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Signal amplification in a lattice of coupled protein kinases†

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The bacterium *Escherichia coli* detects chemical attractants and repellents by means of a cluster of transmembrane receptors and associated molecules. Experiments have shown that this cluster amplifies the signal about 35-fold and current models attribute this amplification to cooperative interactions between neighbouring receptors. However, when applied to the mixed population of receptors of wild-type *E. coli*, these models lead to indiscriminate methylation of all receptor types rather than the selective methylation observed experimentally. In this paper, we propose that cooperative interactions occur not between receptors but in the underlying lattice of CheA molecules. In our model, each CheA molecule is stimulated by its neighbours via their flexible P1 domains and modulated by the ligand binding and methylation states of associated receptors. We test this idea with detailed, molecular-based stochastic simulations and show that it gives an accurate reproduction of signalling in this system, including ligand-specific adaptation.

Introduction

The cluster of receptors and associated proteins at the ‘front end’ of the *Escherichia coli* chemotaxis pathway is a paradigm for membrane complexes in cells. Like focal adhesions and synapses, it acts as a solid-state computational device that amplifies, integrates, and parses signals from the environment and relays the output to the rest of the cell. Unlike most other membrane complexes, however, essentially all of the proteins in the *E. coli* chemotaxis receptor cluster have been identified and we have a detailed knowledge of their structure. It therefore provides an unrivalled opportunity to understand how a sensory structure made of protein operates at the molecular level.

The chemotaxis cluster consists of a large number of receptor dimers—2000–20 000 depending on the strain and medium—closely packed together at one end of the cell. Receptors with different ligand specificities are intermixed in the cluster—Tsr for serine, Tar for aspartate, Tap for dipeptides and pyrimidines, Trg for ribose and galactose, and Aer for redox potential. The most abundant receptors, Tsr and Tar, are very similar in protein terms, having a globular domain on the outside of the cell and a long hair-like region rich in \( \alpha \)-helix in the cytoplasm, the latter folding back on itself to produce a coiled-coil structure about 25 nm long. Regions of distinct structure and function in the receptor molecule include: (i) the sensory domain located in the periplasm, with affinity for particular attractants and repellents; (ii) the transmembrane region and HAMP domain involved in conformational switching; (iii) the methylation region responsible for adaptation; and (iv) the signalling domain containing the hairpin fold of the \( \alpha \)-helix that binds to both CheA and the adapter protein CheW.

Chemotaxis signals originate in the binding of attractants and repellents to the periplasmic domain of receptors. They then propagate to the CheA layer, where they modulate the kinase activity and hence send signals downstream to the flagellar motors. The response of cells to small amounts of attractants such as aspartate is remarkably sensitive and much larger than expected from the change in receptor occupancy. Measurements of the intracellular changes in cells caused by attractants using Förster resonance energy transfer (FRET) reveal a front-end amplification of about 35-fold. This amplification was suggested to arise from cooperative interactions, either between the nearest neighbours as in an Ising lattice or in tightly coupled units operating as a single allosteric complex.

If activity is assumed to spread among contiguous receptors and methylation depends on activity—as it does in either kind of model—it follows that adaptation must also spread from one receptor to the next. In other words, if a group of contiguous receptors becomes inactive due to attractant binding, then all of the receptors in that group will become potential targets for methylation, regardless of their ligand specificity. However, experimental evidence shows clearly that this does not happen in reality. Receptor adaptation is type-specific, so that in a cell exposed to aspartate only Tar receptors increase in methylation; in a cell exposed to serine, only the serine-specific Tsr receptors become more highly methylated.

In this paper, we propose that cooperative interactions occur not between neighbouring receptors but in the underlying lattice of CheA molecules (Fig. 1). This allows signal amplification to be uncoupled from adaptation so that receptor-specific methylation can occur. We envisage neighbouring CheA molecules enhancing each other’s activity (and we suggest a possible molecular mechanism) resulting in clusters of active or inactive CheA molecules and an enhanced output signal, as in an Ising model. Such a system will respond to attractants and repellents by signals that pass from the sensory domain, via the HAMP region and methylation domain, to the

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receptors to bind ligand is calculated from the $K_d$ and starting concentration of ligand, with the assumption that the concentration equal to $K_d$ results in 50% receptor occupancy. Ligands are randomly assigned to receptors and the same number of randomly selected receptors is methylated. Following this, the activity of the CheA lattice is equilibrated as described below.

Once initialization is complete, the simulation runs as a series of iterations, each corresponding to approximately 0.002 seconds of biological time. Each iteration includes three steps: (i) receptor–ligand binding and dissociation, (ii) receptor adaptation, and (iii) a round of CheA activity equilibration. The receptor–ligand binding routine consists of iterating through the collection of receptors to determine the current ligand binding state. This is calculated using simple mass action kinetics based on the following receptor $K_d$ values: serine binding to Tsr = 10 µM; aspartate binding to Tar = 1 µM. An unoccupied receptor will bind its cognate ligand L with the probability $p = \frac{[L]}{[L] + K_d}$, while an occupied receptor will dissociate its ligand with the probability $1 - p$. For simplicity, we ignore cross binding of serine–Tar and aspartate–Tsr as the $K_d$s for these interactions are several orders of magnitude higher than the ones used here. Changes in ligand concentration as the simulation is running are taken into account in the subsequent iterations.

Receptor adaptation involves both methylation and demethylation. First, an unmethylated, liganded receptor (L1M0) is randomly selected. The probability of it becoming methylated (to L1M1) by CheR is calculated from a rate of 0.15 methylation events per iteration, equivalent to 0.03 s$^{-1}$ for each receptor trimer. Next, a methylated receptor is selected and the probability of its demethylation calculated. The latter depends on the total activity of the cluster, since CheB is subject to phosphorylation by CheA and becomes some 60 times more active in its phosphorylated form. We represent this activation by multiplying the basal rate of 0.3 demethylation events per iteration by the fraction of CheA molecules currently active.

We calculated the collective activity in the lattice of CheA molecules using a standard Metropolis Monte Carlo algorithm as in a previous study. Each CheA dimer can adopt either an active or an inactive conformational state and flips from one to the other in a thermally driven manner. The probability of a given CheA dimer being in the active or in the inactive state depended on the difference in energy, $\Delta E$, of the two states. For simplicity, we assume that an isolated, “virgin” CheA dimer unattached to any receptors has a $\Delta E$ of zero so that it is equally likely to assume either conformation.

The free energy of the CheA dimer is unaffected by receptors that are either unmodified L0M0 or both liganded and methylated L1M1. However, each liganded receptor trimer L1M0 raises the energy barrier of the active state of its cognate CheA dimer by 1.6 kT. That is, ligand binding, on average, decreases the likelihood that the associated CheA will be active. Conversely, each methylated receptor trimer L0M1 lowers the energy barrier by 1.6 kT thereby making the CheA dimer more likely to be active.

The energy barrier is also affected by the state of any neighbouring CheA dimers. We assume that coupling between

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**Fig. 1** Schematic of proposed mechanism. (A) Chemotaxis receptors in the bacterial membrane bind attractants such as aspartate and serine in the extracellular medium. The resulting conformational change (green) passes to the underlying layer of CheA and associated molecules where a phosphorylation signal is generated (red). (B) Conventional models ascribe the amplification to cooperative interactions between adjacent receptors, so that a single ligand-binding event creates conformational change in many receptors. (C) In the model proposed here, signals are amplified by cooperative interactions between the underlying CheA molecules. This has the important distinction that each ligand molecule changes just one receptor, so that adaptation is restricted to only those receptors that are occupied.

**Description of computer model**

Each trimer of receptor dimers in our model is represented as a single unit with one binding site for a chemoattractant ligand. This assumption (also adopted by previous models) avoids the complications that arise from potential cross talk within a trimer. For simplicity, we also assume that each trimer has a single site of methylation, so that it can exist in one of four states—L0M0, L1M0, L0M1, or L1M1, where “L” indicates an attractant ligand which can be either unbound (0) or bound (1) and “M” indicates the state of adaptation of the receptor, which can be either not methylated (0) or methylated (1).

At initialization, we assume that ligand is present in low concentration ($0.05 \times K_d$) as a baseline. The number of
CheA dimers is caused by a short-range interaction that depends on only the conformational state. Since each CheA dimer can bind to two receptor complexes, which in turn can bind to two additional CheA dimers (see Fig. 2), a dimer can have up to four neighbours. Energy is lowered by neighbours in the same state and raised by ones in the opposite state, as in an Ising model. For each neighbour that has the same conformation, the energy of a CheA is reduced by \( E_J \); for each neighbour in the alternative conformation, the energy is increased by \( E_J \). The value \( E_J = 1.6 \kappa T \) was chosen to be close to the critical coupling parameter of the two-dimensional Ising model.10

### Results

In our usual configuration, 2562 receptor trimers are arranged on a hexagonal lattice with unit spacing between trimers of 7 nm.21,22 CheA dimers are attached to the signalling domains of the trimers, linking opposite apices of each hexagon of receptor trimers (Fig. 2). This scheme provides the best fit to the known size and shape of the CheA dimer, and allows interaction with sites in clefts on the flank of trimers rather than at the hairpin tip.23 The maximum receptor to CheA ratio with this arrangement is 6 : 1 (counting monomers), which is twice that of the cellular stoichiometry of about 3 : 1 obtained experimentally by Li and Hazelbauer.1 Note however that other published estimates show better agreement: about 6 : 1.24,25

We also tested a second scheme in which CheA molecules were assigned between each pair of trimers, thereby generating a relatively regular lattice and a lower stoichiometry of 2 : 1 (monomers of receptor compared to monomers of CheA). The disadvantage of this scheme is that it appears to require some distortion of the CheA molecule. The same tests, run with this second model, gave closely similar results (not shown).

The initial state of the lattice is shown in Fig. 2A. The receptors—in this case exclusively Tar—are depicted as circular discs on a regular hexagonal lattice, either grey (non-methylated) or purple (methylated). CheA dimers are shown as rectangular bars associated with the cytoplasmic face of the receptors and arranged so as to bridge receptors at opposite vertices. CheA molecules in an active state—that is, actively generating phosphoryl groups—are red whereas those in an inactive state are pale pink. The total number of active (red) CheA dimers thus represents the phosphorylation signal sent into the cell. Because of cooperative interactions between neighbouring CheA dimers, active molecules are not distributed across the cluster completely at random but loosely associated in small groups. Note also that the toroidal boundaries employed in the simulation mean that active groups also wrap around boundaries—between right and left, and between top and bottom.

### Effects of single signals

Fig. 2B shows the effect of adding 1 \( \mu \)M aspartate (equal to the \( K_d \) of Tar receptors). At the instant of time shown (within 1 s of addition), aspartate molecules (green dots) occupy about 50% of Tar receptors causing them to change conformation. The altered receptor state in turn blocks transmission of activity between adjacent CheA molecules in the underlying lattice so that the number of active CheA dimers falls dramatically. The phosphorylation signal sent from the receptor cluster into the cell is thereby reduced.

The state of the receptor cluster following adaptation—about 200 s following addition of aspartate—is shown in Fig. 2C. Although aspartate molecules still occupy about 50% of receptors, a significant proportion of the latter have now become methylated (purple instead of grey). Consequent upon this modification, the ability of receptors to transmit activity between adjoining CheA molecules has been reinstated, so that clusters of active CheA molecules are again seen.

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**Fig. 2** Screen shots of simulated receptor cluster. The receptors—in this case exclusively Tar—are depicted as circular discs on a regular hexagonal lattice, either grey (non-methylated) or purple (methylated). CheA dimers are shown as rectangular bars associated with the cytoplasmic face of the receptors and arranged so as to bridge receptors at opposite vertices. CheA dimers in an active state—that is, actively generating phosphoryl groups—are red whereas those in an inactive state are pale pink. The total number of active (red) CheA dimers thus represents the phosphorylation signal sent into the cell. (A) Lattice before exposure to ligand. (B) Same lattice immediately following exposure to 1 \( \mu \)M aspartate. (C) The same lattice after allowing time for adaptation to reach equilibrium (about 200 s).
The response of the model system to a 0.25 s pulse of aspartate is shown in Fig. 3A. A rapid reduction in the number of active CheA molecules occurs, corresponding to a rapid decrease in the level of phosphorylation signal (and hence suppression of the tumble signal). Termination of the stimulus corresponds with a transient overshoot in the output signals—a characteristic feature seen under experimental conditions. Note that the size of the overshoot was here increased by making the CheR catalytic rate 10 times larger; although present normally, the overshoot is usually hidden in the noise. The relation between the instantaneous drop in CheA activity and the magnitude of the applied stimulus is plotted in Fig. 3B. This shows the level of CheA activity in a cluster following exposure to different concentrations of aspartate without adaptation (that is, with the methylation mechanism inactivated). It may be seen that concentrations as low as 0.01 times the $K_d$, that is 10 nM aspartate, produce a measurable drop in activity. Our computer model therefore shows a similar amplification, or gain, to the experimentally measured $E. coli$ response.

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The response of the system to step increases in attractant, in this case with adaptation, is shown in Fig. 4. A typical time course, seen in Fig. 4A, entails a sudden drop in CheA activity with the onset of the stimulus followed by a slower return to the original level. Larger pulses cause both a deeper fall in initial activity as well as a slower return to baseline. The response to a series of aspartate increments is shown in Fig. 4B, with the average baseline activity before stimulus shown as a broken line. In Fig. 4C, the consequences of the same incremental series of aspartate addition are shown for receptor occupancy (green line) and level of receptor methylation (blue line).

**Effects of mixed signals**

The simulation based on a single type of receptor just described was modified by the addition of a second set of receptors. Serine-binding Tsr molecules were mixed at random locations of the cluster to give a final ratio of 2 : 1 Tsr to Tar, similar to the ratio observed in wild-type $E. coli$. Pulse stimuli consisting of aspartate alone, or serine alone, then generated signals similar to those described above, except that the size of the signal depended on the number of receptors. Thus, if attractants were separately added at the equivalent of 1 $K_d$ (1 μM for aspartate; 10 μM for serine) to a lattice containing 2 : 1 Tsr : Tar then the response to serine was roughly twice that to aspartate. The same was true if the two attractants were added one after the other in sequence, provided sufficient time was allowed for adaptation (Fig. 5A).
When we analyzed the methylation patterns, we found these to be selective for the type of receptor. Thus, when aspartate was added to the mixed lattice, only Tar receptors became occupied and only Tar receptors became methylated (apart from a low level of background methylation that was present before the stimulus—Fig. 5B). The reciprocal finding was true for serine and Tsr, whether the serine was added by itself or after aspartate (Fig. 5C).

Fig. 6 displays the numbers of receptors of each type that become methylated under different conditions. Once again the level of methylation is receptor-specific.

Discussion

In this paper, we propose a new model for the generation of chemotactic signals by *E. coli* bacteria. Our model concerns the first stage in the detection pathway in which receptors

![Fig. 5](image1.png)

**Fig. 5** Response to mixed attractants. A lattice consisting of 2562 receptor trimers in which the ratio of Tsr : Tar is about 2 : 1 was exposed in sequence to 1 μM aspartate followed by 10 μM serine. (A) Each ligand had a characteristic effect on CheA activity independently of the other ligand. (B) Tar receptors became methylated in response to binding of aspartate, and (C) Tsr receptors became methylated in response to binding of serine. Similar effects were seen if serine preceded aspartate (not shown).

![Fig. 6](image2.png)

**Fig. 6** Methylation in a mixed receptor lattice. Bar charts show numbers of (top) Tar and (bottom) Tsr receptors in either unmethylated (grey) or methylated (purple) state. Apart from a background level of methylation that is common to the two receptors, each shows selective methylation that increases with concentration of its cognate ligand.

bind to extracellular diffusing ligands and transmit a conformational change across the membrane to an underlying layer of signalling proteins. The principal new feature we have added concerns the mechanism of amplification. Previous theories all proposed that the “front-end” amplification of approximately 35-fold was due to conformational interactions between neighbouring receptors (see above). By contrast, in the present study, we attribute amplification to interactions in the underlying lattice of CheA kinase molecules instead. The receptors are still involved, inasmuch as they physically associate with CheA molecules and modulate their kinase activity. However, in our new model, each receptor operates individually so that it is influenced solely by its own ligand occupancy. Adaptation, which arises through the methylation machinery recognizing receptor conformations, is therefore type-specific: if the bacterium is exposed to aspartate then only the aspartate receptor Tar will become more highly methylated, and so on. In this respect the model fits more closely to experimental findings.

Although the existence of “front-end” amplification in the *E. coli* chemotaxis pathway is widely accepted, its mechanism is not understood. The prevailing view that it arises through coupling between neighbouring receptors was originally offered on an *ad hoc* basis as the simplest possibility. This assumption was adopted without question in all subsequent theoretical analyses, but without direct experimental evidence. Although numerous studies have shown that cell-free membrane preparations exhibit receptor amplification of CheA activity and inhibition by added attractants (see for example Levit *et al.*28) these investigations do not identify which of the proteins present is responsible for the amplification. Perhaps the most relevant study is that in which purified receptors were embedded in small discs of lipid.29 The authors found that sets of three receptor dimers were more effective at
stimulating CheA kinase—reflecting the known association of dimers to trimers. However no evidence was obtained for cooperativity between neighbouring trimers, so the question whether amplification occurs at the level of receptors or, as suggested here, CheA molecules remains open.

How might CheA molecules be coupled within the lattice? CheA is a large complicated protein that occurs as a dimer, each monomer consisting of five domains, termed P1–P5. Domains P3–P5 make up the core of the molecule, including the regions responsible for dimerization and for ATP binding. Domains P1 and P2 are attached to this core through unstructured lengths of polypeptide which in _E. coli_ consist of 30 (P1 to P2) and 33 (P2 to core) amino acids, respectively. The catalytic cycle of CheA is believed to operate by a two-stroke mechanism in which only one of the monomers is active at any one time. ATP binds to a site on one monomer and the gamma phosphoryl residue then transfers to a histidine residue on the other monomer, and eventually to CheY (or CheB). The phosphoryl transfer just mentioned is made possible by flexible linkers: the P1 domain of one half of the molecule diffuses to the ATP binding site of the opposite half and then captures a phosphoryl group on its crucial histidine residue. The length of the flexible tether (including two lengths of unstructured polypeptide and the intervening P2 domain) is about 22 nm if fully extended. If we consider the dimensions of the CheA dimer and the postulated lattice of receptors then it is clear that the P1 domain of a CheA subunit could interact not only within its own molecule but also with nearby CheA molecules. The kinase activity would thereby be transmitted across the lattice, travelling between the nearest neighbours as in an Ising model.

It is interesting to note that cooperative action is a general feature of many kinds of ATPases. Thus, the subunits of FoF1-ATPase exist as three heterodimers that bind to and hydrolyze ATP in sequence. Subunits of the L Tag helicase function in concert, all simultaneously binding to ATP before hydrolyzing them. The homomeric ring ATPase of the bacteriophage ϕ29 sequentially binds ATP to its subunits during the loading phase and then sequentially hydrolyzes ATP as it translocates the DNA substrate. In these and many other examples, the activities of multiple individual enzymatic units are coordinated so as to refine overall activity. Given the central importance of the CheA kinase in the chemotaxis signal cascade it would not be surprising if it had not also been refined by allosteric interactions.

In our proposed model, the cooperative activity of neighbouring CheA molecules in the receptor cluster is due to their flexible tethers. Other steps in the _E. coli_ chemotactic pathway also employ flexible tethers, such as dephosphorylation of CheYp and receptor methylation. In the latter case, methylation and demethylation are accomplished by enzymes (CheR and CheB, respectively) attached to an unstructured sequence of amino acids at the C terminus of the receptor, which thereby increase the local concentration of reacting species and the catalytic reaction manifold. They create local regions of adaptation, so-called ‘assistance neighbourhoods’. It may be that integration of the activity of different parts of a large multiprotein complex is a widespread function of unstructured polypeptides.

**References**