closely related CheX (Kirby et al., 2001). Some of these have polar flagella and, on negative stimuli, undergo repeated reversals of motion, such as the spirochaete Spirochaeta aurantia (Cercignani et al., 1998; Fosnaugh & Greenberg, 1988) and the archaeon Haloarchaeum salinarum (Spudich et al., 1989). It is clear that the main output of the receptor/CheA complex is to regulate CheY-P levels in these as in all chemotactic bacteria/archaea but how CheY-P levels can regulate switching frequency (it primarily affects bias) has never been understood. In line with the effect of CheC on switching frequency in B. subtilis, we propose a possible mechanism for the increased switching frequency observed in these organisms upon negative stimulation, namely change in CheC (or CheX for S. aurantia) binding at the switch.

To make this argument, we first point out that in the case of H. salinarum, at least, CheY-P has a similar function as in E. coli and B. subtilis in that it permits the nondefault direction of flagellar rotation. In a cheY mutant of H. salinarum, only forward swimming (chronic CW rotation of the flagella at each end of the archaean) occurs (Rudolph & Oesterhelt, 1996). Backward swimming and reversals between directions, however, occur in wild-type. The increased reversals on negative stimulus might be due to changing CheY-P levels affecting affinity of CheC (or CheX) for the switch and possibly also to changing affinity for CheC (or CheX) at the receptors (due to methylation changes that occur there: Nordmann et al., 1994; Spudich et al., 1989). As a further parallel with B. subtilis, we note that a cheB mutant of H. salinarum shows increased frequency of reversals, with no effect on the ratio of CW and CCW rotation of the flagella, compared with wild-type (Rudolph & Oesterhelt, 1996). Since in the B. subtilis cheB mutant the ratio of CW and CCW rotation (that is, bias) of the flagella is unchanged but switching frequency is increased (Table 3), it is not hard to imagine that reversal frequency in H. salinarum and B. subtilis are controlled by the same mechanism, namely, amount of CheC bound at the switch.

One of the hallmarks of B. subtilis chemotaxis is that...
multiple adaptational mechanisms appear to operate concurrently. Besides the CheC mechanism, there is one that involves methylation/demethylation of the receptors (Hanlon & Ordal, 1994; Kirby et al., 1997; Zimmer et al., 2000, 2002) and another that involves phosphorylation of the response regulator CheV (Karatan et al., 2001). We sought to understand how deletion of these adaptational systems affected the unstimulated behaviour of bacteria and their response to attractant. We were already aware of the effect of deleting cheD and of the fact that the phenotype of the cheD and cheCD mutants was quite similar (considerably different from the cheC mutant) (Kirby et al., 2000). We confirmed and extended this previous observation to include strains cheBCD, cheBCD, cheRBCD and cheRBCDV. The low prestimulus biases that occur in these appear to be a result of reduced durations of mean CCW events due, probably, to receptors undeamidated by CheD (Kristich & Ordal, 2002) being fairly inactive at stimulating the CheA kinase. Earlier work had also shown that in both the cheD and cheC mutants about half the cells responded and half did not respond to addition of asparagine (Kirby et al., 2001). We have shown here that this responsiveness was influenced by methylation in the sense that further deletion of cheB made the bacteria all responsive but, curiously, further deletion of cheR made all of them unresponsive. Obviously, the methylation system has a profound effect on the overall geometry and functioning of the receptor/kinase complex.

It should be noted that the failure of the cheRBCD mutant to respond to asparagine in the tethered-cell assay (or on swarm plates; data not shown) is in contrast to the results of Kirby et al. (1999), who reported that oscillations in CCW bias occurred on addition of attractant and stopped on its removal. Those results were reproducible; however, the linkage between cheD and a Spc’ marker in an unidentified gene upstream of the beginning of the fla/che operon (Slack et al., 1995) was later found to be tenfold lower than expected for unknown reasons. The conclusion of the paper that the adaptation was due to CheY-P feedback at the receptors may have been premature since at that time CheV was not recognized as an adaptational protein.

Interestingly, deletion of either gene encoding the coupling proteins cheW or cheV (Karatan et al., 2001; Rosario et al., 1994) to make a cheRBCD or cheRBCDV mutant made the bacteria again responsive to stimuli, and in the former strain also significantly increased the mean prestimulus CCW bias (Table 5). In this sense cheW had a moderating effect on cheD, the only allele that we observed to have this effect. In some way, the conformation of the receptors/ CheA complex was very stable in a refractory state when both coupling proteins were present and became more amenable to changing to an active state when one of the coupling proteins was deleted. Very interestingly, the cheRBCDV mutant lacked all known adaptation systems – the methylation/demethylation mechanism, the CheV mechanism and the CheC mechanism – and yet showed a progressive decrease in CCW bias following the response to the addition of attractant. Very recent experiments have revealed that FliY, one of the switch proteins, can catalyse dephosphorylation of CheY-P (Szurmant et al., 2003) and in future experiments we will investigate the behavioural phenotype of mutants unable to carry out this dephosphorylation.

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REFERENCES


concentrations of attractant in Bacillus subtilis 18610–18616.


