

# Receptor clustering and signal processing in *E. coli* chemotaxis

Victor Sourjik

ZMBH, University of Heidelberg, Im Neuenheimer Feld 282, D-69120, Germany

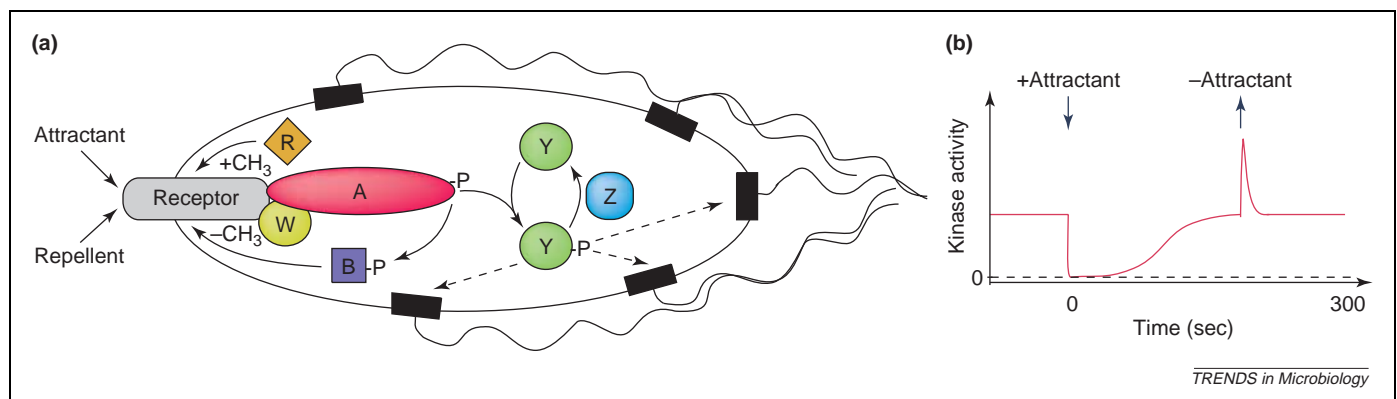
**Chemotaxis in *Escherichia coli* is one of the most thoroughly studied model systems for signal transduction. Receptor–kinase complexes, organized in clusters at the cell poles, sense chemoeffector stimuli and transmit signals to flagellar motors by phosphorylation of a diffusible response regulator protein. Despite the apparent simplicity of the signal transduction pathway, the high sensitivity, wide dynamic range and integration of multiple stimuli of this pathway remain unexplained. Recent advances in computer modeling and in quantitative experimental analysis suggest that cooperative protein interactions in receptor clusters play a crucial role in the signal processing during bacterial chemotaxis.**

Motile chemotactic bacteria are able to move towards higher concentrations of attractants and avoid higher concentrations of repellents by sensing temporal changes in chemoeffector concentrations. The swimming movement of *Escherichia coli* (the best-studied model for bacterial chemotaxis) consists of periods of smooth swimming (or runs) interrupted by short re-orientations (or tumbles), corresponding to counterclockwise (CCW) and clockwise (CW) rotation of the flagellar motors, respectively. The direction of swimming is chosen randomly, but

proceeding in a favourable direction suppresses tumbles, resulting in longer runs [1].

Bacterial chemotaxis is an excellent system for quantitative analysis. Using behavioural, genetic, biochemical and structural data, the signal transduction pathway mediating *E. coli* chemotaxis has been extensively characterized [2,3]. *E. coli* can sense a variety of amino acids, sugars and dipeptides, as well as pH, temperature and redox state. Major receptors, such as those for aspartate (Tar) and serine (Tsr), are highly abundant and number several thousand molecules per cell. Minor receptors, such as those that are specific for dipeptides (Tap), ribose and galactose (Trg), and redox potential (Aer), are much less abundant, with only a few hundred copies per cell [4].

As in many other signalling systems, signalling in chemotaxis relies on protein phosphorylation. The key enzyme in the pathway is a histidine kinase (CheA), the activity of which is modulated by binding of chemoeffector to receptors and by the level of receptor methylation (Figure 1). Changes in receptor methylation levels result in sensory adaptation, enabling the cell to detect further changes in concentration as it swims in chemical gradients. Because the process of receptor modification is



**Figure 1.** Signalling during chemotaxis of *Escherichia coli*. (a) Chemotaxis pathway. Changes in attractant or repellent concentrations are sensed by a protein assembly consisting of transmembrane receptors, an adaptor protein CheW, and a histidine kinase CheA. Autophosphorylation activity of CheA is inhibited by attractant binding and enhanced by repellent binding to receptors. The phosphoryl group is rapidly transferred from CheA to the response regulator CheY. Phosphorylated CheY (CheY-P) diffuses to the flagellar motors and changes the direction of motor rotation from counterclockwise to clockwise to promote tumbles. CheZ phosphatase ensures a rapid turnover of CheY-P, which is essential to quickly re-adjust bacterial behaviour. Adaptation in chemotaxis is mediated by two enzymes, methyltransferase CheR and methyl-erase CheB, which add or remove methyl groups at four specific glutamyl residues on each receptor monomer. Receptor modification increases CheA activity and decreases sensitivity to attractants. Feedback is provided by CheB phosphorylation through CheA that increases CheB activity. (b) The time course of a typical chemotactic response. Step-wise addition of saturating amount of attractant results in an initial fast (less than 0.1 s) decrease in kinase activity that is followed by a slow CheR-dependent adaptation. Adaptation time is proportional to the change in receptor occupancy. Removal of attractant upon adaptation results in an initial fast increase in kinase activity followed by CheB-dependent adaptation.

Corresponding author: Victor Sourjik (v.sourjik@zmbh.uni-heidelberg.de).

## Glossary

**Amplification:** Signal amplification (or gain) in chemotaxis is defined as the fractional change in the output, kinase activity or motor bias, with respect to the fractional change in receptor occupancy.

**Bias:** The fraction of time a cell spends in smooth swimming, or the fraction of time a single motor spends in counterclockwise rotation.

**Cooperative interactions:** Allosteric interactions between the subunits of an oligomeric protein complex, with the ligand occupancy or activity of one subunit affecting the ligand affinity or activity of the other subunits. Depending upon system parameters, cooperative interactions can yield either a highly sensitive response with little apparent cooperativity or a steep response with a large Hill coefficient.

**Dynamic range:** Range of ligand concentrations over which a system responds and adapts.

**Robustness:** The ability of a signalling system to tolerate variations in protein concentrations and reaction rates.

**Perfect adaptation:** Return to the pre-stimulus pathway activity with high precision following a change in the strength of the input stimulus.

**Sensitivity:** Sensitivity of response to ligand can be derived from a dose-response curve as  $K_{1/2}$ , where  $K_{1/2}$  is the ligand concentration at half-maximal response. A different term is parameter sensitivity, defined as the relative variation in output response with respect to the relative variation in ligand concentration. Both response sensitivity and parameter sensitivity depend on amplification and on ligand affinity.

slower than the initial response, receptor methylation provides a short-term memory about past conditions. Abundant quantitative data on the pathway (<http://info.anat.cam.ac.uk/groups/comp-cell/Rates.html>) have inspired several attempts to mathematically model chemotaxis. However, many important features of the pathway, such as an extremely high SENSITIVITY (see Glossary) to stimuli, have not been adequately explained by the theoretical models developed to date [5].

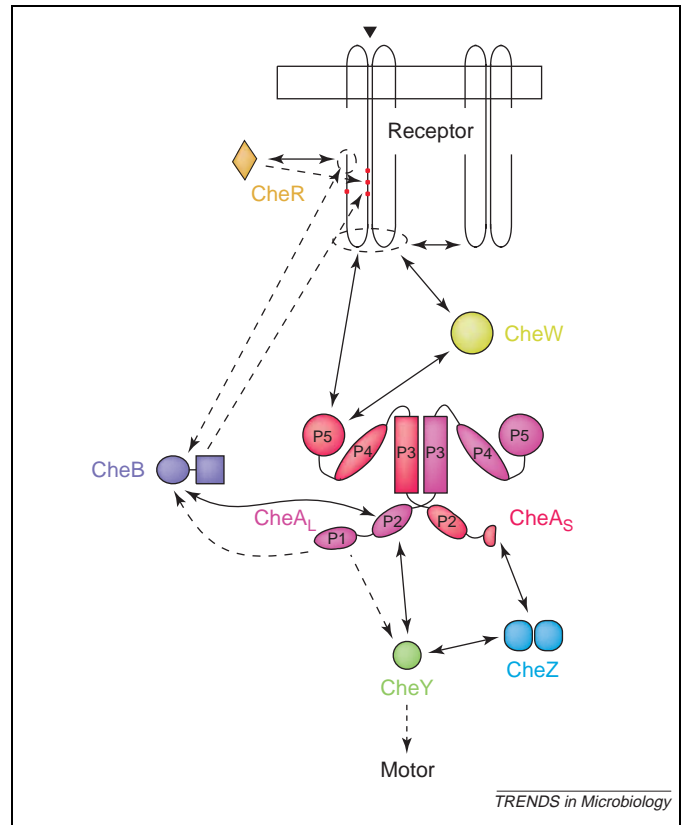
It was observed more than ten years ago that chemotaxis receptors form clusters at cell poles in *E. coli* [6] and *Caulobacter crescentus* [7]. Since then, chemo-receptor clustering has been demonstrated in all bacteria and archaea that have been examined to date [8]. Moreover, it has recently been shown that all other chemotaxis proteins in *E. coli* localize to the cluster of receptors [6,9–12], thereby forming a large sensory complex. This review focuses on recent advances in understanding the composition of the sensory complex and the role of receptor clustering in signal processing during chemotaxis.

## Architecture of receptor clusters

### Protein interactions involved in sensory complex formation

Interactions of chemotaxis proteins involved in assembly of the receptor complex have been extensively studied *in vitro* and *in vivo* (Figure 2). Receptors anchor the complex in the inner membrane and transmit signals from the periplasmic ligand-binding domain to the cytoplasmic part of the complex. The cytoplasmic portion of the receptor dimer forms a four-helix bundle, with a highly conserved signalling domain (known to bind CheW and CheA) at the very tip of the structure [13]. The same region was found to be involved in contact between receptor dimers within the crystal, resulting in the formation of a trimer of dimers.

The exact nature of the receptor–CheW–CheA complex remains controversial. Early studies of complex assembly *in vitro* have suggested that binding of CheA to receptors is mediated by CheW, which acts as an adaptor, and have



**Figure 2.** Protein interactions involved in the assembly and function of the sensory complex. Solid arrows show interactions important for receptor cluster formation and protein localization; dashed arrows show interactions that are not involved in protein localization but rather in the function of the sensory complex. Receptors, CheA and CheZ are each present in dimeric form, whereas CheW, CheY, CheR and CheB are monomeric. CheA has five domains: P1, phosphorylation domain carrying phosphorylation site; P2, CheY- and CheB-binding domain; P3, dimerization domain; P4, catalytic domain; P5, regulatory domain coupling CheA to receptor and CheW. CheA is expressed in two forms, full-length CheA (CheA<sub>L</sub>) and short CheA (CheA<sub>S</sub>). A ternary complex is formed through interaction of the signalling domain at the cytoplasmic tips of receptors with CheW and the P5 domain of CheA. The same region of the receptor is involved in dimer–dimer contacts to form trimers of dimers. CheB consists of a regulatory (CheY-like) domain and a catalytic domain. CheY and the regulatory domain of CheB both bind to the P2 domain of CheA and are phosphorylated by phosphoryl group transfer from the P1 domain of CheA<sub>L</sub>. CheZ localizes to receptor clusters by binding to the N-terminus of CheA<sub>S</sub>. CheZ binds phospho-CheY in the cytoplasm and while bound to CheA<sub>S</sub>, CheR and CheB bind to the pentapeptide sequence at the C-terminus of the major receptors, although CheB binding is not sufficiently strong to localize the full-length CheB to the clusters. Binding to the pentapeptide sequence tethers CheR, and perhaps also CheB, close to four specific glutamyl residues (red dots; two of the residues are originally incorporated as glutamines), which are methylated by CheR and demethylated or deamidated by CheB.

also demonstrated the formation of a ternary complex that has the stoichiometry of one receptor dimer to two CheW monomers to one CheA dimer [14]. However, it was subsequently shown that the receptor–CheA complex could form in the absence of CheW [15,16], and that optimal kinase activation was achieved at the ratio of six receptor dimers to eight CheW monomers to one CheA dimer. Experimental observations, further supported by similarities in the atomic structure of CheW and the P5 domain of CheA, indicated that CheA and CheW might compete for the same binding site on the receptor [16–18]. Whatever its exact structure, the presence of both CheW and CheA is required for receptor cluster formation, and CheW is necessary for binding of CheA to receptors *in vivo* [6,9].

The ternary complex appears to be stable for the duration of the chemotactic response: new CheA and CheW molecules are incorporated into preformed ternary complexes *in vitro* with a half-life of 7 and 17 minutes, respectively [14]. The stability of the ternary complex formed with intact receptors is not, or is only marginally, affected by attractant stimulation and methylation both *in vitro* [14,16,19] and *in vivo* [20], although methylation has a stronger effect on the stability of complexes assembled with soluble receptor fragments [21,22].

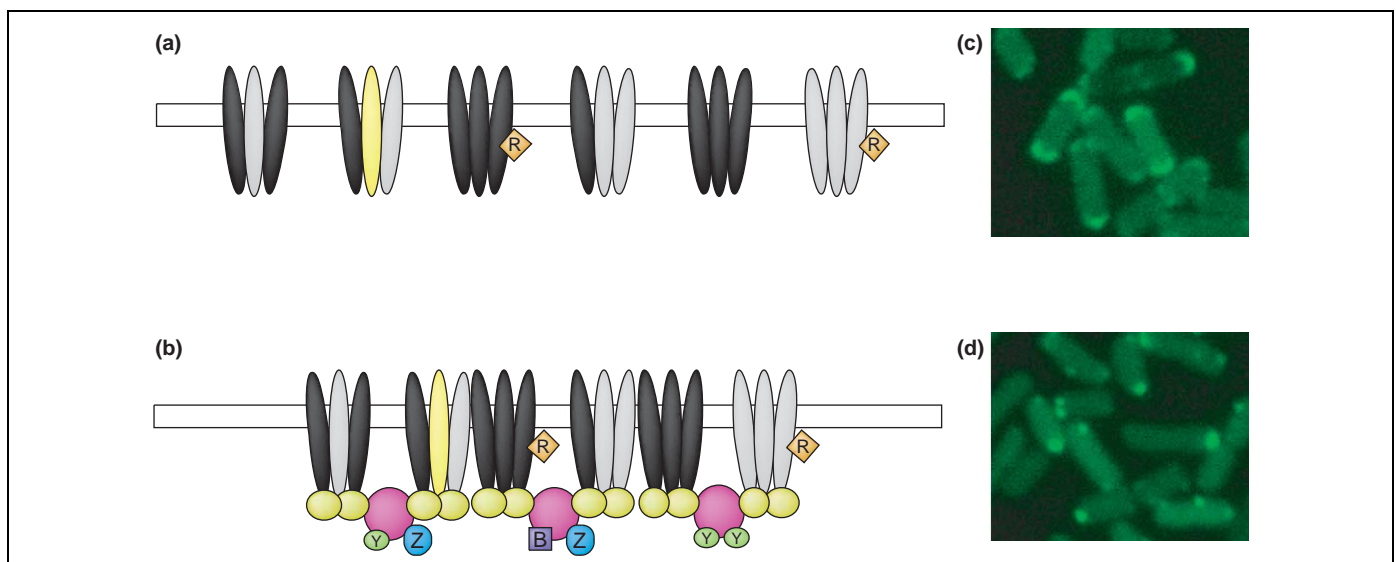
An NWETF pentapeptide sequence at the C-terminus of the major receptors, Tsr and Tar, is a docking site for at least one other protein, the methyltransferase CheR. This sequence, which is absent in minor receptors, is required for CheR localization to receptor clusters *in vivo* [10]. Localization of the other chemotaxis proteins to the cluster is mediated by CheA. CheY and CheB bind competitively to the same P2 domain of CheA [9,12,23]. Additionally, CheB binds to the same pentapeptide sequence as CheR, but with a much lower affinity that is insufficient to localize full-length CheB to the clusters [12,24]. However, some CheA-independent localization has been observed for a CheB fragment that lacks the regulatory domain [12]. CheZ binds specifically to the N-terminus of the short form of CheA (CheA<sub>S</sub>), which lacks the first 97 amino acids (including the phosphorylation site), but not to full-length CheA (CheA<sub>L</sub>) [11,25]. When bound to CheA<sub>S</sub>, CheZ retains its ability to bind phosphorylated CheY, and CheZ-dependent localization of CheY to the pole is observed *in vivo* (A. Vaknin, pers. commun.). Expression of CheA<sub>S</sub> is restricted to enteric bacteria that express CheZ [26], and recruiting CheZ to the receptor cluster appears to be the main function of CheA<sub>S</sub>. The cellular concentration of CheA<sub>S</sub> is about half of that of CheA<sub>L</sub> [4], and CheA<sub>L</sub>:CheA<sub>L</sub>, CheA<sub>S</sub>:CheA<sub>S</sub> and CheA<sub>L</sub>:CheA<sub>S</sub> dimers are thought to co-exist *in vivo* [27].

### Higher-order structure formation

Compared with the binary protein interactions discussed above, the assembly of the higher-order cluster structure is much less understood. It is now widely believed that the trimer of dimers observed in the crystal structure of the cytoplasmic part of the receptor [13] constitutes the basic unit, or 'squad' [28], of cluster structure (Figure 3). The existence of the trimer was recently demonstrated *in vivo* by cross-linking cytoplasmic [29] and periplasmic [30] parts of receptors, although there is no evidence that the trimer is the only possible state of dimer association. Trimer cross-linking in the cytoplasm is independent of CheW and CheA, ligand binding or receptor methylation [29].

Although interactions between receptor dimers are sufficient to form trimers and can drive the formation of large organized structures in membranes at high levels of receptor expression [31], the next level of cluster assembly (at natural protein levels) depends upon CheW and CheA [6,9]. As mentioned above, protein stoichiometry at this next level of structure formation is not firmly established and appears to be variable [16,32], but an assembly consisting of two trimers of receptor dimers, eight monomers of CheW, and one dimer of CheA [16] easily accommodates the trimer structure and might represent a functional unit of the receptor-kinase complex or 'signalling team' [28]. An alternative functional unit, consisting of one trimer of receptor dimers, two monomers of CheW, and one dimer of CheA, was deduced from the cellular stoichiometry of chemotaxis proteins [4].

These receptor-kinase complexes must be linked with each other to form the observed compact clusters. Given the 2.5 nm lateral size of a receptor dimer [13], 7500 dimers [4] tightly packed into a two-dimensional lattice will span ~200 nm, the size of polar receptor clusters observed in immuno-electron and fluorescence microscopy images. However, the nature of the interactions that hold a cluster together is unclear. The only available detailed



**Figure 3.** Model of the higher-order structure of a receptor cluster. (a,b) Receptor homodimers (Tsr, black; Tar, gray; Trg, yellow) are thought to form trimers that, in the absence of CheA and CheW, appear as loose caps at cell poles. CheA and CheW assemble into signalling complexes with trimers to form tight receptor clusters at the pole through a combination of receptor-receptor, receptor-CheW, receptor-CheA, CheA-CheW, and possibly CheW-CheW interactions. (c,d) In fluorescence images, receptor localization is visualized with fluorescently-tagged CheR.

molecular model of a cluster proposes that receptor trimers can be cross-linked by dimers of CheA in a receptor lattice with a fixed stoichiometry of one trimer of receptor dimers to three CheW monomers to 1.5 CheA dimers [33]. Interactions between periplasmic domains of receptor dimers [34] or strand swapping between cytoplasmic domains [35] have been proposed to play a role in the higher-order organization of the complex. CheW might be another candidate for a clustering linker: it has a potential interaction surface that is not involved in binding of receptor or CheA *in vitro* [18,36], and mutant CheW shows cooperativity in receptor binding [37]. Taken together, it is probable that a combination of interactions between receptors, CheW and CheA is required to form a stable cluster with a variable stoichiometry.

### Role of receptor clusters in signal processing

#### *Role of protein localization in signalling*

An obvious function of clustering might be to increase specificity and reaction rates in the pathway by localizing all chemotaxis proteins to one large sensory complex. Consistent with this role, chemotaxis proteins tend to bind close to their functional interaction sites (Figure 2), and two sites of functional interactions of CheB in clusters correspond to two tethering sites. Mutations that affect protein localization but not enzymatic activity can usually be compensated for by expressing higher levels of affected proteins. Deletion of the P2 domain of CheA lowers the levels of phosphorylated CheY and CheB (CheY-P and CheB-P, respectively) [38], but the cells still perform with ~25% efficiency in a standard chemotaxis assay. The defect can be further compensated for by overexpressing the P1 domain of CheA. Deletion of the C-terminal pentapeptide sequence of the major receptors disrupts CheR localization and reduces chemotactic ability but can be complemented by overexpression of CheR [39].

The effect of CheZ localization on the receptor cluster might be more subtle. Mutations abolishing CheZ localization without affecting phosphatase function or CheA activity have very mild effects on chemotaxis under standard laboratory conditions [11,40]. It has been proposed that the localization of CheZ close to the source of CheY phosphorylation might play a role in flattening the concentration gradient of phosphorylated CheY through the cell, so that flagellar motors at different distances from the receptor cluster 'see' similar CheY-P levels (A. Vaknin and H.C. Berg, unpublished) [41,42]. In addition, dephosphorylation of the response regulator CheY in some other bacteria that do not have a CheZ analogue is mediated by reverse phosphotransfer to CheA [43], which also occurs at the receptor cluster.

Phosphorylated CheY, the smallest protein in the pathway, is the only one that has to diffuse through the cytoplasm from the receptor cluster to flagellar motors. Response kinetics data suggest that CheY-P diffusion might be the rate-limiting step in the repellent response, whereas CheY dephosphorylation is the rate-limiting step in the attractant response [41].

### *Sensitivity paradox*

The chemotactic response in *E. coli* is extremely sensitive, and cells respond to the addition of as little as 10 nM aspartate [44]. This corresponds to less than 10 molecules of aspartate in a volume of an *E. coli* cell (approximately 1.4 femtoliter) [41]. An increase in attractant concentration that was estimated to change the receptor occupancy by 0.2% (i.e. of 15 out of ~7500 receptor dimers in the cell) resulted in a 23% change in the BIAS of motor rotation [45], indicating signal AMPLIFICATION (or gain) by a factor of ~100. Moreover, at least for some attractants, cells retain high sensitivity over variations of five orders of magnitude of ambient attractant concentrations. Explaining such high sensitivity combined with a wide DYNAMIC RANGE has posed a major challenge to the modelling of chemotaxis during the past decade.

Signalling in chemotaxis is widely believed to proceed through conformational changes induced in receptor dimers as a result of attractant binding. These changes must then be propagated across the membrane to the cytoplasmic tip of the receptor, which communicates with CheA [46]. Regulation of the autophosphorylation activity of CheA can be described most easily in terms of the two-state model (Box 1). The most impressive achievement of the two-state model was a theoretical analysis of adaptation that demonstrated that the known phosphorylation-mediated feedback from receptor activity (Figure 1) was insufficient to achieve the experimentally observed ROBUST PERFECT ADAPTATION. The conclusion was that an additional feedback must be provided by substrate specificity of the methylation system [47]. Despite these advances, the two-state model could not explain the high sensitivity and wide dynamic range of the chemotactic response. Neither could it explain how minor receptors that constitute only a few percent of the total receptor pool can mediate responses of the same amplitude as major receptors.

A signal amplification mechanism not accounted for by the current knowledge of the pathway must therefore exist. In principle, the signals could be amplified at any of three transduction stages (Figure 1): (i) regulation of kinase activity by attractant binding to receptors; (ii) phosphorylation and dephosphorylation of CheY in the cytoplasm; and (iii) regulation of flagellar motor bias by CheY-P. Recent advances in fluorescence microscopy have enabled quantitative measurement of amplification at the stages of stimulus detection and motor regulation. An assay based on fluorescence resonance energy transfer (FRET) was used to monitor CheA activity in living cells (Box 2). It showed an approximate 35-fold amplification of chemotactic signals by receptor-kinase complexes [48] that was independent of the ambient attractant level, meaning that a 1% increase in attractant binding to receptor results in a 35% inhibition of kinase activity. Another experiment, in which concentrations of fluorescently labelled CheY were determined in a single cell by fluorescence correlation spectroscopy (FCS), demonstrated a highly non-linear dependence of motor switching on CheY-P concentration, with a Hill coefficient of ~10 [49]. When combined, these two amplification steps, one at the beginning and one at the end of the chemotaxis

### Box 1. Two-state and allosteric models of kinase regulation in chemotaxis

The two-state model of the receptor–kinase complex [5,15,47,61] assumes that the receptor exists in two conformational states, active or inactive, which either promote or inhibit the activity of associated CheA (Figure 1a). The model further assumes that the receptor–kinase complex is stable on the timescale of the chemotactic response and that kinase associated with active receptor is always active and vice versa. Binding of attractant increases the probability that receptor is inactive, whereas methylation increases the probability that it is active. In the two-state model, a cluster consists of independent receptor–kinase complexes (Figure 1b; only receptors are shown), and changes in their activity directly reflect changes in receptor occupancy. It is thus unable to explain signal amplification.

Allosteric models of multi-subunit receptor–kinase complexes can be seen as an extension of the two-state model, including interactions between receptors. One key assumption of allosteric models is that the inactive state of a receptor homodimer has a higher affinity to attractant than the active state. Another assumption is that the inactive state can be stabilized by either attractant binding or the conformational states of neighbouring receptors. A complex of interacting receptors thus has a tendency to exhibit a switch-like behaviour. If

activity of such a complex (or its subunits) in absence of ligand is moderate, binding of attractants to only a few receptors stabilizes the entire complex in the inactive state. The sensitivity of the response therefore grows dramatically with increasing numbers of subunits [32]. By contrast, if the initial bias toward the active state is high, the complex does not make the transition to the inactive state until most subunits are occupied, producing a steep response with a large Hill coefficient.

An allosteric receptor cluster can be thought to exist either as several independent all-or-none complexes, as in the classical Monod–Wyman–Changeux (MWC) model [62] (Figure 1c), or as a single extended two-dimensional lattice of several thousand receptors in which each receptor interacts with its neighbours with a finite coupling strength [56] (Figure 1d). The number of effectively interacting receptors corresponds to the size of the complex in the former case and the extent of the conformational spread in the latter case. Allosteric behaviour of such a lattice could be modelled using either the stochastic model of the conformational spread [53,56] or a mean-field solution of the Ising model, which is commonly applied to explain ferromagnetism [52,55].

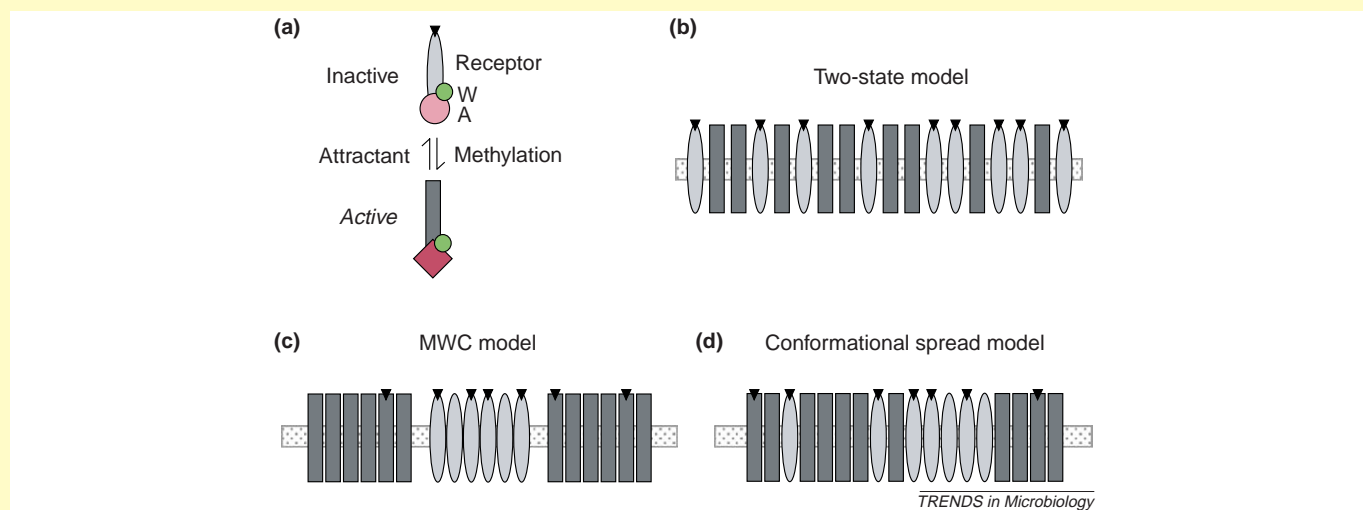


Figure 1. Models of receptor–kinase complexes in chemotaxis.

pathway, are sufficient to explain the observed gain [48]. Amplification does not appear to happen during the cytoplasmic stage of signal transduction. There is an approximately linear relationship between CheA activity and CheY-P concentration; CheZ-dependent dephosphorylation of CheY-P can be also ruled out as a signal amplification mechanism, because *cheZ* mutants were shown to retain high sensitivity [41,50].

#### Signal amplification by receptor clusters

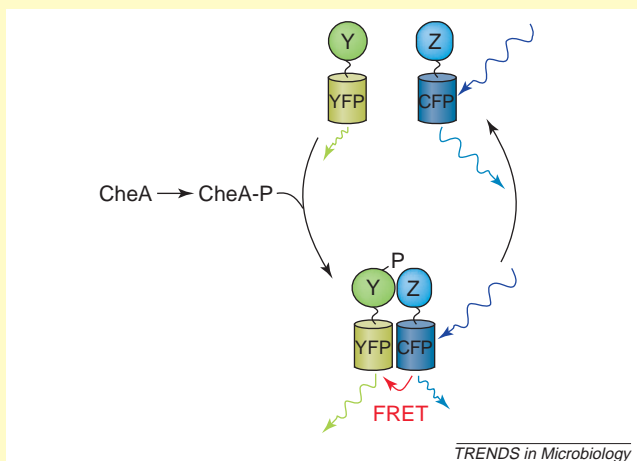
What is the nature of signal amplification in chemotaxis? One possibility is that COOPERATIVE INTERACTIONS might occur between receptors in clusters and between subunits of the motor switch complex [51–56]. Functional interactions between receptors have been demonstrated *in vitro* [57,58] and *in vivo* [27,32,59]. *In vivo* studies using a FRET-based assay showed that signals are processed by large allosteric receptor–kinase complexes [32], predicted to consist of at least 25 receptor homodimers. It appears to be in agreement with the size of receptor patches observed in electron micrographs

(R. McAndrew and M. Manson, pers. commun.). The response of cells with a homogeneous receptor population could fit the classical Monod–Wyman–Changeux (MWC) model of allosteric proteins (Box 1), assuming that a receptor cluster is composed of isolated multi-subunit complexes [32]. An alternative representation of the receptor cluster is that it comprises an extended two-dimensional lattice containing several thousand interacting receptors. The allosteric behaviour of such a lattice has been modelled stochastically [53,56].

Allosteric interactions between receptors can explain signal amplification and the integration of different stimuli. Receptor dimers of different types interact and appear to be randomly mixed in receptor complexes [27,29,32]. In a mixed allosteric receptor complex, addition of aspartate increases the sensitivity to serine, and vice versa [32]. To a first approximation, receptor dimers of all types in a mixed lattice can be considered ‘equal’, in that the output of the system depends simply upon the total number of receptors occupied by ligand. Attractant stimuli are thus integrated at the level of regulation of kinase

### Box 2. Monitoring kinase activity *in vivo* using FRET

Fluorescence resonance energy transfer (FRET) is a technique that measures the separation of two fluorescently labelled proteins (and hence their interaction) in cells. It relies on distance-dependent energy transfer from an excited donor fluorophore to an acceptor fluorophore. Because FRET-based measurements are quantitative and non-invasive, FRET is particularly useful for observing transient protein interactions involved in signal transduction. For *in-vivo* FRET, proteins of interest can be expressed as fusions to cyan fluorescent protein (CFP, donor) and yellow fluorescent protein (YFP, acceptor), and energy transfer can be measured by exciting CFP fluorescence and monitoring fluorescence intensity in two spectral channels corresponding to CFP and YFP emissions. Interactions between fusion proteins result in energy transfer from CFP to YFP, thereby quenching CFP fluorescence and inducing YFP fluorescence (Figure 1). In the chemotaxis pathway, phosphorylation-dependent interactions of the response regulator CheY fused to YFP (CheY–YFP) with its phosphatase CheZ fused to CFP (CheZ–CFP) were used to monitor the activity of the receptor–kinase complex [32,48]. At steady-state or close to steady-state, CheY is phosphorylated by CheA at the same rate at which CheY–P is dephosphorylated by CheZ. The kinase activity in the cell can therefore be inferred from the concentration of the CheZ–CheY–P enzyme–substrate complex measured by FRET. The kinase activity is limited by the rate of CheA autophosphorylation and thus reflects the number of active receptor–kinase complexes.



**Figure 1.** Fluorescence resonance energy transfer (FRET)-based assay of intracellular kinase activity in chemotaxis.

activity, and changing the occupancy of 100 aspartate receptors is equivalent to changing the occupancy of 100 ribose receptors, which is equivalent to changing the occupancy of 50 aspartate and 50 serine receptors, and so on. Such a picture greatly simplifies our understanding of signal processing in chemotaxis; high sensitivity of responses mediated by major receptors and disproportionately large amplitude of responses mediated by minor receptors can be explained by the same process of allosteric signal amplification in the receptor cluster.

#### Role of the methylation system

To enable high sensitivity over a range of attractant concentrations, the signal amplification mechanism has to be coupled to adaptation. In the absence of adaptation, a system with 100-fold signal amplification will saturate at 1% receptor occupancy and will not behave differently from a system without amplification but with a higher

### Box 3. Conservation and diversity of chemotaxis machinery among prokaryotes

All motile prokaryotes, with a notable exception of *Mycoplasma* and possible some archaea, use similar chemotaxis systems to regulate their motility; see Ref. [63] for a recent comparative analysis. The chemotaxis pathway can be roughly divided into (i) the virtually invariable core, consisting of CheW, CheA, CheY, CheR and CheB; (ii) the sensory chemoreceptors; and (iii) the 'auxiliary' proteins, involved in signal termination, that differ between species. Pathway diversity arises from the variation in the last two parts and from the duplications and fusions of the core proteins. The chemotaxis machineries of many prokaryotes are significantly more complex than the paradigm pathway of *Escherichia coli*, which has a small number of chemoreceptors, only two 'auxiliary' chemotaxis proteins, CheZ and a short form of CheA, and no protein duplications or fusions.

The greatest variation among species appears in the sensory apparatus. Based on analysis of the sequenced prokaryotic genomes, most prokaryotes appear to have a highly variable number of chemoreceptor species (up to 45 in *Vibrio cholerae*) of largely unknown ligand specificity; some receptors are cytoplasmic rather than membrane-bound [60]. Another variation comes from several 'auxiliary' chemotaxis proteins that are absent in *E. coli* but found in different combinations in other prokaryotes. One of the most complete sets of non-enteric chemotaxis proteins is present in *Bacillus subtilis*. Although most of these proteins are poorly characterized, some appear to play a role in the dephosphorylation of CheY–P, whereas others might assist adaptation.

In addition, many bacteria have multiple sets of the core chemotaxis proteins: three in *Rhodobacter spaeroides*, five in *Pseudomonas aeruginosa*, and nine in *Myxococcus xanthus*. Such multiple sets might control different types of motility [63] and/or their expression might be environmentally regulated [64]; some might be involved in the regulation of processes other than chemotaxis [65].

affinity for attractant. Consequently, the methylation system is required for adaptation and high sensitivity *in vivo* [48,50] and *in vitro* [19]. The allosteric model of signal processing by the receptor complex can provide a natural explanation for the relationship between sensitivity and adaptation. As discussed in Box 1, allosteric interactions of multiple subunits produce high sensitivity only if the bias of the complex to an active state is moderate (i.e. if the complex has a similar probability of being active or inactive in the absence of attractant). Such balanced activity of the receptor–kinase complex is assured in wild-type cells by feedback through the methylation system; adapted cells can respond by either decreasing or increasing kinase activity (Figure 1). Therefore, the role of the methylation system in signal amplification appears to be the same as in adaptation [32].

#### Perspectives

Our understanding of bacterial chemotaxis has benefited from recent advances in computer modelling together with quantitative experimental analysis of the pathway *in vitro* and *in vivo*, but much remains to be learned. Although the existence of cooperative interactions between receptors and their role in signal processing is widely accepted, the physical nature of these interactions remains obscure. Nor is it clear how receptor clusters localize to the cell poles and whether localization of signalling proteins in bacteria is a general feature or a special feature of chemotaxis. On the modelling side, allosteric models of

receptor interactions in the receptor–kinase complex, when combined with kinetic models of the cytoplasmic part of the pathway, are able to account for most observations in chemotaxis [54,55], but they still have to be ‘tuned’ to match experimental data more closely.

The chemotaxis pathway in *E. coli* appears to be relatively simple compared with most other bacteria and archaea, which have additional chemotaxis proteins and a larger number of receptor types (Box 3) that are able to form spatially disjunct clusters [60]. However, the core of the pathway is conserved, and signal processing by the clusters of chemoreceptors is expected to play a crucial role in all chemotactic prokaryotes. A quantitative understanding of chemotaxis in *E. coli* will make it possible to analyze the more complicated chemotaxis systems in other bacteria with the same rigour.

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