Review

The three adaptation systems of *Bacillus subtilis* chemotaxis

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Adaptation has a crucial role in the gradient-sensing mechanism that underlies bacterial chemotaxis. The *Escherichia coli* chemotaxis pathway uses a single adaptation system involving reversible receptor methylation. In *Bacillus subtilis*, the chemotaxis pathway seems to use three adaptation systems. One involves reversible receptor methylation, although quite differently than in *E. coli*. The other two involve CheC, CheD and CheV, which are chemotaxis proteins not found in *E. coli*. Remarkably, no one system is absolutely required for adaptation or is independently capable of generating adaptation. In this review, we discuss these three novel adaptation systems in *B. subtilis* and propose a model for their integration.

Bacterial chemotaxis and adaptation

Chemotaxis is the process by which cells sense chemical gradients in their environment and then move towards more favorable conditions. This gradient-sensing mechanism involves a form of sensory adaptation. In other words, the response to ligand, either chemo-attractants or -repellents, is phasic: the sensory response always returns to prestimulus levels despite the sustained presence or absence of ligand [1]. This sensory adaptation underlies the temporal gradient-sensing mechanism employed by bacteria. In peritrichously flagellated bacteria such as *Escherichia coli* and *Bacillus subtilis*, cells move up gradients of attractants and down gradients of repellents by modulating the duration and probability of smooth runs and reorientating tumbles. If a cell finds itself traveling in a favorable direction, up a gradient of attractant or down one of repellents, then it will tend to continue along its current trajectory [2]. This strategy enables cells to bias their motion towards more favorable chemical environments. The crucial aspect of this binary decision process underlying gradient sensing is that the response, the tendency to run or tumble, is proportional to the rate of change in the average number of ligand-bound receptors and not their absolute levels [3].

The *E. coli* chemotaxis pathway uses a modified two-component system for chemotaxis (Box 1). Briefly, the swimming behavior is determined by the concentration of phosphorylated CheY (CheYp), which binds to the flagellar motors [4] and increases the likelihood of tumbles [5]. When attractants bind to the receptors, the associated CheA kinase is inhibited, leading to decreased levels of CheYp and an increased likelihood of a run [6]. This response, however, is only transitory as the cells eventually adapt to the attractant [1] (Figure 1). The adaptation process is mediated by receptor methylation. Two enzymes, CheR [7] and CheB [8], add or remove methyl groups, respectively, at conserved glutamate residues on the receptors in response to changes in receptor and kinase activity [9]. The key element of this regulation is that methylation of the receptors causes the associated CheA kinases to become more active [10]. In addition to affecting CheA kinase activity, attractant binding also increases the rate of methylation by CheR and decreases the rate of demethylation by CheB [11]. These changes in enzyme activity counteract the kinase-inhibiting effect of attractant binding by increasing the levels of receptor methylation. In the case of repellents (e.g. nickel) or the loss of attractants, the reciprocal process occurs [11]. Receptor methylation is partially regulated in a feedback loop involving CheB phosphorylation. To be active, CheB must be phosphorylated by CheA [12]. Thus, CheB activity is proportional to kinase activity. In addition, the activities of both CheR and CheB are thought to be responsive to receptor conformation, where CheR preferentially methylates receptors in an inactive conformation and phosphorylated CheB preferentially demethylates receptors in an active conformation [10,13,14].

Although many important questions remain unanswered, particularly with regard to how receptor methylation tunes kinase activity, there is no longer any doubt regarding the basic mechanism of adaptation in *E. coli*. However, until recently, the same could not be said for *B. subtilis*. We propose that *B. subtilis* has three adaptation systems. These include the methylation system [15], the CheC–CheD–CheYp system [16] and the CheV system [17]. The long-standing puzzle is that no one system is necessary for adaptation, although completing the adaptation process over the full range of concentrations requires all three. Here, we review the current understanding of these three adaptation systems and attempt to provide a model for their integrated action.

The methylation system

B. subtilis employs receptor methylation for adaptation [18,19]. However, the mechanism is quite different from the receptor-methylation system of adaptation in *E. coli* despite the same enzymes, CheR and CheB, being used in both organisms [18,19]. In *B. subtilis*, the receptors are rapidly demethylated in response to the addition of

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Box 1. Comparison of the *Escherichia coli* and *Bacillus subtilis* chemotaxis pathways

Both *E. coli* and *B. subtilis* use a modified two-component system involving the CheA histidine kinase and CheY response regulator for transmitting signals from the receptors to the flagellar motors [24,51]. In both organisms, the receptors, CheA kinases and CheW adaptor proteins form stable ternary complexes localized primarily at the poles of the cell [26,52,53]. In the case of *B. subtilis*, there is an additional coupling protein, CheV, that is also involved in adaptation and that is functionally redundant to CheW, indicating that quaternary complexes form in this organism [31]. Both organisms use phosphorylated CheY (CheYp) to alter the frequency of runs and tumbles through direct binding to the flagellar motors. However, the effect is different: CheYp binding causes counter-clockwise rotation of the motor in *B. subtilis* [54] and clockwise rotation in *E. coli* [55,56]. Note that counter-clockwise rotation correlates with runs and clockwise rotations with tumbles in both organisms. Despite the different effects, the response to attractants is the same. In *E. coli*, the binding of attractant to the receptors inhibits CheA kinase activity, thereby reducing CheYp concentrations and increasing the likelihood of a run. In *B. subtilis*, attractant activates the CheA kinase, thereby increasing both CheYp concentrations and the likelihood of a run. Both organisms also use phosphatases to control the concentration of CheYp and improve the dynamic response. In *E. coli*, there is a single CheYp phosphatase, CheZ, that is localized with the receptors [51,57]. In *B. subtilis*, there are two CheYp phosphatases; one, CheC, is localized with the receptors, and the other, FliY, is an integral part of the flagellar motor C-ring [34]. Both CheZ and FliY are constitutively active, whereas the phosphatase activity of CheC is regulated by CheD. *E. coli* and *B. subtilis* also have two enzymes, CheR and CheB, that methylate and demethylate specific glutamate residues on the receptors, respectively [7–9,58,59]. In addition, CheB must be phosphorylated by CheA to be active [12]. The two enzymes function in adaptation, although the mechanisms are different in the two organisms (see main text for details). Finally, *B. subtilis* also possesses a receptor deamidase, CheD, that functions with CheC in one of the three *B. subtilis* adaptation systems [16,36].

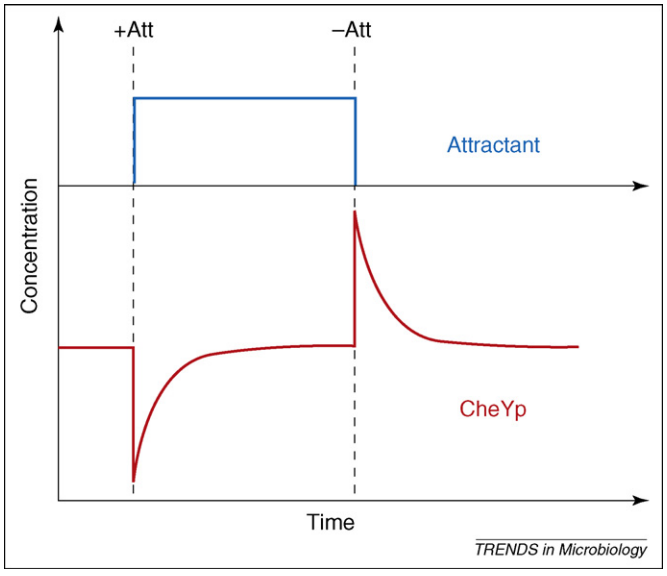

Figure 1. The adaptation process. The addition of attractant (+Att) causes a transient decrease in the concentration of CheYp in *Escherichia coli*, which leads to an increased likelihood of a smooth run. However, the change in CheYp concentrations is transitory because the pathway eventually adapts the concentration of CheYp to the addition of the attractant. In other words, the concentration of CheYp returns to prestimulus levels despite the presence of attractant. The removal of attractant (–Att), by contrast, increases the concentration of CheYp, which leads to a decreased likelihood of a smooth run or, alternatively, an increased likelihood of a reorientating tumble. As with addition, the pathway eventually adapts the concentration of the CheYp to the loss of attractant. The reciprocal process occurs in *Bacillus subtilis*: the addition of attractant causes an increase in CheYp concentration, and the removal of attractant causes a decrease in CheYp concentration. In *B. subtilis*, the frequency of runs is proportional to the concentration of CheYp, whereas, in *E. coli*, the frequency of runs is inversely proportional to the concentration of CheYp.

attractant and then slowly remethylated as the bacterium continues to be incubated in the presence of attractant. The same process also occurs when the attractant is removed. Moreover, the net level of methylation is approximately constant at steady state irrespective of the ambient concentration of attractant. Consistent with these changes in methylation, methanol, the by-product of the demethylation reaction catalyzed by CheB, is released both in response to the addition and removal of attractant [20]. As a comparison, receptors are methylated in *E. coli* when attractant is added and demethylated when attractant is removed. Likewise, methanol is only released in *E. coli* when the attractant is removed, which is consistent with a decrease in the level of receptor methylation [21]. The methylation dynamics in *B. subtilis* indicate that methyl groups are shuttled between different sites on the receptor in response to the addition or removal of attractant. Such a model would predict that the methylation of certain residues activates the kinase, whereas the methylation of others deactivates it.

Evidence for such a mechanism in *B. subtilis* comes from the analysis of the individual methylation sites on McpB, the asparagine receptor. This receptor has three methylation sites located at Glu371, Glu630 and Glu637 (residue 371 is initially encoded as a glutamine, but is converted to glutamate via deamidation). Aspartate substitutions at these positions yielded the following results. An Asp630 substitution decreases the activity of the receptor complex, whereas an Asp637 substitution increases it. However, the Asp371 substitution does not seem to affect activity [22]. In terms of methylation, aspartate substitution prevents a residue from being methylated by CheR [23] and, thus, it will remain negatively charged. A simple model based on these results would indicate that either methylating residue 630 or demethylating residue 637 activates the receptor, whereas demethylating residue 630 and methylating 637 inactivates it. Such a model could also explain the methylation data by the following sequence of hypothetical events. Initially, residue 630 is methylated and residue 637 is unmethylated. When an attractant binds the receptor, the kinase becomes hyperactive [24] (as opposed to the reverse scenario in *E. coli* [6]). To compensate for the increase in activity, residue 630 is demethylated and 637 methylated because these two changes would decrease kinase activity [22]. This step would also explain why methanol is released by the demethylation–remethylation cycle [20]. Likewise, when the attractant is removed, the kinase becomes inactive. To compensate for the decrease in activity, residue 630 is methylated and residue 637 demethylated [20]. These changes would increase activity. They would also lead to another demethylation–remethylation cycle and the release of methanol.

Although this model of selective methylation is appealing, the data indicate a more complex pattern regarding shuttling of methyl groups between residues. In experiments in which different combinations of methylation

sites were changed to unmethylatable aspartate residues, methanol was found to be released from residues 371 and 630 upon the addition of attractant and from residues 630 and 637 upon removal of attractant [22]. Thus, residue 630 is demethylated both upon the addition and removal of attractant. These results, however, might not directly mimic the natural system because the aspartate is one methylene group shorter than the native glutamate. A more problematic aspect of this simple model concerns the relative timing of the demethylation and remethylation cycle. When *B. subtilis* is exposed to attractant, the receptors are rapidly demethylated within the first minute [25]. However, nearly 20 minutes pass before the receptors are completely remethylated [20]. To put these times in perspective, the adaptation process takes less than one minute, as determined by the frequency of runs and tumbles, even at high concentrations of attractant. Somewhat longer times are observed when the attractant is removed (although still much shorter than the time required for remethylation) [26] (C.V. Rao and G.W. Ordal, unpublished). These results seem to indicate, based simply on relative timing of the events, that the demethylation step is sufficient for adaptation, whereas the remethylation step has some other role. In fact, the remethylation step could coordinate the three adaptation systems as described later.

Receptor methylation increases the activity of the CheA kinase in *E. coli*. Charge–charge repulsion between the unmethylated glutamates is thought to destabilize the coiled-coil structure of the receptor, leading to weaker activation of the associated kinase [27]. Methylation neutralizes the negative charges on the glutamates. The tighter receptor conformation afforded by the neutralization of these negative charges produces greater CheA kinase activity. The salient feature of methylation in *E. coli* is that the contribution of methylated receptor residues to the free-energy bias between the kinase active and inactive states is additive [28,29]. Although many details are unknown, methylation in *B. subtilis* is clearly selective: methylation of certain residues activates the kinase, whereas methylation of others deactivates it [22]. Some insight into how selective methylation tunes kinase activity is afforded by the recent crystal structure of the cytoplasmic domain of the chemotaxis receptor Tm1143 from *Thermotoga maritima*, a receptor that closely aligns with those from *B. subtilis* [30]. Homology modeling indicates that the three methylation sites on McpB form a tight cluster on the outward face of the receptor (Figure 2). Based on the relative proximity of the glutamates to one another, charge–charge repulsion between them is likely to affect receptor stability and associated kinase activity. In particular, the selective methylation of these glutamates probably induces either a destabilizing outward or a stabilizing inward rotation of the helices within the receptor homodimer. Homology modeling also indicates that residue 371 tunes the magnitude of these conformational changes.

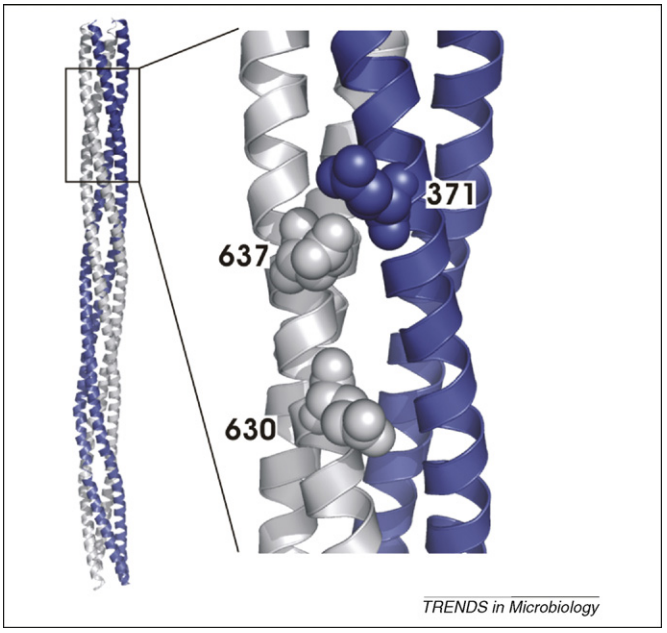

Figure 2. Model of the McpB structure in *Bacillus subtilis*. Based on homology to the Tm1143 chemoreceptor, the methylation sites (highlighted) form a tight cluster. Note that residue 371 is on a separate monomer of the chemoreceptor homodimer, opposite that of residues 630 and 637.

The CheC–CheD–CheYp system

B. subtilis has two chemotaxis proteins, CheC and CheD [31,32], that are not found in *E. coli*. CheC is a CheYp phosphatase [33,34]. However, a *cheC* null mutant does not have elevated levels of CheYp [35], as is the case with a *cheZ* phosphatase mutant in *E. coli* [5]. Rather, a *cheC* mutant is unable to complete the adaptation process [35]. Deletion of the CheYp-binding domain of FliY, a homolog of CheC, yields a phosphatase phenotype similar to CheZ, indicating that CheC functions primarily as a regulator of adaptation rather than as a phosphatase [34]. CheD is a receptor deamidase that converts conserved glutamine residues to glutamates [36]. In addition to its enzymatic function, CheD also regulates CheA kinase activity, as *cheD* null mutants have increased tumbling, which is indicative of low levels of CheY phosphorylation [35]. CheC and CheD interact with one another [37]. How CheC as a CheYp phosphatase could function with CheD in adaptation was recently determined when the phosphatase activity of CheC was decoupled from its ability to interact with CheD [16].

Members of the CheC–CheX–FliY phosphatase family share a common domain (PFAM PF04509) [38]. Both CheC and FliY have two of these domains, whereas CheX, a related chemotaxis phosphatase found in many bacteria, has only one [39]. Systematic mutagenesis of the active site within each domain in CheC revealed crucial glutamate (Glu17 and Glu118) and asparagine residues (Asn20 and Asn121) (Figure 3). A CheC mutant with Glu→Ala substitutions at both positions was unable to bind CheYp. Moreover, a CheC mutant with Asn→Ala substitutions at both positions could still bind CheYp but had absolutely no phosphatase activity. With regard to chemotaxis, the Glu17Ala Glu118Ala mutant showed the same poor chemotaxis as a null mutant in capillary assays (~20% of wild-type chemotaxis). The Asn20Ala Asn121Ala mutant, however, was able to perform taxis at ~50% wild-type levels. Thus, chemotaxis seems to depend more on the ability of CheC to bind CheYp than its CheYp phosphatase activity [16].

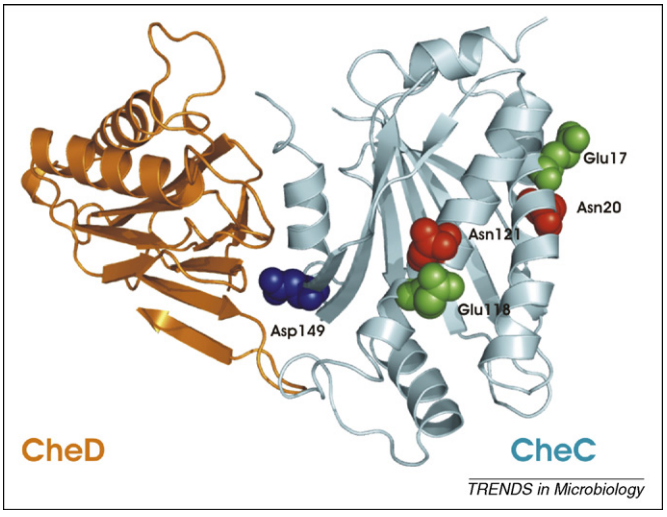

Figure 3. CheD and CheC interaction in *Bacillus subtilis*. Based on the *Thermotoga maritima* structure, Asp149 (blue) lies on the face of CheC that binds to CheD. The active site glutamate (green) and asparagine (red) residues are on the opposite side of the molecule, presumably where CheC interacts with CheYp.

The binding of CheC to CheYp, however, is only half the picture because it does not explain the role of CheC in adaptation. To understand this aspect, CheD must be accounted for as it interacts with CheC. CheD binds to CheC and increases its CheYp phosphatase activity approximately fivefold [34]. Furthermore, the affinity of CheC for CheD is increased by the presence of CheYp [16,40]. However, the phosphatase activity of CheC is not crucial for adaptation. To understand the potential role of CheD, CheC was mutated at a crucial Asp149 residue positioned along the CheC–CheD interface (Figure 3). Alanine and lysine substitutions at this position did not affect phosphatase activity. However, these CheC mutant proteins were unable to bind CheD. Furthermore, the corresponding mutant strains showed the same chemotaxis defects in capillary assays as the *cheC* null mutant [40]. These results seem to indicate that the role of CheC in adaptation is through its interaction with CheD.

Based on these results, CheC and CheD probably function in a negative-feedback loop involving CheYp (Figure 4). Prior to stimulation with attractant, a fraction of CheD is bound to the receptors. (Recall that CheD is a positive activator of CheA kinase.) Although the mechanism for this activation is unknown, CheD is known to interact with the receptors [24,32,37] and this interaction increases the tendency of the receptors to be in a conformation that activates the CheA kinase. When the cells are exposed to attractant, CheA kinase activity increases, leading to an increase in CheYp levels. CheYp then binds to CheC. This complex provides an alternative binding target for CheD and, thus, recruits CheD away from the receptors. As a consequence, the kinase is inhibited. This mechanism amounts to a negative-feedback loop involving CheYp. Likewise, when the attractant is removed or the cell is exposed to a repellent, CheA kinase activity and CheYp levels decrease. As a consequence, fewer CheC–CheYp complexes form and more CheD is free to bind the receptors and reactivate them. One alternative mechanism is that receptor activation causes CheD to disassociate from the receptors. Free CheD could then bind CheC and increase the rate of CheYp dephosphorylation. Such a 'feed-forward' mechanism would adapt the CheYp levels rather than CheA kinase activity as described. However, this mechanism is less likely because CheC mutants with reduced phosphatase activity are able to adapt [16]. It is also possible that a combination of the two mechanisms is involved in modulating CheYp levels.

In addition to their role in adaptation, it seems – based on various mutant data – that CheC, CheD and CheYp also regulate receptor methylation. For example, the receptors are weakly methylated in a *cheD* null mutant, whereas they are methylated at levels approximately twice wild type in a *cheC* null mutant [32,37]. Moreover, the receptors in a *cheY* null mutant do not remethylate after the addition of attractant. Rather, they only remethylate after the removal of attractant [20]. Finally, the remethylation step in a *cheC* null mutant occurs more rapidly than in wild type (J. Kirby, PhD thesis, University of Illinois at Urbana-Champaign, 1998). Collectively, these results indicate that the CheC–CheD–CheYp adaptation system also coordinates selective methylation. It is likely that some feedback mechanism is necessary to control which residues are selectively methylated and which ones are selectively demethylated in response to positive and negative stimuli.

One last puzzle concerning the CheC–CheD–CheYp adaptation system regards McpC, the proline receptor in *B. subtilis* chemotaxis. Cells expressing only the McpC receptor absolutely require CheD for taxis. However, cells expressing only the McpB receptor do not. Using McpB–McpC receptor chimeras, it was found that the HAMP domain (a widely conserved motif that links input and output domains in many sensory receptors [41]) from McpC was the crucial determinant for whether CheD was required [42]. More specifically, McpB–McpC receptor chimeras possessing the HAMP domain from McpC require CheD, whereas chimeras possessing the first (AS1) part of the HAMP domain from McpB and the remainder from McpC do not. The HAMP domain links the extracellular sensing (ligand-binding) and intracellular signaling domains of the receptor [43]. Presumably, CheD interacts with the HAMP domain, either directly or indirectly, and facilitates signal transmission from the sensing domain to the kinase. This facilitation also does not involve the deamidase activity of CheD, so it probably occurs through protein binding and the stabilization of specific receptor conformation. Finally, all taxis absolutely requires CheD in an ensemble of receptors [35].

The CheV system

CheV has two domains, an N-terminal CheW-like coupling domain and a C-terminal response-regulator domain that is phosphorylated by CheA [31,44]. In all chemotactic bacteria, CheW facilitates the coupling between the receptors and the CheA kinase [6,45,46]. In *B. subtilis*, CheV is redundant to CheW in the sense that both *cheW* and *cheV* null mutants are still capable of adaptation and gradient sensing (although not as well as wild type). However, *cheW cheV* null mutants show increased tumbling, which is consistent with the inability of the receptors to interact with and activate CheA in such strains. When the

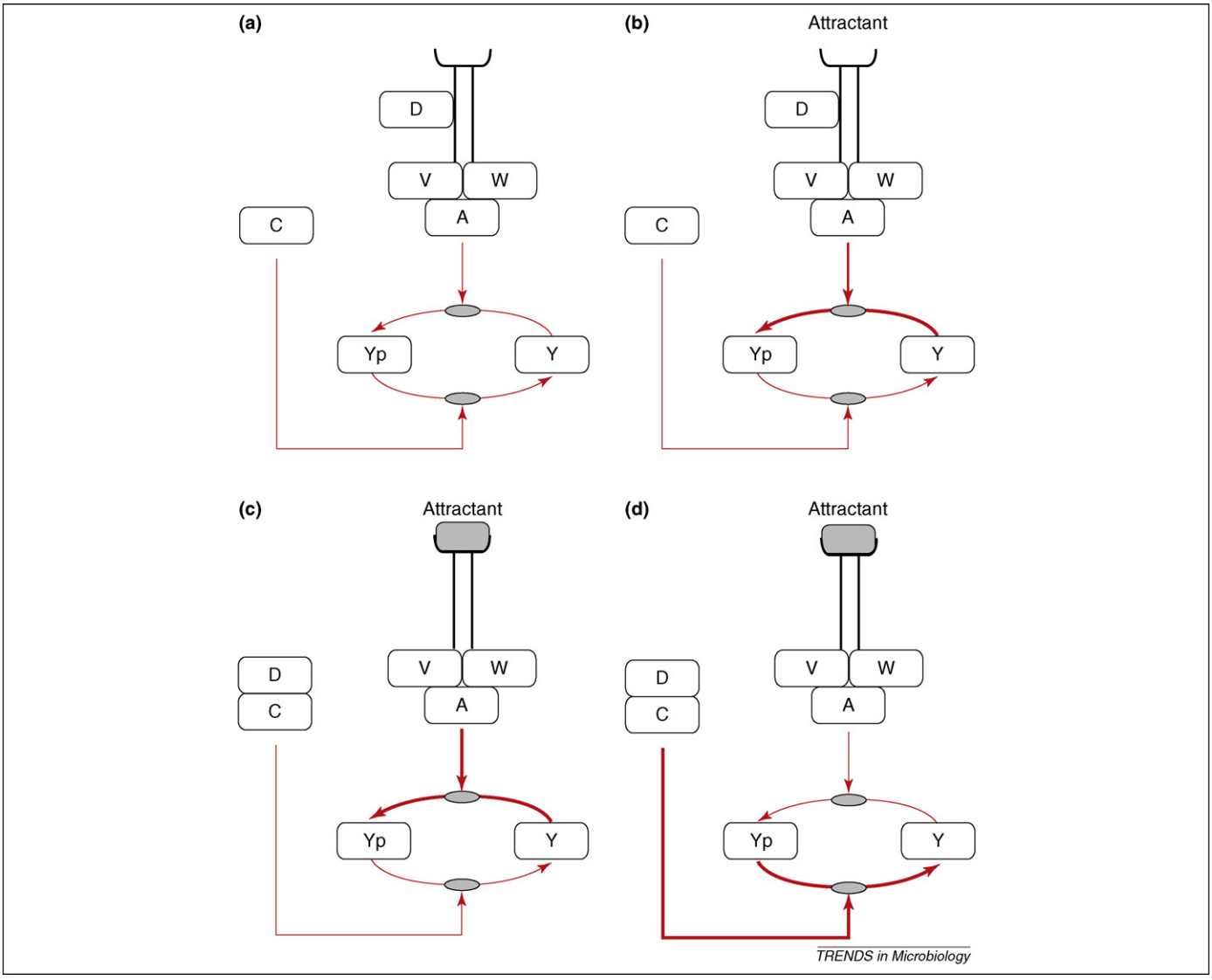

Figure 4. Model for the CheC–CheD–CheYp adaptation system in *Bacillus subtilis*. **(a)** Prior to simulation with attractant, CheD is bound to receptors. **(b)** When attractant binds to the receptors, the CheA kinase is activated and more CheYp is formed. **(c)** Higher levels of CheYp lead to more CheC–CheYp complexes, which then attracts CheD away from the receptors. **(d)** Receptors unbound with CheD only weakly activate the CheA kinase, causing less CheYp to be formed (adaptation). In addition, the greater binding of CheD to CheC caused by the presence of CheYp also enhances CheYp dephosphorylation and thus 'sharpens' the response. Note that this mechanism is incapable of full adaptation because the strength of the negative feedback is proportional to CheA kinase activity. It is likely that only some of the CheD leaves the receptors, although the extent of association between CheD and the receptors remains unknown. Abbreviations: A, CheA; C, CheC; D, CheD; V, CheV; W, CheW; Y, CheY; Yp, CheYp.

phosphorylatable aspartate residue within the response regulator domain of CheV is changed to alanine, the bacteria have a nearly wild-type bias (fraction of time that the flagella rotate counter-clockwise for running), as determined by the frequency of runs and tumbles, and respond to addition of attractant by running. However, there is only a small degree of return to prestimulus bias, reminiscent, in fact, of the phenotype in a *cheC* null mutant. Deletion of the entire response regulator domain has the same phenotype, with perhaps an even slighter degree of return to prestimulus bias [17]. Based on these observations, phosphorylation of the response regulator domain by CheA could yield a conformational change that inhibits CheA kinase activity, probably by disrupting the interaction between the attractant-bound receptor and the kinase (Figure 5). Such a mechanism would provide a negative-feedback loop similar to the CheC–CheD–CheYp system, where an active kinase phosphorylates CheV, which, in turn, inhibits kinase activity.

Integration of the three *B. subtilis* adaptation systems

An open question is why does *B. subtilis* need three adaptation systems when organisms such as *E. coli* require only one. Possibly, the additional adaptation systems in *B. subtilis* provide some added degree of robustness. Indeed, deletion of any one system leads only to a moderate inhibition of chemotaxis. However, deletion of any two severely inhibits taxis at all attractant concentrations (G.W.O., unpublished). In fact, CheV is useless by itself and *cheV* null mutants show ~50% wild-type chemotaxis [31]. However, the fact that taxis is markedly reduced when two systems are disabled indicates that the three truly do function as an ensemble and are not simply redundant to one another [47].

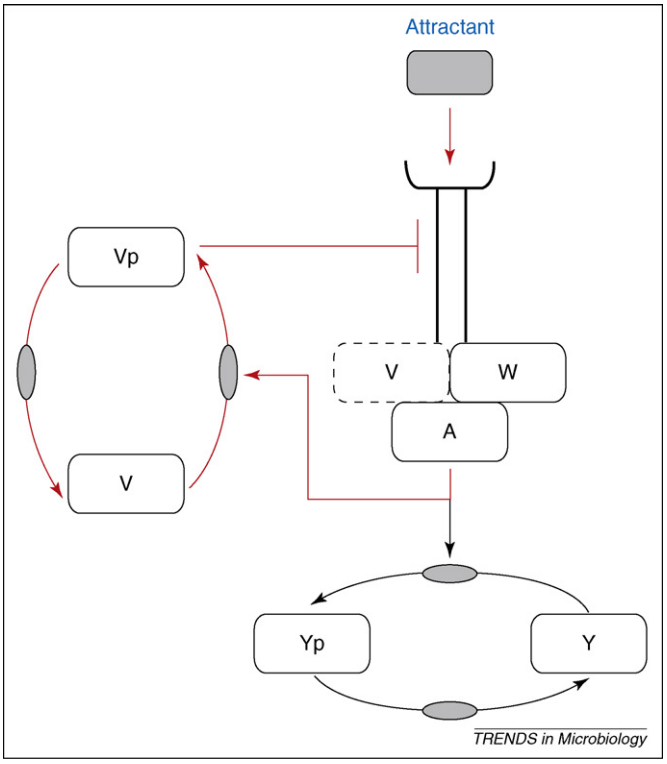

Figure 5. Model for CheV adaptation system in *Bacillus subtilis*. Attractant binding activates the CheA kinase, leading to increased phosphorylation of CheY and CheV. Phosphorylated CheV (Vp) is then thought to inhibit kinase activity by disrupting the coupling between the receptors and CheA. The dashed box is used to emphasize that CheV is probably stably coupled to the receptors CheA and CheW. Abbreviations: A, CheA; V, CheV; W, CheW; Y, CheY; Yp, CheYp.

Neither the CheC–CheD–CheYp system nor the CheV system alone can complete the adaptation process. In both cases, the concentrations of the CheC–CheYp complex and CheVp will reflect the level of CheAp owing to the phosphoryl group on both CheY and CheV being readily hydrolyzed. Therefore, these systems can only partially adapt their response to the attractant because the strength of the inhibition induced by them falls with decreasing kinase activity, thus reversing the adaptation process. However, *cheB* null mutants have normal chemotaxis at low concentrations of attractant [19], where both the CheC–CheD–CheYp and CheV systems are operating. Thus, some additional event, such as a diminished affinity of CheD for attractant-bound receptors, must occur; the nature of this event is under investigation. As a comparison, the methyl glutamates on the receptor are stable [9] and thus capable of sustaining the adaptation. Based on these results, these two systems might facilitate adaptation to low concentrations of attractant, whereas methylation facilitates adaptation to high concentrations. In support of this conjecture, the amount of methanol released increases exponentially as a function of the change in receptor occupancy [48]. In other words, binding attractant to a considerable proportion of the available receptor is needed to elicit any change in receptor methylation. Such a model would argue that there are two adaptation modes operating in *B. subtilis*, one for small gradients involving the CheC–CheD–CheYp and CheV systems and the other for large gradients involving the methylation system.

Finally, in addition to its role in mediating gradient sensing, adaptation also enables cells to sense gradients over a wide range of ambient concentrations. In *E. coli*, cells are able to sense gradients in ambient concentrations spanning five orders of magnitude [49]. Results from similar experiments indicate that *B. subtilis* cells are able to sense gradients over similar range of concentrations [50]. Based on the model described, it is unlikely that either the CheC–CheD–CheYp or CheV system is capable of tuning the sensitivity of the response. More likely, the remethylation step serves to desensitize the receptors to attractant (or sensitize them in the case of attractant removal). This process would serve to reset the CheC–CheD–CheYp and CheV systems so that they can respond to additional changes in the concentration of attractant or repellent. If this is the case, then selective methylation can independently tune the receptor activity and affinity of attractant. Such a mechanism is also quite different to the model proposed for *E. coli*, where the two are coupled [28].

Concluding remarks and future perspectives

The outstanding question regarding chemotaxis in *B. subtilis* is how the three adaptation systems function as an ensemble. Although we have succeeded in identifying the individual systems, we can now only speculate as to how they are integrated. Furthermore, many questions remain regarding the individual systems. For example, we still do not know for certain which receptor residues are methylated in response to the addition or removal of attractant. We also lack a clear understanding of how CheV functions. One of the many challenges is that it is difficult to study these systems in isolation because cells lacking two adaptation systems are incapable of taxis and often respond poorly to attractant. Much of our recent success can be attributed to the determination of structures for homologs to CheC, CheD and the chemotaxis receptors in *T. maritima*. These structures have been immensely useful in formulating hypotheses and designing experiments.

One of the remarkable aspects of bacterial chemotaxis is the diversity of mechanisms observed in different species [39]. Indeed, bacteria have evolved a host of different approaches for solving essentially the same basic tasks – among them, adaptation. For example, *B. subtilis* is not alone in using multiple adaptation systems. Furthermore, in cases such as receptor methylation, similar enzymes and their associated biochemistries are used with different effects, yet they achieve the same end goal. This diversity in the chemotaxis pathways among the different bacterial species is still an unexplored arena. Although it is unlikely that any additional adaptation systems exist in *B. subtilis*, the same cannot be said for other species. The chemotaxis pathway in *B. subtilis* is similar to those found in archaea [39] and perhaps the *B. subtilis* chemotaxis pathway is close to the progenitor pathway and all other pathways have evolved from it. Support for this hypothesis comes from the fact that *B. subtilis* has orthologs to all known chemotaxis proteins, with the exception of CheZ [51], which is the CheYp phosphatase found primarily in the γ-proteobacteria [39]. Of course, chemotaxis has been explored in too few organisms and new surprises await us.

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