Changing Cellular Location of CheZ Predicted by Molecular Simulations

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In the chemotaxis pathway of the bacterium Escherichia coli, signals are carried from a cluster of receptors to the flagellar motors by the diffusion of the protein CheY-phosphate (CheYp) through the cytoplasm. A second protein, CheZ, which promotes dephosphorylation of CheYp, partially colocalizes with receptors in the plasma membrane. CheZ is normally dimeric in solution but has been suggested to associate into highly active oligomers in the presence of CheYp. A model is presented here and supported by Brownian dynamics simulations, which accounts for these and other experimental data: A minority component of the receptor cluster (dimers of CheA_short) nucleates CheZ oligomerization and CheZ molecules move from the cytoplasm to a bound state at the receptor cluster depending on the current level of cellular stimulation. The corresponding simulations suggest that dynamic CheZ localization will sharpen cellular responses to chemoeffectors, increase the range of detectable ligand concentrations, and make adaptation more precise and robust. The localization and activation of CheZ constitute a negative feedback loop that provides a second tier of adaptation to the system. Subtle adjustments of this kind are likely to be found in many other signaling pathways.

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Introduction

Experimental results of the past several years reveal that the bacterial cytoplasm is more complex and sophisticated than previously thought. To cite a recent review on the prokaryotic cell cycle: “Many signal transduction proteins are dynamically localized to specific subcellular addresses ... and proper localization is essential for their function” [1]. The well-studied bacterial chemotaxis pathway is now known to depend on two kinds of large multiprotein complexes: inputs are detected by a cluster of receptors and associated proteins at one end of the cell, while flagellar motors elsewhere in the cell generate the system’s output [2,3]. A small protein, CheY, achieves communication between these two complexes by diffusing freely through the cytoplasm. This protein receives its phosphate from the histidine kinase CheA, associated with the inner face of the receptor cluster, at a rate that depends on chemotactic stimulation. From there, phosphorylated CheY (CheYp) diffuses to the four-or-so motors, where it causes a change in rotational switching frequency (i.e., duration of swimming or tumbling behavior) according to its local concentration. The signal is initiated and terminated through the level of the kinase activity. It adapts to constant stimulus levels and returns to its steady-state value through changes in receptor methylation by the enzymes CheR and CheB. The signal is also stopped directly through dephosphorylation of CheYp, which is promoted by the protein CheZ.

The present report adds to this picture by proposing that CheZ is a second molecule of the pathway, which changes its location during the signal transduction process. According to this model, the relocation coincides with changes in dephosphorylation activity and leads to a second tier of adaptation, by regulating the termination of the signal. Reminiscent of the migration of proteins of the Min system that ensures the correct positioning of the bacterial cell division plane [4], the changing location of CheZ should serve to sharpen responses of the cell to attractants and repellents and make adaptation more precise. The presented proposal is based on published data, supported by quantitative computer simulations, and makes specific predictions that can be tested by experiment.

Results

Hypothesis and Biological Background

A translational variant of the CheA kinase, CheA_short, is known to be required for polar localization of CheZ [5]. A crucial element of our model is that homodimers of CheA_short nucleate CheZ oligomers. We predict that CheZ molecules move from freely diffusing in the cytoplasm to the receptor cluster according to the current level of stimulation of the cell, with repellents favoring the bound form and attractants favoring the soluble, cytoplasmic form (Figure 1). The balance between these two states is proposed to depend on the current rate of formation of CheYp at the receptor cluster. Because nucleation is entirely dependent on dimers of CheA_short, in this model oligomers will form only on the receptor cluster and not in the cytoplasm.

Three known features of the pathway form a basis for our hypothesis. (1) CheA_short (A_S) is a truncated variant of CheA

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Abbreviations: A, CheA; A_S, CheA_short; CheYp (Yp), phosphorylated CheY; Z, CheZ dimer

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generated by translation from an in-frame start site of the cheA locus [6]. AS can form dimers either with itself or with CheAshort (AL) [7]. Both forms have the same dimerization domain, so it seems reasonable to assume that they form dimers with equal probability. Since AS lacks the histidine phosphorylation site, homodimers are enzymatically inactive. (2) In solution, AS stimulates the activity of CheZ, the enzyme that promotes dephosphorylation of CheYp [8,9]. (3) CheZ exists in solution as a dimer (Z2) [10,11] but has been proposed to self-associate into an oligomer containing approximately ten molecules of CheZ (Z10) in the presence of CheYp [10]. The oligomeric form has elevated activity and dephosphorylates CheYp an order of magnitude faster than Z2 [12]; this means that CheYp production leads to the very change that causes its breakdown by hydrolysis. Indirect support for the presence and importance of this feedback loop was recently provided by a combined experimental/theoretical study, which showed that CheYp-mediated activation of CheZ increases the robustness of the pathway and thus chemotactic efficiency and shows better agreement with experimentally measured noise levels [13]. Our model also provides a basis for methylation-independent adaptation, as discussed later.

Biochemical and cytological assays have shown that CheZ binds selectively to AS and at best weakly to AL [5,8,14]. We propose that this interaction is specific for the homodimer made of two molecules of CheAshort (ASAS) and that the heterodimer ASAL does not bind, or binds only weakly, to CheZ. We arrived at this conclusion from the published biochemical data showing that immunoprecipitation with antibodies to CheZ yields only AS. If heterodimers were bound, this experiment should yield both AS and AL dimers [8]. The significance of our proposal lies in the stoichiometry of the chemotaxis proteins. Based on recent estimates of the numbers of proteins in the chemotaxis pathway, we calculate that a typical Escherichia coli cell contains about 1,500 ASAS, 1,500 ASAL, and 360 ASAS dimers (numbers based on strain RP437 in rich medium and an assumed equal binding) [15]. Of these, the first two (ASAS and ASAL) have catalytic activity and are able to generate phosphoryl groups [7,16–18]. The third species, ASAL, comprising approximately 10% of the total, will be inactive and thus unable to participate directly in the generation of signals. According to our hypothesis, however, these 360 inactive molecules of ASAL could act as nuclei to attach up to 360 molecules of Z2 to the receptor cluster. We propose that a proportion of the cellular total of 1,600 CheZ dimers [15] will be recruited to the receptor cluster as highly active oligomers (Figure 1).

Structurally, the oligomerization could be achieved if CheYp molecules bind to the catalytic domain of one CheZ dimer and the C-terminal binding domain of another, connected by the unstructured tether which links both CheZ domains [11]. Each CheZ dimer can be attached to four CheYp monomers, and each CheYp to two CheZ dimers (Figure 2). We envisage a network of CheZ molecules on the inner face of the cluster with the remaining CheZ molecules diffusing freely as relatively inactive dimers.

What will be the distribution of CheZ molecules at any instant of time, and how will this be affected by the

Figure 1. Schematic of the Dynamic CheZ Hypothesis

(A) A layer of CheA dimers is positioned at the cytoplasmic face of the polar chemoreceptor cluster. Interspersed with the catalytically active CheA dimers are CheAshort homodimers, which act as anchoring points for CheZ dimers. In the absence of CheYp, a condition produced by saturating concentration of attractants, the remaining CheZ dimers diffuse freely in the cytoplasm. (B) Upon increased phosphorylation of CheY, which occurs after exposure to repellent, CheZ dimers bind CheYp and oligomerize by assembly at the CheAshort-CheZ nuclei. These clustered oligomers have a greatly increased CheYp dephosphorylation activity, providing negative feedback to the system.

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chemotactic signals entering the cell? To address these questions, we employed a recently developed computer program, Smoldyn, which allows the movement and interaction of a large number of individual molecules in a structured environment to be simulated [19]. In a recent study, we used Smoldyn to construct a three-dimensional model of an E. coli cell and examined the diffusion of CheYp from the cluster of receptors to the flagellar motors, under control conditions and in response to attractant and repellent stimuli. The high spatial resolution available to us with the Smoldyn program allowed us to calculate the locus of individual CheYp molecules in a cell and the distribution of their lifetimes under different cellular conditions [20]. In this way, we have already found that the position of CheZ can affect chemotaxis. When this protein is distributed throughout the cytoplasm, it generates a shallow gradient of CheYp concentration that is highest next to the receptor cluster, as has also been observed in parallel FRET experiments and analytical studies [20–22]. When CheZ molecules are positioned at the receptor cluster, they change the lifetime profile and reduce the cytoplasmic gradient of CheYp, ensuring equal occupancy of flagellar motors throughout the length of the cell [20].

Model Specifications

Smoldyn was created to stochastically simulate chemical and biochemical reaction networks in a spatially detailed environment [19]. This is achieved by modeling each individual molecule and its exact position in a series of short time intervals. Diffusing molecules assume a new, random direction at every time step, similar to Brownian motion. They will react when finding themselves in close proximity to a reaction partner or, for unimolecular reactions, at a certain probability. Firmly based on physical chemistry, the diffusive distances, reaction radii, and probabilities are calculated from the user-defined rate constants and the time-step length (see also Materials and Methods).

Here, we have used the Smoldyn program to explore possible changes to the location and state of oligomerization of CheZ within an E. coli cell. To do this, we generated a model of a bacterium with an array of AL and AS dimers at one pole, flagellar motors on the lateral sides, and diffusible molecules within the cell volume (Figure 3A). We set up a series of binding and catalytic equations (Table 1); these are based on known interactions between CheZ, CheYp, and CheAS but include many binding and rate constants that are not presently known (see also Discussion). In these reactions, CheZ dimers in solution (Z2) bind to CheYp (Yp) to form the complex Z2Yp, which we consider the building block from which oligomers are built. Units of Z2Yp (or free Z2) then associate with ASAS dimers and thereby nucleate assembly at the receptor cluster. In our model, additional Z2Yp units add in a linear fashion up to a maximum of five, with the largest complex consequently having the composition ASAS(Z2Yp)5. Note that this mechanism ensures that Yp promotes oligomer formation, as shown experimentally (most convincingly through protein crosslinking [23]). The stoichiometry of the complexes, with one Yp per Z2 dimer and up to five Z2 dimers per oligomer (and per ASAS), is consistent with published data [8,10,15]. Although we envisage a network in which almost all CheZ molecules could be linked together (Figure 2B), at saturation there will be on average five CheZ dimers

Figure 2. Proposed Structure of the CheZ,Yp-Oligomeric Clusters

(A) In the (CheY–BeF$_3$–Mg$^{2+}$)$_2$CheZ$_2$ co-crystal structure (PDB entry 1KM1, [11]), CheZ (green and gray) exists as a stable dimer. On each side of its four-helix bundle is an active site with loose affinity for a CheYp monomer (orange). The main binding affinity for CheYp is in a short C-terminal helix, which is connected to the main body of CheZ by a flexible peptide tether (dashed lines). Instead of bending back on itself, the unstructured domain, which is invisible to the crystallographer, could connect a CheYp molecule bound to the C-terminus of one CheZ dimer to the catalytic site of a neighboring one. This allows for the formation of extended oligomers. Anchorage to the polar cluster could occur via the CheZ-apical helices to CheAS homodimers (salmon-colored ovals), as suggested by mutagenesis [5].

(B) In oligomeric networks, each CheZ dimer can be connected to a maximum of four neighboring CheZ dimers, via flexible tethers and CheYp. A looser network will exist if not all CheYp binding sites are occupied. View from below, as compared to (A). Created with MacPyMOL (DeLano Scientific LLC, San Carlos, California, United States). DOI: 10.1371/journal.pcbi.0020039.g002
per CheA dimer in the cluster. One $A_5$-anchored $Z_2Y_p$ molecule can be directly attached to four other $Z_2Y_p$ molecules—a $Z_2Y_p$ pentamer is therefore likely to be quite stable.

In the absence of more concrete information, we tested a variety of reaction schemes. We found, for example, that the inclusion of reactions in which oligomers simultaneously hydrolyze $Y_p$ and dissociate did not make any substantial difference (unpublished data). For simplicity, we therefore assumed that the hydrolysis of $CheY_p$ promoted by $CheZ$ is separable from the oligomerization. This can occur if only one $CheY_p$ monomer per $CheZ$ dimer is sufficient to stabilize an oligomer, as in Figure 2. In this case, vacant sites on a $Z_2Y_p$ unit will fill and empty in an iterated cycle without causing disruption of the oligomer. Note that hydrolysis of $CheY_p$ that is separable from oligomerization is required for the presence of negative feedback. This way, unlimited numbers of $CheY_p$ can be hydrolyzed by each clustered (and highly active) $CheZ_2$. If, on the other hand, each hydrolysis resulted in the break up of oligomers, only one $Y_p$ would be hydrolyzed per clustered $CheZ$ dimer, which would be no advance over doing it in solution.

Rates of hydrolysis increase in the oligomers in accordance with published observations [23] and are proportional to the number of free active sites (Table 1). We chose rate constants so as to generate the experimentally estimated level of $CheY_p$.
in unstimulated cells [24,25] and to fit the activity profile of *A. chei* cells [26]. (These mutants serve to distinguish the effects of CheZ oligomerization from other adaptive mechanisms, see below.) Recent FRAP measurements of the CheZ diffusion coefficient are consistent with a low molecular weight species in the cytoplasm (M. A. DePristo, L. Chang, K. Lipkow, R. D. Vale, and S. Khan, unpublished data). Consequently, in the simulations presented here, the formation of CheZ oligomers takes place exclusively at the receptor cluster, unless stated otherwise. Simulations in which oligomerization occurs independently of CheA α in the cytoplasm or not at all were done as controls.

**Dynamics of the Model**

Responses of our simulated bacterial cell to repeated addition and removal of attractant are shown in Figure 4A–4C. Changes in stimulus produce corresponding changes in the level of activation of CheA and hence changes in the level of CheYp, as seen in experiments and reproduced in previous computer models [20,27–29]. The traces show considerable noise due to the relatively small numbers of molecules under examination (there are 8,200 CheY molecules per cell, including both phosphorylated and unphosphorylated species) [15]. Because of the spatial detail included in the Smoldyn simulations, both the formation and most of the hydrolysis of CheYp are localized to the immediate vicinity of the receptor cluster. In response to stimulation by repellent (or removal of attractant) the concentration of CheYp rises, initially in the cluster. In response to stimulation by repellent (or removal of attractant) the concentration of CheYp rises, initially in the cluster. In response to stimulation by repellent (or removal of attractant) the concentration of CheYp rises, initially in the cluster.

### Table 1. Reactions

| Number | Description | Equation | Rate (Forward) | Rate (Reverse) | Reference |
|--------|-------------|----------|----------------|---------------|-----------|
| 1      | Kinase activity with explicit autophosphorylation | $A_3 \rightarrow A_3^+$ | Immediate equilibration |  |  |
|        | $A_{3p} \rightarrow A_{3}^+$ | Immediate equilibration |  |  |  |
|        | $A_{3}^+ \rightarrow A_{3p}$ | $34 \text{ s}^{-1}$ | — |  | [53,54] |
|        | $Y = A_{3}^+ \rightarrow Yp + A_3^+$ | $1.0 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ | — |  | [55] |
|        | $Y = A_{3p} \rightarrow Yp + A_3$ | $1.0 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ | — |  | [55] |
| 2      | Kinase activity with implicit autophosphorylation | $A_3 \rightarrow A_{3p}$ | Immediate equilibration |  |  |
| 3      | Y autophosphorylation and motor binding | $Y = Yp$ | $7.7 \times 10^{-3} \text{ s}^{-1}$ | $0.085 \text{ s}^{-1}$ | [56,57] |
| 4      | Clustered CheZ: Yp hydrolysis | $Yp + Z_2 \rightarrow Y + Z_2$ | $1.6 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ | — | [15,57] |
| 5      | Free CheZ: Yp hydrolysis | $Yp + Z_2 \rightarrow Y + Z_2$ | $1.6 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ | — | [15,57] |
| 6      | Oligomerizing cytoplasmic CheZ: oligomer formation | $Yp + Z_2 \rightarrow Z_2 Yp$ | $2.5 \times 10^{-10} \text{ M}^{-1} \text{ s}^{-1}$ | $0.5 \text{ s}^{-1}$ | [10,59] |
| 7      | Oligomerizing cytoplasmic CheZ: Yp hydrolysis | $Yp + Z_2 Yp \rightarrow Y + Z_2 Yp$ | $2.4 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ | — | [8,59] |
| 8      | Dynamic CheZ: oligomer formation | $Yp + Z_2 \rightarrow Z_2 Yp$ | $2.5 \times 10^{-10} \text{ M}^{-1} \text{ s}^{-1}$ | $0.5 \text{ s}^{-1}$ | [10,59] |
| 9      | Dynamic CheZ: Yp hydrolysis | $Yp + Z_2 Yp \rightarrow Y + Z_2 Yp$ | $2.4 \times 10^{-10} \text{ M}^{-1} \text{ s}^{-1}$ | — | [8,59] |

Only a subset of reactions was used for each simulation run; see figure legends. Numbers and descriptions also refer to subsequent lines without text. Reactions labeled with “Immediate equilibration” are the system’s input: every 10 ms throughout the simulation, the ratio of the two indicated molecular species was adjusted stochastically. Boldface symbols in column 3, such as $A_3(Z_2 Yp)$, are used to indicate protein complexes that are attached to the membrane and therefore nondiffusing. For others, the following diffusion rates were employed: $Y$, $Yp$, $10 \text{ μm}^2 \text{ s}^{-1}$; $Z_2$, $5.4 \text{ μm}^2 \text{ s}^{-1}$; $Z_2 Yp$, $4.8 \text{ μm}^2 \text{ s}^{-1}$; $Z_2 Yp p$, $3.4 \text{ μm}^2 \text{ s}^{-1}$; $Z_2 Yp p p$, $2.8 \text{ μm}^2 \text{ s}^{-1}$; $Z_2 Yp p p p$, $2.4 \text{ μm}^2 \text{ s}^{-1}$; $Z_2 Yp p p p p$, $2.1 \text{ μm}^2 \text{ s}^{-1}$ (extrapolated from molecular weights: $D = M^{1/2} / 62$). In columns 4 and 5, boldface numbers indicate values that were directly taken from (black) or derived from (blue) experimental measurements (references given). Y, CheY; Yp, CheYp; A2, CheA dimer, inactive; A2*, CheA dimer, active; A2p, phospho-CheA dimer, inactive; A2p*, phospho-CheA dimer, active; As2, CheAshort dimer; Z2, CheZ dimer; Z2Yp, complex of CheZ and CheYp; M, FIM (motor subunit).

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opposite sense: now the rate of CheYp production falls and there is a net release of CheZ which moves from an oligomeric state bound to the receptors to a freely diffusing state in the cytoplasm (Figure 4A–4C). With the parameters used here, the shifts in location are only partial, with approximately one third of the bound CheZ being released by a strong attractant stimulus.

Temporal changes in CheYp level in response to stimulation were examined in greater detail in a series of simulations employing the maximum possible change in CheA activation (corresponding to a receptor occupancy change from 100% to 0% and back to 100%) (Figure 5). The traces were averaged over 25 simulation runs in order to reduce noise. Here it is clear that the rapid rise and subsequent fall in CheYp level correlate with the formation of oligomeric CheZ clusters (Figure 5D). Note that these slower changes in the level of CheY phosphorylation constitute an adaptation of the signal that is independent of receptor methylation, since both the methylating enzyme CheR and the demethylating enzyme CheB are not present in these simulations. This feedback of free CheYp concentration in the cell is observed whether oligomers are formed in the cytoplasm or at the receptor cluster (Figure 5C and 5D). For comparison, traces for the traditional scheme, with fixed CheZ position and constant CheZ activity, are presented (Figure 5A and 5B). Here the CheYp profile adopts the shape expected of a saturation curve.

It is interesting to note that the overshoot in CheYp concentration seen in Figures 4B and 5D corresponds closely to in vivo data published 20 years ago [26,30] (Figure 4D). In these studies, cheR cheB mutant bacteria exhibited a partial adaptation of flagellar rotation within 1 to 2 min of chemoeffectors addition or removal, explaining findings that these mutants retain some chemotactic capability [31–33]. Our model of dynamic CheZ relocalization and activity can completely account for these experimental results. Both methylation-defective bacteria and our simulations are unable to compensate for a complete shutdown of kinase activity but adapt perfectly to smaller attractant stimuli (not shown).

**Implications for Signaling Properties**

Because our simulations follow all of the CheYp molecules in the cell, we are able to monitor the changes in binding of CheYp to the flagellar motors. Detailed changes in the occupancy of motors at two different locations in the cell—one near the polar cluster (0.2 μm) and the other at the opposite end of the cell—are shown in Figure 5E–5L. These records indicate that the dynamic trafficking of CheZ produces an improved temporal response: a rise in the production of CheYp is delayed most effectively to flagellar motors if the CheZ is localized to the receptor cluster, since otherwise the phosphatase in the cytoplasm attenuates the level of the production of CheYp before it can diffuse to distant motors (Figure 5E and 5F). Conversely, a sudden fall in CheYp is best relayed to the flagellar motors if CheZ is diffusing freely in the cytoplasm since this ensures a rapid fall in local CheYp concentration (Figure 5I and 5J). In the scheme with a dynamically assigned CheZ, the occupancy-level changes give the best of both worlds (Figure 5H and 5L), i.e., it allows the cell to react to both repellent and attractant stimuli with maximum speed. This scheme also prevents the formation of intracellular CheY gradients, which result in differences in the occupancy and bias of anterior and posterior motors when CheZ is restricted to the cytoplasm—either dimers or oligomers (Figure 5F–5H and 5J–5L).

Analysis of the dose-response of our simulated cell revealed another consequence of CheZ redistribution. The performance of a cell in which CheZ was dynamically relocated in the manner described above was compared to a cell with either all fixed or all diffusing CheZ molecules (Figure 6). In all
schemes, the level of CheYp rises initially with rising CheA activity—due, for example, to increased exposure to repellent (Figure 6A–6D). In cells with entirely polar or entirely cytoplasmic CheZ (with or without oligomerization), the CheYp level quickly saturates (Figure 6A–6C), but with dynamic CheZ localization, the level of CheYp continues to rise throughout the entire activity range (Figure 6D). This feature should allow a cell to distinguish repellent levels even at high concentrations. For decreasing activity or increasing attractant concentrations, all schemes perform equally well (Figure 6E–6H).

Finally, our system is relatively robust to exact rate constants. For example, with a 100-fold increase of the oligomerization and deoligomerization constants (last four lines of Table 1, reactions 8), the CheYp levels adapt after only 2 s, but motor occupancy and dose-response curves retain the advantages described above (not shown). This also leaves room for incorporation of new experimental data, such as a higher proportion of A₅A₅ homodimers [14].

**Discussion**

An early hint that CheZ might redistribute between the cytoplasm and the membrane was obtained almost three decades ago. In 1977, Ridgway and colleagues reported that this newly described protein was present in both the cytoplasmic and the membrane fractions of disrupted *E. coli* [34]. Direct visual evidence, however, came only in 2000, when Sourjik and Berg found that green fluorescent protein–labeled CheZ colocalizes with the polar cluster of receptors [35], an association shown to depend on the presence of CheAshort [5]. A common feature of these and all subsequent analyses is that considerable cell-to-cell heterogeneity exists in the amounts of CheZ located at the cell poles [36].
**Figure 6. Dose-Response Curves**
CheA activity (orange) was increased in ten equal steps from steady-state to maximum level to mimic an increasing repellent concentration (A–D) or decreased to mimic an increase in attractant (E–H). Orange, ratio of active CheA; red, free CheYp; black, oligomeric CheZ.

(A, E) CheZ all dimeric and fixed at the cluster. Reactions 1, 3, 4 (Table 1).

(B, F) CheZ all dimeric and cytoplasmic. Reactions 1, 3, 5 (Table 1).

(C, G) Cytoplasmic CheZ oligomerization. Reactions 1, 3, 6, 7 (Table 1).

(D, H) Dynamic CheZ clustering. Reactions 1, 3, 8, 9 (Table 1).

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The presented model predicts that the amount of CheZ associated with receptor clusters shifts in response to external stimulation. Detection of this movement will not be trivial, as the predicted changes are small, short-lived, and dependent on the formation of complex oligomeric structures, which might be disrupted by labeled fusion proteins. At this point, rate constants for association/dissociation of and hydrolysis by the different oligomeric forms of $\Delta$CheZ are not known, and may be difficult to determine, as the dynamic nature of the proposal implies that cells and in vitro reactions will always contain a mixture of oligomers. Without these numbers, precise quantitative predictions cannot be made with any certainty—this, however, was not the aim of this study. Although a lot of care has been taken to incorporate and match known data, the goal was a proof of principle—to show how a novel loop and spatial reorganization in the well-studied network of bacterial chemotaxis can function and benefit the cell.

If changes in localization are small, what significance can they have for cellular function? The most striking consequence will be to sharpen the chemotactic response, which is demonstrated in Figure 5. Sudden exposure to attractant will initiate a rapid fall in CheYp (due to the activity of the accumulated CheZ dimers) and cause dispersal of CheZ into the cytoplasm. The departure of CheZ will limit the extent of hydrolysis of CheYp at the receptor cluster while at the same time it will enhance CheYp capture in the cytoplasm. This could enable CheZ to scavenge CheYp from regions close to the flagellar motors, thereby ensuring that these respond in a timely fashion to the external stimulus. Note that CheYp only has to be bound by CheZ, and not necessarily hydrolyzed, to be unavailable to the motors. In reverse fashion, if the cell encounters a repellent, this will engender a rapid rise in CheYp concentration, closely followed by a movement of CheZ to the membrane. The rise in CheYp in the vicinity of the cluster will thereby be limited in duration while, at the same time, it will be accentuated at the motors.

Another consequence of the changes in localization and oligomeric state is that they will provide an additional layer of adaptation. The ability to adapt to attractants on a relatively slow time scale (slower than the initial phosphorylation of CheY) is a crucial element in chemotaxis, since it allows the organism to detect chemical gradients over a wide range of concentrations. *E. coli*, for instance, can detect aspartate at concentrations below 10 nM but continues to move up gradients that reach almost 1 mM [37,38]. This remarkable capacity is possible only because the system returns to its initial position after each increment of attractant. The principal mechanism for adaptation is the well-characterized methylation of receptors, which acts as a counterbalance for the inhibitory effects of the attractant [39]. However, evidence from studies of bacterial mutants lacking the methylation enzymes shows that an additional level of adaptation exists that is independent of methylation [26]. It has been suggested previously that this second tier of adaptation could be due to CheZ oligomerization [12,23]. Almogy et al. [40] showed analytically that a delayed response of CheZ to changes in CheYp would ensure a more rapid and precise return to initial conditions and hence amplify the range over which chemotaxis could work. In contrast to their work, our model proposes that CheZ is the mobile element that moves between cytoplasm and membrane, and not CheAS. Our model does not require that CheAS’s affinity to the cluster is dependent on receptor activity, although it does not rule out that this could further enhance and refine CheZ-based adaptation. However, whereas active receptors in their model directly promote the release of cluster-bound molecules, in ours they indirectly promote attachment to the polar clusters. It thereby localizes the maximum dephosphorylation activity to the cluster and not the cytoplasm, which is consistent with recent FRET data [21]. Moreover, the application of a whole-cell simulation, in which the spatial location of each molecule is considered, takes the analysis to a new level of confidence. A previously unmentioned function of this second tier of adaptation is as a back-up system in conditions in which the methylation system is impaired due to toxins, mutation, or stochastic fluctuations in the low-copy enzymes CheR and CheB [15,41–43].

The described changes and advantages are quite small, but benefits do not need to be large to be selectable in evolution, especially when there is no additional cost: our model uses exactly the same components and amount of energy as the traditional scheme. Considering the astronomical numbers of...
generations in the lineage of present-day bacteria and their
dominant interactions could be easily made. They could improve
given in [19]. Briefly, Smoldyn rewritten as a stochastic master equation:
continuous space but no volume, shape, or inertia. They diffuse in
appears that both of these components do exist to some degree
molecules of CheA—are stable and unchanging. In fact it
has an altered function (enhanced dephosphorylation). This
is the probability that a specific B molecule is within a volume
CheAlong to CheAshort. It was shown that during growth of a
is the probability that a specific B molecule is within a volume
from the anterior end (Figure 3). 156 CheAlong dimers, the nucleation
parameters that can vary is the ratio of
molecule numbers (see below), it took approximately 8 to 24 h to
selves are point objects and have no dimensions. At each time step, all
potential reactions and reaction probabilities. The molecules them-
may be zero if it is membrane-associated) and a color and size for the
others (those that are freely diffusing) are initially assigned random
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Changing Cellular Location of CheZ
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The author has declared that no competing
Materials and Methods
Simoldyn. Smoldyn source code, executable program, manuals, and
detailed documentation are downloadable from http://sahara.lbl.gov/
sandrell/software.html (Steven Andrews) and http://www.pdn.cam.
aq/groups/comp-cellSmoldyn.html (Dennis Bray’s group). A de-
tailed report of the theory and assumptions underlying Smoldyn is
given in [19]. Briefly, Smoldyn employs the Smoluchowski level of
detail, i.e., molecules have an identity and an exact position in
continuous space but no volume, shape, or inertia. They diffuse in
random directions by distances calculated from Fick’s second law
rewritten as a stochastic master equation: $p_B(x,t) = D_B \nabla^2 p_B(x,t)$, with
product $p_B(x,t)$, spatial probability density of a single B molecule at position $r$
and time $t$; $D_B$, diffusion coefficient for a B molecule. The product
is the probability that a specific B molecule is within a volume
about position $r$ at time $t$. Solving the above equation shows that
the probability density for the displacement of a molecule after a
time step has a Gaussian profile on each Cartesian coordinate. These
results form the basis of the simulation method called Brownian
dynamics in which diffusion is simulated by picking a normally
distributed random displacement for each molecule at each step.
Since space is continuous, not compartmentalized, the level of detail
can be adjusted by a suitable choice of step time $dt$.
To run a Smoldyn simulation, the user writes a configuration file.
The coordinates of the simulation volume are specified, and identified molecules are placed at specific positions within the framework of this
cell box. Some molecules are anchored just inside the walls, whereas
others (that are freely diffusing) are initially assigned random
locations. Each molecular species has a diffusion coefficient (which may be zero if it is membrane-associated) and a color and size for the
Graphical animation. The configuration files include a list of
potential reactions and reaction probabilities. The molecules them-
sew objects and have no dimensions. At each time step, all
mobile molecules undergo a diffusive step in a random direction.
Diffusive distances are calculated from Fick’s law, converted into
potential reaction events. At the first simulation step molecules are
moved to their new positions. Any molecule that crosses the boundary
of the cell box is reflected back in like a billiard ball. Unimolecular
reactions now occur with a probability calculated from the specified
rate constant. Bimolecular reactions are decided by the proximity of
two potential reactants: two stable molecules that come within a
Other radii are calculated to give the correct reaction rates following diffusive encounter. The user
can specify intermittent changes, such as instantaneous reactions or the
probabilistic conversion of one molecular species to another, and
record the state of the system as required.

Simulations. Simulations were performed on an Apple Power Mac
G5 (2 CPUs, 2 GHz, 3.5 GB RAM), an AMD Athlon 2000+ cluster (26
CPUs, 1.67 GHz, 1 GB RAM each), and on an AMD Athlon MP cluster
(22 CPUs, 1.5 GHz, 1 GB RAM each), all running smoldyn version 1.56.
Times of 0.1 ms were used throughout, after finding that the
simulation outcome at this level was the same as with slightly
larger and much smaller time steps (“rule-of-thumb-test”)—steps of
this length are not expected to confound any significant inaccuracies
[19]. Simulations were performed at the maximum level and
small boxes of 150 nm side length. With this setup and molecule numbers (see below), it took approximately 8 to 24 h to simulate 1 min.

The simulation systems were rectangular cells of 2 μm length and 0.84 μm thickness, with a cluster of 1,250 CheA kinase dimers 20 μm
from the anterior end (Figure 3). 156 CheAlong dimers, the nucleation
points for CheZ oligomers, were 40 nm from the end. These lower
numbers compared to those in the text reflect the finding that, on
average, less than 50% of total CheA localizes to the pole [44]. Two motors, each a ring of 34 FIM molecules, were included in each
analysis: motor #1 situated 0.2 μm and motor #2 situated 1.8 μm from
the anterior end. 8,200 CheY monomers were randomly placed and
diffuse in the cytoplasm. 1,600 CheZ dimers were either randomly
diffusing (Table 1, reactions 5, 6, and 8) or placed in a lattice 40 nm
from the anterior end (Table 1, reactions 4). Reactions from Table 1,
as specified in the figure legends, were included. See [29] for further
details of the simulation procedure.

Conversion of experimental data. For Figure 4D, the measured
values of counterclockwise bias (% CCW) were read in from [26].
Figure 10 reaction of tethered cells that continues to rotate CCW
during the indicated 15-s intervals). Values were transformed to
numbers of CheYp molecules with
$Y_p = \beta_{CCWbias}/(1 - \beta_{CCWbias})$ [46] (rearranged from the Hill equation in [50]), where $Y_p$ is the number of
CheYp molecules in the cell at each timepoint; $\beta_{CCWbias}$, number of CheYp molecules in an unstimulated cell = 1.640 = 0.2 + 8.20C CheYp
clockwise bias = 1 — (100-CCW); $H$, Hill coefficient, degree of cooperativity between CheYp concentration and motor bias =
$5.0$ [51, 52] or $10.3$ [25].

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1. Ryan KR, Shapiro L (2003) Temporal and spatial regulation in prokaryotic cell cycle progression and development. Annu Rev Biochem 72: 367–394.

2. Wadhams GH, Armitage JP (2004) Making sense of it all: bacterial chemotaxis. Nat Rev Mol Cell Biol 5: 1024–1037.

3. Sourjik V (2004) Receptor clustering and signal processing in Escherichia coli. Trends Microbiol 12: 569–576.

4. Hale CA, Meinhardt H, de Boer PA (2001) Dynamic localization cycle of the cell division regulator MinC in Escherichia coli. EMBO J 20: 1563–1572.

5. Cantwell BJ, Draheim RR, Weart RB, Nguyen C, Stewart RC, et al. (2003) CheZ phosphatase localizes to chemoreceptor vesicles via CheA-dependent shortening of the CheA protein. J Bacteriol 185: 2554–2561.

6. Smith RA, Parkinson JS (1980) Overlapping genes at the cheA locus of Escherichia coli. Proc Natl Acad Sci U S A 77: 5370–5374.

7. Wolfe AJ, Stewart RC (1993) The short form of the CheA protein restores kinase activity and chemotactic ability to kinase-deficient mutants. Proc Natl Acad Sci U S A 90: 1518–1522.

8. Wang H, Matsumura P (1996) Characterization of the CheAS/CheZ complex: A specific interaction resulting in enhanced dephosphorylating activity on CheY. J Bacteriol 179: 693–703.

9. Hess JF, Oosawa K, Kaplan N, Simon MI (1988) Phosphorylation of three proteins in the signaling pathway of bacterial chemotaxis. Cell 55: 79–87.

10. Blat Y, Eisenbach M (1996) Oligomerization of the phosphatase CheZ upon interaction with the phosphorylated form of CheY. The signal protein of bacterial chemotaxis. J Biol Chem 271: 1226–1231.

11. Zhao R, Collins EJ, Bourret RB, Silversmith RE (2002) Structure and catalytic mechanism of the E. coli chemotaxis phosphatase CheZ. Nat Struct Biol 9: 579–583.

12. Blat Y, Gillespie B, Bren A, Dahlquist FW, Eisenbach M (1996) Regulation of phosphorylase activity in bacterial chemotaxis. J Mol Biol 284: 1191–1199.

13. Kollmann M, Bartholome K, Laidov L, Timmer J, Sourjik V (2005) Design principles of a bacterial signaling network. Nature 438: 504–507.

14. Li M, Hazelbauer GL (2004) Cellular stoichiometry of the components of the chemotaxis signaling complex. J Bacteriol 186: 3607–3619.

15. Liberman L, Berg HC, Sourjik V (2004) Effect of chemoreceptor modification on assembly and activity of the receptor-kinase complex in Escherichia coli. J Bacteriol 186: 6643–6646.

16. Mesibov R, Ordal GW, Adler J (1975) The range of attractant concentrations for bacterial chemotaxis and the threshold and size of response over this range. Weber law and related phenomena. J Gen Physiol 62: 203–226.

17. Bray D (2002) Bacterial chemotaxis and the question of gain. Proc Natl Acad Sci U S A 99: 7–9.

18. Springer MS, Goy MF, Adler J (1979) Protein modification in behavioural control mechanisms and in signal transduction. Nature 280: 279–284.

19. Almogy G, Stone L, Ben-Tal N (2001) Multi-stage regulation, a key to reliable adaptive biochemical pathways. Biophys J 81: 3016–3028.

20. Spudich JL, Koshland DE Jr (1976) Non-genetic individuality: Chance in the single cell. Nature 262: 467–471.

21. Levi MD (2003) Noise in gene expression as the source of non-genetic individuality in the chemotactic response of Escherichia coli. FEBS Lett 556: 135–138.

22. Sokolova E, Emonet T, Vilar JM, Shimizu TS, Cluzel P (2004) From molecular noise to behavioural variability in a single bacterium. Nature 428: 574–578.

23. Maddock JR, Shapiro L (1993) Polar location of the chemoreceptor complex in the Escherichia coli cell. Science 259: 1717–1723.

24. Bunn MW, Ordal GW (2003) Transmembrane organization of the Bacillus subtilis chemoreceptor McpB deduced by cysteine disulfide crosslinking. J Mol Biol 331: 941–949.

25. Bunn MW, Ordal GW (2004) Receptor conformations enhance methyltransferase activity during chemotaxis by Bacillus subtilis. Mol Microbiol 51: 721–728.

26. Homma M, Shimoi D, Kawagishi I (2004) Attractant binding alters arrangement of chemoreceptor dimers within its cluster at a cell pole. Proc Natl Acad Sci U S A 101: 3462–3467.

27. Amann RA, Ordal GW, Kiessling LL (2005) Large increases in attractant concentration disrupt the polar localization of bacterial chemoreceptors. Mol Microbiol 57: 774–785.

28. Wang H, Matsumura P (1997) Phosphorylating and dephosphorylating protein complexes in bacterial chemotaxis. J Bacteriol 179: 287–289.

29. Bray D, Bourret RB (1995) Computer analysis of the binding reactions leading to a transmembrane receptor-linked multiprotein complex involved in bacterial chemotaxis. Mol Biol Cell 6: 1367–1380.

30. Kuo SC, Koshland DE Jr. (1989) Multiple kinetic states for the flagellar motor switch. J Bacteriol 171: 6279–6287.

31. Scharf BE, Fahrner KA, Turner L, Berg HC (1998) Control of direction of flagellar rotation in bacterial chemotaxis. Proc Natl Acad Sci U S A 95: 201–206.

32. Francis NR, Levit MN, Shaik TR, Melancon LA, Stock JB, et al. (2002) Subunit organization in a soluble complex of tar, CheW, and CheA by electron microscopy. J Biol Chem 277: 36753–36759.

33. Shroot AL, Montefusco DJ, Weiss RM (2003) Template-directed assembly of receptor signaling complexes. Biochemistry 42: 13379–13385.

34. Stewart RC, Jahnres K, Parkinson JS (2000) Rapid phosphotransfer to CheY from a CheA protein lacking the CheY-binding domain. J Bacteriol 182: 11157–11165.

35. Schuster M, Silversmith RE, Bourret RB (2001) Conformational coupling in the chemotaxis response regulator CheY. Proc Natl Acad Sci U S A 98: 6003–6008.

36. Sourjik V, Berg HC (2002) Binding of the Escherichia coli response regulator CheY to its target measured in vivo by fluorescence resonance energy transfer. Proc Natl Acad Sci U S A 99: 12669–12674.

37. Sagi Y, Khan S, Eisenbach M (2003) Binding of the chemotaxis response regulator CheY to the isolated, intact switch complex of the bacterial flagellar motor: lack of cooperativity. J Biol Chem 278: 25867–25871.

38. Silversmith RE, Smith JG, Guanga GP, Les JT, Bourret RB (2001) Alteration of a nonconserved active site residue in the chemotaxis response regulator CheY affects phosphorylation and interaction with CheA. J Biol Chem 276: 18478–18484.

39. Elowitz MB, Suvirette MG, Wolf P-E, Stock JB, Leibler S (1999) Protein mobility in the cytoplasm of Escherichia coli. J Bacteriol 181: 197–203.

40. Segall JE, Ishihara A, Berg HL (1988) Chemotactic signaling in filamentous cells of Escherichia coli. J Bacteriol 161: 51–59.

41. Nelson P (2004) Biological physics: Energy, information, life. New York: W. Freeman. 598 pp.