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REVIEW ARTICLE

Bacterial chemotaxis: *Rhodobacter sphaeroides* and *Sinorhizobium meliloti* – variations on a theme?

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Overview

More than half the known bacterial species swim, with swimming being used to direct their overall movement towards an optimum environment for growth, which could include sites of invasion for pathogens and symbionts. A great deal is now known about swimming and its environmental control in enteric species (for recent reviews see Amsler & Matsumura, 1995; Blair, 1995), but only recently has there been a significant increase in the study of non-enteric species. From these studies it has become apparent that the chemosensory system must have a very ancient origin as it is present in some form not only in all the genera examined, but also in both bacteria and archaea (Alex & Simon, 1994). The central theme, an ion-driven motor rotating a semi-rigid helix, controlled by a phosphorelay system to bias the overall movement of the bacterium to its optimum environment for growth, is common to all species. However, as might be expected of a system with such a long history, it has been adapted by different bacterial subgroups in a way which may relate to the different environmental niches into which they evolved. The chemosensory system in enteric species is relatively straightforward, but that of other species is turning out to be much more complex, and more varied.

This variation on a central theme is illustrated well if the behaviour of different members of the α-subgroup of bacteria is examined. This group, characterized by DNA with a high G+C content (over 60 mol%), includes species found in a very wide range of natural environments, for example *Agrobacterium*, *Azospirillum*, *Caulobacter*, *Sinorhizobium* and *Rhodobacter*, with most of these species showing great metabolic flexibility. Many can grow as aerobes or anaerobes, as phototrophs or heterotrophs, a number fix nitrogen and some are pathogens, particularly plant pathogens (for a general overview of the subgroup see Balows *et al.*, 1992). Examination of their motility and chemosensory systems shows how the basic chemosensory pathway has been adapted for different environments, and also shows that within a subgroup such as the α-subgroup there can be variation, with some, such as *Caulobacter crescentus*, being somewhat closer to the enteric paradigm than others, such as *Sinorhizobium* (formerly *Rhizobium*) *meliloti* and *Rhodobacter sphaeroides*, which are more distant.

Patterns of swimming

Chemotaxis is used to bias the overall swimming direction of bacteria towards or away from particular stimuli. Bacteria are, in general, too small to sense a gradient along their bodies and must therefore sample their environment with time (Block *et al.*, 1982). In *Escherichia coli*, the six or so flagella tend to rotate counterclockwise (CCW), forming a bundle which pushes the cell forward. Periodically, a number of flagella switch to clockwise (CW) rotation and this forces the handedness and wavelength of the flagella to change. This in turn drives the bundle apart and the cell tumbles on the spot. When the bundle reforms, the cell is usually facing in a different direction. In a uniform environment this system of smooth swimming punctuated with tumbles results in a three-dimensional random pattern of swimming (Fig. 1b). When faced with a gradient of an attractant or repellent, however, the cells swim for longer when moving towards the attractant or away from the repellent, biasing the overall movement in a favourable direction (Berg & Brown, 1972).

Unlike the enteric species, many members of the α-subgroup of bacteria appear to have flagellar motors that only rotate in one direction, rather than switching direction. Some species do however have flagella that

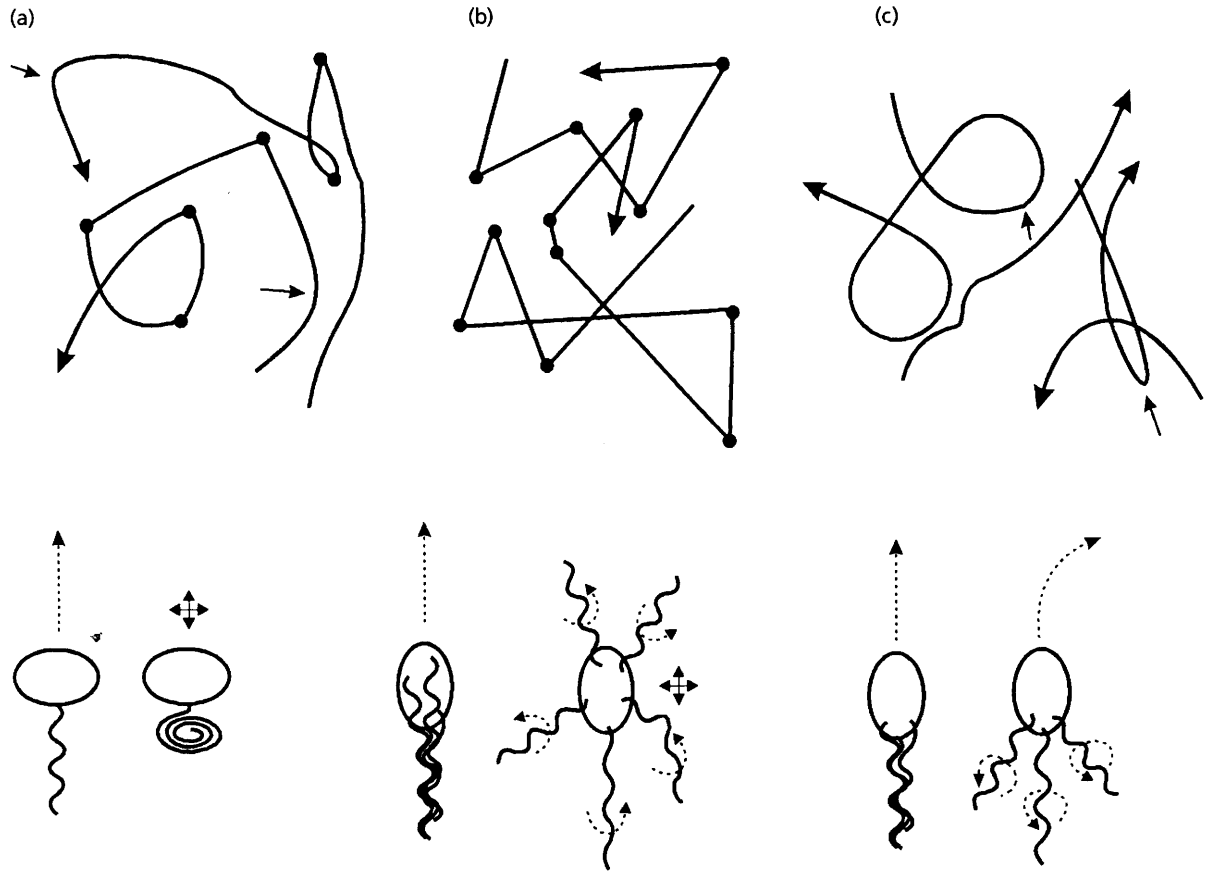

Fig. 1. Flagellar arrangement and mechanism of swimming of (a) *R. sphaeroides*, (b) *E. coli* and (c) *S. meliloti*. *R. sphaeroides* has a unidirectional motor which both changes speed and stops periodically. When the motor stops the flagellum relaxes to a short-wavelength, large-amplitude coil, the slow rotation of which reorients the cell. *E. coli* swims smoothly when its CCW-rotating flagella come together as a bundle; periodically they switch to CW rotation and the bundle flies apart, the cell tumbles and reorients for the next period of smooth swimming. The direction of swimming also changes without stops when the speed changes (arrows). *S. meliloti* has a bundle of flagella that only rotate CW. The speed of the individual motors can change and this causes the filaments to separate and the cell to turn (arrows); the flagella never stop or switch. All mechanisms result in a three-dimensional random swimming pattern.

switch, for example the CW-rotating flagellum of *C. crescentus* switches briefly to CCW rotation to change its swimming direction (Gomes & Shapiro, 1984). The questions arise, what is the pattern of swimming produced by a unidirectionally rotating flagellum and how does this pattern change when cells are chemotactically stimulated?

Flagellar rotation is driven by the protonmotive force (Δp) across the membrane (Manson *et al.*, 1977). If *E. coli* is examined, either free-swimming or tethered, under condition of saturating Δp, there is little evident change in the rotation rate of the flagella. On the other hand, *S. meliloti* and *R. sphaeroides* can show major changes in the rate of flagellar rotation under conditions where the Δp can be considered saturating.

R. sphaeroides has a single flagellum arising not from the pole, but laterally (Fig. 1a). The mechanisms involved in localizing the single flagellum are unknown, but it is always in the non-photosynthetic cytoplasmic membrane, whatever the growth conditions. The *R. sphaeroides* flagellum only rotates in one direction (Armitage & Macnab, 1987). This is usually CW but occasionally variants have been identified with a CCW flagellar motor; in neither case have the motors been seen to switch (Packer & Armitage, 1993).

If anaerobically grown *R. sphaeroides* is tethered in a flow chamber and the cells subjected to increases and decreases in attractant concentration, no change in rotational behaviour is seen when the attractant concentration is increased, but all the cells stop transiently when an attractant is removed (Packer *et al.*, 1996). This suggests that rather than swimming and tumbling *R. sphaeroides* swims and stops, re-orienting during a stop, but the response is 'pessimistic' with the cells sensing the reduction in attractant (or its metabolic consequence), whereas *E. coli* responds primarily to the increase in an attractant concentration. The behaviour of aerobically grown cells is more complex, and probably reflects the more complex sensory pathways induced under these conditions. Direct observation of

the flagellum on free-swimming cells shows that during stops flagella relax from the distal end to form a short-wavelength, high-amplitude coil against the cell body. During this period, the cell is re-oriented and when the helix reforms, from the cell body out, the cell swims off in a new direction. It was assumed that Brownian motion must reorient the cell during the stop, but the relative viscosity of the medium would make this an inefficient way of reorienting. Recently, differential interference-contrast (DIC) microscopy has made it possible to see the flagellum *in vivo* much closer to the cell body than was possible with high-intensity dark-field microscopy. DIC microscopy shows that the coiled flagella can still slowly rotate and this contributes to reorientation. In addition to the functional helix and the coiled form during a stop, DIC microscopy also shows that the *R. sphaeroides* flagellum can switch into a rapid rotating, apparently straight form (J. P. Armitage & T. P. Pitta, unpublished data). Whether this switching in conformation is related to changes in speed remains to be investigated.

When free-swimming *R. sphaeroides* cells are tracked in three dimensions the rate at which they accelerate out of stops is found to be variable, and often much slower than *E. coli* coming out of a tumble. This may be the result of the filament reforming a fully functional helix. However, cells also change speed while actively swimming. *R. sphaeroides* shows considerable variation in swimming speed under unstimulated conditions, accelerating and decelerating during periods of swimming and apparently changing direction without an obvious stop or pause (Packer *et al.*, 1997; Packer *et al.*, 1996, 1997; R. Ford, M. A.-S. Vigeant & J. P. Armitage, unpublished). The mean swimming speed of *R. sphaeroides* is much faster than that of *E. coli*. *E. coli* tends to swim at about 20 $\mu m\ s^{-1}$, but *R. sphaeroides* swims at a mean speed of 35 $\mu m\ s^{-1}$.

In addition to the spontaneous alterations in speed measured in free-swimming cells, there is also a stimulus-dependent increase in the rate of flagellar rotation in *R. sphaeroides* (Packer & Armitage, 1994). This increase in speed, which can amount to as much as 25% and also reduces the stopping frequency, occurs on addition of a limited number of chemicals, mainly weak organic acids and potassium ions. The speed increase is sustained over tens of minutes and, in *R. sphaeroides*, the speed only returns to prestimulus levels after the stimulus has apparently been metabolized and the concentration reduced below a critical level (Brown *et al.*, 1993). As adaptation, a return to unstimulated swimming, has been shown to be essential for gradient-sensing in enteric species, long-term chemokinesis is unlikely to have a direct role in chemotaxis. Modelled in its simplest form, chemokinesis would result in dispersal, not accumulation (Packer & Armitage, 1994). The increase in speed is apparently independent of any increase in Δp or electron transport rate and is probably the result of an intracellular signal controlling motor behaviour. It is possible that in the natural environment in which most of these species find themselves an increase in a major nutrient is a signal to disperse. It does not inhibit the sensing of different gradients and it may help spread a species in an environment where there are limited resources. Chemokinesis is shown not only by *R. sphaeroides*, but also by the related *Azospirillum brasilense* (Zhulin & Armitage, 1993; Zhulin *et al.*, 1995) and *S. meliloti*, although in this case the role of speed control may be different.

S. meliloti also has right-handed helical flagella, but unlike *R. sphaeroides* there are several flagella per cell and the flagellar filaments have a rigid, 'complex' structure which cannot undergo polymorphic changes from right-handed to left-handed helices (Krupski *et al.*, 1985; Pleier & Schmitt, 1989; Trachtenberg & Hammel, 1992). It has been shown (Götz *et al.*, 1982) that *Sinorhizobium* cells with peritrichously or lophotrichously inserted complex flagella swim efficiently through viscous media. This may therefore provide an environmental advantage for these soil bacteria. The flagella come together to form a unidirectional CW-rotating bundle (Götz & Schmitt, 1987). However, unlike *R. sphaeroides*, there is no obvious stopping in that rotation. How can a continuously rotating unidirectional flagellar motor govern both forward runs and directional changes in a swimming *S. meliloti* cell? Video-enhanced DIC microscopy allowed the direct observation of the two to six short, complex peritrichous flagella of a swimming *S. meliloti* cell. Two swimming modes were seen, a rapid forward swimming with the flagella forming rotating bundles, and direction changes in which the flagella were seen to rotate separately at different rates (R. Schmitt & T. P. Pitta, unpublished). Thus, by implication, changes in the direction of swimming may be the result of periods of asynchronous flagellar rotation, the different rates of flagellar rotation driving the flagellar bundle apart (Fig. 1c). Single peritrichously inserted flagella contribute differentially, therefore, to the swimming behaviour of individual cells. This is very different from the rotational switching of enterics and requires a new model for control of motor rotation at constant Δp, both under unstimulated conditions and modulated by positive and negative signals in response to chemoattractants. The possible mechanisms involved in controlling speed are discussed below.

Chemosensing

Genetic organization

The genetic organization of the chemosensory genes of several members of the α-subgroup has been characterized and found to be very similar (Fig. 2) (Greck *et al.*, 1995; Ward *et al.*, 1995a; M. R. K. Alley, personal communication). The organization is, however, very different from that seen in *E. coli* (Fig. 2c) (Macnab, 1992). In all members of the α-subgroup so far studied at least two copies of the *cheY* gene have been found, but no *cheZ* gene. In *R. sphaeroides* a second operon has been identified with a second functional set of the chemotaxis genes *cheA*, *cheW* and *cheR* and a

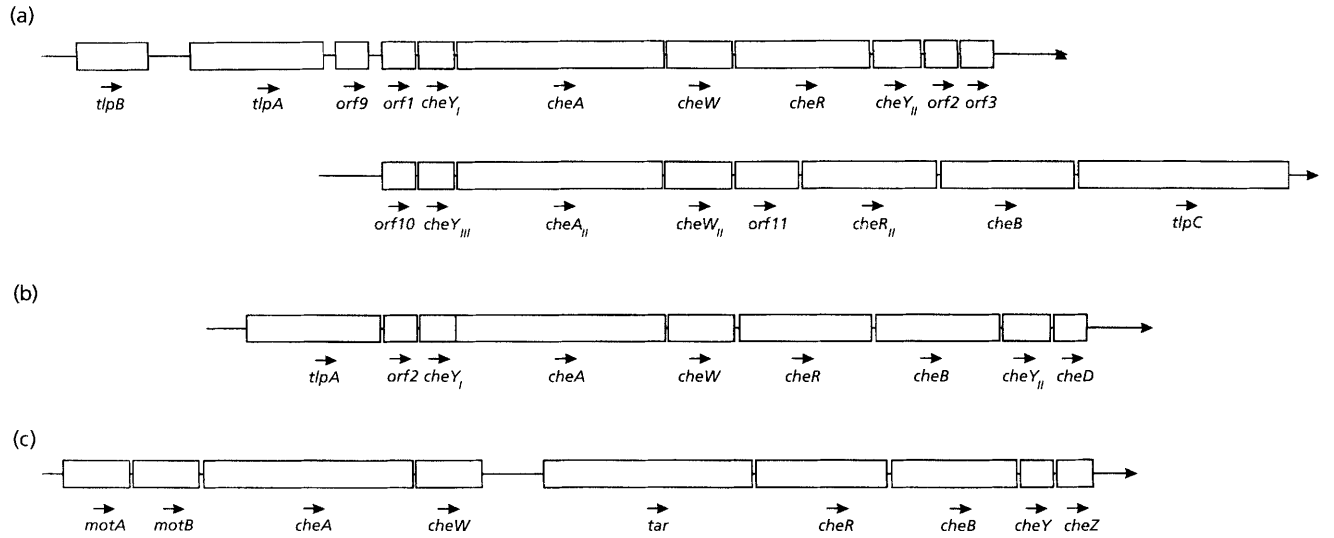

Fig. 2. The arrangement of chemotaxis and motility genes in (a) *R. sphaeroides*, (b) *S. meliloti* and (c) *E. coli*. *R. sphaeroides* has at least two independent and functional gene clusters encoding homologues of the enteric genes, with at least two copies of *cheA* and three of *cheY*. *S. meliloti* has a single cluster of chemosensory genes in a similar arrangement to the two clusters of *R. sphaeroides*. A cluster of flagellar genes has also been identified that are involved in controlling motor speed. The *E. coli* gene clusters show very different arrangements. *C. crescentus* shows a similar arrangement to that of both *R. sphaeroides* and *S. meliloti*, but also has three copies of *cheY* (M. R. K. Alley, personal communication). *tlp*, genes encoding transducer-like proteins (which have the cytoplasmic signalling domain of MCPs but lack classical transmembrane and periplasmic domains); *motA* and *motB*, genes encoding proteins involved in transducing electrochemical energy into motor rotation; *tar*, MCP gene. Other genes are as described in the text.

third copy of the *cheY* gene (Hamblin *et al.*, 1977*b*, Fig. 2a). A third copy of *cheY* has also been found in *C. crescentus* (M. R. K. Alley, personal communication). The *che* operon of *S. meliloti* (Fig. 2b), as well as containing the chemotaxis genes encoding CheA, CheW, CheR, CheB and two response regulators, $CheY_I$ and $CheY_{II}$, also contains a promoter-distal ORF (named *cheD*) encoding a 20 kDa polypeptide reminiscent of CheD from *Bacillus subtilis* and believed to augment methylation by CheR of methyl-accepting chemotaxis proteins (MCPs) involved in primary signal reception (Rosario *et al.*, 1995).

The *mcp* genes are not located in the major clusters of chemosensory genes in many species, suggesting that the MCPs may have been added to an already present phosphorelay system. This could account for the range of diverse *mcp* homologues in *S. meliloti* and *R. sphaeroides* apparently encoding a more diverse set of sensory proteins than those identified in *E. coli* (see below).

The obvious major questions are: why do these species have this increased number of chemosensory protein homologues, what do these proteins do and why should these species be so much more complex than the enteric species?

Gradient sensing

Gradient sensing in *E. coli* relies on the change in conformation of membrane-spanning receptor dimers, MCPs, to sense the change in the external concentration of a limited number of chemoeffectors (Hazelbauer, 1992). MCPs are not involved in transport, and deletion of any one results in loss of chemotaxis to a few compounds, but no effect on responses to other attractants. The conformational change resulting from a change in attractant or repellent binding is signalled across the membrane to a phosphorelay system, which is one of a large family of phosphorelay systems found in prokaryotic and eukaryotic species responsible for sensing environmental change (see Hoch & Silhavy, 1995). Chemotaxis relies on short-term sensing to respond to changes within a gradient. Therefore, to be able to sense whether the concentration of chemoeffector is increasing or decreasing, the receptor must be reset periodically. This occurs by methylating specific glutamate residues on the cytoplasmic face of the MCP, putting the protein back into a non-signalling conformation. Methylation occurs within a few minutes of stimulation and is controlled by the relative activity of two enzymes, a methyl transferase, CheR, and a methyl esterase, CheB. The activity of CheB is controlled by the strength of the chemotactic stimulus.

In addition to chemotaxis through the sensory MCPs, which cause a response whatever the current growth requirements of the cell, enteric species also respond to phosphotransferase (PTS) sugars. This response is dependent on transport through the PTS system (Lengeler & Vogler, 1989; Lengeler & Jahreis, 1996). Phosphorylation of the sugar as it is transported through the specific EII/EIII proteins relies on a common

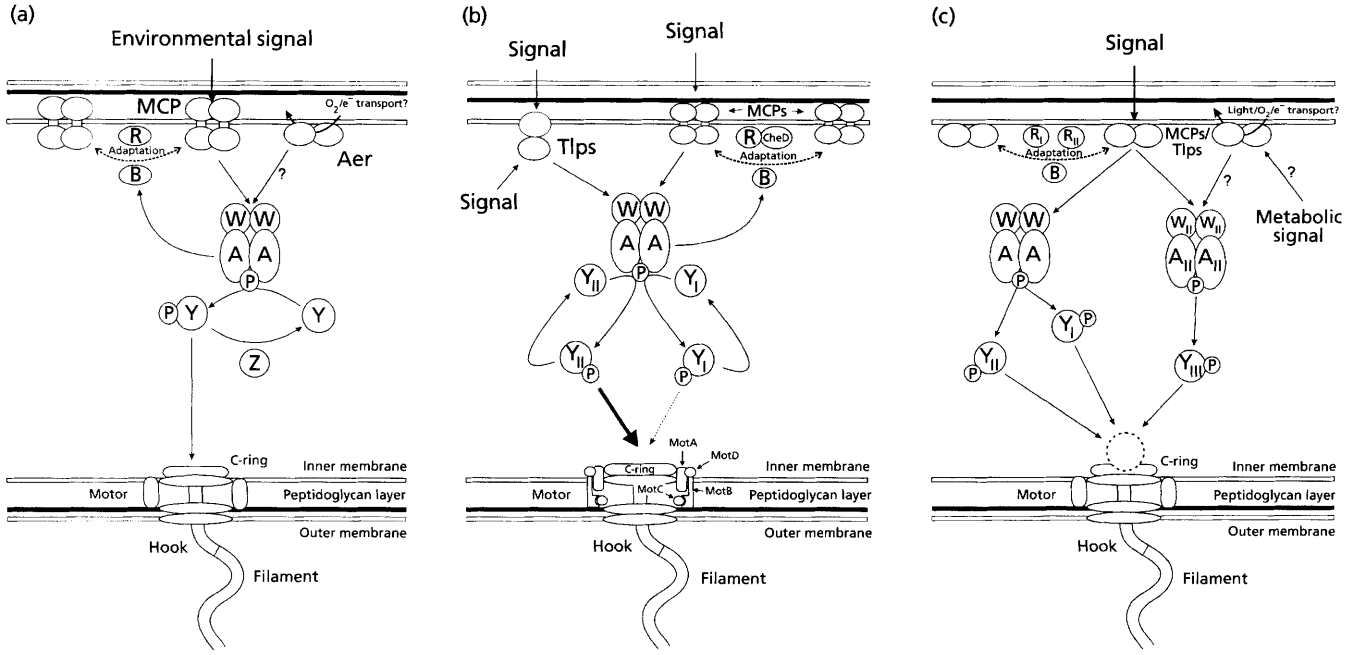

Fig. 3. Possible sensory pathways of (a) *E. coli*, (b) *S. meliloti* and (c) *R. sphaeroides*. A, B, R, W, Y and Z represent CheA, CheB, CheR, CheW, CheY and CheZ, respectively. P denotes phosphorylation. Other abbreviations are as outlined in the text.

cytoplasmic EI transferring phosphate from phosphoenolpyruvate via the HPr protein to the sugar. EI can also control the phosphorylation level of the chemosensory histidine protein kinase, CheA (see below) and, therefore, as a sugar is transported and phosphorylated the level of CheA-P falls, causing smooth swimming (Lux *et al.*, 1995; Lengeler & Jahreis, 1996). In this case, the chemosensory response is independent of MCPs, and adaptation presumably depends on the resetting of the metabolic state. The PTS sensory pathway may represent one of the original sensory systems, with chemotaxis controlled by metabolic activity. This may have been modified later by the addition of 'dedicated' chemosensory receptors.

E. coli also swims towards optimum concentrations of oxygen. Evidence suggests that the response depends on the activity of the respiratory electron transport chain and recent data suggest that the redox state of a component of the chain is sensed via an MCP homologue, Aer. Aer is a cytoplasmic protein probably anchored into the membrane. The N-terminal domain of the protein shows homology to the oxygen-sensing proteins, NifL and FixL, while the C-terminal domain looks very like the signalling domains of MCPs, but without the methylation sites required for chemosensory adaptation. The N-terminal domain has been shown to non-covalently bind FAD, suggesting that this may have a redox sensing role. Mutants in *aer* show reduced responses to oxygen gradients (Bibikov *et al.*, 1997; Rebbapragada *et al.*, 1977). It seems probable that Aer senses the redox state of the electron transport chain via the bound FAD and signals a change in electron transfer through a conserved MCP-like signalling domain to CheA. As temperature and pH changes are also sensed via MCPs it appears that in *E. coli* all sensory signals are channelled through CheA. CheA-P controls the phosphorylation level of CheY, a small 14 kDa response regulator protein. When phosphorylated, CheY can bind to the switch complex of the flagellar motor and cause it to change its rotational direction and the cell to tumble. The level of CheA-phosphate and thus CheY-phosphate controls the switching frequency of the motor and biases the swimming direction of the cell towards attractants or away from repellents (Fig. 3a).

The mechanism of sensing an environmental change seems to vary across the α-subgroup, from *C. crescentus*, which has membrane-spanning MCPs related to those in *E. coli* but expressed only in the swarmer cells (Gomes & Shapiro, 1984), to *R. sphaeroides*, which primarily senses its metabolic state using both cytoplasmic and membrane-bound sensors to signal changes to the flagellar motor, with *S. meliloti* taking an intermediate position.

There is substantial evidence that *R. sphaeroides*, a purple non-sulphur bacterium which can grow aerobically as a heterotroph or anaerobically as a photoheterotroph, senses its metabolic state in some way (Ingham & Armitage, 1987; Poole & Armitage,1989; Poole *et al.*, 1993). All attractants are metabolites, the predominant ones being organic acids such as succinate and acetate, but it will also respond to a wide range of sugars and polyols and to amino acids. No repellents have been identified. The specific metabolic pathway for the attractant needs to be induced for a response to

occur and a period of starvation enhances the responses, with well-fed cells showing much weaker responses than starved cells. The attractant response to glutamate is lost in a transport mutant, but is restored by expressing a completely unrelated *E. coli* glutamate transporter, supporting the hypothesis that, for at least some chemoeffectors, there are no dedicated receptors and transport cannot be the source of the sensory signal (Jacobs *et al.*, 1995). Similarly, responses to sugars such as mannitol metabolized through the Entner–Doudoroff pathway are lost in a glucose-6-phosphate dehydrogenase mutant, although the sugars are still transported, whilst the responses to sugars metabolized through the Embden–Meyerhoff pathway, such as fructose, are still present (Hamblin *et al.*, 1997b).

What is the metabolic signal controlling *R. sphaeroides* swimming behaviour and how is the metabolite sensed? As yet, the nature of the metabolic signal remains elusive. Homologues of *mcp* genes have been identified in *R. sphaeroides*, two of which are located upstream from one of the chemosensory operons (Ward *et al.* 1995b). They code for two unusual MCP homologues. One, TlpA, (named transducer-like protein,Tlp, rather than McpA because of its lack of membrane-spanning domains) is about 70 kDa (compared to the 55 kDa of *E. coli*) but with only one possible membrane-spanning domain, rather like Aer from *E. coli*. Antibody studies suggest that it is primarily a cytoplasmic protein. The other MCP homologue, TlpB, is small, less than 40 kDa, and comprises a single signalling domain, homologous to the signalling domain of an MCP, with a short hydrophobic stretch that may serve as a membrane anchor. The lack of any putative 'sensory' domain may indicate that it interacts with another, possibly membrane-bound, protein. Both proteins appear to be cytoplasmic, and probably sense specific cytoplasmic metabolic intermediates; however, athough it shows some homology to Aer from *E. coli*, the protein does not appear to bind FAD or be involved directly in oxygen-sensing.

Unlike deletion of an *mcp* gene in *E. coli*, which would only result in the loss of a limited number of responses, deletion of the gene for TlpA results in a reduction in chemotaxis to all compounds under aerobic, but not anaerobic, conditions. This again supports the idea that a change in a metabolic intermediate, probably one produced primarily by aerobic metabolism, is creating a sensory signal (Ward *et al.*, 1995b). A role for TlpA in aerobic chemosensing is supported by transcription/translation studies which show that the level of expression of TlpA, although always low, increases in aerobically grown cells. TlpB appears to be expressed primarily under anaerobic conditions, but deletion of *tlpB* has little effect on behaviour either aerobically or anaerobically. Transcriptional and translational fusions indicate increased expression under the different growth conditions (D. A. Harrison & J. P. Armitage, unpublished). The mechanisms controlling expression have not been identified but *tlpA* includes a large number of rare codons, which suggests a mechanism for translational control. The different MCPs in *E. coli* may also be expressed to different extents, with Tsr (the serine sensor) being present at higher levels than Tap (the dipeptide sensor) or Aer (the redox sensor). The reasons for this are unknown. How closely related these two *R. sphaeroides* MCP homologues are to enteric MCPs was examined by expressing them in both *mcp*-minus strains of *E. coli* and wild-type cells. Although neither gene could complement the mutations they inhibited chemotaxis in wild-type cells, showing that they do have a role in chemosensing.

Deletion of both *tlp*A and *tlpB* from *R. sphaeroides* does not result in the loss of all bands cross-reacting with antibodies raised to TlpA from *R. sphaeroides* or Tsr from *E. coli*. This strongly suggests at least one additional MCP, and a new *mcp* gene has recently been located in a region distant from the other *mcp* genes and the two *che* operons, which are on the large chromosome of *R. sphaeroides* (*R. sphaeroides* has two chromosomes, a large ~ 4 Mb chromosome and a second of ~ 0·9 Mb, Choudhary *et al.*, 1994). The new *mcp* gene is probably on the small chromosome. The structure and role of this MCP have yet to be determined although Western blots suggest that it is present at higher concentrations than either TlpA or TlpB, is more closely related to *E. coli* MCPs and is also environmentally regulated. Immunoelectron microscopy shows high levels of expression under aerobic conditions, resulting in polar clusters of MCPs, but much lower expression in photosynthetically grown cells, with few MCPs localized within the cell (D. M. Harrison, J. P. Armitage & J. R. Maddock, unpublished).

Are the MCP homologues in *R. sphaeroides* methylated, suggesting receptor adaptation similar to the enterics? Two genes encoding homologues of the methyl transferase CheR and one encoding the methyl esterase CheB have been identified. Methylation of MCPs has been measured, but not following the pattern familiar in *E. coli*. *In vivo* experiments show that bands of the correct molecular mass for MCPs, and cross-reacting with anti-Tlp and anti-MCP antibody, are post-translationally methylated, and this depends on CheR. However, measurable methylation only occurs after a long period of starvation followed by the addition of complete medium. There is no measurable change in the pattern of methylation when individual attractants are either added alone or removed. It also takes tens of minutes for methylation to occur, whereas in *E. coli* it occurs after a brief exposure (D. M. Harrison & J. P. Armitage, unpublished). Taking the *E. coli* paradigm, adaptation has to occur rapidly for gradient sensing. If the long-term change in the pattern of methylation is linked to adaptation, it is possible that *R. sphaeroides* has a methylation system more closely related to *B. subtilis*. In *B. subtilis* it is not the absolute level of methylation that controls adaptation but the turnover of methyl groups via an additional cytoplasmic protein. Both *R. sphaeroides* and *S. meliloti* do contain an ORF in the *che* operon which resembles the *cheD* gene from *B. subtilis*

(Rosario *et al.*, 1995), although the homology is greater between the *S. meliloti* and *B. subtilis* genes than the one from *R. sphaeroides*. CheD is responsible, in combination with the transferase CheR, for adaptation-dependent methylation in *B. subtilis*. Moreover, a *cheD* mutant of *S. meliloti* is chemotaxis-deficient and exhibits a smooth swimming pattern, as would be expected in a non-methylating mutant (P. Muschler & R. Schmitt, unpublished).

S. meliloti is metabolically more constrained than *R. sphaeroides* and this may be reflected in a more straightforward chemosensory pathway. It is an obligate aerobic soil bacterium capable of nitrogen fixation when in symbiosis with certain leguminous plants. The infection of host rootlets by *S. meliloti* is preceded by a tactic response to root exudates, such as amino acids, carbohydrates and flavones (Dharmatilake & Bauer, 1992). The mechanisms of sensing chemoattractants, of signal transduction and response regulation by *S. meliloti* cells have much in common with those of the related *R. sphaeroides*, but with only one chemosensory pathway rather than the multiple pathways of *R. sphaeroides*. The chemotactic responses shown by *S. meliloti* are, as with *R. sphaeroides*, much stronger if the cells have been starved before being chemotactically stimulated and metabolites such as L-amino acids are the most potent chemoattractants (Götz *et al.*, 1982). The data suggest that metabolism (and possibly transport) is also required for *S. meliloti* to respond chemotactically to at least some attractants.

S. meliloti possesses a cytoplasmic sensor, TlpA, encoded by the first ORF of the chemotaxis (*che*) operon, which looks very similar to the *tlpA* gene of *R. sphaeroides* (Fig. 2). The encoded 57 kDa protein contains a conserved signalling domain (and one possible methyl-accepting site), but again neither hydrophobic transmembrane domains nor a periplasmic receptor domain are present, both features of 'classical' MCPs (Greck *et al.*, 1995; Bourret *et al.*, 1991). On the other hand, conservation of residues needed for signal output and interaction with CheW (Liu & Parkinson, 1991) strongly suggests a role for TlpA in signal transduction. In fact, in the same way as overexpression of TlpA from *R. sphaeroides* reduces *E. coli* chemotaxis, the overexpression of TlpA from *S. meliloti* in *E. coli* also reduces chemotaxis to aspartate or *N*-acetylglucosamine (V. Sourjik & R. Schmitt, unpublished). It is probable that the expressed MCP homologues compete for the endogenous cytoplasmic chemotaxis proteins CheW and CheA.

In-frame deletions of *che* genes in *S. meliloti* also produce distinct mutant phenotypes, but different from the *che* mutants of either *E. coli* or *C. crescentus*. A mutant in which *tlpA*, the putative cytoplasmic sensor, has been deleted exhibits a 10–15% reduced response to proline in capillary assays. The reduction is more pronounced (40%), however, if a deletion is introduced in *tlpB*, the second gene in the *che* operon encoding a small 11 kDa protein that contains a moderately conserved MCP-like signalling motif (*tlpB*-like genes are present in the same position in *R. sphaeroides* and *C. crescentus* but have not been mutated). A double mutant, Δ(*tlpA–tlpB*), exhibits the lowest response (50% of the wild-type) suggesting that the two components may produce an additive signal. When free-swimming cells are examined, TlpA-deficient cells exhibit 10% reduction and TlpB-deficient cells 30% reduction in swimming speed. Possibly both TlpA and TlpB differentially inhibit CheA autokinase activity, thereby preventing CheY phosphorylation, and this results in an increase in the rate of flagellar rotation (see following section on motor control). Both TlpA and TlpB are candidates for sensing internal stimuli that reflect the metabolic or energy state of a cell; however, as with *R. sphaeroides*, the nature of the ligands that elicit these signals remains to be elucidated.

Southern and Western blots indicate that *S. meliloti* may possess at least four MCPs, in addition to the two cytoplasmic sensor proteins. The gene encoding one of them, *mcpC*, has recently been cloned and sequenced, and was found to possess both a periplasmic and a cytoplasmic domain. It is located outside the 40 kb cluster containing most of the approximately 60 *che*, *fla* and *mot* genes, and it may reside on one of the large plasmids responsible for either nitrogen fixation or symbiosis. This again suggests a similarity between *S. meliloti* and *R. sphaeroides*, with related genes on different chromosomes/megaplasmids. An *mcpC* knock-out mutant was not impaired in its response to L-amino acids or the carbohydrate attractant mannitol. These receptors may be more specialized, possibly being involved in sensing host plant components, such as flavones.

Signalling pathway

In *E. coli* any change in MCP binding alters the activity of a histidine protein kinase, CheA, the change being signalled through a linking protein, CheW (Fig. 3a). An increase in attractant binding, which means that the cell is going in a positive direction and therefore continued smooth swimming is required, apparently inhibits CheA autophosphorylation (Hazelbauer, 1992). If the level of attractant bound to the MCP falls, indicating the cell is swimming in the wrong direction, there is increased autophosphorylation. CheA-phosphate transfers its phosphate to one of two response regulators (Barak & Eisenbach, 1992). CheY is a small 14 kDa protein, with the classical conserved aspartate acid pocket of a phosphorelay response regulator (Lukat & Stock, 1993; Volz, 1993; Stock *et al.*, 1993). When phosphorylated, CheY interacts with a switch protein, FliM, on the cytoplasmic face of the flagellar motor and thus switches the direction of rotation from CCW to CW, causing a tumble (Welch *et al.*, 1994). To terminate the signal, a small protein, CheZ, forms multimers with CheY-phosphate, competing with FliM and increasing the rate of CheY dephosphorylation (Eisenbach, 1996; Huang & Stewart, 1993). The activity of CheB, which also has the

conserved aspartate pocket of a response regulator as well as the esterase domain, is also controlled by phosphorylation via CheA (Li *et al.*, 1995; Stewart *et al.*, 1990). It operates in concert with a constitutive methyl transferase, CheR, and together they control the level of methylation of the specific glutamate residues on the cytoplasmic face of the MCP, resetting the conformation of the MCP into a non-signalling state and thus bringing about adaptation (Fig. 3a).

What is the relationship between the Che homologues identified in the α-subgroup species and their enteric counterparts? Again, *C. crescentus* appears to be more closely related to enteric species than many of the other members of the α-subgroup. As with *E. coli*, deletion of some Che components produces a very obvious phenotype (Gomes & Shapiro, 1984; M. R. K. Alley, personal communication). In *E. coli*, mutations in *cheA*, *cheY* or *cheR* produce an exclusively smooth swimming phenotype with no tumbling, whilst mutations in *cheZ* or *cheB* produce mutants that constantly tumble. If *cheR* is mutated in *C. crescentus*, the cells swim smoothly forwards, rotating their flagella CW whereas *cheB* mutants swim smoothly but backwards, pushed by a CCW-rotating flagellum.

In-frame deletions in the four *S. meliloti* signalling genes and in *cheD* all result in chemotaxis-deficient phenotypes, with Δ*cheB* strongly affecting chemotaxis towards mannitol and succinate, but not towards proline, suggesting a more complex signalling mechanism for proline. The fact that defined mutants in the *che* operon have clear phenotypes suggests that there is only a single copy of the *che* operon in *S. meliloti*. By analogy to the corresponding *E. coli* strains, the *S. meliloti* mutants either produce the equivalent of a 'smooth' (Δ*cheW*, Δ*cheA*, Δ*cheR*, Δ*cheD*) or a 'tumbly' (Δ*cheB*) mutant (the actual effect on the behaviour of the flagellar motor is described below). CheB and CheR, perhaps in consort with CheD, are likely to de-methylate and methylate the MCPs encoded elsewhere on the *S. meliloti* genome as McpC contains four typical methylation sites. Whether other components of the signalling chain (like TlpA) are also targets for these adaptation proteins has yet to be identified.

The situation is more complex in *R. sphaeroides*. When the individual genes in the first chemosensory operon identified in *R. sphaeroides* were deleted in-frame, individually, in pairs or finally the complete operon, there was no major change in either unstimulated swimming behaviour or chemosensory responses (Hamblin *et al.*, 1977b). However, a second round of transposon mutagenesis on the mutant in which the first *che* operon had been deleted identified the second operon, encoding a complete second set of *che* homologues, with a second *cheA*, a third *cheY* and two more *cheW*s (Fig. 2a). This operon contains the only copy of *cheB* identified so far. Deletion of the second CheA, $CheA_{II}$, results in the loss of chemotactic behaviour. However, not only is chemotaxis lost, but the phototactic response is altered (Hamblin *et al.*, 1977b). This suggests that a signalling pathway from both chemoreceptors and electron transport goes through this histidine protein kinase, rather than the first CheA identified. Tethered $CheA_{II}$ mutant cells still show responses to chemo- and photostimuli, but the responses are inverted with respect to the wild-type response, with the cells stopping on addition of the stimulus and swimming when it is removed, suggesting that the normal response may be the result of signal integration which is unbalanced in the mutant. Preliminary data suggest there may be yet another copy of *cheA*, interruption of which inhibits both chemosensory and photosensory behaviour. Interestingly, even after a mutation that results in the complete loss of chemotaxis and phototaxis, the mutant cells still swim normally when examined under the microscope. They do not exhibit the smooth swimming or tumbly phenotypes of *E. coli* or *S. meliloti che* mutants, suggesting that some aspects of motor control may be independent of the chemosensory pathway.

Given the lack of a phenotype when the first chemotaxis operon identified in *R. sphaeroides* was deleted, the role of the genes in chemotaxis was investigated by examining the effect on the swimming behaviour of *E. coli che* mutants and wild-type cells. *cheW* was the only *R. sphaeroides* gene to complement an *E. coli* mutant, but expression of the other genes in wild-type *E. coli* interferred with a normal chemosensory response without altering growth (Hamblin *et al.*, 1997a). The protein products, therefore, can interfere with the chemotactic pathway, probably by sequestering or competing with the proteins of the *E. coli* pathway, causing smooth swimming. The sensory proteins must, therefore, be related to those in *E. coli*, but the proteins beyond CheW, CheA and the CheY homologues must be unable to interact with their homologous target motor proteins, suggesting that the sequence of events after the conformational change in CheW is different.

Motor control and swimming behaviour

In enteric species the frequency of periodic switching in the direction of flagellar rotation is controlled by the concentration of CheY-phosphate and is usually considered to cause the bias in the swimming direction of bacteria, and thus chemotaxis. Recent work with *S. meliloti* and *R. sphaeroides* suggest, however, that in these species direction changing is more complex.

When proline, a strong chemoattractant, is added to *S. meliloti* cultures a sustained increase in absolute swimming speed (chemokinesis) is seen, the increase in smooth-swimming rates resembling the suppression of tumbles in enterobacteria (Sourjik & Schmitt, 1996). Thus, *S. meliloti* cells respond to tactic stimulation by modulating the flagellar rotation rate, unlike enteric bacteria which respond by switching the direction of rotation. *S. meliloti* has a single copy of CheA and two copies of the response regulators $CheY_I$ and $CheY_{II}$ and it seems probable that phosphorylated $CheY_I$-phosphate and $CheY_{II}$-phosphate proteins act to slow the flagellar

motor (Sourjik & Schmitt, 1996). Behavioural studies using *cheY* deletion mutants suggest that $CheY_{II}$ is the protein primarily involved in regulating the rate of flagellar rotation. A mutant strain overexpressing $CheY_{II}$ shows an increase in the rotation rate of the motor on addition of proline and decreases upon its removal. It is possible that the interaction of $CheY_{II}$ with the motor is modulated by $CheY_I$, perhaps by competition for phosphate from CheA and probably by differential interaction at the cytoplasmic surface of the motor. In a working model consistent with the experimental data (Sourjik & Schmitt, 1996) the CheA autokinase activity is assumed to be directly controlled by signals from the sensors, the addition of an attractant resulting in inhibition of CheA autophosphorylation and thus increasing the motor speed as no CheY-phosphate is formed to interact with the switch. Conversely, the removal of attractant would cause activation of CheA, with the consequent production of $CheY_I$-phosphate and $CheY_{II}$-phosphate. In this model, $CheY_{II}$-phosphate is the major regulator interacting with and slowing the motor (Fig. 3b). The differential effect exerted by $CheY_I$-phosphate and $CheY_{II}$-phosphate may depend both on their relative affinity for the motor and on the stability of the phosphorylated state. The autodephosphorylation rates of $CheY_I$-phosphate and $CheY_{II}$-phosphate, phosphorylated by acetyl phosphate or CheA-phosphate, were measured *in vitro* and the half-life of their decay (determined by the loss of ^{32}P) found to be about 12 s. If independently phosphorylated by CheA-phosphate, the steady-state level of $CheY_I$-phosphate was about half that of $CheY_{II}$-phosphate. However, if phosphorylated together, more than 90% of the phosphate was found on $CheY_I$, and less than 10% on $CheY_{II}$. Addition of either $CheY_I$ or CheA did not increase $CheY_{II}$-phosphate, dephosphorylation rates, suggesting that $CheY_I$ may be the preferred substrate for CheA-phosphate rather than CheA acting as a phosphatase. Also, reversed phosphotransfer from $CheY_{II}$-phosphate (but not $CheY_I$-phosphate) to CheA was observed and in the presence of both CheA and $CheY_I$ the level of $CheY_{II}$-phosphate was seen to decrease more rapidly (V. Sourjik & R. Schmitt, unpublished). It thus appears that in *S. meliloti* $CheY_I$ serves as a sink for phosphate from CheA-phosphate and $CheY_{II}$-phosphate, and this serves to terminate the signal, $CheY_{II}$-phosphate being the major 'slow' signal for the motor (Fig. 3c).

The variation in flagellar rotation rate (at constant Δp) seen in cells tethered by a single flagellum may also depend on the product of two novel motor genes identified in *S. meliloti*, *motC* and *motD* (Platzer *et al.*, 1997). MotC is a secreted protein probably acting at the periplasmic surface of the motor, whereas MotD may be associated with and interact with the cytoplasmic surface of the motor. Both these proteins are required for normal motor function, as knockout mutants are flagellate but paralysed. Mutations that alter the stoichiometry of MotB and MotC result in extreme fluctuations in flagellar rotation rates and uncoordinated ('jiggly') swimming. Experiments designed to test protein–protein interactions in the yeast two-hybrid system suggest that the periplasmic domains of MotC and MotB interact, indicating that association-dissociation dynamics may be involved in the variation in rotation rate. It has also been shown that the speed of flagellar rotation increases with the dosage of MotD (Platzer *et al.*, 1997); however the role of this cytoplasmic component has yet to be established. Homologues of *motC* and *motD* have not yet been identified in *R. sphaeroides*.

In *R. sphaeroides*, speed changes have been measured during unstimulated swimming, but have not been investigated in stimulated cells. The role of the three CheY homologues in *R. sphaeroides* chemotaxis has not been characterized, but $CheY_I$ and $CheY_{II}$ can be deleted without any change in swimming behaviour or chemotactic response under conditions tested.

Aerotaxis and phototaxis

Electron-transport-dependent signalling in the α-subgroup has only been studied in any detail in *R. sphaeroides* and, to some extent, in *Rhodospirillum centenum* (see Armitage, 1997 for a recent review). Early experiments had shown that photosynthesis and phototaxis are very closely linked. Reaction-centre mutants no longer show phototaxis, and bacteria accumulate in wavelengths corresponding to the wavelengths of light absorbed by the photosynthetic pigments. Observation of swimming behaviour of a range of photosynthetic species when they swim over a light/dark boundary showed that they reverse back into the light, but show no response to a dark/light boundary. This type of behaviour obviously traps cells in the light, although it can result in a stopping species, such as *R. sphaeroides*, being trapped in the dark! (Sackett *et al.*, 1997). *Rhodospirillum rubrum* responds to a change in intensity of as little as 1% of the starting intensity, but the response depends on the strength of the background light (Clayton, 1958). More recent work has shown that a range of specific inhibitors of photosynthetic electron transport interferes with photoresponses without altering motility, again suggesting a link between photosynthesis and photoresponses. Whether cells respond to a step-down in light depends on the light intensity in which they are grown. Cells grown in high light with few light-harvesting complexes respond to 97% reduction in light intensity from a wide range of starting intensities, whereas low-light-grown cells with a high concentration of light-harvesting complexes only respond to a 97% reduction in light intensity if the starting intensity is already very low. Whether or not cells respond to a defined step-down in light therefore depends on the number of light-harvesting complexes, and thus whether the photosynthetic system remains saturated after the step-down. The cells are therefore not measuring the actual change

in light intensity but the change in photosynthetic activity (Grishanin *et al.*, 1997).

Aerotaxis was also found to depend on active electron transport, inhibitors of electron transport similarily inhibiting the aerotactic response. Experiments with DMSO, an electron acceptor under dark anaerobic growth conditions, showed that all electron transport sensory systems are connected. *R. sphaeroides* shows chemotaxis towards DMSO when incubated anaerobically in the dark, but the presence of either light or oxygen inhibits the response, although the DMSO reductase is still present. Similarly, the presence of oxygen inhibits the photoresponse. In all cases the electron flow through the electron transfer pathway to the acceptor is reduced or lost (Gauden & Armitage, 1995). This supports previous suggestions that components of the electron transfer chain are shared between pathways. It also suggests that when there are no specific receptors for light, oxygen or DMSO *per se*, with bacteria simply responding to a change in the activity of their electron transfer chain, their overall swimming direction is biased to the environment in which their electron transport rate is maximal.

Does the cell sense a change in the protonmotive force (Δp) resulting from a change in photosynthetic or respiratory activity, as has been suggested before or do they respond to a change in electron transport rate altering the redox state of an electron transport component, as has been suggested for ArcA and Aer? Anaerobically grown *R. sphaeroides* only responds to a decrease in an attractant concentration and no repellents have been identified; they respond to a step-down in light intensity not a step-up. This 'pessimistic' approach to gradient sensing was used to identify whether the cells respond to a change in Δp or a change in the rate of electron transfer (Grishanin *et al.*, 1997). The membrane-bound carotenoids in *R. sphaeroides* can be used as a natural monitor of the size of the Δp. It was possible to add small amounts of the uncoupler FCCP such that there was a decrease in the size of Δp with a concomitant increase in the rate of electron transfer, but the cells remained motile. When FCCP was removed in a flow chamber the reverse happened, the Δp increased but the rate of electron transfer decreased. *R. sphaeroides* did not respond to the addition of FCCP, which decreased the Δp but increased the rate of electron transport. A decrease in light intensity that produced a similar fall in Δp did however cause a response, but in this case the electron transport rate also fell. On the other hand, the removal of FCCP, which increased the Δp but decreased the rate of electron flow, did cause the cells to respond by stopping. This implies that *R. sphaeroides* responds to a change in electron transfer rate rather than a change in Δp, presumably sensing the redox state of an electron transport intermediate. Whether *R. sphaeroides* contains a homologue of the Aer protein recently identified in *E. coli* remains to be seen, but it seems probable. The change in electron transport rate is then signalled through $CheA_{II}$ and the second chemosensory phosphorelay system to control the stopping frequency of the motor, all signals integrating at the single motor to produce a balanced response (Fig. 3c).

Summary

We are only beginning to understand the mechanisms involved in tactic sensing in the α-subgroup of bacteria. It is clear, however, from recent developments that although the central chemosensory pathways are related to those identified in enteric species, the primary signals and the effect on flagellar behaviour are very different. The expression of chemoreceptors is under environmental control, and the strength of a response depends on the metabolic state of the cell. This is very different from enteric species which always respond to MCP-dependent chemoeffectors, and in which the expression of the receptors is constitutive. Chemotaxis in *R. sphaeroides* and *S. meliloti* is therefore more directly linked to the environment in which a cell finds itself. The integration of chemosensory pathways dependent on growth state may be much more suited to the fluctuating environment of these soil and water bacteria. There is still a great deal that needs to be understood about the mechanisms involved in motor control. The presence of at least two CheY homologues and the finding that the swimming speed of these bacteria can vary, and, in the case of *S. meliloti*, vary with chemosensory stimulation, suggests a different control mechanism at the flagellar motor where speed can be altered, or the motor stopped, with a full Δp still present. Why *R. sphaeroides* should have at least two functional sets of genes encoding homologues of the enteric chemosensory pathway remains to be determined. The major differences in sensory behaviour between the two α-subgroup species so far studied in detail and the differences from the enteric species suggests that many more variations of the chemosensory pathways will be found as more species are studied.

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