# MAKING SENSE OF IT ALL: BACTERIAL CHEMOTAXIS

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Abstract | Bacteria must be able to respond to a changing environment, and one way to respond is to move. The transduction of sensory signals alters the concentration of small phosphorylated response regulators that bind to the rotary flagellar motor and cause switching. This simple pathway has provided a paradigm for sensory systems in general. However, the increasing number of sequenced bacterial genomes shows that although the central sensory mechanism seems to be common to all bacteria, there is added complexity in a wide range of species.

HAMP DOMAIN ('Histidine kinases, adenylyl cyclases, methyl-binding proteins and phosphatases' domain). A domain that is broadly conserved in histidine protein kinases, chemoreceptors and phosphatases. It contains two amphipathic helices, and is presumed to have a role in signal transduction.

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In an ever-changing environment, it is essential that organisms are able to sense these changes and to respond appropriately. Possible responses include alterations in gene expression and/or active movement towards or away from an environment. Most sensory pathways in eukaryotic organisms rely on serine, threonine or tyrosine protein kinases, whereas the most common sensory pathways in prokaryotes use a histidine–aspartate phosphorelay (HAP) system. HAP systems have at least two components — a dimeric histidine protein kinase (HPK) and a response regulator (RR)[1]. HAP systems are also found in many lower eukaryotes, in which they control processes such as osmoregulation in, for example, *Saccharomyces cerevisiae*[2], *Candida albicans*[3] and *Dictyostelium discoideum*[4]. They are also found in plants, in which they control, for example, ethylene-mediated fruit ripening[5] and blue-light responses. The *Arabidopsis thaliana* genome has genes for 16 HPK and 24 RR homologues.

Bacteria can sense a vast range of environmental signals, from the concentrations of nutrients and toxins to oxygen levels, pH, osmolarity and the intensity and wavelength of light. Genome sequencing has identified over 600 HAP systems and some individual bacterial species contain more than 130 (REF. 6) — for example, *Myxococcus xanthus* has about 140 HAP systems (J. R. Kirby, personal communication). Despite the extremely high homology between the components of HAP systems, numerous pathways operate in individual bacteria without significant crosstalk. Although most of these HAP systems are involved in the regulation of gene expression, one of the most well-understood pathways regulates motile behaviour. Chemotaxis operates as part of a complex network of signals that balance to produce a physiological response that is suited to a specific environment (BOX 1). Here, we describe the basic bacterial chemosensory pathway, as well as some of the variations of this system. However, first we provide a general overview of HAP systems.

## Histidine–aspartate-phosphorelay systems

HAP systems rely on the *trans*-autophosphorylation of a His residue that resides in one monomer of the HPK dimer by the γ-phosphoryl group of an ATP molecule that is bound to the kinase domain of the other monomer. This phosphoryl group is then transferred to an Asp residue on a separate RR protein to alter its activity and generate a response. Some systems incorporate several His–Asp phosphorylation steps, which possibly allows for complex regulatory control as discussed below[7,8].

Numerous HAP systems have been identified in different bacteria (FIG. 1). Most of the HPKs involved (for example, EnvZ; FIG. 1a) have an N-terminal region that spans the membrane twice, which results in an intervening periplasmic domain that can sense an extracellular signal. The C-terminal cytoplasmic region comprises a linker or HAMP DOMAIN ('histidine kinases, adenylyl cyclases, methyl-binding proteins

## Box 1 | Chemotaxis as part of a complex network of signalling pathways

**Pathogenicity**

Motility and chemotaxis are required for many pathogenic species to colonize and invade a host. Bacteria sense a wide range of signals — including temperature and pH changes, nutrient concentrations, osmolarity and oxygen — and they integrate this information to generate the pathogenic response. Chemotaxis can, for example, guide *Helicobacter pylori* to the mucus lining of the stomach[97], free-living *Vibrio anguillarium* to the surface of fish[121], and *Agrobacterium tumefaciens* to wounded plants[122]. Each species responds to specific, relevant chemoattractants and other sensory signals then control the expression of virulence genes. Interestingly, it is not clear whether *Vibrio cholera* — which can live in biofilms in aquatic environments or in the human small intestine[123], and has a spectacular range of histidine–aspartate-phosphorelay systems (~ 30), methyl-accepting chemotaxis proteins (~40) and chemotaxis protein (Che) homologues (3 CheW, CheA, CheB, CheR and CheV proteins, 4 CheY proteins and 1 CheZ protein) — uses chemotaxis to swim to the villi of the small intestine. However, motility is known to be essential for pathogenicity[124].

**Symbiotic associations**

Chemotaxis probably guides free-living rhizobia to the environment around legume root hairs, which contains a higher concentration of nutrients. The correct host–symbiont contact then triggers the formation of NODULES and the subsequent nitrogen fixation by bacteria[125]. In a different type of symbiotic association, the squid *Euprymna scolopes* uses bioluminescence to confuse preditors. The marine bacterium *Vibrio fischeri* is only bioluminescent at high cell densities (~$10^{10}$ $ml^{-1}$), and chemotaxis guides free-living bacteria to the squid light organs, in which the colony size increases. Eventually, the concentration of a diffusible autoinducer (*N*-acyl homoserine lactone) reaches the level that is required to induce the expression of bioluminescence genes[126].

**Biofilms**

Many bacterial species are not normally free living, but are associated with surfaces in complex polysaccharide biofilms, in which microcolonies of different species coexist and compete for diffusing nutrients[127]. Biofilms are involved in fouling — for example, on oil rigs, teeth and medical implants, and in the lungs of cystic-fibrosis patients[128] — and they protect bacterial colonies from antimicrobial agents. Biofilm formation and development results from large numbers of interacting sensory signals — for example, QUORUM SENSING[129], osmolarity and charge sensing, and also chemotaxis. In biofilms, many species lose flagella, but induce the expression of type-IV PILI, which allow them to 'twitch' along surfaces (BOX 2). Biofilms are far from static entities and they mature, develop and shed bacteria, which swim to new locations.

NODULE
A swelling on the roots of nitrogen-fixing plants that contains symbiotic nitrogen-fixing bacteria.

QUORUM SENSING
The ability of bacteria to sense their own cell density by detecting the concentration of signalling molecules that have been released in their environment.

PILI
Short, polymerized-protein projections that protrude from the bacterial cell and are used for surface attachment and twitching motility.

PAS DOMAIN
(PER, ARNT, SIM domain). A domain that is involved in recognizing stimuli such as light, oxygen, redox potential, energy status and small ligands.

HPt DOMAIN
(Histidine-containing phosphotransfer domain). These domains are ~120 amino acids long and contain a histidine residue that can participate in phosphoryl-transfer reactions. They function as phosphoreceivers and phosphodonors, and can therefore be used to create multistep phosphorelay systems.

and phosphatases' domain), a dimerization domain and a kinase domain that interacts with its cognate RR. Some HPKs lack the periplasmic and transmembrane domains, for example, CheA (chemotaxis protein A; FIG. 1c) and NtrB. CheA interacts with transmembrane receptors (see below), whereas NtrB interacts with cytoplasmic sensors. Some transmembrane HPKs have several transmembrane regions, for example, PrrB (also known as RegB) has four transmembrane helices and KinB has six[8,9] (FIG. 1d). Other HPKs have further cytoplasmic domains — for example, aerobic-respiration-control sensor protein-B (ArcB) has a cytoplasmic PAS (PER, ARNT, SIM) DOMAIN, a fused RR domain and an extra histidine-containing phosphotransfer (HPt) DOMAIN (FIG. 1b).

HAP-system signalling is initiated by *trans*-autophosphorylation within the HPK dimer. As mentioned above, the γ-phosphoryl group of an ATP molecule that is bound to the kinase domain of one monomer is transferred to a His residue in the other monomer. The phosphoryl group is then transferred to the conserved Asp residue of the relevant RR, which results in the activation of the output domain of the RR (FIG. 1). RRs can undergo spontaneous dephosphorylation by transferring the phosphoryl group to water, and the half-lives of the different phosphoryl–Asp RRs vary from a few seconds to hours. However, in many cases, the half-life is reduced by the presence or activation of a phosphatase, which might be the HPK itself. More complex versions of HAP systems with numerous His–Asp–His–Asp phosphotransfer steps are widespread, particularly in eukaryotes, and such systems have been characterized in detail in relation to osmoregulation in *S. cerevisiae*, as well as sporulation in *Bacillus subtilis*[8] (FIG. 1d). HPt domains, which can add extra phosphotransfer steps, have no catalytic activity and the cognate RR functions to take or transfer the phosphoryl group from the HPt domain. The reason for numerous steps is unclear, but studies on the oxygen-sensing Arc pathway (FIG. 1b) indicate that it might assist in complex regulatory control[8].

## Bacterial chemotaxis as a model system

Bacterial chemotaxis is the biasing of movement towards regions that contain higher concentrations of beneficial, or lower concentrations of toxic, chemicals. The signalling pathway that is involved has been most extensively studied in the enteric bacteria *Escherichia coli* and *Salmonella enterica* serovar Typhimurium[10,11]. Structural and biochemical details are now available for every step of the pathway, which makes it one of the most well-understood of all sensory pathways.

Most sequenced bacterial genomes include homologues of genes that are known to encode components of flagella and chemosensory pathways, which indicates that motility is widespread (BOX 2) and probably provides a selective advantage, especially in non-homogeneous, nutrient-limiting environments. However, all bacteria in which motility has been studied seem to be

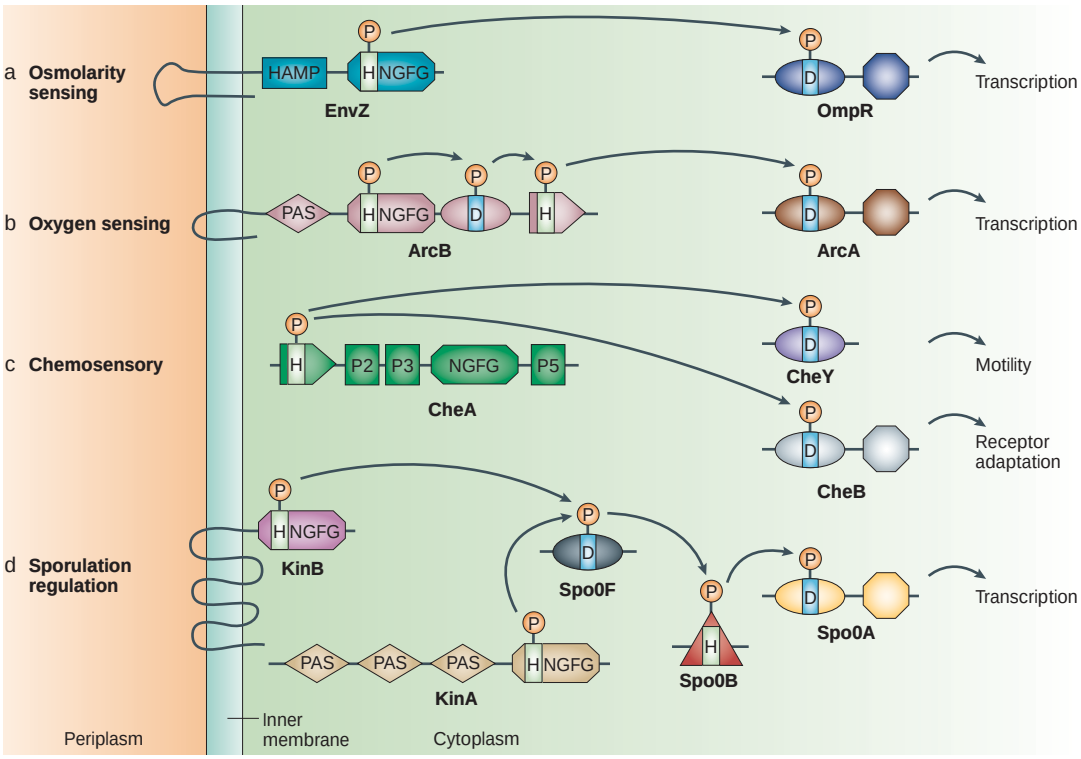



Figure 1 | **The domain organization of selected histidine–aspartate-phosphorelay systems.** Some of the different combinations of histidine protein kinase (HPK) and aspartate response regulator (RR) domains in histidine–aspartate phosphorelay (HAP) systems. **a** | The EnvZ/OmpR pathway of *Escherichia coli*, which is involved in regulating the expression of the two outer-membrane porins OmpF and OmpC. A membrane-bound HPK (EnvZ) controls the activity of the RR OmpR in response to changes in osmolarity. **b** | The complex ArcB–ArcA HAP system of *E. coli*. The membrane-bound HPK ArcB senses changes in the redox state of components of the respiratory electron-transport chain through its PAS (PER, ARNT, SIM) domain. The phosphoryl group is then passed from the conserved His in the ArcB kinase domain to a fused RR domain, then to a fused histidine-containing phosphotransfer (HPt) domain and finally to a DNA-binding RR ArcA. ArcA regulates microaerophilic gene expression. **c** | The chemosensory pathway of *E. coli.* The soluble HPK chemotaxis protein (Che)A has five domains per monomer that are designated P1–P5 from the N terminus to the C terminus. CheA senses changes through transmembrane chemoreceptors, which induce the *trans*-autophosphorylation of dimeric CheA on a His residue of the HPt domain. Two RRs compete for this phosphoryl group: CheY, a single-domain, motor-binding protein, which controls flagellar motor switching, and CheB, which controls the adaptation of the chemoreceptors. **d** | Part of the complex system that regulates sporulation in *Bacillus subtilis*. A single-domain RR, Spo0F, is regulated by two HPKs, one of which has numerous transmembrane domains (KinB), the other of which is soluble with numerous PAS domains (KinA). Spo0F indirectly phosphorylates a DNA-binding RR, Spo0A, by way of a His residue in Spo0B. Throughout this figure, light-green rectangles highlight conserved, phosphorylatable His residues, light-blue rectangles highlight conserved, phosphorylatable Asp residues, and orange circles highlight phosphoryl (P) groups. NGFG represents the kinase domain and, with the exception of CheA, the conserved His residue that precedes the kinase domain is contained within the dimerization domain. Despite being dimeric in nature, HPKs are shown here as monomers for simplicity, and the HAMP domain ('histidine kinases, adenylyl cyclases, methyl-binding proteins and phosphatases' domain) is a linker domain.

chemotactic (although two sequenced Archaea with putative flagellar genes have no obvious chemosensory genes[12]). So, the ability to move is not, in itself, beneficial — it must be biased to result in the accumulation of bacteria in particular environments. Bacteria are thought to be too small to sense a concentration gradient along their length and therefore use temporal sensing to bias their overall direction of movement[13] (although for a contrasting view, see REF. 14).

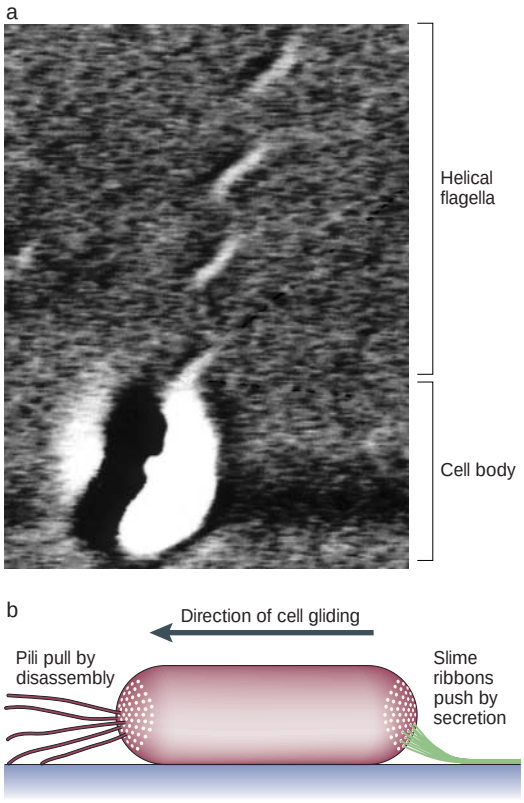
*E. coli* swim by rotating their five to eight helical flagella anticlockwise, which causes them to come together into a bundle that propels the cell forwards. Switching the rotational direction of some flagellar motors to clockwise disrupts this bundle and causes the cell to tumble. When the motors return to anticlockwise rotation, the cell is reorientated and swims off in a new direction[15] (BOX 3). In homogeneous environments, swimming bacteria change direction about once a second, which produces random movement. In non-homogeneous environments, the frequency of direction changing is controlled by positive or negative stimuli to bias the overall direction of movement. Although the number and location of flagella vary between bacterial species, the biasing of swimming always relies on chemosensory regulation of the rotary activity of the flagella motor (switching, stopping or slowing down)[16].

Although it is slightly modified compared to the HAP systems that regulate transcription, the chemotaxis pathway has long been viewed as a paradigm of

## Box 2 | Bacterial motility

Most bacterial species are motile and move either by swimming through liquid environments or by moving across surfaces. Free-swimming bacteria use the electrochemical $H^+$ (or $Na^+$) gradient to power the rotation of transmembrane motors at speeds of up to 300 Hz (for $H^+$) or 1300 Hz (for $Na^+$)[130,131]. These motors drive the rotation of semi-rigid, helical flagella that move bacteria at speeds of 15–100 μm $s^{-1}$ (REF. 130) — that is, up to 100 body lengths per second (see figure, part **a**, which shows a micrograph of a swimming *Rhodobacter sphaeroides* cell). The number and position of flagella varies between species. The Reynold's number — $R = lv\rho/\eta$ (where l = length, v = velocity, ρ = density and η = viscosity) — is ~100 for man but is $10^{-6}$ for microorganisms, which means that bacteria experience a high viscosity and almost no inertia. So, when bacteria stop, they stop immediately. They do not coast or displace liquid.

On surfaces, some species become HYPERFLAGELLATE, which allows movement as the flagella rotate together as numerous bundles. Other species use gliding or twitching to move over surfaces. This can involve type-IV pili, which extend from the poles (sometimes for several body lengths), bind to the surface and then retract, pulling the cell forward[132]. Other gliding bacteria seem to extrude slime through special pores at their poles, with only the rear pores being used at any one time to push the cells forward. The mechanisms behind this type of movement are less clear. Some species, for example, *Myxococcus xanthus* might use both pili and slime[133] (see figure, part **b**). Unlike free-swimming cells, gliding cells move slowly (~1 μm $min^{-1}$), and the fastest movement is the end-over-end movement of *Flavobacterium johnsoniae*[134]. Species of the *Spiroplasma* wall-less bacteria can move through viscous environments, using a dynamic internal cytoskeleton to induce movement through deformations in cell shape. This cytoskeleton is attached to the cytoplasmic membrane and is composed of a flat, monolayered ribbon, which is constructed from seven contractile fibrils that can change their length in a coordinated manner[135]. Part **a** of the figure was modified with permission from REF. 136 © (1999) the American Society for Microbiology. Part **b** of the figure was modified with permission from *Nature Reviews Microbiology* REF. 133 © (2003) Macmillan Magazines Ltd.




HYPERFLAGELLATE
An increased number of flagella.

GAIN
A parameter of chemotactic behaviour that describes the relationship between the stimulus and the response. It is defined as the fractional increase in anticlockwise motor bias divided by the fractional change in receptor occupancy.

CHEMORECEPTOR
A sensory receptor that responds to chemical stimuli. The term covers transmembrane receptors (such as the methyl-accepting chemotaxis proteins) and cytoplasmic receptors (such as transducer-like proteins).

METHYL-ACCEPTING CHEMOTAXIS PROTEIN
(MCP). A transmembrane chemoreceptor that shows methylation-dependent adaptation.

HAP signalling and it is one of the most well-understood physiological processes in biology. This is probably because it has been easy to isolate a wide range of behavioural mutants as they spread differently through soft nutrient agar[17]. The wealth of experimental data that has been obtained for the chemotaxis pathway provides details of the number of molecules of each chemosensory protein within the cell, the localization of each protein and their binding kinetics. From these data, cells can be mathematically or computationally simulated and concentrations of attractants or repellents varied. It is then possible to compare the behaviour of the model cell with experimental data and determine how well the model performs[18]. These models have led to the reinterpretation of data that were obtained using certain mutants, and have provided a basis for making other mutants. However, even though so much is understood, many aspects are still unclear, including details of how the amazing sensitivity, GAIN and adaptation are achieved. Testable models have been, and are being, developed to identify whether these aspects depend on, for example, receptor clustering, robust adaptation or motor kinetics[19–21].

### The chemosensory system

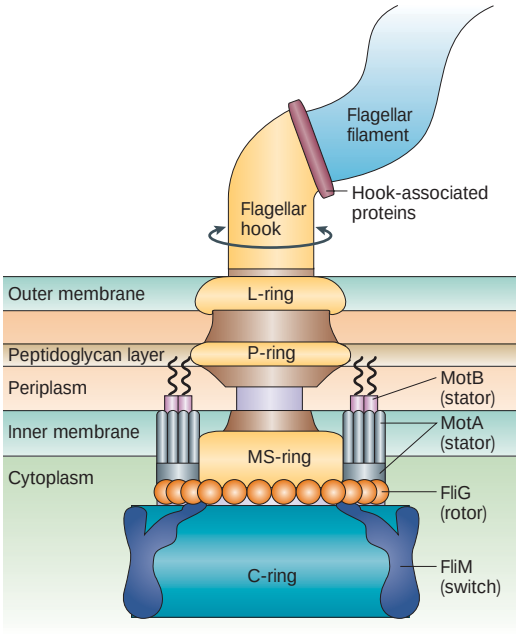
The *E. coli* chemotaxis pathway is so sensitive that it is able to sense a change in a few molecules — perhaps just a change in ligand binding to a single receptor — in the presence of background concentrations that can vary over at least five orders of magnitude[22,23].

Chemotactic signals are detected by dedicated transmembrane CHEMORECEPTORS — the METHYL-ACCEPTING CHEMOTAXIS PROTEINS (MCPs) and an MCP-like protein — rather than by a fused sensory domain of an HPK as is found for most HAP systems that regulate transcription. An adaptor protein, CheW, helps to link the MCPs to the cytoplasmic HPK, CheA, and two RRs compete for binding to CheA (FIGS 1,2). One RR is a single-domain, flagellar-motor-binding protein, CheY (although see BOX 4), whereas the other, CheB, has two domains, one of which functions as a methylesterase and controls the adaptation of the MCPs[24,25]. Phosphorylated CheY (CheY–P) binds the switch protein FliM (see BOX 3 figure) on the flagellar motor and causes a reversal in the direction of motor rotation[26,27]. In *E. coli*, the phosphatase CheZ is required to increase the spontaneous dephosphorylation rate of CheY–P and allow rapid signal termination[28] (FIG. 2).

**Box 3 | The bacterial flagellar motor**

The bacterial flagellar motor is the most complex structure in a bacterial cell, with protein components in the cytoplasm, across the cytoplasmic membrane, the periplasmic space, the outer membrane and in the external environment (see figure for the *Escherichia coli* structure). It is the product of the controlled expression of about 50 genes[137]. Expression of the genes is tightly regulated to assure ordered protein assembly and whereas the early transmembrane proteins are transported through the classic Sec pathway, the later proteins, including those of the hook and filament, use a flagellar-specific export pathway that is closely related to that used for the type-III secretion of toxins by pathogenic species[138].

The filament assembles from the end that is distal to the bacterium and forms a semi-rigid helix, the wavelength and handedness of which alters with changes in the direction of motor rotation. Protons move through roughly eight independent force-generating units (Mot complexes), which are anchored to the peptidoglycan layer to form the stator (the stationary part) of the motor. The protons interact with the ring of ~32 FliG proteins that are associated with the cytoplasmic component of the rotor, and this drives rotation. The mechanism by which the electrochemical gradient is coupled to mechanical rotation is unclear, but it probably involves electrostatic interactions between the stator and the rotor proteins[130]. Phosphorylated CheY, a chemotaxis response regulator, binds FliM proteins that are associated with the FliG rotor to bring about a switch in direction of motor rotation. The figure was modified with permission from REF. 139 © (2003) Elsevier.




Recent *in vivo* imaging and fluorescence resonance energy transfer studies have started to identify the interaction of the proteins in this sensory pathway inside living cells[21,29]. Combining *in vivo* data with mutational and *in vitro* data from the past 40 years indicates a specific sequence of events (FIG. 2). A decreased concentration of attractant results in decreased attractant binding to the MCPs, which stimulates CheA *trans*-autophosphorylation. This results in an increase in the concentration of CheY–P. CheY–P then binds to the flagellar motor and causes it to switch to clockwise rotation, which results in cell tumbling and direction change. The CheY–P signal is terminated by the phosphatase CheZ. CheB is also phosphorylated by CheA–P, which results in an increased methylesterase activity and an increased demethylation of the MCPs. Demethylated MCPs have a reduced ability to induce CheA autophosphorylation (even in the presence of a low concentration of attractant), so the rate of CheA autophosphorylation and therefore the rate of direction changing returns to the pre-stimulus level. The system has now adapted and is primed to sense any subsequent increases or decreases in ligand binding.

An increased concentration of attractant inhibits the autophosphorylation of CheA, which reduces the concentration of CheY–P and therefore the frequency of motor switching. This causes the bacterium to swim in this positive direction for longer. CheB phosphorylation and therefore activity is also reduced, which allows the constitutive methyltransferase CheR to increase the methylation of the MCPs. Highly methylated MCPs are better able to stimulate CheA autophosphorylation, even in the continued presence of a chemoattractant, so this returns CheA autophosphorylation to the pre-stimulus level and the bacterium to a normal frequency of direction changing.

### The chemoreceptors

The number of putative chemoreceptor genes in sequenced genomes ranges from 1 in *Mezorhizobium loti*, to 5 in *E. coli*, to more than 60 in *Magnetospirillum magnetotacticum*. So far, the data indicate that most species have more than 10 chemoreceptor genes and that many have more than 20. Whereas the sequences of the cytoplasmic domains of all these receptors are highly conserved (and are, in fact, used to identify these genes)[30], the sequences of the periplasmic sensing domains vary significantly from species to species and from receptor to receptor. This is unsurprising, as each receptor must bind different ligands and different species have different optimum niches. Why some species require over 50 different receptors is unclear, although different receptors might be expressed under different growth conditions and might tune behaviour to particular environments.

Escherichia coli *methyl-accepting chemotaxis proteins.* *E. coli* has four MCPs (the fifth *E. coli* chemoreceptor is an MCP-like protein; see below), which form homodimers that span the bacterial cell membrane[31]

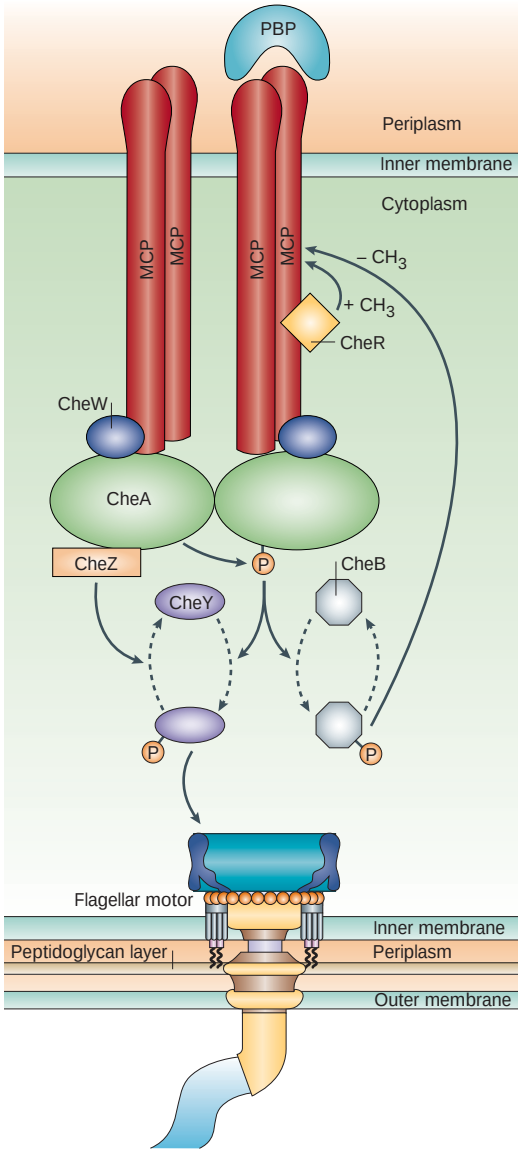



Figure 2 | **Schematic diagram of the chemosensory system of *Escherichia coli*.** Two dimeric chemoreceptors — methyl-accepting chemotaxis proteins (MCPs) — are shown, one of which is interacting with a periplasmic binding protein (PBP). In addition, two chemotaxis protein (Che)W monomers and a CheA dimer are shown interacting with the highly conserved signalling domain of the MCPs in the cytoplasm. It should be noted that, given the packing within MCP clusters and the calculations of the number of chemosensory proteins, the actual arrangement will be different. One CheA monomer will probably not interact with one MCP dimer, and instead a CheA dimer might span several receptors. A decrease in attractant concentration induces *trans*-autophosphorylation of the CheA dimer, which phosphorylates the response regulator CheY. Phosphorylated CheY then binds to the flagellar motor to bring about a change in direction. Phosphorylated CheA also phosphorylates another response regulator — the methylesterase CheB. Phosphorylated CheB competes with a constitutive methyltransferase, CheR, to control the degree of methylation of specific glutamates in the MCPs. This resets the signalling state of the receptors and allows them to adapt to the present concentration of attractant and to sense subsequent changes. The dephosphorylation of phosphorylated CheY is accelerated by the phosphatase CheZ. P, phosphoryl group.

(FIG. 2). X-ray crystallography of isolated periplasmic and cytoplasmic domains of MCPs shows that they are formed almost completely from α-helical coiled-coil structures[32,33] (FIG. 3). An entire *E. coli* MCP is ~380-Å long, with an 80-Å periplasmic domain, a 40-Å transmembrane domain and a 260-Å cytoplasmic domain. The periplasmic domains of the *E. coli* MCPs each consist of a four-helix bundle. However, the fold of the periplasmic domains of MCPs from other species tends to be highly diverse, which reflects the wide range of ligands that are sensed by these receptors.

Ligands bind to the periplasmic domain of MCPs at the interface between the two monomers of the dimer[34], and residues from both monomers are involved in ligand binding. Some MCPs, for example *E. coli* Tar, can bind to two different ligands — to one directly, and to the other through a periplasmic binding protein that can also interact with the appropriate transporters[35]. Ligand binding alters the interactions between the periplasmic domains of the MCPs, and also changes the interactions between the transmembrane four-helix bundle of the MCP dimer. Mutational analyses, tryptophan-fluorescence and cysteine-crosslinking studies of the transmembrane domains, together with NMR measurements, indicated that a 1.4-Å 'piston-like movement' of one transmembrane helix against another in the MCP dimer occurs on ligand binding, which might be important for signal propagation across the bacterial membrane[31,36–40]. However, the propagation of such a small shift through such a large coiled-coil protein and the difference in the rapid kinetics of attractant binding and the slow kinetics of CheA autophosphorylation indicate that this might only be part of the signal. Isolated MCP cytoplasmic domains from *E. coli* can still activate CheA, which indicates that a change in the interactions of the periplasmic domains on ligand binding probably also alters the packing of the highly conserved cytoplasmic domains to cause signalling[41]. In addition, many bacterial species seem to contain cytoplasmic chemoreceptors, which lack transmembrane domains and are active in clusters[42,43] (the role of receptor packing is discussed below). *E. coli* also responds to a range of repellents and will swim towards its optimum growth temperature. Although the mechanisms that are involved in these responses are unclear, data indicate that some signals might be sensed directly through the cytoplasmic signalling domain, rather than the periplasmic domain, of the MCPs[44].

In MCPs, a structurally conserved HAMP domain links the transmembrane helices to the cytoplasmic signalling domain. This region is lacking in crystal structures and its relationship to the rest of the protein structure remains unclear. However, disruption of this region results in a loss of signalling and the maintenance of this structure is crucial in the production of chimeric signalling proteins[45]. The HAMP domain might therefore be important in regulating the coiled-coil interactions that seem to be involved in signal propagation[46].

The signalling domain contains two adaptation regions, each with four to six glutamate residues that can

**Box 4 | Do all putative chemosensory components control motility?**

*Myxococcus xanthus* is a social, gliding bacterium that displays two types of motility — social and adventurous. In addition, in response to starvation, *M. xanthus* cells aggregate to form multicellular fruiting bodies. Motility and chemotaxis are essential for these responses[140], and require two chemotaxis operons — *frz* and *dif*[141]. A third operon, *che3*, has been shown to be required for differentiation[142]. The latter apparent chemosensory pathway senses an environmental signal through a classic methyl-accepting chemotaxis protein (MCP)-like receptor that controls the phosphorylation of the chemotaxis protein (Che)A. Phosphorylated CheA phosphorylates either a CheB homologue or, rather than CheY, a transcriptional regulator, which drives the expression of genes that are required to switch the cells from a vegetative to a sporulation state. Similarly, one of the four *Pseudomonas aeruginosa che* operons seems to be essential for the expression of pathogenicity genes, and one operon in *Pseudomonas fluorescens* has been proposed to be involved in cellulose biosynthesis. Recent sequencing of the *M. xanthus* genome indicates that it might have as many as eight chemosensory operons in total. The role of these other operons remains to be elucidated (J. R. Kirby, personal communication).

This indicates that analysing genome sequences without the use of supporting physiological data could lead to the misannotation of genes and to their subsequent misinterpretation. Do all species with four or five CheA proteins and numerous MCPs use all of them for chemotaxis? The roles of the numerous homologues of the other chemotaxis proteins that are found in many non-enteric bacteria remain an enigma. Many bacterial species live in diverse environments and it is possible that, under different conditions, different chemotaxis operons might be expressed to allow the bacteria to tune their chemotactic responses to their existing environment.

be reversibly methylated[47]. Some of these glutamates are actually encoded as glutamines, which mimic methylated glutamates and probably ensure that newly translated MCPs are inserted into the membrane in a neutral signalling state. CheB is actually a dual-function enzyme, as it can deamidate as well as demethylate MCPs. Deamidation of the glutamine residues to glutamates means that all of these residues become potential targets for methylation by CheR and, therefore, for adaptation[48]. A pentapeptide motif (NWETF) at the C terminus of the two most abundant *E. coli* MCPs, Tsr and Tar, recruits the methyltransferase CheR to the MCP to bring about methylation[49]. The two low-abundance *E. coli* MCPs, Trg and Tap, lack the NWETF motif and therefore require the presence of Tsr or Tar for adaptation to their ligands[50]. This indicates that the different receptors must be close enough to interact *in vivo*. Indeed, there is direct evidence for inter-dimer methylation[51].

*Localization of methyl-accepting chemotaxis proteins.* Transmembrane MCPs have been shown to cluster in patches that are thought to be ~200 nm in diameter and that might form higher-order arrays at the poles of cells[52,53]. Similar higher-order arrays of receptors and channels have been observed in eukaryotic cells, for example, in lymphocytes and neurons[54], and significant efforts are underway to try to understand the implications of such arrays for signal generation and processing in general.

Clustering depends not only on the MCP itself — which might be targeted, to some extent, to the poles — but also on the MCP-associated cytoplasmic proteins CheW and CheA[52,55,56]. Structural and biochemical studies indicate that MCPs might pack as trimers of dimers[33,57,58] (FIG. 4a). However, certain cryo-electron-microscopy studies and quantitative data indicate that the clusters could be formed from dimers that are packed into a higher-order array, which involves interactions between periplasmic domains and/or the swapping of anti-parallel strands of the individual MCPs within the cluster[59]. This packing pattern would allow the necessary CheW and CheA interactions at the base of the MCPs. It has been proposed that the clustering of MCPs might allow the high signal sensitivity and gain of receptors that is seen, as well as integration between receptors[60] — the binding of a ligand to one receptor would influence neighbouring, unbound receptors. This model also postulates that the number of neighbouring receptors that are affected decreases as the overall proportion of ligand-bound receptors increases. The model elegantly encompasses two of the key features of chemotaxis — the high degree of sensitivity in the system and the wide range of background concentrations over which differences can be sensed. Unfortunately, at present, there is little evidence to indicate how such lateral interactions might be established and altered within such a receptor cluster, although changing the charge of the receptors through methylation and demethylation might have a key role.

Using a lattice of interacting chemosensory proteins allows the integrated control of the output of relatively few CheA molecules[61]. The use of multivalent ligands to physically cluster MCPs *in vivo* showed that signalling information is transferred between MCPs in a cluster[62]. *In vivo* crosslinking has also proved that there are direct physical interactions between different MCPs in a cell[58,63]. Taken together, these data indicate that the receptor clusters are composed of signalling teams, possibly of mixed trimers of MCP homodimers, with the different MCPs functioning collaboratively to regulate the activity of CheA molecules that are bound to the receptors (FIG. 4a). Interestingly, quantitative western-blot measurements of the number of the different chemosensory proteins in different strains of *E. coli*[64] and of *Rhodobacter sphaeroides* (M. Gould and J.P.A., unpublished data) under different growth conditions showed that, whereas the absolute concentrations of the different chemotaxis proteins vary significantly, the stoichiometries remain almost constant. This finding has key implications for our understanding of chemosensory signalling and for the modelling of these

FLAVIN ADENINE DINUCLEOTIDE (FAD). FAD functions as a redox centre in many proteins.

SRC-HOMOLOGY-3 DOMAIN (SH3 domain). A 60-amino-acid domain that mediates the assembly of specific protein complexes through binding to proline-rich peptides. It is found in many proteins that are involved in signal transduction and membrane–cytoskeleton interactions.

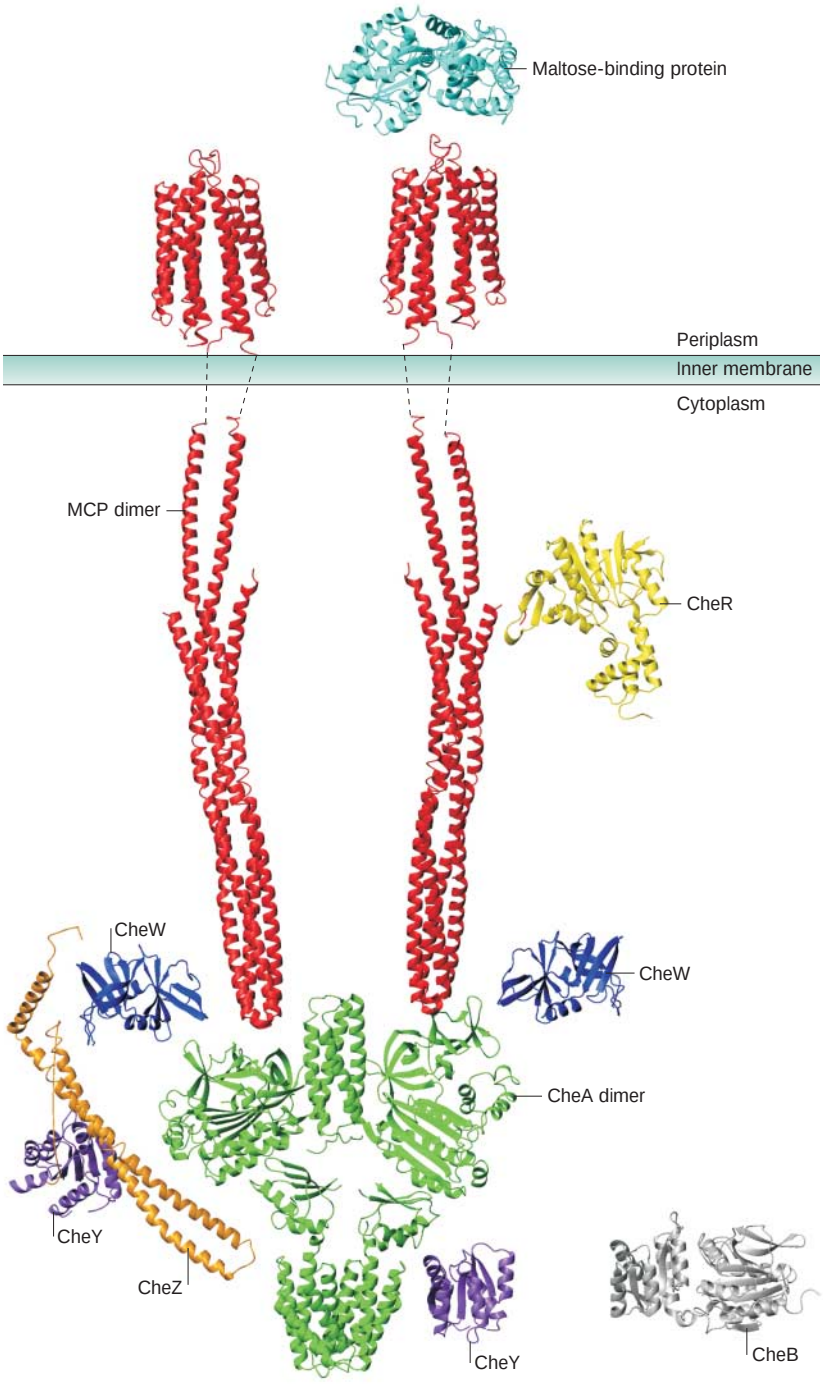



Figure 3 | **The structures of components of the chemosensory system of *Escherichia coli*.** The figure shows the structures of the components of the chemosensory system in the same stoichiometry and localization as is shown in FIG. 2 — that is, two methyl-accepting chemotaxis protein (MCP) dimers are shown interacting with two chemotaxis protein (Che)W monomers and a CheA dimer, and CheY, CheB and CheR are all depicted as monomers. The relevant Protein Data Bank accession numbers are: maltose-binding protein (1ANF); MCP periplasmic domain (1WAT) and cytoplasmic domain (1QU7); the CheA P1 domain (1I5N), P2 domain (1EAY) and P3–P5 domains (1B3Q); the CheW averaged NMR structure (1KOS); CheY (1EHC); CheR (1BC5); CheB (1A2O); and CheZ with phosphorylated CheY bound (1KMI). The figure is a graphical representation and is not meant to imply that the domains or proteins interact in the manner depicted or that their orientations are as shown. The dashed lines represent the transmembrane domains and the HAMP domains ('histidine kinases, adenylyl cyclases, methyl-binding proteins and phosphatases' domains), for which there are, as yet, no structures. This figure was prepared using the program MOLMOL[143].

and possibly other biochemical pathways. *In vitro*, the maximum kinase activity occurs when there is a ratio of 6 MCPs to 4 CheW molecules to 1 CheA molecule[61]. However, modelling predicts maximum activity when there is 1 trimer of MCP dimers (6 MCPs) to 3 CheW molecules to 1.5 CheA molecules[65], and western-blot analysis indicates 3.4 MCP dimers (6.8 MCPs) to 1.6 CheW molecules to 1 CheA dimer[64] (2 CheAs). Something close to these ratios probably exists in extended arrays, possibly with non-integral stoichiometries and with CheW and CheA both interacting with the receptor tips. The formation of targeted local complexes that are composed of particular ratios of these proteins might turn out to be central to signalling regulation in this and other biochemical pathways.

*Variations within* Escherichia coli. The fifth *E. coli* chemoreceptor is the MCP-like transmembrane protein Aer, which mediates aerotactic responses (that is, movement in response to changes in oxygen levels). This protein has the conserved cytoplasmic signalling domain that is found in all MCPs, but it is fused to a cytoplasmic FLAVIN ADENINE DINUCLEOTIDE (FAD)-binding domain and a PAS domain and it lacks methylation sites[66,67]. This protein seems to sense the rate of respiratory electron transport, which allows *E. coli* to respond to changes in oxygen concentrations. Oxygen was probably absent from the atmosphere when MCPs evolved, and the responses of different species to oxygen varies depending whether they are aerobes, microaerophiles or anaerobes. Different species have used different input modules to control the activity of the conserved cytoplasmic signalling domain, including FAD (as mentioned above), haem and cytochrome oxidase, which can either directly or indirectly detect oxygen levels[68,69].

Not all chemotactic responses in *E. coli* are mediated by chemoreceptors. *E. coli* also shows a chemotactic response towards carbohydrates that are transported by the phosphoenolpyruvate-dependent phosphotransferase system. A cytoplasmic component, EI (enzyme I), which is common to all of the transporters of this system, transfers phosphate from phosphoenolpyruvate, by way of an E1–P intermediate, to the carbohydrate-specific EII and EIII transporters. This phosphate is then transferred to the carbohydrate during its transport into the cell. An increasing concentration of carbohydrates outside the cell increases the rate of carbohydrate transport into the cell and therefore lowers the concentration of EI–P in the cell. Unphosphorylated EI interacts with CheA and prevents its autophosphorylation, which, in turn, reduces the amount of CheY–P and prolongs swimming in this positive direction[70].

## Other chemotaxis proteins

*CheW.* CheW is composed of two SRC-HOMOLOGY 3 (SH3)-like subdomains and is an 18-kDa protein that interacts with both the signalling domain of the MCP and the HPK CheA[71] (FIGS 2,3). It is required for the formation of the quaternary MCP–CheW–CheA complex at the poles of cells[52,56], and is essential for signal transduction.

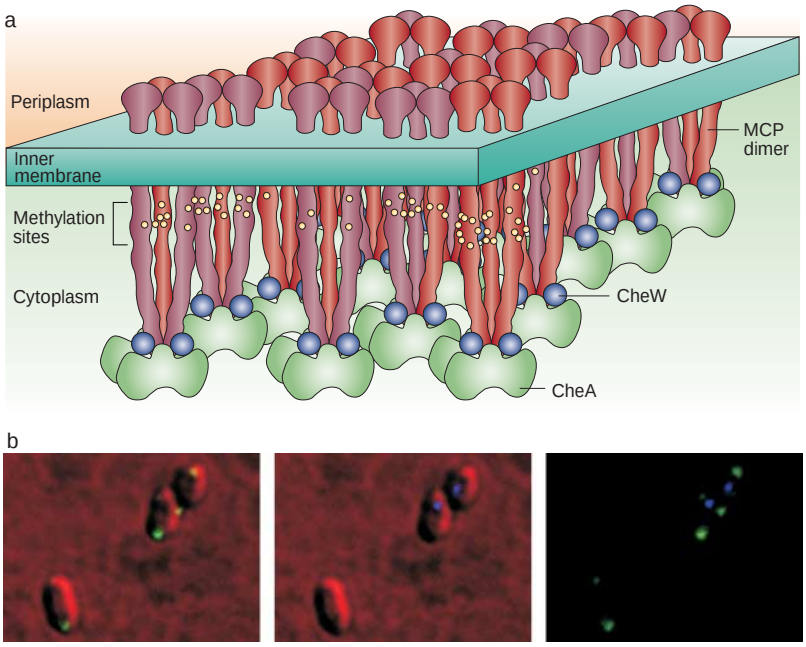



Figure 4 | **Chemotaxis-protein localization. a** | The possible pattern of interaction between different components of the chemosensory pathway at the poles of bacterial cells, which produces signalling clusters. The clusters are shown as being formed of mixed trimers of methyl-accepting chemotaxis protein (MCP) homodimers. These trimers might interact laterally through changes in their packing, as well as through alterations in their interactions with, and between, the chemotaxis protein (Che)W and CheA. The stoichiometry could be ~three MCP dimers to two CheW monomers to one CheA dimer, as is shown in the figure, but the exact stoichiometry is still unclear. The rough positions of the methylation sites are highlighted in yellow. **b** | The localization of chemotaxis proteins *in vivo*. *Rhodobacter sphaeroides* has two chemosensory pathways. Components of one pathway, which includes $CheW_3$, localize to a polar cluster (green labelling in the first and third panels) that contains the transmembrane MCPs, whereas components of the other pathway, which includes $CheW_4$, localize to a cytoplasmic cluster (purple labelling in the second and third panels) that contains the transducer-like proteins. The physical and biochemical separation of the components of these two signalling pathways (see the third, merged panel) might be important for preventing crosstalk between closely related proteins *in vivo*. Part **a** was modified with permission from REF. 144 © the American Association for the Advancement of Science. Part **b** was reproduced with permission from REF. 119 © Blackwell Publishing Ltd.

CheW has no known catalytic activity and is therefore thought to be a scaffold protein that transduces the signals that are generated by the MCP to CheA. The structure of CheW from *Thermotoga maritima* has been solved by solution NMR and it has two five-stranded β-barrel domains with an extensive hydrophobic core[72] (FIG. 3). This structure is very similar to that of the P5 domain of CheA, with which it interacts (CheA has five domains per monomer that are designated P1–P5 from the N terminus to the C terminus; FIGS 1,3; see below).

Despite its supposedly simple scaffold role, the primary sequence of CheW is highly conserved between species, and in many species there are several homologues that are involved in chemotaxis. Interestingly, there does not seem to be a direct relationship between the number of CheW homologues and the number of CheA homologues, which indicates that there is not just one specific CheW for each CheA. Some CheW proteins from the α-subgroup bacterium *R. sphaeroides* (Proteobacteria can be divided into five main subgroups) are capable of restoring switching in an *E. coli cheW* mutant[73,74]. So, as *E. coli* is a γ-subgroup bacterium, this indicates that the structure and function of CheW is conserved between different species. CheW might therefore have a more significant role in the process of signal transduction than just providing a static link between the MCPs and CheA.

*The histidine protein kinase CheA.* CheA is an HPK that functions as a dimer, which comprises two 71-kDa monomers and, as mentioned above, it interacts with ~three MCP dimers and ~two CheW monomers to form oligomeric complexes at the poles of cells. Although CheA is known to interact with CheW, and CheW is known to interact with the MCPs, there is also evidence for a direct CheA–MCP interaction[75]. The X-ray structures of the various domains of CheA have been determined[76,77] (FIG. 3). Each CheA monomer has five domains that are separated by linker regions and hinges (FIG. 1c). Studies have shown that the P1 domain is structurally and functionally homologous to the HPt domains of hybrid kinases such as ArcB (FIG. 1). In the presence of ATP and $Mg^{2+}$, the CheA dimer shows a basal level of *trans*-autophosphorylation, which increases significantly in the presence of receptors. MCPs and CheW bind to the P5 regulatory domain of CheA[78,79], and can both inhibit and activate phosphorylation relative to the basal level by controlling the access of the P1 domain of one monomer to the kinase domain of the other[77]. CheA shows increased autophosphorylation in response to a decrease in attractant binding or an increase in repellent binding to MCPs. Such stimulation results in the γ-phosphoryl group of ATP that is bound to the P4 kinase domain of one monomer being transferred to His48 of the HPt domain of the other monomer in a *trans*-autophosphorylation reaction.

CheY and CheB compete for binding to the P2 domain of CheA, and the phosphoryl group is transferred from His48 of CheA to Asp57 of CheY, or to Asp56 of CheB[80,81]. Interestingly, CheA that lacks a P2 domain can still phosphorylate CheY, although this occurs at a much slower rate than for full-length CheA[82]. This indicates that the P1 domain of CheA might also contribute to the rate of phosphotransfer. Phosphotransfer to CheY is faster than to CheB, which ensures that a response is generated before adaptation occurs[83]. Deletion of either *cheW* or *cheA* in *E. coli* results in a smooth-swimming phenotype, because CheY remains unphosphorylated.

*The response regulator CheY.* CheY is a single-domain, 14-kDa RR protein. Several high-resolution crystal and NMR structures of CheY are now available[84,85]. In addition, there is a structure of CheY bound to the CheA P2 domain[81] and a structure of the active conformation of CheY, in which $BeF_3$ was used to mimic the bound phosphoryl group[86]. CheY has the classic RR five-α-helix, five-stranded-β-sheet structure (FIG. 3). Phosphorylation occurs on the conserved Asp57 residue, which is in an acidic binding pocket that comprises Asp12, Asp13 and Lys109. Phosphorylation of

this residue allows CheY to interact with FliM in the switch complex of the flagellar motor[87–89] (BOX 3). The backbone structure of the protein does not change significantly on phosphorylation, but the exposed molecular surface can alter substantially. The binding of CheY–P to FliM promotes clockwise flagellar rotation, which causes the cell to tumble and change direction[26,88]. *In vitro*, CheY can be phosphorylated by small inorganic phosphodonors, such as acetyl phosphate[90], and it is also subject to acetylation at numerous sites[91]. The potential biological relevance of acetylation and phosphorylation by such inorganic phosphodonors is not known, but mutants in acetyl-CoA synthetase show abnormal behaviour[91], which indicates that some aspects of chemotactic behaviour might be modulated by metabolic activity.

*The phosphatase CheZ and signal termination.* Signal termination is essential for chemotaxis. In the γ-subgroup of proteobacteria, CheZ is an allosteric activator of CheY dephosphorylation, which decreases the half-life of CheY–P from ~20 seconds to ~200 milliseconds — a rate that is required to allow temporal sensing. CheZ is probably a dimer, although large multimeric complexes might form in the presence of CheY–P (REF. 92). The activity of CheZ seems, at least in part, to be controlled by the concentration of its substrate, CheY–P (REF. 93). Co-crystals of CheZ and CheY bound to a phosphate analogue indicate that the Gln147 side chain of CheZ inserts into the CheY active site and might orientate a water molecule for nucleophilic attack[94]. *E. coli* mutants that lack CheZ have a 'tumbling' phenotype, because they accumulate high concentrations of CheY–P.

Interestingly, CheZ seems to be restricted to the γ-subgroup of proteobacteria, so most species must use different mechanisms for signal termination. Several CheY homologues have been found in many species that lack CheZ, so they might function as phosphate sinks, as was shown for the two CheY proteins of *Sinorhizobium meliloti*. However, there are species that have both several CheY proteins and a CheZ homologue, so the story might be more complex. In *S. meliloti*, both CheY proteins can be phosphorylated by CheA–P, but only one of them binds to the flagellar motor. Reverse phosphotransfer from the motor-binding $CheY_2$–P, through CheA, to the second, non-motor binding $CheY_1$ increases the rate of signal termination[95]. Yet other species have CheY domains that are fused to other chemosensory proteins. For example, CheV is a CheW–CheY fusion that is found in *Helicobacter pylori* and *B. subtilis*[96,97], and a CheA–CheY fusion protein has been identified in a number of species, including *Rhodospirillum centenum*[98]. Although the precise role of these fusion proteins is not known, the CheY domain cannot diffuse to the flagellar motor and might therefore function as a phosphate sink. *R. sphaeroides* has six CheY homologues and, interestingly, these all have rapid dephosphorylation rates[99], which might eliminate the need for CheZ.

*Receptor adaptation.* As mentioned above, bacteria must adapt to a background level of attractants and/or repellents by both rapid signal termination and by resetting the receptor proteins to a non-signalling state. Adaptation depends on the degree of methylation of specific, conserved glutamate residues in the cytoplasmic signalling domain of an MCP. Methyl groups are transferred from a cytoplasmic pool of *S*-adenosyl methionine to an MCP by the constitutively active methyltransferase CheR[100], and they are removed and released as methanol by the methylesterase CheB[101,102]. The crystal structure of CheR bound to *S*-adenosyl homocysteine (the methylation reaction product) has been determined in the presence[103] and absence[104] of the C-terminal NWETF motif from the *E. coli* MCP Tar. CheR has a small N-terminal domain that is linked to a large C-terminal domain (FIG. 3). The latter forms most of the interactions with *S*-adenosyl homocysteine. CheR is localized to MCP clusters mainly through hydrophobic interactions between the β-subdomain of CheR and the CheR-docking NWETF motif in the *E. coli* MCPs Tar and Tsr. These interactions position CheR close to the sites that are to be methylated[105]. As the CheR:MCP ratio is thought to be about 1:100 (REF. 64), these interactions must be dynamic.

The crystal structure of CheB has also been determined (FIG. 3). It has an N-terminal regulatory domain linked to a C-terminal catalytic domain[106]. Phosphorylation of the N-terminal domain causes a conformational change that exposes the active site in the catalytic domain to its substrate and stimulates methylesterase activity 100-fold[106,107]. The changes in the methylation level of the conserved glutamates of an MCP — which are brought about by changes in the level of phosphorylation and, therefore, the activity of CheB — reset the signalling state of an MCP and return CheA activity to the pre-stimulus level. The bacterium resumes its original pattern of swimming and the MCP can now respond to any subsequent increases or decreases in chemoeffector concentration. The mechanism by which methylation exerts its effect on CheA activity remains unclear. However, the neutralization of the negative charges of the glutamate residues that occurs on methylation, and the subsequent return of these charges on demethylation, might affect helix packing, and might therefore affect interactions within the oligomeric MCP clusters[108].

## Other bacterial species: variations on a theme

The *E. coli* chemosensory pathway forms the basis of our understanding of bacterial chemotaxis. However, it is becoming increasingly apparent that chemotaxis in other bacteria, although based on similar principles, might be far more complex. In particular, many species have far more putative chemoreceptors than the five that are present in *E. coli*. Some of these receptors lack obvious transmembrane domains and have been shown to be cytoplasmic[43]. Other species also have further chemotaxis proteins[109]. Although we have data on a wide range of species, two of the most well-studied will be used here to illustrate some aspects of this diversity — *B. subtilis*,

TRANSDUCER-LIKE PROTEIN (Tlp). Tlp proteins are cytoplasmic chemoreceptors that have the cytoplasmic domain of methyl-accepting chemotaxis proteins and lack transmembrane domains.

because it has a single chemosensory pathway that contains the largest number of different types of chemosensory protein, and *R. sphaeroides*, because it has several chemosensory pathways.

*Chemotaxis in* Bacillus subtilis. *B. subtilis* is one of the most well-studied Gram-positive bacteria and it seems to have more types of chemosensory protein than any other species that has been studied so far. This indicates either that the *E. coli* system represents a 'streamlined' pathway, or that more proteins arose to refine the *B. subtilis* system during evolution. The *B. subtilis* pathway has a great deal in common with that of Archaea and might represent an alternative paradigm[109]. The chemoreceptors are larger than those of *E. coli* and have a more complex cytoplasmic α-helical structure that is also conserved in Archaea. The phosphorylation reactions are reversed with respect to those in *E. coli*, with an increasing concentration of attractant resulting in an increased (rather than decreased) activity of CheA and therefore an increase (rather than a decrease) in CheY–P concentration. However, in *B. subtilis*, the binding of CheY–P to the flagellar motor decreases (rather than increases) the tumbling frequency and therefore prolongs smooth swimming[110].

Unlike in *E. coli*, in which methylation simply compensates for a decreased CheA activity, methylation in *B. subtilis* is more subtle, with different glutamate residues carrying out specific roles. In McpB, Glu637 methylation helps to bring about adaptation to the presence of an attractant, whereas Glu630 methylation (with the accompanying demethylation of the nearby Glu637 site) helps to bring about adaptation to the removal of an attractant[111]. So, although there is no change in the overall level of methylation, methylation at each site has different consequences[111]. Methanol is released on both attractant addition and removal, which is unlike *E. coli* but is the same as in Archaea[112–114]. However, this only occurs when the attractant concentration is high, which reflects a nutrient-rich environment[115]. When attractant levels are low and the loss of methanol might be energetically costly, another relatively common system (in terms of genome sequences) can be used for adaptation[96]. In this system, CheV (a CheW–CheY fusion protein) can be phosphorylated by CheA–P. Furthermore, two other proteins — CheC and CheD — are present in *B. subtilis*, many other bacterial species and all Archaeal chemotaxis pathways[116,117]. CheC and CheD might interact to regulate the adaptation pathway and CheC is also involved in the dephosphorylation of CheY–P, and therefore represents yet-another signal-termination pathway.

*Chemotaxis in* Rhodobacter sphaeroides. *R. sphaeroides* is a purple, non-sulphur, photoheterotrophic bacterium. It has three operons that potentially encode complete chemosensory pathways. Two of these operons are essential for chemotaxis under laboratory conditions[118], and DNA arrays and proteomic analysis have shown that both operons are expressed, but at different levels in aerobic versus anaerobic conditions (S. Kaplan, personal communication).

*R. sphaeroides* has nine transmembrane chemoreceptors (MCPs) and four putative cytoplasmic chemoreceptors (TRANSDUCER-LIKE PROTEINS (Tlps)). These chemoreceptors localize to discrete regions of the bacterial cell — MCPs localize to the poles of the cell, whereas Tlps are found in a defined cluster in the bacterial cytoplasm[43,53,119] (FIG. 4b). This indicates that clustering might be an essential part of chemoreceptor signalling for both membrane-localized and cytoplasmic receptors. It also indicates that some bacteria might respond chemotactically to changes in the concentration of internal compounds — which might reflect the general metabolic state of the cell — as well as to external compounds.

*R. sphaeroides* has four CheA proteins — three of which are essential for chemotaxis — together with six CheY proteins and two CheB proteins[118]. *In vitro* phosphotransfer experiments have shown that the different CheA proteins can phosphotransfer to different subgroups of RRs[99]. $CheA_2$ phosphorylates all of the RRs, whereas $CheA_3$ and $CheA_4$ phosphorylate $CheY_6$ and $CheB_2$, which are all encoded by the same operon, as well as $CheY_1$ from operon 1. $CheA_3$ and $CheA_4$ are highly unusual, as $CheA_3$ contains P1 and P5 domains, whereas $CheA_4$ contains P3, P4 and P5 domains, but no P1 domain. Neither $CheA_3$ nor $CheA_4$ has the P2 CheY/CheB-binding domain, and a $CheA_4$ homodimer can phosphorylate the P1 domain of $CheA_3$ (REF. 120).

Although $CheA_2$ can phosphotransfer to all of the RRs *in vitro*, it does not compensate for $CheA_3$/$CheA_4$ deletions *in vivo*[118]. This might be because the proteins that are encoded by one chemosensory pathway ($CheW_2$, $CheW_3$, $CheA_2$ and $CheR_2$) localize with MCPs at the poles of cells, whereas the proteins that are encoded by the other operon ($CheW_4$, $CheA_3$, $CheA_4$ and $CheR_3$) localize with the cytoplasmic receptors in a discrete cluster in the cytoplasm[119] (FIG. 4b). The cytoplasmic cluster is actively segregated on cell division, and this process requires a protein homologue of a ParA type-1 DNA-partitioning protein, PpfA, which is encoded in the centre of the related chemosensory operon (S. R. Thompson, G.H.W. and J.P.A., unpublished data). This physical separation of the components of the two pathways might prevent crosstalk *in vivo*. It also provides intriguing evidence that proteins are targeted and segregated within the bacterial cytoplasm, which perhaps indicates a more complex bacterial cytoskeleton than was previously thought. Unlike in *E. coli*, the two CheB proteins do not seem to be specifically localized in *R. sphaeroides*, whereas the CheR proteins are partially localized[119]. The physiological advantage of physically segregating these two chemosensory pathways and the structural basis for the kinase discrimination is unclear, but genome sequences indicate that this segregation of different pathways will not be uncommon in bacteria.

## Summary

The numerous studies that have been carried out on the chemotaxis pathways of bacteria have provided us with invaluable data concerning the structure, kinetics

and regulation of signalling systems in biology. The vast array of physiological data that has been collected from assaying this system is now being combined with the biochemical data to allow mathematical models of chemotaxis and signal kinetics to be generated and evaluated. From these models, general principles are emerging that might be applicable to many other signalling pathways both in prokaryotes and in higher eukaryotes. Chemotaxis is just one of the sensing systems that bacteria possess to monitor and respond to changes in their environment. The correct interplay between this system and other sensing systems is essential for numerous sensory signals to result in a balanced behavioural response.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## Competing interests staement
The authors declare no competing financial interests.

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