A model of excitation and adaptation in bacterial chemotaxis

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ABSTRACT  Bacterial chemotaxis is widely studied because of its accessibility and because it incorporates processes that are important in a number of sensory systems: signal transduction, excitation, adaptation, and a change in behavior, all in response to stimuli. Quantitative data on the change in behavior are available for this system, and the major biochemical steps in the signal transduction-processing pathway have been identified. We have incorporated recent biochemical data into a mathematical model that can reproduce many of the major features of the intracellular response, including the change in the level of chemotactic proteins to step and ramp stimuli such as those used in experimental protocols. The interaction of the chemotactic proteins with the motor is not modeled, but we can estimate the degree of cooperativity needed to produce the observed gain under the assumption that the chemotactic proteins interact directly with the motor proteins.

Chemotaxis (or more accurately, chemokinesis), the process by which a cell alters its speed or frequency of turning in response to an extracellular chemical signal, has been most thoroughly studied in the peritrichous bacterium Escherichia coli. E. coli exhibits sophisticated responses to many beneficial or harmful chemicals. In isotropic environments the cell swims about in a random walk produced by alternating episodes of counterclockwise (CCW) and clockwise (CW) flagellar rotation. CCW rotation pushes the cell forward in a fairly straight “run,” CW rotation triggers a random “tumble” that reorients the cell. The durations of both runs and tumbles are exponentially distributed, with means of 1.0 s and 0.1 s, respectively (1). In a chemoeffectector gradient, the cell carries out chemotactic migration by extending runs that happen to carry it in favorable directions. Using specific chemoreceptors to monitor its chemical environment, E. coli perceives spatial gradients as temporal changes in attractant or repellent concentration. The cell in effect compares its environment during the past second with the previous 3–4 s and responds accordingly. Attractant increases and repellent decreases transiently raise the probability of CCW rotation, or “bias,” and then a sensory adaptation process returns the bias to baseline, enabling the cell to detect and respond to further concentration changes. The response to a small step change in chemoeffector concentration in a spatially uniform environment occurs over a 2–4 s time span (2). Saturating changes in chemoeffector concentration can increase the response time to several minutes (3).

Many bacterial chemoreceptors belong to a family of transmembrane methyl-accepting chemotaxis proteins (MCPs) (reviewed in ref. 4). Among the best-studied MCPs is Tar, the E. coli receptor for the attractant aspartate. Tar has a periplasmic binding domain and a cytoplasmic signaling domain that communicates with the flagellar motors via a phosphorelay sequence involving the CheA, CheY, and CheZ proteins (see Fig. 1). CheA, a histidine kinase, first autophosphorylates and then transfers its phosphoryl group to CheY.

Phospho-CheY interacts with switch proteins in the flagellar motors to augment CW rotation, CCW being the default state in the absence of Phospho-CheY. CheZ assists in dissipating CW signals by enhancing the dephosphorylation of CheY. Tar controls the flux of phosphate through this circuit by forming a stable ternary complex (Tar/CheW/CheA) that modulates CheA autophosphorylation in response to changes in ligand occupancy or methylase state. CheA phosphorolytes more slowly when the receptor is occupied than when it is not.

Changes in MCP methylation state are responsible for sensory adaptation. Tar has four residues that are reversibly methylated by a methyltransferase, CheR, and demethylated by a methylesterase, CheB. CheR activity is unregulated, whereas CheB, like CheY, is activated by phosphorylation via CheA. Thus, receptor methylation level is regulated by feedback signals from the signaling complex, which can probably shift between two conformational states having different rates of CheA autophosphorylation. Attractant binding and demethylation shift the equilibrium toward a low CheA activity state; attractant release and methylation shift the equilibrium toward a high CheA activity state. A receptor complex that is bound to attractant but not highly methylated can be thought of as a “sequestered” state (5) which is unable to autophosphorolyte at a significant rate.

E. coli can sense and adapt to ligand concentrations that range over five orders of magnitude (5). In addition, the machinery for detection and transduction is exquisitely sensitive to chemical stimuli. The cell can respond to exponential increases (“ramps”) in attractant levels that correspond to rates of change in fractional occupancy of only 0.1% per second. Consequently, the cell can respond even if only a small fraction of its receptors have changed occupancy state during a typical sampling period.

These experimental observations raise several questions. First, how does the cell achieve the extreme sensitivity that is observed? Second, why are there multiple methylation states of the receptor? In this paper we describe a mathematical model based on known kinetic properties of Tar and the phosphorelay signaling components that accounts for this exquisite sensitivity. In the model, excitation results from the reduction in the autophosphorolyte rate of CheA when Tar is bound to a ligand, and adaptation arises from methylation of the receptor. The disparity in the time scales of these processes produces a “derivative-” or “temporal-sensing” mechanism with respect to the ligand concentration. The model makes essential use of the multiple methylation states to achieve adaptation, and can be used to derive quantitative relations between certain key rates and the behavioral responses to experimental protocols, and to predict the cooperativity needed to achieve the observed gain.

A Qualitative Description of the Response to Different Stimuli

We assume that Tar is the only receptor type, that the Tar–CheA–CheW complex does not dissociate, and that Tar, CheA, and CheW are found only in this complex. We also assume that methylation of the multiple sites occurs in a specified order (6, 7).

Abbreviations: CCW, counterclockwise; CW, clockwise; MCP, methyl-accepting chemotaxis protein.

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First consider the response of the network to a step increase in attractant. The ligand-binding reactions are the fastest, so the first component of the response is a shift in the distribution of states toward the ligand-bound states (i.e., from states at the top to those at the bottom in Fig. 2). This increases the fraction of receptors in the sequestered states (top to those at the bottom in Fig. 2). This increases the fraction in attractant. The ligand-binding reactions are the fastest, so this constitutes the excitation response of the system.

Next methylation and demethylation, which are the slowest reactions, begin to exert an effect. CheR methylates ligand-bound receptors more rapidly than unbound receptors, and the decrease in CheAp that results from excitation causes a decrease in the level of CheBp, thereby reducing the rate of demethylation. As a result, the distribution shifts toward higher methylation states. However, autophosphorylation of CheA is faster, the higher the methylation state of the Tar–CheA–CheW complex, and therefore in the last phase of the response there is a shift toward the states containing CheAp via transitions along the right face of the network. Consequently, the net effect of an increase in attractant is to shift the distribution of receptor states toward those which are ligand-bound and more highly methylated, but the total level of receptor complex containing CheAp (the sum of the states at the rear face of the network) returns to baseline. As a result, the total phosphotransfer rate from CheAp species to CheY returns to the prestimulus level, which means that CheYp returns to its prestimulus level. Under the assumption that only CheYp and CheY interact with the motor complex, this in turn implies that the bias returns to its prestimulus level. Thus the cell can respond to an increase in ligand by a transient decrease in tumbling, but it adapts to a constant background level of ligand and retains sensitivity to further changes in the ligand concentration.

Of course this qualitative description must be supplemented by numerical results which demonstrate that the model can also reproduce quantitatively correct results using experimentally based rate coefficients. This is done in the following section.

**Fig. 2.** The ligand-binding, phosphorylation, and methylation reactions of the Tar–CheA–CheW complex, denoted by $T$, LT indicates a ligand-bound complex. Vertical transitions involve ligand binding and release, horizontal transitions involve methylation and demethylation, and front-to-rear transitions involve phosphorylation and the reverse involve dephosphorylation. The details of the phosphotransfer steps are depicted in Fig. 3. Numerical subscripts indicate the number of methylated sites on Tar, and a subscript p indicates that CheA is phosphorylated. The rates for the labeled reaction pairs are given in Table 3.
Methylation (first order to the rate of change beneath the corresponding rate expression. A large range of ligand concentrations. The rate constants of the full system by trial and error so that it adapts at the bias returns precisely to baseline in the face of any constant parameters which guarantee perfect adaptation, by which we mean that the motor interacts only with CheYp and possibly CheY, and in view of the small number of motor complexes in a cell, we further assume that the motor does not affect the budgets of these species. It then follows from the conservation of Y that perfect adaptation of the bias only requires perfect adaptation of CheYp.

The responses obtained from this three-methylation-state model are shown in Fig. 4A–C and the concentrations and rates used are listed and compared with measured values in Tables 2 and 3. The system exhibits both a significant response to a slow ramp and an appropriate adaptation time for a small step. It also exhibits the correct adaptation time to a saturating step in ligand concentration.

Analysis of the Gain

In the preceding figures we have chosen the step size so that the maximum deviations of the CheYp concentration from baseline are equal in Fig. 4A and B, and thus the maximum change in bias in response to the corresponding ramp (0.015 s⁻¹) and step (11% change in receptor occupancy) will be the same. Experimentally it is found that the maximum change in bias in response to this ramp is about 0.3 (figure 4A in ref. 16), and so our model, coupled with a scheme for the interaction of CheYp with the motor, would produce a maximum gain of about 0.3/0.11 = 2.73 in response to the step used in Fig. 4B. This is consistent with the finding (17) that the maximum gain is about 6, though not with the much higher gain of 55 reported (18). The model thus demonstrates that ramp experiment results are consistent with the lower estimates regarding the gain for small steps. However the source of the gain remains undetermined, because even though the deviations of CheYp concentration from baseline are significant (∼9%), they are small compared with the reported change (0.3) in bias for this ramp stimulus (16). Thus one or more mechanisms to amplify the internal CheYp signal must be involved, and the major possibilities are cooperative effects in the interaction of CheY species with the motor and modulation of cheZ activity.

To explore these possibilities, we define the gain as

$$g = -\frac{db}{d \ln p}$$

where $b$ is the bias, or probability that a flagellum will be rotating CCW, and $p = k_1 P$ is the pseudo-first order rate constant for phosphorylation of CheY. We have chosen this definition of gain for mathematical convenience, but it can be shown (10) that for small steps this definition of $g$ is consistent with the definition of gain given in ref. 17, namely, the change in bias per percent change in receptor occupancy.

When the phosphorylation reactions are at pseudo-equilibrium, one can show that

$$y = \frac{p}{p + z},$$

where $y = Y_p/Y_0 \in [0,1]$ is the dimensionless amount of CheY in phosphorylated form, and $p$ and $z = k_3 Z$ are the production and loss coefficients, respectively, of $y$. We note that

$$\frac{dy_p}{dt} = k_1 P(Y_0 - Y_p) - k_{-3} Z Y_p,$$
that both CheYp and unphosphorylated CheY may bind (19), binding to the flagellar switch, and we allow for the possibility of cooperativity acting multiplicatively. The expression makes it clear that the two possible sources of amplification are via cooperative binding, or possibly via some cooperative interaction among subunits of the switch which bind individually to CheY molecules. Here we consider the first possibility (the second is treated in ref. 10), and we assume that a motor unit binds either CheY or CheYp according to

\[ jY_p + M \rightleftharpoons M(Y_p)_j \]

and

\[ ky^+ + M \rightleftharpoons M(Y)_k. \]

Table 2. Conserved quantities used in the model (see ref. 3)

<table>
<thead>
<tr>
<th>Species</th>
<th>(T)</th>
<th>(R)</th>
<th>(B)</th>
<th>(Y)</th>
<th>(Z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration, (\mu)M</td>
<td>8</td>
<td>0.3</td>
<td>1.7</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

Here \(j\) and \(k\) are the number of molecules of CheYp and CheY, respectively, which bind to the motor, \(M\) represents a motor unbound, and \(M(Y_p)_j\) and \(M(Y)_k\) respectively, in complex with CheYp and CheY.

We assume that CheYp exists in CCW mode rapidly, and study only the steady-state quantities

\[ m = \frac{M}{M_0} = \frac{1}{1 + A_j y^J + A_k (1-y)^K}, \]

\[ m_j = \frac{M(Y_p)_j}{M_0} = \frac{A_j y^j}{1 + A_j y^J + A_k (1-y)^K}, \]

\[ m_k = \frac{M(Y)_k}{M_0} = \frac{A_k (1-y)^k}{1 + A_j y^J + A_k (1-y)^K}. \]
both CheY species (fractional change in receptor occupancy is possible, indicating that both CheY species should have strong binding affinities for the flagellar switch. In the limit as \( \frac{k_{1b}}{k_{1a}}, \ldots, \frac{k_{4b}}{k_{4a}} \) is maximized with respect to \( *Methylation rates of different methylation sites vary by a factor of up to 50. †Ligand binding increases methylation rates of different methylation sites by a factor of 15–30. ‡Demethylation rates of different methylation sites vary by a factor of up to 3. §Ligand binding has little effect on demethylation rate. ‡Estimated from figure 10 in ref. 3. ‡Estimated from figure 3 in ref. 11.

\[ b = m + f jm_j + f_k m_k = \frac{1 + f_j A_j y^j + f_k A_k (1 - y)^k}{1 + A_j y^j + A_k (1 - y)^k}. \]  

One can show that the optimal values of \( f_j \) and \( f_k \) are 0 and 1, respectively (10), and using these \( v \) is given by

\[ v(\bar{y}) = \frac{1}{4} \left( j(1 - \bar{y}) + k\bar{y} \right). \]  

where \( a_k = A_k(1 - y)^k \) and \( \bar{y} \) is the baseline CheYp concentration. Because this expression is monotonically increasing in \( a_k \), \( v(\bar{y}) \) is maximized with respect to \( a_k \) when \( a_k \) is as large as possible, indicating that both CheY species should have strong binding affinities for the flagellar switch. In the limit as \( a_k \to \infty \), Eq. 14 becomes

\[ v(\bar{y}) = \frac{1}{4} \left( j(1 - \bar{y}) + k\bar{y} \right). \]  

Finally, maximizing Eq. 15 with respect to \( \bar{y} \) (subject to \( \bar{y} \in [0, 1] \)) shows that the maximum possible gain at the flagellar switch is

\[ v(\bar{y}) = \frac{1}{4} n, \quad n = \text{max}(j, k). \]  

Thus, a gain of 2.7 requires a Hill coefficient of at least 11 if the only cooperativity is in the interaction of CheY species with the switch. In Fig. 4 D–F we show the bias resulting from the CheYp response in Fig. 4 A–C using a Hill coefficient of 11 for both CheY species (\( j = k = 11 \)).

The full expression for the gain now becomes

\[ g \leq \frac{1}{4} n(1 + w), \]  

and so the fractional change in CheZ activity relative to the fractional change in receptor occupancy is

\[ \frac{w}{2} \geq \frac{g}{n} - 1. \]  

Thus, without cooperative effects at the switch, we must have \( w \geq 10 \), and for moderate cooperativity at the switch, for example \( n = 6 \) (20), we must have \( w \geq 0.8 \). A gain of 6 (17) requires \( w \geq 23 \) and \( w \geq 3 \) in these respective cases.

Finally, we note that an additional cooperative step probably occurs in the interactions between flagella, since the bias of an individual flagellum in the absence of stimulation \([t=0.64(2)] \) is less than that of a swimming cell \([t=0.9, \text{ assuming mean run and tumble durations of 1.1 s and 0.14 s, respectively (21)} \]. We can estimate what the threshold number of flagella might be for this cooperative interaction if we adopt the “voting hypothesis” (21, 22), whereby the biases of the individual flagella are identical and independent, and the probability that the flagella will form a bundle is one when the number of flagella turning CCW equals or exceeds a threshold \( \theta \) and zero otherwise. Then the bias \( B \) of the cell is given by

\[ B = \sum_{j=0}^{N} \binom{N}{j} b^{j}(1 - b)^{N-j}, \]  

\[ \text{not } B = \sum_{j=0}^{N} b^{j}(1 - b)^{N-j}, \text{ as claimed by Weiss and Koshland (22)}, \text{ where } N \text{ is the total number of flagella and } b \text{ is the bias of an individual flagellum. For } b = 0.64 \text{ and either } n = 6 \text{ or } n = 8 \text{ (23), we find that } B = 0.9 \text{ when } \theta = N/2, \text{ and thus a simple majority rules.}

**Discussion**

In our model of aspartate signal transduction via Tar, excitation is the result of the reduced autophosphorylation rate of the ligand-bound state of the receptor, which reduces the level of CheAp and CheYp, thereby reducing the tumbling rate. Adaptation results from the enhanced rate of methylation of bound states and the fact that methylation increases the rate of autophosphorylation, which returns CheAp and CheYp to their prestimulus
levels. The results we present demonstrate that the model can reproduce the experimentally observed responses to both step increases and slow ramps using experimentally determined values for most of the parameters. The disparity between the time scale of excitation, which is fast, and that of adaptation, which is slow, implies that the transduction system can function as a “derivative sensor” with respect to the ligand concentration: the DC component of a signal is ultimately ignored if it is not too large. This provides a bacteria with a temporal sensing mechanism without the need for any type of memory beyond that embodied in the disparity in time scales between excitation and adaptation.

In ref. 10 we show that, as is seen experimentally (16), the magnitude of the response to a slow ramp is an increasing function of ramp rate, and so we may understand the ramp response as the result of a difference between the rate at which receptors enter the sequestered state via increases in receptor occupancy, and the rate at which they exit via transitions between methylation states. Steeper ramps result in larger differences between the entrance and exit rates, and thus in larger responses. The threshold ramp response (16) occurs when these rates are equal. In response to a ramp, the deviation from baseline of the level of sequestered receptor is an approximately linear function of ramp rate. If the downstream steps in the transduction pathway operate in a linear range, then the bias response will in turn be approximately linear in the ramp rate, consistent with the finding of Block et al. (16).

Three methylation states were necessary to accurately reproduce the responses to both step and ramp stimuli, because these responses place competing restrictions on the effective methylation and demethylation rates. For step stimuli given at a baseline attractant concentration of zero (17, 18), adaptation is dominated by the fast transitions between the two lowest methylation states, which provide a sufficiently fast adaptation response. At the higher attractant concentrations of ramp experiments (16, 18), receptors are on average more highly methylated, and so the slower transitions between the two highest methylation states are more prominent, providing a significant ramp response.

The sensitivity, or gain, of the signal transduction system is found experimentally to be quite high, but the source of this sensitivity is unknown. We have shown that the sensitivity observed in response to ramp stimuli (16, 18) is consistent with the moderate estimate of 6 for the maximum gain of the system (17) obtained from step experiments, but that cooperativity equivalent to a Hill coefficient on the order of 11 is necessary to produce the desired gain. There are several potential sources of cooperativity. Binding of CheYp to the flagellar switch is thought to be cooperative (20), and we have shown that competitive inhibition by unphosphorylated CheY can enhance the sensitivity. Because the motor contains 26 or 27 subunits of switch protein FliF (19), it is conceivable that high cooperativity occurs either in binding to the switch or else in interactions among switch subunits bound individually to CheYp. CheZ phosphatase activity may also be modulated in a manner that exhibits cooperativity. The system gain could be significant if CheZ activity were positively correlated with the level of sequestered CheA, because small fractional changes in receptor occupancy can correspond to large fractional changes in sequestered CheA. As an example, the cells possess a short form of CheA (CheA) which may bind several molecules of CheZ (24). If it does so when the associated transducer is unbound to attractant or highly methylated, and releases these molecules into the cytoplasm upon attractant binding, the effective concentration of CheZ will be raised following the latter event. Alternatively, the CheA–CheZ complex may amplify CheZ phosphatase activity [Wang (1996) cited in ref. 25], which could produce high gain if the complex were to form upon attractant binding. Polymerization of CheZ in the presence of CheYp is another potential mechanism for signal amplification, though it is more likely that this reaction instead enhances the adaptation response (25). A further source of gain might be found in cooperative interactions among receptors in close proximity to one another, a possibility raised by the existence of a ‘nose spot’ of elevated receptor density (26).

We have shown that the effects of cooperativity at different locations in the signal transduction pathway are likely to be multiplicative, and thus the total system gain may be the result of moderate cooperativity occurring at two or more of the above-mentioned locations. For example, a Hill coefficient of 6 at the flagellar switch (20) and a moderate degree of CheZ modulation are sufficient to produce the desired gain.

Although the model analyzed herein is specific to signal transduction in bacterial chemotaxis, the structure of the network in Fig. 2 is very similar to those of other signal transduction processes, such as those modeled in refs. 27–29. This suggests that the type of analysis done here will have applicability to other systems. A more complete discussion of aspects of adaptation not treated here, including an evaluation of general models of adaptation, is given in ref. 10.

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Robustness in simple biochemical networks

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Cells use complex networks of interacting molecular components to transfer and process information. These "computational devices of living cells" are responsible for many important cellular processes, including cell-cycle regulation and signal transduction. Here we address the issue of the sensitivity of the networks to variations in their biochemical parameters. We propose a mechanism for robust adaptation in simple signal transduction networks. We show that this mechanism applies in particular to bacterial chemotaxis. This is demonstrated within a quantitative model which explains, in a unified way, many aspects of chemotaxis, including proper responses to chemical gradients. The adaptation property is a consequence of the network's connectivity and does not require the finely-tuned values will ruin the network's performance. Another possibility is the key properties of biochemical networks should be robust in order to ensure their proper functioning.

Cellular biochemical networks are highly interconnected: a perturbation in reaction rates or molecular concentrations may affect numerous cellular processes. The complexity of biochemical networks raises the question of the stability of their functioning. One possibility is that to achieve an appropriate function, the reaction rate constants and the enzymatic concentrations of a network need to be chosen in a very precise manner, and any deviation from the finely-tuned values will ruin the network's performance. Another possibility is that the key properties of biochemical networks are robust; that is, they are relatively insensitive to the precise values of biochemical parameters. Here we explore the issue of robustness of one of the simplest and best-known signal transduction networks: a biochemical network responsible for bacterial chemotaxis. Bacteria such as Escherichia coli are able to sense (temporal) gradients of chemical ligands in their vicinity. The movement of a swimming bacterium is composed of a series of 'smooth runs', interrupted by events of 'tumbling', in which a new direction for the next run is chosen randomly. By modifying the tumbling frequency, a bacterium is able to direct its motion either towards attractants or away from repellents. A well established feature of chemotaxis is the property of adaptation: the steady-state tumbling frequency in a homogeneous ligand environment is insensitive to the value of ligand concentration. This property allows bacteria to maintain their sensitivity to chemical gradients over a wide range of attractant or repellent concentrations.

The different proteins that are involved in chemotactic response have been characterized in great detail, and much is known about the interactions between them (Fig. 1a). In particular, the receptors that sense chemotactic ligands are reversibly methylated. Biochemical data indicate that methylation is responsible for the adaptation property: changes in methylation of the receptor can compensate for the effect of ligand on tumbling frequency. Theoretical models proposed in the past assumed that the biochemical parameters are fine-tuned to preserve the same steady-state behaviour at different ligand concentrations. We present an alternative picture in which adaptation is a robust property of the chemotaxis network and does not rely on the fine-tuning of parameters.

We have analysed a simple two-state model of the chemotaxis network closely related to the one proposed previously. The two-state model assumes that the receptor complex has two functional states: active and inactive. The active receptor complex shows a kinase activity: it phosphorylates the response regulator molecules,
which then bind to the motors and induce tumbling. The receptor complexes can be either in the active or in the inactive state, although with probabilities that depend on both their methylation level and ligand occupancy. The average complex activity can be considered as the output of the network, whereas its input is the concentration of the ligand. A quantitative description of the model consists of a set of coupled differential equations describing interactions between protein components (Box 1).

The two-state model correctly reproduces the main features of bacterial chemotaxis. When a typical model system is subject to a step-like change in attractant concentration, $I$ (Fig. 2), it is able to respond and to adapt to the imposed change. The adaptation is nearly perfect for all ligand concentrations. The addition (removal) of attractant causes a transient decrease (increase) in system activity, and thus of tumbling frequency. We observe a strong asymmetry in the response to the addition compared with the removal of ligand. This asymmetry has been observed experimentally\(^1\). The chemotactic response of the system has been measured by the average drift velocity in the presence of a linear gradient of attractant (Fig. 2, inset). The system is very sensitive: an average change in the receptor occupancy of $\sim 1\%$ per second is enough to induce a drift velocity of $\sim 1$ micron per second.

Figure 3A illustrates the most striking result of the model: we have found that the system shows almost perfect adaptation for a wide range of values of the network’s biochemical parameters. Typically, one can change simultaneously each of the rate constants several-fold and still obtain, on average, only a few per cent deviation from perfect adaptation. For instance, over 80 per cent of model systems, obtained from a perfectly adaptive one by randomly changing all of its biochemical parameters by a factor of two, still show $<15\%$

### Box 1 Two-state model of the bacterial chemotactic network

The main component of the two-state model\(^2,19\) is the receptor complex, MCP + CheA + CheW (Fig. 1A), considered here as a single entity, $E$. The complex is assumed to have two functional states—active and inactive. A receptor complex in the active state shows a kinase activity of CheA, by phosphorylating the response regulators, CheY, it sends a tumbling signal to the motors. The output of the network is thus the average number of receptors in the active state, the system activity $A$. It is assumed that this quantity determines the tumbling frequency of the bacteria. The transformation between $A$ and the tumbling frequency depends on the kinetics of CheY phosphorylation and dephosphorylation, as well as on the interaction of CheY with the motors, which are not considered explicitly in the present model.

The receptor complexes are assumed to exist in different forms. Consider a complex methylated on $m$ sites ($m = 1 \ldots M$). Such a complex can either be occupied or unoccupied by the ligand. We denote the concentration of these complexes by $E_{m}^{a}$ and $E_{m}^{b}$, respectively. Each form of the receptor complex can be in the active state with a probability depending on both its methylation level and its ligand occupancy. We assume that an occupied receptor complex has the probability $a_{m}^{a}$ of being in the active state; for an unoccupied receptor, this probability is $a_{m}^{b}$. If $B$ is the ligand concentration, $B(R)$ the concentration of CheB (CheR), and $E_{m}^{a,b}$ the concentration of the $E_{m}^{a,b}$CheB complex and so on, the model reactions can then be illustrated schematically (see figure).

The differential equations describing our model can be written in a standard way from the figure. For instance, the kinetic equation for $E_{m}^{a}$ is

\[
\frac{dE_{m}^{a}}{dt} = -k_{a}E_{m}^{a} + k_{b}E_{m}^{b} + \left(1 - b_{m}\right) \left(-a_{m}E_{m}^{a}B + d_{m}E_{m}^{a}B + k_{a}E_{m} \cdot R\right) + \left(1 - b_{m}\right) \left(-a_{m}E_{m}^{b}B + d_{m}E_{m}^{b}B + k_{a}E_{m} \cdot R\right) + \sum_{n=1}^{M} \left(1 - b_{n}\right) \left(-a_{n}E_{n}^{a}B + d_{n}E_{n}^{a}B + k_{a}E_{n} \cdot R\right) + \sum_{n=1}^{M} \left(1 - b_{n}\right) \left(-a_{n}E_{n}^{b}B + d_{n}E_{n}^{b}B + k_{a}E_{n} \cdot R\right)
\]
deviation from perfect adaptation (Fig. 3a, lower panel). When varied separately, most of the rate constants may be changed by several orders of magnitude without inducing a significant deviation from perfect adaptation.

In our model we have assumed Michaelis–Menten kinetics for simplicity. However, we have found that cooperative effects in the enzymatic reactions can be added without destroying the robustness of adaptation. Similarly, robust adaptation is obtained for systems with different numbers of methylation sites. Multiple methylation sites are thus not required for robust adaptation, but possibly are for allowing strong initial responses for a wide range of attractant and repellent stimuli (N.B. et al., manuscript in preparation).

The adaptation itself, as measured by its precision (Fig. 3a), is thus a robust property of the chemotactic network. This does not mean, however, that all the properties are equally insensitive to variations in the network parameters. For instance, Fig. 3b shows that the adaptation time, \( \tau \), which characterizes the dynamics of relaxation to the steady-state activity, displays substantial variations in the altered systems. Robustness is thus a characteristic of specific network properties and not of the network as a whole: whereas some properties are robust, others can show sensitivity to changes in the network parameters.

Plots similar to the ones depicted in Fig. 3 can be obtained in quantitative experiments. A large collection of chemotactic mutants can be analysed for variations in the biochemical rate constants of the chemotactic network components. Alternatively, the rate constants of the enzymes could be systematically modified or their expression varied. At the same time, their various physiological characteristics can be measured, such as steady-state tumbling frequency, precision of adaptation, adaption time, and so on. In this way, the predictions of the model can be quantitatively checked.

What features of the chemotactic network make the adaptation property so robust? We propose here a general and simple mechanism for robust adaptation. Let us introduce this mechanism for one of the simplest networks (Fig. 1b), which can be viewed either as an ‘adaptation module’, or as a simplifying reduction of a more complex adaptive network, such as the one presented for bacterial chemotaxis. Consider an enzyme, E, which is sensitive to an external signal \( l \), such as a ligand. Each enzyme molecule is at equilibrium between two functional states: an active state, in which it catalyses a reaction, and an inactive state, in which it does not. The signal level \( l \) affects the equilibrium between two functional states of the enzyme: we suppose that a change in \( l \) causes a rapid response of the system by shifting this equilibrium. Thus, \( l \) is the input of this signal transduction system and the concentration of active enzymes (that is, the system activity, \( A \)) can be considered as its output. The enzyme E can be reversibly modified, for example by addition of methyl or phosphate groups. The modification of E affects the probabilities of the active and inactive states, and hence can compensate for the effect of the ligand. In general, then, \( A(l) = \alpha(l)E + \alpha_m(l)E_m \), where \( E_m \) and \( E \) are the concentrations of the modified and unmodified enzyme, respectively, and \( \alpha_m(l) \) and \( \alpha(l) \) are the probabilities that the modified and unmodified enzyme is active. After an initial rapid response of the system to a change in the input level, \( l \), slower changes in the system activity proceed according to the kinetics of enzyme modification.

The system is adaptive when its steady-state activity, \( A^{ss} \), is independent of \( l \). A mechanism for adaptation can be readily obtained by assuming a fine-tuned dependence of the biochemical parameters on the signal level, \( l \). This kind of mechanism has been proposed for an equivalent receptor system\(^7,16\). A mechanism for robust adaptation, on the other hand, can be obtained when the rates of the modification and the reverse-modification reactions depend solely on the system activity, \( A \), and not explicitly on the concentrations \( E_m \) and \( E \). This system can be viewed as a feed-back system, in which the output \( A \) determines the rates of modification.
reactions, which in turn determine the slow changes in $A$. With such activity-dependent kinetics, the value of the steady-state activity, $A^\ast$, is independent of the ligand level, therefore the system is adaptive. Activity-dependent kinetics can be achieved in a variety of ways. As a simple example, consider a system for which only the modified enzyme can be active ($\alpha = 0$); the enzyme $R$, which catalyses the modification reaction $E \rightarrow E_m$, works at saturation, and the enzyme $B$, which catalyses the reverse-modification reaction $E_m \rightarrow E$, can only bind to active enzymes. In this case, the modification rate is constant at all times, whereas the reverse modification rate is a simple function of the activity

$$\frac{dE_m}{dt} = V^{R\max} - V^{B\max} \frac{A}{K_b + A}$$

where $V^{R\max}$ and $V^{B\max}$ are the maximal velocities of the modification and the reverse-modification reactions, respectively, and $K_b$ is the Michaelis constant for the reverse modification reaction; we have assumed $V^{R\max} < V^{B\max}$. For simplicity, we have assumed that the enzymes follow Michaelis–Menten (quasi-steady-state) kinetics. The functioning of the feedback can now be analysed: the system activity is continuously compared to a reference stead-state value

$$A^\ast = K_b \frac{V^{R\max}}{V^{R\max} - V^{B\max}}.$$  

For $A < A^\ast$, the amount of modification increases, leading to an increase in $A$; for $A > A^\ast$, the modification decreases, leading to a decrease in $A$. In this way, the system always returns to its steady-state value of activity, exhibiting adaptation. Moreover, with these activity-dependent kinetics, the adaptation properties is insensitive to the values of system parameters (such as enzyme concentrations), so adaptation is robust. Note, however, that the steady-state activity itself, which is not a robust property of the network, depends on the enzyme concentrations. Thus, the mechanism presented here still provides a way to control the system activity on long timescales, for example by changing the expression level of the modifying enzymes while preserving adaptation itself on shorter timescales.

A quantitative analysis demonstrates that, on methylation timescales, the kinetics of the two-state model of chemotaxis can, for a wide range of parameters, be mathematically 'reduced' to the simple activity-dependent kinetics shown in equation (1) (N.B. et al., manuscript in preparation). Robust adaptation thus follows naturally as consequence of the simple mechanism described above. The deviations from perfect adaptation (Fig. 3) are in fact connected to departures from the assumptions underlying this mechanism (such as $V^{R\max} < V^{B\max}$). This simple mechanism suggests that the various detailed assumptions about the system's biochemistry can be easily altered, provided that the activity-dependent kinetics of receptor modifications is preserved. All variants of the model obtained in this way still exhibit robust adaptation (N.B. et al., manuscript in preparation).

Two main observations argue in favour of a robust, rather than a fine-tuned, adaptation mechanism for chemotaxis. First, the adaptation property is observed in a large variety of chemotactic bacterial populations. It is easier to imagine how a robust mechanism allows bacteria to tolerate genetic polymorphism, which may change the network's biochemical parameters. In addition, in genetically identical bacteria some features of the chemotactic response, such as the values of adaptation time and of steady-state tumbling frequency, vary significantly from one bacterium to another, while the adaptation property itself is preserved. This 'individuality' can be readily explained in the framework of the present model. The concentrations of some cellular proteins, for example the methylating enzyme CheR, are very low, and thus may be subject to considerable stochastic variations. In consequence, both adaptation time and steady-state tumbling frequency, which are not robust properties of the network, should vary significantly. Moreover, the present model predicts that both these quantities should show a strong correlation in their variation (Fig. 3c), which has been observed experimentally.

How general are the results presented here? In addition to explaining response and adaptation in chemotaxis, the present model accounts, in a unifying way, for other taxis behaviour of bacteria mediated by the same network. Indeed, as the network's dynamics is solely determined by the system activity, the system will respond and adapt to any environmental change that affects this activity. Mechanisms of robust adaptation similar to the one introduced above could apply to a wider class of signal transduction networks. Robustness may be a common feature of many key cellular properties and could be crucial for the reliable performance of many biochemical networks. Robust properties of a network will be preserved even if its components are modified through random mutations, or are produced in modified quantities. Systems whose key properties are robust could have an important advantage in having a larger parameter space in which to evolve and to adjust to environmental changes.

The degree of robustness in many biochemical networks can be quantitatively investigated. This can be achieved by characterizing a behavioural, a physical or biochemical property while varying systematically the expression level and the rate constants of the network's components. The complexity of biological systems introduce several conceptual and practical difficulties, however. Among the most important is the difficulty of isolating smaller subsystems that could be analysed separately. For instance, in the present analysis, we have neglected the existence of different types of receptors and any crosstalk between them. We have also disregarded the interactions between the chemotaxis network and other components of the cell. In addition, the complexity and stochastic variability of biological networks may preclude their complete molecular description. Rate constants and concentrations of many enzymes can only be measured outside their natural cellular environment and many other network parameters remain unknown. Robustness may provide a way out of both these quandaries: robust properties do not depend on the exact values of the network's biochemical parameters and should be relatively insensitive to the influence of the other subsystems. It should then be possible to extract some of the principles underlying cell function without a full knowledge of the molecular detail.

**Methods**

Numerical integration of the kinetics equations defining the two-state model (see Box 1) was used to investigate its properties. Computer programs in C language were executed on an SGI(R4000) workstation using a standard routine (ode45 from LLNL). Typical CPU time for finding a numerical solution of a model system is of the order of 1 min. A particular model system was obtained by assigning values to the rate constants and the total enzyme concentrations. Most of our results were obtained for a reference system defined by the following biochemical parameters: the equilibrium binding constant of ligand to receptor is 1 $\mu$M and the time constant for the reaction is 1 ms ($k_l = 1 \text{ms}^{-1} \mu\text{M}^{-1}$, $k_e = 1 \text{ms}^{-1}$). CheR methylates both active and inactive receptors at the same rate, with a Michaelis constant of 1.25 $\mu$M, and a time constant of 10 s ($a_a = 80 \text{s}^{-1} \mu\text{M}^{-1}$, $d_a = 100 \text{s}^{-1}$, $k_0 = 0.1$). CheB (CheR$^-$) demethylates only active receptors with a Michaelis constant of 1.25 $\mu$M and a time constant of 10 s ($a_a = 800 \text{s}^{-1} \mu\text{M}^{-1}$, $d_a = 1,000 \text{s}^{-1}$, $k_0 = 0.1$). The number of enzyme molecules per cell are: 10,000 receptor complexes, 2,000 CheB and 200 CheR (cell volume of 1.4 x 10$^{-12}$). The probabilities that a receptor with $m = 1, \ldots 4$ methylated sites is in its active state are: $\alpha_0 = 0$, $\alpha_1 = 0$, $\alpha_2 = 0.75$, $\alpha_3 = 1$ if it is unoccupied, and $\alpha_4^0 = 0$, $\alpha_4^1 = 0.1$, $\alpha_4^2 = 0.5$, $\alpha_4^3 = 1$ if it is occupied.
Response and adaptation. In a typical assay, a model system was subject to a step-like change in attractant concentration. A system in steady-state, characterized by the system activity $A^s$, was perturbed by an addition or removal of attractant. As a result, the system activity changed abruptly and then relaxed, with the characteristic adaptation time, $\tau$, to a new steady-state value $A^p$. Here $p$ measures the precision of adaptation; perfect adaptation corresponds to $p = 1$ (see inset in Fig. 3a).

Robustness of adaptation. The sensitivity of adaptation precision and adaptation time to variations in the biochemical constants defining a model system was investigated. An ensemble of altered systems was obtained from the reference system by random modifications of its reaction rate constants and enzymatic concentrations, $K_i$. Each alternation of the reference system was characterized by the total parameter variation, $\delta$, which is defined as:

$$
\log(\delta) = \sum_{i=1}^{n} \log(k_i/K_i),
$$

where $k_i$ are the biochemical parameters of the altered system. The altered system was subject to a step-like addition of saturating concentrations of attractant (1 mM), and both the precision of adaptation, $p$, and the adaptation time, $\tau$, were measured. The assay was repeated for various reference model systems, with different values of biochemical parameters and of $\alpha$, and different variants of the model. The robustness of adaptation (Fig. 3) is independent of these choices.

Chemotactic drift velocity. The behaviour of a model system in the presence of a linear gradient of attractant, $\nabla A$, was simulated. The movement of the system was assumed to be composed of a series of smooth runs at a constant velocity of 20 $\mu$m s$^{-1}$, interrupted by tumbling events. The tumbling frequency was taken to be a sigmoidal function of the system activity (Hill coefficient, $q = 2$. Different values of $q$ lead to the same qualitative picture; the sensitivity increases with $q$). The trajectories were also subject to a rotation diffusion, with $D = 0.125$ rad$^2$ s$^{-1}$ (ref. 9). Attractant concentration was increasing along the $x$ direction, with $f = 1$ M$^2$ s$^{-1}$ at $x = 0$. The chemotactic drift velocity was estimated by measuring the average $x$ position of a hundred identical simulated systems as a function of time.

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A family of cytokine-inducible inhibitors of signalling


Cytokines are secreted proteins that regulate important cellular responses such as proliferation and differentiation1. Key events in cytokine signal transduction are well defined: cytokines induce receptor aggregation, leading to activation of members of the JAK family of cytoplasmic tyrosine kinases. In turn, members of the STAT family of transcription factors are phosphorylated, dimerize and increase the transcription of genes with STAT recognition sites in their promoters2–4. Less is known of how cytokine signal transduction is switched off. We have cloned a complementary DNA encoding a protein SOCS-1, containing an SH2-domain, by its ability to inhibit the macrophage differentiation of M1 cells in response to interleukin-6. Expression of SOCS-1 inhibited both interleukin-6-induced receptor phosphorylation and STAT activation. We have also cloned two relatives of SOCS-1, named SOCS-2 and SOCS-3, which together with the previously described CIS (ref. 5) form a new family of proteins. Transcription of all four SOCS genes is increased rapidly in response to interleukin-6, in vitro and in vivo, suggesting they may act in a classic negative feedback loop to regulate cytokine signal transduction.

To identify cDNAs encoding proteins capable of suppressing cytokine signal transduction, we used an expression cloning approach. The strategy used the murine monocytic leukaemic M1 cell line that differentiates into mature macrophages and ceases proliferation in response to various cytokines, including interleukin-6 (IL-6), and in response to the steroid, dexamethasone5. Parental M1 cells were infected with the RUFneo retrovirus, into which a library of cDNAs from the factor-dependent haemopoietic cell line FDC-P1 had been inserted6. Retrovirally infected M1 cells that were unresponsive to IL-6 were selected in semi-solid agar culture by their ability to generate compact colonies in the presence of IL-6 and geneticin. One stable IL-6-unresponsive clone, 4A2, was obtained after examining $10^7$ infected cells (Fig. 1). A 1.4 kilobase pair (kb) cDNA insert, which we have named suppressor of cytokine signalling-1, or SOCS-1, was recovered by polymerase chain reaction (PCR) from the retrovirus that had integrated into genomic DNA of 4A2 cells. The SOCS-1 PCR product was used to

Figure 1 Phenotype of IL-6 unresponsive M1 cell clone, 4A2. Colonies of parental M1 cells (left panel) and clone 4A2 (right panel) cultured in semi-solid agar for 7 days in saline or 100 ng ml$^{-1}$ IL-6.