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REVIEW ARTICLE

Overview of Mathematical Approaches Used to Model Bacterial Chemotaxis I: The Single Cell

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Abstract Mathematical modeling of bacterial chemotaxis systems has been influential and insightful in helping to understand experimental observations. We provide here a comprehensive overview of the range of mathematical approaches used for modeling, within a single bacterium, chemotactic processes caused by changes to external gradients in its environment. Specific areas of the bacterial system which have been studied and modeled are discussed in detail, including the modeling of adaptation in response to attractant gradients, the intracellular phosphorylation cascade, membrane receptor clustering, and spatial modeling of intracellular protein signal transduction. The importance of producing robust models that address adaptation, gain, and sensitivity are also discussed. This review highlights that while mathematical modeling has aided in understanding bacterial chemotaxis on the individual cell scale and guiding experimental design, no single model succeeds in robustly describing all of the basic elements of the cell. We conclude by discussing the importance of this and the future of modeling in this area.

Keywords Bacterial chemotaxis · Single cell · Review

1. Introduction

Bacterial chemotaxis refers to the ability of bacteria to sense changes in their extracellular environment and to bias their motility towards favorable stimuli (attractants) and away from unfavorable stimuli (repellents). Generally too small to sense a change in extracellular gradient along their own length, bacteria use a system of membrane receptors and intracellular signals to sense, adapt, and respond to changes in their environment.

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Bacterial chemotaxis was first observed in the late 1800s (Beyerinck, 1895; Engelmann, 1881a, 1881b; Pfeffer, 1888). The motivation for studying such small organisms lies in the belief that elucidating the mechanisms controling their behavior will help in understanding more complex biological pathways and organisms. Hence today, in part thanks to the pioneering work of Adler (1966), bacterial chemotaxis is one of the most studied and well-documented systems in biology, serving as a powerful model for higher organisms. There remain, however, a number of important, unanswered questions about this intriguing system.

There exist a number of different but related bacterial chemosensory systems (Eisenbach et al., 2004), of which the most widely studied is *Escherichia coli* (*E. coli*).[1] *E. coli* is a rod-shaped bacterium with polar clusters of membrane-spanning methyl-accepting chemotaxis proteins (MCPs) situated at either end of the cell as shown in Fig. 1 (Maddock and Shapiro, 1993; Lybarger and Maddock, 2001). Receptor types (responding to different chemoeffectors) vary both within each bacterium as well as across the various species. In *E. coli*, the most common receptors are Tar (responding to aspartate) and Tsr (serine), with Tap (dipeptides), Trg (galactose) and Aer (oxygen) being less abundant (Bren and Eisenbach, 2000). In the case of *E. coli*, the four or so flagella are located at random points on the bacterial membrane which during smooth swimming, collectively bundle together to propel the bacterium through the medium.

Bacteria swim in a random walk in the absence of a gradient of attractant or repellent. In the presence of attractant gradients, this random walk becomes biased so that movement towards a better environment is favored. *E. coli* alternates between counter-clockwise (CCW) rotations of its flagella and clockwise (CW) rotations. CCW rotations result in runs while CW rotations are responsible for tumbling and reorientation of the bacterium. CCW rotations cause the flagella to come together forming a single flagellar bundle which propels the bacterium through the surrounding medium. When the cells are swimming up an attractant gradient, CCW rotation is favoured resulting in longer periods of directed motion (runs).

The signal transduction between the receptors and the flagellar motors is controlled by a set of well defined intracellular protein–protein interactions (Wadhams and Armitage, 2004). In the case of *E. coli*, one of the most common attractants used to study the bacterial response is aspartate. A number of intracellular proteins (known as chemotaxis (Che) proteins) provide the necessary signaling cascade which links the membrane receptors to the flagellar motor as shown in Fig. 1. Both CheW and CheA are localized to the receptors. CheW is thought to act as a linker between the receptor and CheA although CheA appears to also directly interact with the receptors. To bring about tumbling, the receptors activate CheA autophosphorylation on a conserved histidine in response to decreased attractant or increased repellent concentration. One of the phosphoryl groups is transferred to CheY. $CheY_P$ shows a reduced affinity for CheA and a higher affinity for the flagellar motor protein FliM, and thus diffuses through the cytoplasm to the motors. CheZ acts to dephosphorylate $CheY_P$ at the receptors to regulate the rate of signal termination.

[1]The work presented in this manuscript focuses mainly on *E. coli* given that most experimental and thus theoretical work has been undertaken using this system. The reader should consult Garrity and Ordal (1995), Armitage (1999), Eisenbach et al. (2004), and Wadhams and Armitage (2004) for details on other chemotactic species.

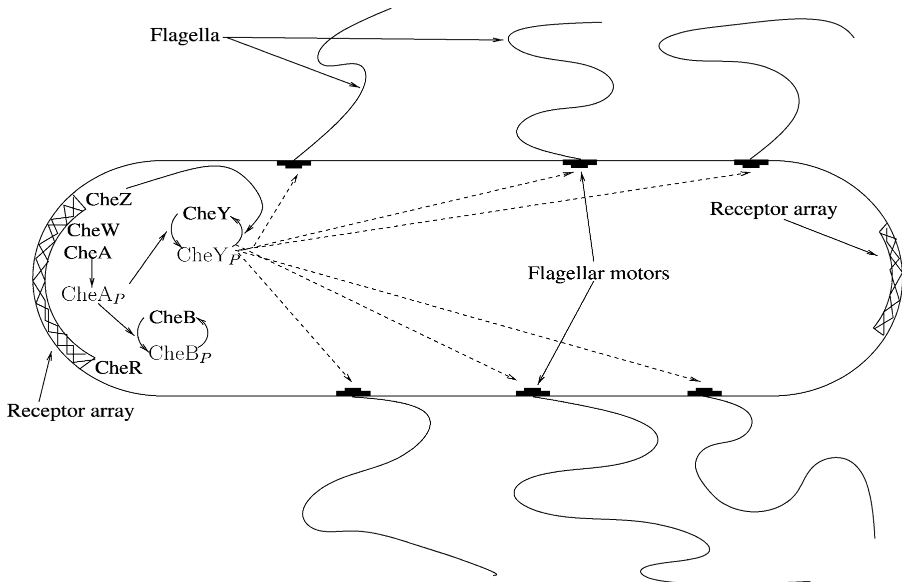

Fig. 1 A schematic representation (not to scale) of a typical *E. coli* bacterium (approximately 3 μm long) showing the location of the membrane receptors and flagella. At the cell poles, the receptor proteins are clustered along with the linker protein CheW and the kinase CheA. A decrease of attractant causes CheA to autophosphorylate, the phosphorylated CheA, CheA$_P$, is a phosphodonor for both CheY and CheB. The subsequently phosphorylated CheY diffuses (indicated schematically by the dotted lines) to the flagellar motors where it causes them to rotate in a clockwise direction. CheY$_P$ can be dephosphorylated by CheZ. The rise in CheB$_P$ levels reduces the receptor methylation state which mediates adaptation by reducing the ability of the receptors to activate CheA autophosphorylation. With the addition of attractant, the rate of autophosphorylation of CheA is reduced, and thus CheY$_P$ levels fall. This fall causes the motors to revert to CCW rotation allowing the flagella to bundle together to produce runs.

Phosphotransfer does not only occur between CheA$_P$ and CheY, a phosphoryl group can also be transferred from CheA$_P$ to CheB. CheB$_P$ mediates the process of adaptation. The phosphotransfer between CheA$_P$ and CheB occurs more slowly than the phosphotransfer between CheA$_P$ and CheY. CheB is key to resetting the receptors into the unstimulated state. The phosphorylated CheB acts to demethylate the receptors counteracting the effect of constant methyltransferase (CheR) activity which methylates specific glutamate residues on the receptor. CheY and CheB both compete for a specific binding domain of CheA. In the case of unstimulated bacteria, this process results in a dynamic equilibrium between CheR and CheB$_P$, and thus no net methylation change of the respective receptors occurs. The most abundant receptor Tar has five methylation sites per monomer (commonly denoted $m = 0, 1, 2, 3, 4$ in the modeling literature), which when modified affect the activity of the receptors. State $m = 4$ is the highest methylation level whereas $m = 0$ denotes no methylation.

The addition of attractant to the membrane receptors reduces the rate of CheA autophosphorylation. This has a twofold effect. First, no phosphoryl groups are available for phosphotransfer to CheY. The levels of CheY$_P$ in the cytoplasm of the bacteria thus fall, CheY dominates and the flagellar motors rotate in the CCW direction. Second, a drop

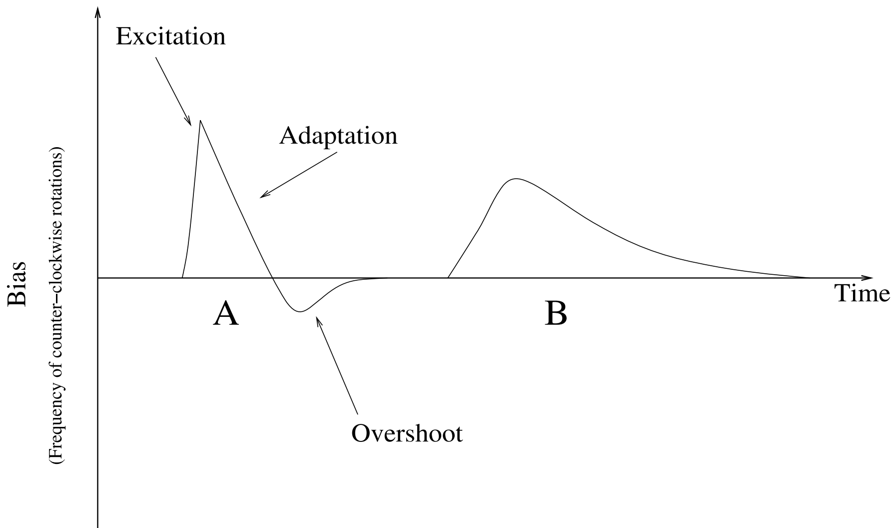

Fig. 2 Schematic representation of typical adaptation curves to impulse (A) and step (B) attractant gradients as adapted from Segall et al. (1986). In each case, the adaptation curve comprises a period of excitation and a period of adaptation. The measured response is generally the motor bias (measure of CCW) or level of intracellular protein concentration, for instance, the concentration of CheY or CheY_P. The observed overshoot is often explained by the negative feedback of the methylation/de-methylation process (Shimizu et al., 2003) and was first observed by Block et al. (1982) and Segall et al. (1986).

in CheB_P allows the receptors to be methylated by CheR. The rise in receptor methylation increases the rate of CheA auto-phosphorylation, thus returning the system to its prestimulus state. The effect of a typical attractant stimulus is shown in Fig. 2.

The following are common characteristics of bacterial systems:

Adaptation: Many bacterial systems show an inherent ability to adapt to local changes in the levels of extracellular attractant or repellent over quite wide ranges (approximately five orders of magnitude) of background concentrations (Wadhams and Armitage, 2004). Exact adaptation in a bacterial chemotaxis context means the ability to respond to changes in the external environment and return the intracellular protein phosphorylation levels to their pre-stimulus levels. Adaptation is not necessarily always exact as in the case of *E. coli* responding to serine. Following this process, the bacteria are then able to detect any further changes in the attractant concentration. A typical adaptation curve is shown in Fig. 2.

Sensitivity: Studies have shown (Segall et al., 1986) that even small changes in the local extracellular environment of bacteria, as small as ten attractant molecules per cell, can initiate a chemotactic response from the bacteria.

Gain: The ability to sense small changes in the extracellular environment means the bacterium must be able to amplify the received signal, so as to modulate the intracellular signaling cascade (Bray, 2002). Gain is generally defined as the change in motor bias with respect to the change in occupancy of the receptors.

Robustness: In order to cope with cell-to-cell variations in levels of the signal transduction proteins the intracellular signaling network must be robust (Alon et al., 1999).

All of the above concepts are closely interwoven. For instance, gain requires the sensitivity of the system to be high enough to initiate the downward cascade of biochemical signals. The system must be robust to cope with variations in levels of the signal transduction proteins between cells in order to be able to adapt across an extremely wide range of background concentrations. It is important to note that these events all occur on different timescales. Attractant binding occurs over the order of milliseconds whereas demethylation is of the order of 1/10ths of seconds. In stark contrast, adaptation can take up to seconds or minutes dependent upon the magnitude of the stimulus (Wadhams and Armitage, 2004).

Understanding the mechanisms which drive adaptation, sensitivity, gain, and robustness, both individually and collectively, has been the focus of both experimentalists and theoreticians for the past four decades. In many cases, experimental work has informed mathematical models derived to understand concepts such as adaptation (Block et al., 1983). In addition, mathematical models have allowed various aspects of the bacterial system to be studied without the need for intensive experimental work, and proposed a range of testable hypotheses for the way in which certain elements of the bacterial system may function (Bray et al., 1998).

The work reviewed here focuses on mathematical models developed to understand the bacterial chemotactic response on the single cell scale. We begin by considering the early work of experimentalists and theoreticians who sought to elucidate the reasons for the observed chemotactic response by considering the idea of temporal "memory." Section 3 focuses on the large number of models which have been proposed to explain the observed adaptation of bacterial systems. The modeling of the biochemical phosphorylation cascade is discussed in Section 4 before the models which have sought to elucidate sensitivity via receptor interactions are discussed in Section 5. Both the importance of and various approaches to modeling methylation and demethylation are briefly reviewed in Section 6. Recently developed models which account for the spatial movement of proteins within the bacterial cytoplasm are discussed in Section 7. We conclude our discussion on intracellular signaling with an overview of some common modeling assumptions and outcomes that have arisen from our review process. We do not provide an overview of modeling of flagella motors given the recent comprehensive reviews, both experimental and theoretical, of Berg and Berry and Armitage in this area (Berg, 2000, 2003; Berry and Armitage, 1999).

While the biochemical reactions controlling chemotaxis within bacteria are now well understood, no single mathematical model based upon experimental findings can, as yet, produce an accurate and comprehensive description of adaptation, sensitivity, and gain as well as being robust to changes in parameter values governing the respective processes. Furthermore, a number of interesting questions remain regarding certain elements of how the bacterial system functions. These issues and possible areas of future research are discussed in Section 9 of this review.

2. Early work

Early mathematical modeling work (Segel, 1976; Koshland, 1977; Block et al., 1982, 1983) focused on the adaptive behavior individual bacteria exhibited when subjected to

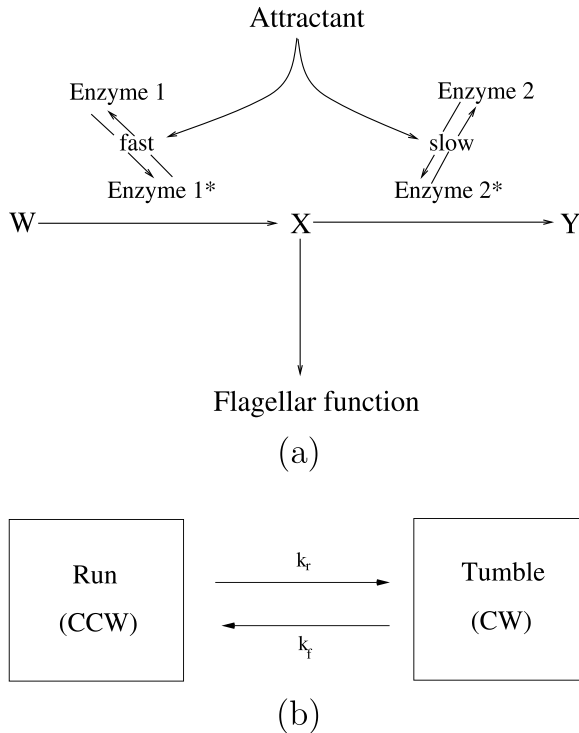

Fig. 3 (a) The response regulator of Macnab and Koshland (1972) as adapted from their original manuscript. An intracellular control factor X is upregulated by W, due to fast enzyme activity, and downregulated by Y. Koshland (1977) later postulated the activity to be controlled by receptor methylation. (b) The two-state model of Block et al. (1982) as adapted from their original paper.

changes in their extracellular environment. Such work was often combined with experimental results (Block et al., 1982, 1983). In understanding such work, it is important to remember that much of the detailed knowledge we currently have of the biochemistry within *E. coli* was unknown at the time.

Macnab and Koshland (1972) were the first to postulate that bacteria use an internal "memory" to respond to both positive and negative stimuli. They postulated the existence of an intracellular compound W (essentially CheY) which was converted to a state X (CheY_P), as shown in Fig. 3(a). Conversion was by enzymatic processes whose activity was dependent upon the concentration of the external attractant. The first enzyme acts quickly to ensure a rapid response by the bacteria to the external stimulus, while the post-adaptation response is regulated by a slower acting enzyme (predecessor to CheB_P) which returns X to its pre-stimulus level by degrading it to Y (CheY). In the case of positive attractant, the first enzyme is more active than the second and this leads to X exceeding a critical level which leads to chemotactic runs. In the case of a negative stimulus, X is degraded and tumbling of the bacteria ensues. This notion of an intracellular or response regulator was further extended by Koshland (1977). He noted that covalent modification of

the receptors due to methylation was most likely responsible for controlling the proposed enzymatic activity of the response regulator X.

The work of the Berg laboratory (Block et al., 1982, 1983) involved undertaking a number of experiments, combined with theoretical predictions, to understand the bacterial response. In their 1982 paper, they considered the bacterial response to pulses of attractant and repellent. They averaged the CW and CCW responses of individual cells to attractant and considered the average respective probability of CCW motor rotation versus time. Analysis of the resulting response showed that excitation and adaptation occurred on somewhat different timescales, sufficiently separate to infer that each was controlled by a different process. The time taken for a bacterium to respond to an attractant gradient indicated that it was able to integrate stimuli over a number of seconds in order to determine its response. The biphasic nature of the bacterial response also showed that bacteria were sensitive to stimuli which had occurred over the past few seconds and any variations in concentration that occurred on a longer timescale were simply averaged out. From their results, Block et al. (1982) postulated the existence of a two-state system, as shown in Fig. 3(b). The respective rates, the probability of terminating a run or tumble per unit time, were determined from plots of the CW and CCW responses.

In their 1983 paper, Block et al. considered the bacterial response to increasing concentration levels (ramps) of attractant, both exponential and sinusoidal. They showed receptor activity corresponded to the rate of change of receptor occupancy, thus indicating the bacteria must be able to compare current and previous levels of receptor occupancy. This was comparable to results found by Segel (1976, 1977) in modeling populations of chemotactic bacteria. In numerical simulations of the response regulator model of Koshland (1977), Block et al. (1983) showed that resultant run and tumble distributions from their model did agree with the experimentally derived ones, thus indicating that the transition between either state was not random, but depended upon adaptation to the sensory input. By extending their two-state model to include a description of adaptation and receptor occupancy, they showed that their model agreed well with experimental evidence.

Block et al. (1983) were the first to formulate a basic model of the bacterial response which included adaptation and alluded to the possible role of methylation in affecting the bacterial response. They adapted the model of Delbrück and Reichardt (1956) (initially used to show light adaptation in *Phycomyces*) to chemotaxis, in which the degree of adaptation was proposed to follow that of receptor occupancy

$$\frac{dA}{dt} = \frac{1}{\tau}(P - A),$$

where A is the concentration of an internal (adapting) cell variable which changes according to the receptor occupancy P. In essence, A lags P and the difference between them is referred to as the error in the signal. Here τ is an adaptation time constant. As $\tau \to 0$, $A \to P$ and the system shows perfect adaptation. Block et al. (1983) noted that the original Delbrück–Reichardt model could not account for the response thresholds the bacteria exhibited to attractant and repellent, but a revised model which includes methylation can. They proposed that methylation could be the bacterium's adaptation mechanism, and hence the error signal could be accounted for by the difference in receptor occupancy and methylation.

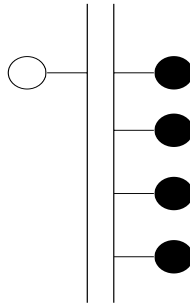

Fig. 4 A conceptual representation of how methylation of receptors may occur. Receptors are methylated and demethylated with a maximum of up to five sites on each receptor complex being available for methylation. The above diagram shows a receptor which has four sites methylated (black circles) whilst the fifth remains unmethylated. Receptors are methylated by CheR and demethylated by CheB$_P$.

3. Adaptation

Following the work undertaken by members of the Berg laboratory (Block et al., 1983), a number of researchers have sought to understand adaptation within bacterial systems (Goldbeter and Koshland, 1982; Asakura and Honda, 1984; Segel and Goldbeter, 1986; Hauri and Ross, 1995; Barkai and Leibler, 1997; Almogy et al., 2001; Mello and Tu, 2003a; Arocena and Acerenza, 2004), with many of the models focusing on the role of methylation in describing the process. Such models have generally used the framework of ordinary differential equations (ODEs) to describe the underlying processes. In order to successfully describe adaptation, the mathematical models have had to ensure that the steady-state bacterial response is independent of the attractant concentration. In addition, models should also show "robustness." Goldbeter and Koshland (1982) were the first to propose a receptor modification model which included methylation (as demonstrated in Fig. 4). They proposed a four state model whereby free receptors are bound by attractant and may subsequently undergo modification as shown in Fig. 5. They noted that for perfect adaptation to be achieved within this model, the steady-state concentration of attractant-bound receptors is independent of the attractant concentration. From their ODE model, the authors derived analytical estimates of the adaptation time and the degree to which receptor modification occurs as a function of methylation rate in either state, namely k_d and k_m. Analysis of various forms of the reaction rates, both dependent and independent of the attractant concentration and overall receptor occupancy, and comparison with experiment showed that the simplifying assumption $k_m = k_d =$ constant did not give good model-experimental agreement. Reasonable comparison was, however, obtained when receptor demethylation was assumed to depend on receptor occupancy.

Asakura and Honda (1984) proposed a two-state model in which multiple methylation occurred in a preferred order. Based on experimental evidence from a number of sources, their model assumes unbound receptors S accept attractant molecules whereby they move to a state T, such that $T_i = S_i A$ where S_i is the ith methylated unbound receptor state and A is the attractant concentration. T state receptors can accept repellent molecules to move back to S. Only unbound receptors can be methylated whilst bound receptors only undergo demethylation, processes which were assumed to occur on a longer timescale

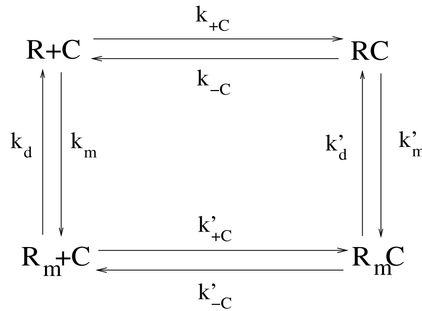

Fig. 5 The model of Goldbeter and Koshland (1982) as adapted from their original paper. Here R represents the concentration of free receptors, C the concentration of attractant, RC the concentration of bound receptors and $R_m C$ the concentration of bound receptors which have undergone modification. See text for further details.

than that of attractant binding. A dynamic equilibrium of S and T receptors can thus exist whereby methylation causes a shift of the receptors toward the T state as shown in Fig. 6. This simple model allows for up to n methylated states of either receptor state. Analysis of the model showed that it exhibited both excitation and adaptation and the T bound states are independent of bound attractant concentration for certain parameter values. Asakura and Honda (1984) noted that their model could maintain a constant steady-state fraction of T states over a wide range of attractant and repellent concentrations so long as: (i) "the S/T equilibria of unmethylated and fully methylated species heavily favored S and T conformations, respectively", i.e., $L_0^0 = S_0/T_0 \gg 1$ and $L_n^0 = S_n/T_n \ll 1$, where L_0^0 and L_n^0 represent the zeroth and nth methylation state, respectively; and (iii) all methylation steps were characterized by the same rate constant ratio, i.e., $\mu = a_i/b_i = T_i/S_{i-1}$. Analysis for $n = 1$ and $n = 6$ methylation states revealed that in the case of $n = 1$ the bacterium could still maintain a steady-state over a wide range of attractant concentrations. Increasing the number of methylation states increased the sensitivity of the bacterium to the attractant concentration, but the model sensitivity decreased more rapidly with increasing attractant concentration when $n = 1$. From these results, Asakura and Honda (1984) concluded that methylation plays a dual role. Firstly, it allows the bacterium to adapt to changes in the attractant concentration and secondly, it increases the excitation sensitivity to small changes in it. They also noted that their model responded in a similar way to the experimental findings of Block et al. (1983) in the case of a sinusoidal input.

Segel and Goldbeter (1986) defined a four state model which achieved perfect adaptation. Although conceptually similar to the work of Goldbeter and Koshland (1982), their model allowed receptor modification to occur before or after attractant binding, in essence allowing the effect of methylation and de-methylation on unbound and attractant-bound receptors to be considered. Similar to Asakura and Honda (1984), Segel and Goldbeter (1986) assumed that attractant binding occurs on a faster timescale than receptor modification, an assumption also adopted by many later models (see Appendix B for an overview). Considering Fig. 7, the total activity of the system was defined as

$$A(t) = a_1 R(t) + a_2 X(t) + a_3 Y(t) + a_4 D(t), \tag{1}$$

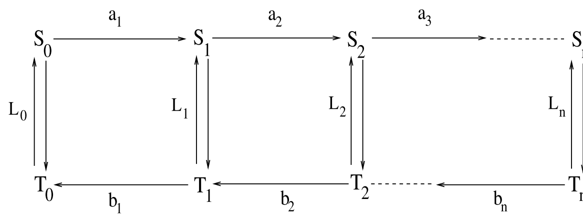

Fig. 6 The multiple receptor methylation model of Asakura and Honda (1984). Here S_i and T_i are the ith methylated attractant-free and attractant-bound states, respectively. See text for further details. Adapted from Asakura and Honda (1984).

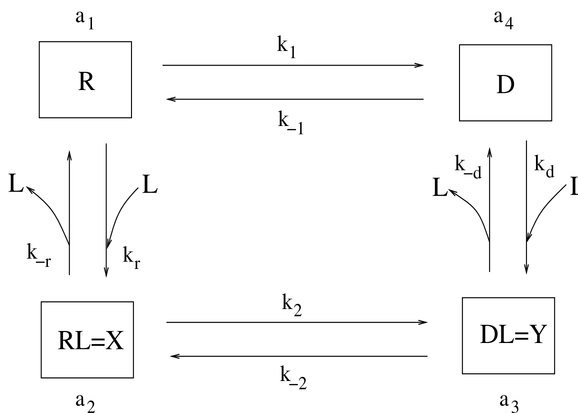

Fig. 7 The four state model of Segel and Goldbeter (1986) as adapted from their manuscript. Here R represents the concentration of attractant-free, unmodified (unmethylated) receptors, D is the concentration of attractant-free, modified (methylated) receptors, L is the ligand concentration and X and Y denote the concentration of attractant-bound, unmodified, and modified receptors, respectively.

where a_i $(i = 1, 2, 3, 4)$ are constants. We note that the model does not differentiate between varying levels of methylation, as later models would, but simply that receptors are either unmodified (unmethylated) or modified (methylated). By choosing appropriate values of a_i, based on deriving relationships assuming exact adaptation, Segel and Goldbeter (1986) were able to show that the model exhibited exact adaptation. Whether this model is robust, however, remains unclear, as no sensitivity analysis was undertaken. Segel and Goldbeter (1986) noted that the level of receptor modification increased with increasing levels of attractant binding and that demodification acts as a counter-weight to changes in the attractant concentrations. The authors also derived a number of analytical estimates for the requirements for inexact adaptation, time for adaptation, the extent of receptor modification and the effect of quickly removing the applied stimulus following excitation on the system response.

The work of Segel and Goldbeter (1986) has been extended by Hauri and Ross (1995) and more recently by Arocena and Acerenza (2004). The extensions in the former included a description of the phosphorylation pathway (and its interaction with the receptor complexes) and increasing the number of receptor states from four to ten (five attractant-

free, five attractant-bound). Each receptor complex T constituted an MCP receptor and CheA and CheW molecules, an assumption since adopted by a number of researchers as detailed in Appendix B. The Hauri and Ross model of the phosphotransfer pathway went so far as describing the phosphorylation of CheY and CheB and their subsequent dephosphorylation. They did not include a description of the CheY$_P$-flagellum motor interaction, as little was understood about the respective interactions at the time. Instead, they noted the frequency of counter-clockwise rotations of the flagellum (f_{CCW}) could be related to the CheY$_P$ levels (Y_P), as experimentally observed by Kuo and Koshland (1989)

$$f_{CCW} = \frac{1}{1 + hY_P^{5.5}}, \tag{2}$$

where h is a constant. A description of receptor methylation by CheR was also included. Hauri and Ross (1995) further assumed that the rate of phosphorylation was dependent upon the methylation level of the receptors and whether or not an external attractant was bound to them. Their model sought to differentiate between two different mechanisms for achieving adaptation, namely: (1) the effect of decreasing the rate of receptor demethylation by CheB$_P$; and (2) increasing the rate of receptor methylation upon attractant binding, where the difference is due to conformational change at the receptor. Hauri and Ross (1995) noted that their model agreed well with experimental findings, in particular the predicted timescale of initial excitation is similar and the model demonstrates exact adaptation for both attractants and repellents or a combination of both. However, their model failed to account for the observed sensitivity and gain and the timescale of adaptation was not significantly dependent upon the concentration of the stimulus. In undertaking sensitivity analysis of their model by altering the rate constants, the ability of the model to adapt is removed, thus the model is not robust.

The recent work of Arocena and Acerenza (2004) has sought to understand the effect of receptor modification, via attractant binding (using mass action kinetics), in comparison to covalent modification via, for example, methylation or phosphorylation, over the bacterial response range. Their work shows that covalent modification of the receptors allows the bacterium to respond across five orders of magnitude of attractant binding, a wider range than in the case of receptor modification via attractant binding. Arocena and Acerenza (2004) note that two conditions are required for a high and a repeatedly similar adaptive response for varying attractant concentrations: (1) by varying the number of receptor states in the model, it is found that the receptor modifying reactions need to include a similar number of reaction cycles to the orders of magnitude of attractant concentration; and (2) the receptor is able to operate at steady-state. They used an optimization scheme to obtain estimates for their parameter values and noted that the optimized parameters gave higher values than the experimentally reported ones. As with other models of adaptation, they are unable to obtain the observed experimental level of sensitivity. They do, however, postulate that a wider response range may be due to the ability of some receptors to form clusters (for more detail, see Section 5).

In 1997, Barkai and Leibler (1997) developed a model using a single receptor species which included the effects of phosphorylation and methylation on the receptors, but not phosphotransfer from CheA$_P$ to CheY or CheB. As shown in Fig. 8, receptor methylation was assumed to be by CheR and demethylation by CheB$_P$, which only acted on active receptors. As with previous models, the receptors existed in either an attractant-bound or

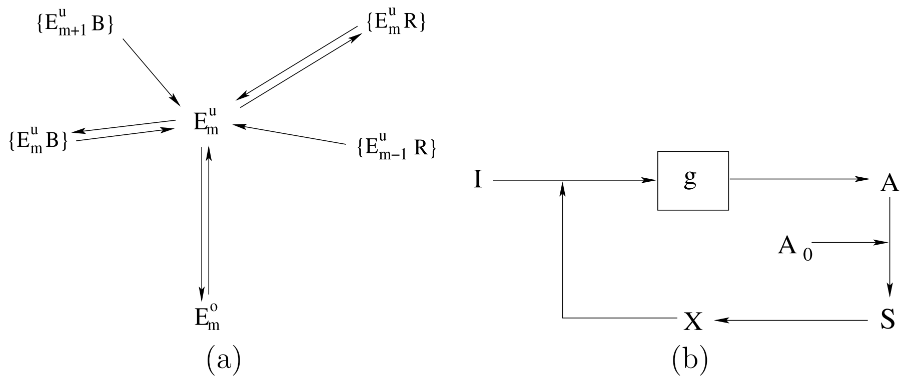

Fig. 8 (a) Schematic of the adaptation model of Barkai and Leibler (1997) which exhibits perfect adaptation and is robust for a large range of parameter values. Here, E_m^u represents the receptor complex (MCP–CheA–CheW) in the mth methylation state which is either unbound u or bound with attractant o. Methylation of inactive receptors is by CheR (R) and demethylation of active receptors is by CheB$_P$ (B). (b) The integral feedback control mechanism which Yi et al. (2000) argued is an inherent characteristic of the Barkai and Leibler (1997) model. Here, I represents the model input which undergoes a functional change according to g. The resultant difference between the actual output (A) and its steady-state value (A_0) is defined by S, which is then integrated to give X which is fed back into the input stream. In comparison to Barkai and Leibler's model, the chemoattractant is the input, the output is the receptor activity and X represents the receptor methylation level. Both figures have been adapted from the original manuscripts.

unbound state, and could form any one of m methylated states. They were able to show that the model exhibited perfect adaptation and was robust for a wide range of parameter values. This latter result was important for this was the first model of perfect adaptation that showed robustness over a wide range of parameter values. Yi et al. (2000) stated that robustness was an inherent characteristic of an integral feedback control system, commonly observed in engineering systems, and showed that the model of Barkai and Leibler could be written in a form analogous to such a system. Hence, they argued, it was not surprising that the Barkai and Leibler model exhibited robustness. Furthermore, this work showed that the biological system operates like many man-made engineering systems, e.g., amplifiers.

Methylation-free adaptation has been the subject of work undertaken by Almogy et al. (2001). They considered modeling the phosphorylation and de-phosphorylation pathway within *E. coli*, but did not include receptor modification. Following experimental results reported by Wang and Matsumura (1997) they assumed either active CheZ or a complex formed of CheA$_s$[2] and CheZ (CheA$_s$–CheZ) dephosphorylated CheY$_P$, the latter more active than the former. Their model exhibits adaptation in the case where both CheZ and CheA$_s$–CheZ dephosphorylate CheY$_P$, but not when either acts individually. The model also exhibits robustness. One important feature of the model is the assumption that CheA molecules may dissociate preferentially from active receptors and associate with inactive ones. Interestingly, while both concentrations of CheY$_P$ and CheZ show

[2]CheA$_s$ (the subscript "s" denotes short) is a truncated version of CheA only found in species with CheZ as the CheY$_P$ phosphatase. It is expressed from an internal start site in CheA producing a protein lacking the histidine phosphorylation site, but containing all other CheA domains.

almost perfect adaptation, the concentration of CheA$_s$–CheZ does not, tending instead to a different steady-state value than that of the initial value. Almogy et al. (2001) note that methylation dependent or independent adaptation processes do not alone give perfect and robust adaptation, but combinations of them do. This, they argue, is the overall result of the interaction between the regulatory mechanisms seeking to adapt due to changes in one another.

More recent work by Mello and Tu (2003a) has modeled the effect of attractant binding through to the phosphorylation of CheY. The authors derive a set of governing ODEs by applying the law of mass action to the known reactions. Both attractant-bound and free receptors may undergo up to n of levels of methylation and demethylation, although the model analysis considers only a total of five states (one unmethylated, four methylated). From both model results and further analysis, Mello and Tu (2003a) define six conditions for perfect adaptation.

1. Attractant binding occurs on a faster timescale than methylation/de-methylation and phosphorylation/dephosphorylation. Thus, the attractant binding reaction can be assumed to be in quasi-equilibrium.
2. The association rates for methylation and demethylation, by CheR and CheB$_P$, respectively, are linearly related to the receptor activity and are zero for the unmethylated and the fourth methylation states. The dissociation rates[3] are independent of the receptor state, either bound or unbound.
3. The receptor activity of the unmethylated and fourth methylated receptors is independent of the receptor state.
4. The ratio of the rate of methylation for the nth methylation state by CheR to that of the $(n+1)$th de-methylation rate by CheB$_P$ is independent of the methylation state.
5. The rate of phosphorylation by CheA of CheB and CheY is proportional to the rate of CheA autophosphorylation.
6. The condition

$$\left(-\frac{[R^F]}{K^R}+\frac{[B^{PF}]}{K^B}\right)\sum_{n=0}^{4} P_n^2\left[T_n^F\right]=0,$$

must be met where $[R^F]$ is the concentration of free (not receptor bound) CheR, $[B^{PF}]$ is the concentration of free phosphorylated CheB, T_n^F is the concentration of free (nth methylated) receptor complexes, K^R and K^B are the dissociation constants for CheR and CheB, respectively, and P_n is the relative receptor activity ($0 \le P_n \le 1$).

Mello and Tu (2003a) explore the robustness of their model system by considering the effect of violating each of these conditions. They find that violating conditions (5) and (6) has little effect on adaptation, violating condition (4) leads to the largest deviation away from perfect adaptation, deviation away from perfect adaptation increases with increasing difference in activity of the zeroth and fully methylated receptor for condition (3) and

[3] In a reaction of the form $A+B \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C$, the dissociation constant is given by $K=\frac{k_-}{k_+}$. A small dissociation constant means A binds to B to form C more quickly than it is produced by the breakdown of C. A large dissociation constant means the opposite, i.e., C breaks down more quickly than it is formed.

10–15% deviation away from adaptation occurs when condition (2) is violated. Violation of condition (1) is considered to be unrealistic given experimental evidence. By incorporating receptor methylation governed by CheR and CheB_P as well as certain aspects of the phosphorylation cascade, the results in this model provide a clear and comprehensive check-list of conditions which are required in order for the signaling pathway within *E. coli* to exhibit robust adaptation.

4. Modeling the phosphorylation cascade

Increasing experimental knowledge of the phosphorylation cascade within *E. coli*, particularly during the 1990s, led a number of investigators to model the cascade.

Bray et al. (1993) were the first to produce a mathematical model, called BCT, of the phosphorylation cascade. As they note, their work is an extension of Block et al. (1982, 1983). The model extensions included incorporating the motor–CheY_P interaction and the effects of pausing between tumbling and swimming (the stationary motor case). The authors undertook numerical simulations of their ODE model of the phosphorylation reactions, but excluded receptor methylation. Autophosphorylation of CheA was considered to be governed by a Michaelis–Menten reaction, while phosphotransfer was governed by a first order reaction and dephosphorylation was assumed to be linear. Motor bias was considered to be dependent upon the number of CheY_P molecules which bind to the motor. The model was populated with data from the experimental literature and considered the motor response to step changes in aspartate (attractant) and nickel (repellent). Much of the model analysis attempted to reproduce the results of mutant experiments, the model results agreeing well with experimental evidence. However, the model was unable to reproduce the gain reported by Segall et al. (1986) and did not exhibit adaptation.

In 1997 Spiro and coworkers (Spiro et al., 1997; Spiro, 1997) sought to incorporate the effects of attractant binding, methylation, phosphorylation, and motor bias into a single model. Their model, as shown in Fig. 9, accounted for a total of three methylation states, the rate of phosphorylation increasing with each state. The methylation reaction was assumed to obey Michaelis–Menten kinetics, given the saturating effect of CheR on the receptors, while demethylation was described by first order reaction kinetics. While it was further assumed that the rate of demethylation for both attractant-bound and unbound receptors remains the same, the rate of methylation for attractant-bound receptors is much greater than for unbound receptors. While the Spiro et al. model was able to show adaptation, corresponding to various levels of attractant, it could not reproduce the experimentally reported sensitivity or gain, where gain was defined as $g = -db/d(\ln p)$, with b being the motor bias and p the rate of CheY phosphorylation. The authors do hypothesize that cooperation between certain elements in the biochemical pathway, e.g., binding of CheY_P to the motor and CheZ activity, could be enough to obtain the desired gain observed experimentally.

The effect of variation in the concentration of each intracellular protein on the overall concentration of CheY_P was the subject of work by the Bray group (Levin et al., 1998). This work was motivated by the observations of the Koshland group (Spudich and Koshland, 1976) who observed variational differences in the response of a cloned population of *E. coli* to attractant. At the time, they hypothesized that such a variation could be due to stochastic variation in a small number of molecules, but further investigation

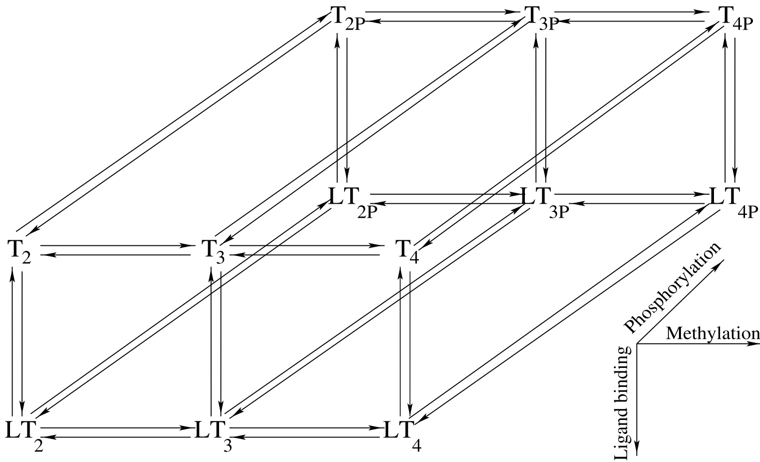

Fig. 9 The model of Spiro et al. (1997) which incorporates attractant binding, methylation and phosphorylation. Here T_i $(i = 2, 3, 4)$ represents the concentration of receptor complex (MCP–CheA–CheW) in the ith methylation state, LT_i represents an attractant-bound receptor, T_{iP} a phosphorylated receptor complex and LT_{iP} an attractant-bound phosphorylated receptor complex. Methylation is governed by Michaelis–Menten kinetics and is assumed to be more rapid when the receptors are phosphorylated than when they are not. Figure adapted from Spiro et al. (1997).

was not possible given the lack of detail on the biochemical work. Levin et al. considered the effect that both individual and collective protein variation (Tar, CheA, CheW, CheB_P, CheR, CheY, CheY_P) has on the CheY_P concentration. They developed two models which extended the early work of Bray and Bourret (1995) (see Section 5) to include the adaptation reactions of CheR and CheB_P. The first, the "fine-tuned model," incorporates a similar adaptation mechanism as proposed by Segel and Goldbeter (1986). The second, the "robust model", includes receptor state kinetics as detailed by Asakura and Honda (1984) and methylation as described by Barkai and Leibler (1997). Individual protein variation effects show that increasing Tar, CheW, or CheA concentrations cause the CheY_P concentration to pass through a maximum, while increases in CheR or CheY result in rising levels of CheY_P and increases in CheB or CheZ the reverse. The refined model does not exhibit adaptation of CheY_P, but the robust model does. Noting that protein concentration in eukaryotic cells can vary by a standard deviation of as much as 10% of the mean in any one population (Darzynkiewicz et al., 1982; Crissman et al., 1985), Levin et al. (1998) show that the spread in CheY_P concentration increases with the variance in protein concentration. The model shows that differences in bacterial behavior are, therefore, possible if the standard deviation of the mean in concentration is approximately 10%. The variation in CheY_P concentration is shown to be reduced for mutants lacking CheR and CheB or Tar, CheW, and CheA, given the lack of receptor modification and receptor complexes, respectively.

The Bray group (Morton-Firth and Bray, 1998) also considered a stochastic approach to modeling the intracellular signaling pathway. Their approach was motivated by the inability of previous deterministic models to reproduce the reported stochastic behavior of motor switching as reported by Block et al. (1982, 1983) and Eisenbach (1990), and was based on the two models of Asakura and Honda (1984) and Barkai and Leibler (1997).

The developed stochastic simulation programme StochSim considers individual protein molecules and the interactions which occur between them. The program chooses two molecules at random, considers the likelihood of interaction between them and assigns a reaction rate, randomly chosen from a predefined probability density distribution specific to the respective reaction, should the reaction be deemed to take place. StochSim can also account for both uni- and bi-molecular reactions as well as for molecules in various different states, e.g., attractant-bound or unbound receptors. Morton-Firth and Bray (1998) applied StochSim to the methylation and phosphotransfer reactions governing bacterial chemotaxis, where the resulting bias in terms of CheY_P was defined by a Hill function similar to that of Eq. (2). They found that the concentration of CheY_P fluctuated around the deterministically calculated values, even when the system was in a steady-state, the fluctuations decreasing with increasing number of molecules. The length of the fluctuations (∼80.7 ms) was found to be less than that of clockwise and counter-clockwise rotations of the flagella (∼2.6 s). They concluded that while their model was unable to account for motor switching, filtering of the CheY_P fluctuations could produce temporal run and tumble distributions closer to the experimentally observed behavior, thereby raising the possibility that the flagellar motors may filter the CheY_P signal.

Recent work by Rao et al. (2004b) has focused on comparing the intracellular signaling pathway of *E. coli* and *Bacillus subtilis* (*B. subtilis*). *B. subtilis* has a similar intracellular pathway to *E. coli* but, importantly, has a number of "extra" chemotaxis signaling proteins (Ches), namely, CheC and CheD, localized to the receptors and responsible for negative and positive receptor methylation, respectively, and CheV, which is predicted to down-regulate receptor activity by impeding CheW function. Rao et al. (2004b) modeled the intracellular pathway of *E. coli* by combining the two-state model of Barkai and Leibler (1997) with the phosphorylation cascade model proposed by Sourjik and Berg (2002a), the latter extended to include the phosphorylation of CheB. The *B. subtilis* pathway was modeled on a variation of the *E. coli* pathway in which receptors were considered to exist in either an active, inactive, weakly active, or weakly inactive state. Results from the two models showed that both *E. coli* and *B. subtilis* can adapt to external stimuli and the respective pathways are robust, a somewhat unsurprising result given the model is based upon that of Barkai and Leibler (1997). *B. subtilis* was found to be no more sensitive than *E. coli* to changes in attractant concentration. Rao et al. (2004b) also showed that deletion of CheR and/or CheB (methylation independent chemotaxis) in *B. subtilis* still led to oscillations in CheY_P due to the presence of the CheV and CheY feedback loops. From this result, they hypothesized that the CheY pathway provides adaptation and methylation was later added (as an evolutionary step) to provide a more robust, adaptive pathway in *B. subtilis*. In all, whilst both species of bacteria have related intracellular signaling pathways it is unclear why *B. subtilis* is apparently far more robust than *E. coli*.

5. Sensitivity and gain—the role of receptor clustering

Surprisingly, while many of the models detailed in the previous sections have increased our understanding of the role of methylation and phosphotransfer in respect of bacterial excitation and adaptation, they repeatedly fail to reproduce the experimentally observed sensitivity and gain (Segall et al., 1986; Sourjik and Berg, 2002b). In more recent years, experiments have shown that receptor clustering is a possible mechanism for explaining

this sensitivity and gain. Mathematical models have played an important role in assessing the plausibility of such a mechanism, the details of which are discussed in this section.

5.1. Early work

Work by Bray and Bourret (1995) on receptor biochemistry sought to elucidate the binding steps involved in the formation of the Tar–CheW–CheA complex (denoted TTWWAA for short, where CheW is a monomer and CheA is a dimer), receptor complex. By considering an ODE network model of the various formations that the complex could assume, they found from a total of ten possible complexes that the smallest working reaction network consisted of six complexes. This receptor formation model was then coupled to their 1993 phosphorylation model (Bray et al., 1993) to investigate how differences in corresponding receptor complex formation affected the phosphorylation rate of CheY, and hence the respective motor bias. By considering seven, ten, and twelve reaction networks, Bray and Bourret (1995) showed the bias was sensitive to the concentrations of Tar, CheW and CheA and the bias was lowest when the concentration of the complex TTWWAA was at a maximum. All of the networks showed that the bias differed from one only over small changes in the concentration of CheW. Bray and Bourret (1995) noted that the binding constants for all of the receptor forming networks were three to four orders of magnitude smaller than the experimentally determined values. Fixing the rates at their experimental levels failed to produce the expected bias.

The work of Bray et al. (1998) made an important contribution in understanding the role receptor clustering may play in providing a bacterium with the ability to sense small changes in its extracellular environment. They hypothesized that receptors do not work individually, but collectively. Although receptor clustering had been demonstrated experimentally as early as 1993 (Maddock and Shapiro, 1993) Bray et al. (1998) provided a theoretical interpretation of the effect such clustering may have on the sensitivity and response of a bacterial system. By considering each receptor as a discrete entity, which existed in either an attractant-bound or unbound state (the latter being more active than the former), they assumed that the inactivation of a single receptor caused a number of its neighbors to become inactive. Thus, the inactivation of a single receptor is magnified and can inhibit the phosphorylation cascade to the flagellum. By considering an array of 2,000 receptors, Bray et al. (1998) showed a number of important results. Firstly, the ability of the receptor network to respond to attractant decreased as the number of receptors which were inactivated by binding increased. This is a result of the majority of unbound receptors within the network assuming the reduced activity of the bound state. Hence, sensitivity increases, but with a loss in attractant range to which the bacteria could respond. The solution to this is for the bacteria to adapt, thereby freeing receptors to respond to further changes in attractant concentration. Bray et al. (1998) further showed that the best bacterial response (a range of five orders of magnitude) is achieved when receptor clustering is maximized at low attractant concentration and minimized at high concentrations.

5.2. The Ising model approach

Following the work of Bray et al. (1998), an increasing number of models, using different modeling approaches, have sought to elucidate the effect of receptor coupling on the

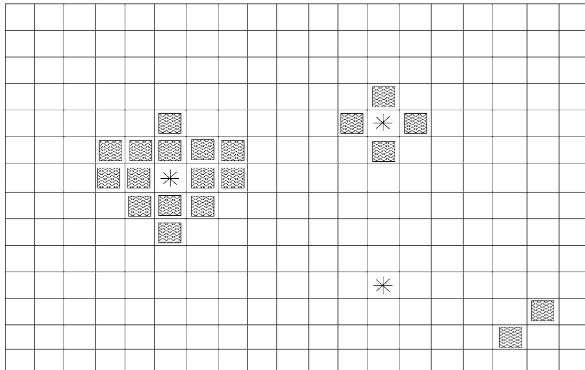

Fig. 10 A schematic representation of the Ising model applied to receptor–receptor interactions on a two-dimensional lattice array. Receptors exist in either an inactive (patterned box) or active state (clear box) and can flip between them randomly (thermal fluctuations). The binding of an attractant molecule inactivates the bound receptor and surrounding receptors become inactive dependent upon the coupling strength between them. The four cases above show: significant coupling (middle left), some coupling (upper right), no coupling (bottom right) and random thermal fluctuations (far lower right). Note that attractant-bound receptors (denoted by a star) are in this case inactive, but for diagrammatic purposes are not shaded.

bacterial response. A number of authors (Shi and Duke, 1998; Duke and Bray, 1999; Shi, 2000, 2001, 2002) have applied the Ising model (Toda et al., 1983) to study receptor–receptor interactions and the effect of attractant binding on the activity of a receptor array. The Ising model is a statistical mechanics description of how an array of particles, for instance electrons, behave when a magnetic field is applied to them. Most importantly it allows for a description of particle coupling, i.e., the effect of the state of one particle being allowed to change the state of neighboring particles, dependent upon the strength of a coupling energy.

The Ising model is, therefore, an appropriate analog of receptor–receptor coupling as shown in Fig. 10; the average magnetization of a lattice of particles corresponds to the activity of the receptor array and the strength of the local magnetic field represents the attractant concentration. The receptors exist in either one of two states (commonly denoted $i = \pm 1$) between which they flip. The energy of the array is defined by a Hamiltonian

$$H(t) = -\sum_{\langle ij \rangle} J_{ij} S_i S_j - \sum_i B_i(t) S_i, \tag{3}$$

where S_i and S_j represent the spin of the ith and jth particles (in this case, the ith and jth receptor dimers), respectively, J_{ij} is the coupling strength between them (generally taken to be constant) and $B_i(t)$ is the strength of the local magnetic field (in this case attractant concentration). In the absence of attractant binding (the applied magnetic field), the receptors are free to flip randomly between their states. These are thermal fluctuations or noise. Given the large difference in timescales of attractant binding, phosphorylation, and adaptation, these fluctuations can be considered to be in quasi-equilibrium. Ising models have also been used to describe receptor–receptor interactions in other cellular systems (Guo and Levine, 1999, 2000).

Shi and Duke (1998) were the first to apply the Ising model to receptor–receptor interactions in bacterial chemotaxis. Using mean-field analysis and considering various limits of the thermal fluctuation, their work showed that the sensitivity of the receptor array depended greatly on the strength of the receptor–receptor coupling, but less so on the strength of the attractant binding. While adaptation is also included in the model by simply inducing a "magnetic field" opposite to that induced by attractant binding, it is not studied in detail. Shi and Duke (1998) note that receptor–receptor coupling enhances the sensitivity of the bacterium in detecting attractant gradient changes.

Duke and Bray (1999) undertook Monte Carlo simulations of the model of Shi and Duke (1998) using an array of 50 × 50 receptors. Here, each receptor was coupled to its four nearest neighbors and the paper considered the array response to varying levels of attractant over differing timescales. Results showed that the receptor array could detect a relative change in attractant concentration over five orders of magnitude. A 30% reduction in signal is achieved when doubling the attractant concentration over four orders of magnitude, in comparison to 10% for an uncoupled array of receptors. Duke and Bray (1999) argued that a linear system, i.e., one in which the receptors are uncoupled, can not give the required sensitivity at high concentrations given the difference in coupling energies is too large and can not be accommodated without affecting the response at lower levels of concentration. However, in the nonlinear coupled model, the number of receptors which have been modified by methylation and are attractant-bound cancel each other out and the receptors are not biased toward an active or inactive state. They are thus free to be influenced by further localized receptor coupling at higher attractant concentrations. It was further noted that the geometry of the array is important—the receptors need to be arranged in a well-ordered lattice given the sensitivity of the response to the coupling energy.

Shi (2000) later extended this earlier work (Shi and Duke, 1998) by incorporating the effects of CheB_P and CheR. Here, an adaptive Ising model was used in which a source of negative feedback from the receptor system, in this case the effects of methylation by CheR and demethylation by CheB_P, was fed back into the output from the system (the phosphorylation of CheA_P). Attractant binding to the receptors is assumed to be the input. In effect, this adds a second governing equation to Eq. (3)

$$\frac{dS_i}{dt} = -\sigma S_i(t - t_r), \tag{4}$$

where t_r is the delay time, the sum of the time taken for demethylation and phosphoryl transfer from CheA_P to CheB. Given the difference in timescales of attractant binding, phosphorylation, and adaptation, Shi (2000) coarse-grained the governing Hamiltonian and showed that the feedback effect of CheR and CheB_P was sufficient to naturally attenuate the receptor activity back to zero following an initial stimulus, i.e., perfect adaptation. They also noted that the feedback relaxed certain conditions on the strength of the receptor–receptor coupling, thus making the model more robust. The model also remained sensitive to repeated changes in the attractant concentration.

Shi later considered (Shi, 2001) how the models presented in Shi and Duke (1998) and Shi (2000) could be compared with experimental findings. He compared theoretical predictions of the ratio of attractant binding to receptor–receptor interactions, the adaptation time, and the ratio of pre- versus post-stimulus CheA phosphorylation with experimental

evidence and found that all agreed well with experiment. Thus, he argued, the statistical mechanics approach adopted by Shi and Duke (1998) and Shi (2000) provides a good and plausible description of receptor–receptor interactions. It was noted that unlike Hill functions, where the physical meaning of non-integer values of the Hill coefficient (Murray, 1993) are unclear, each of the expressions obtained from this statistical mechanics approach are physically descriptive.

The effect of receptor movement on the receptor array activity was considered in Shi (2002). Using mean-field analysis, Shi (2002) obtained expressions for the receptor array activity and the average number of receptors. Monte Carlo simulations showed that clustering is important in order for there to be interactions amongst the receptors, but that the correlation amongst receptors died off exponentially quickly in terms of interactions with non-nearest neighboring receptors, i.e., nearby receptors are closely correlated and those at a distance are not. These results were unaffected by the receptor–receptor coupling strength and the fraction of attractant-bound receptors. It was noted that receptors in the same state clustered together to decrease the total energy of the system, and thus Shi (2002) argued it is the receptor state, rather than whether they are attractant-bound or not, which is most likely to affect the interaction between receptors.

5.3. Recent modeling work

The Bray group (Morton-Firth et al., 1999) extended the work of Barkai and Leibler (1997) by modeling the Tar receptor and the related methylating and phosphorylating reactions using StochSim (Morton-Firth and Bray, 1998; Novère and Shimizu, 2001). Like Barkai and Leibler (1997), Morton-Firth and Bray (1998) assumed that only active receptors were demethylated by CheB_P. Early simulations showed that if CheR is active at saturation then adaptation cannot be exact, i.e., there is a 6–7% variation in post- versus pre-stimulus levels of CheY_P. They also noted that the experimentally determined concentration of CheR was not high enough for the Michaelis–Menten kinetics assumption of the substrate being in excess of the enzyme to hold for a receptor–CheR interaction. It was thus assumed that CheR only affects inactive receptors, which also meets the requirement that methylation is activity dependent. Whilst model results for the duration of bacterial response agree well with experimental data over four orders of magnitude, the model fails to show sensitivity at low aspartate concentrations and shows a 2% error in the pre- versus post-stimulus levels of CheY_P. The adaptation error was found to arise from: (1) the level of activation of the receptor complex (receptors bound with either CheB or CheR can not bind CheR or CheB, respectively, thus a number of inaccessible receptors is created. This means the rates of methylation and demethylation are not exactly proportional to the number of active and inactive receptors, and hence any error in adaptation will increase with increasing attractant concentration); and (2) deviations in the rate of CheY phosphorylation, due to differences in phosphorylation and dephosphorylation of active and inactive receptor complexes.

Morton-Firth et al. (1999) also considered the effect of aspartate binding on methylation of the Tar and Tsr receptors. As expected, methylation of the Tar receptor increased with aspartate binding, but unexpectedly decreased in the case of the Tsr receptor. They noted that aspartate binding to the Tar receptors lowers CheB_P affinity for the Tar receptors such that it is released into the cytoplasm. The reduction in CheB_P formation due to the phosphotransfer from CheA_P means the increase in CheB_P occurs over a very short

timescale, the same timescale over which there is an observed decrease in the Tsr receptor methylation. Analysis of whether methylation of Tar receptors occurs in a sequentially ordered or independent (random) manner showed that ordered methylation is important for adaptation; the randomly methylated systems failed to adapt and showed a 50% deviation in pre- versus post-stimulus activity.

Levin et al. (2002) used deterministic simulations and StochSim (Morton-Firth and Bray, 1998; Novère and Shimizu, 2001) to simulate the diffusion and binding of CheR within a cluster of receptors. They devised a generalized "dumbell" model of enzyme-receptor binding, whereby each end of the molecule contains a binding site separated by a flexible chain. The probability of particle attachment was explored when either one or both ends of the molecule were attached to a surface. A more detailed analysis of this situation was recently provided by Windisch et al. (2006). The attachment and partial detachment of the molecule from the surface meant it was able to wander across the receptor surface, a process termed "molecular brachiation." Levin et al. (2002) noted that this generalized model was analogous to CheR binding to and detaching from a receptor cluster and showed that when the dissociation constant is of the order of that observed experimentally, the CheR molecules had sufficient time to both visit and methylate the receptor sites. When receptor–molecule association was increased to mimic binding to a corresponding single high-affinity site, the delayed time meant fewer receptors were visited and was not in agreement with experimental evidence. Levin et al. (2002) further noted that CheB was likely to behave in a similar manner. However, the modeling could not account for the effects of receptor size or shape and the likelihood of some receptors not being able to bind CheR, i.e., Trg and Tap. They also noted that the dissociation constant may vary with attractant binding; CheR preferentially methylates receptors that inactivate CheA while CheB_P preferentially demethylates receptors that activate CheA. This may also lead to the two enzymes excluding one another from different domains.

Bornhorst and Falke (2003) have addressed the issue of the inability of a two-state model of receptor modification to predict the experimentally observed changes in attractant affinity for varying ranges of adaptation. The general two-state model describes receptors as existing in either an on- or off-state. The authors propose a model whereby altering the attractant affinity of the receptor off-state gives an appropriate fit to the experimental data. They conclude that this result produces a heterogenous off-state population of receptors and note that changes in attractant-receptor affinity for on-state receptors, maximal kinase stimulation of either state, receptor–receptor interactions, or the formation of a receptor-kinase signaling complex do not provide adequate matches of model output with experimental data.

The work of Shimizu et al. (2003) considered a spatially extended version of the StochSim algorithm coupled with an Ising type model of receptor–receptor interactions. Their model included a description of methylation and adaptation based upon the work of Barkai and Leibler (1997) in which receptors can be in up to five different methylation states. Shimizu et al. (2003) examined the importance of the receptor array geometry (hexagonal, trigonal, or square) and size on the overall sensitivity, gain and signal-to-noise ratio of the receptor array. They found that the array sensitivity increases up to a certain lattice size and gain is largest for trigonal lattice arrays, i.e., when the number of nearest neighbors is highest. However, increasing the interactions amongst receptors decreases the signal-to-noise ratio, but increases the gain. Both the signal-to-noise ratio and gain are insensitive to the attractant concentration. Shimizu et al. (2003) chose the most stable

array configuration, a square lattice, and showed that coupling amongst receptors gives a better comparison with experimental data (motor bias) than the uncoupled case. Gain is larger for smaller changes in receptor occupancy when the receptors are coupled. Coupling is also shown to be important in describing the observed overshoot phenomenon. The uncoupled model can only produce overshoot in cases where the methylation and demethylation levels are increased 100-fold, whereas overshoot is easily reproduced for only small changes in methylation and demethylation in the coupled model. Coupling between receptors was also predicted to generate spatial patterns of methylation in the two-dimensional array of receptors, something that would be impossible to test experimentally with present techniques. Even with coupling there are still, as Shimizu et al. (2003) observe, significant differences between the model and the experimentally reported gain.

Bray and Duke have summarized experimental evidence indicating that conformational changes can propagate through protein lattices (Bray and Duke, 2004). They propose a statistical mechanics model, analogous to the Ising model, to consider the dynamical propagation of conformational change within a ring of proteins (Duke et al., 2001). Their model shows that conformational spread progresses over a number of steps, i.e., the ring does not automatically switch between an "off" and "on" state, but varying numbers of proteins become turned "on" by propagation or "diffusion" of a single signal through neighboring proteins.

Goldman et al. (2004) undertook Monte Carlo simulations of a lattice gas model of protein–protein (receptor–receptor) interactions with a defined two-dimensional lattice. Their model considered the effects of geometry between the individual proteins, i.e., the bond angle, and also the overall geometry of the lattice array (trigonal, square, or hexagonal). They showed that protein clusters of a single species formed nonlinearly and the bonding and clustering of the proteins was spread heterogenously across the two-dimensional space. As with the previous Ising models (Shi and Duke, 1998; Duke and Bray, 1999), their work demonstrated that this clustering depended upon the local protein concentration and the bond strength between each protein. This result was unaffected by the different lattice types. In the case of the two protein species within the two-dimensional lattice, changing the initial ratio between the two species affected the number of bonds formed, and thus the respective clustering. From this result, Goldman et al. (2004) were able to determine a maximum aggregation ratio.

Recent work by a number of authors (Albert et al., 2004; Mello and Tu 2003b, 2005; Mello et al., 2004; Rao et al., 2004a) has sought to elucidate the mechanisms responsible for receptor interactions inferred by the work of Sourjik and Berg (2002b, 2004). Sourjik and Berg (2002b) studied the sensitivity of the chemotactic response, in particular focusing on the role of CheR and CheB$_P$. Their work suggested that signal amplification is dependent upon CheB$_P$ and the degree to which receptors are modified. They also noted that receptor–receptor interactions may provide an explanation for the observed sensitivity of the cell. In Sourjik and Berg (2004), they studied the effect on CheA activity that variations in receptor concentrations (Tar and Tsr) had, both individually and together. They noted that the activity of the cells containing both Tar and Tsr was greater than could be explained by the activation of just one receptor type when either aspartate or serine was present. They inferred coupling between Tsr and Tar receptor clusters to explain the observed activity.

Mello and Tu (2003b) and Mello et al. (2004) extended the Ising model to include a description of receptor methylation and demethylation. Their model does not include a

description of CheR or CheB_P, but assumes that receptors can occupy up to five different methylation states. Following on from the work of Barkai and Leibler (1997), Mello and Tu (2003b) and Mello et al. (2004) assumed that only inactive receptors could be methylated and active ones demethylated. Their initial work (Mello and Tu, 2003b) focused on reproducing the experimental results of Sourjik and Berg (2002b) for both mutant (CheR and CheB deleted) and wild type *E. coli*. Undertaking mean-field analysis of their model, they showed that mutant results agreed well with the experimental data. However, matching the wild-type data required different parameters and this, the authors noted, was most likely due to the multiple methylation levels of the wild-type bacteria versus the single methylation levels of the mutants. Mello and Tu (2003b) noted that the interesting result of Sourjik and Berg (2002b) on Tsr receptor activity at concentration levels below which such receptors are generally active can be explained by strong coupling between Tar and Tsr receptors and the associated methylation of each. Analysis of the sensitivity and gain showed that the model could only give a 20-fold increase in gain versus the 36-fold increase reported by Sourjik and Berg (2002b). It was also shown that attractant binding is dependent upon receptor activity; a result of methylation and the assumption that attractant can only bind active receptors and release from inactive ones.

Mello and Tu (2003b) further extended their work in Mello et al. (2004) where they undertook both mean-field analysis and Monte Carlo simulations of the model in which multiple methylation states were allowed. An important finding of this later work was that receptor coupling has a large effect on the activity of the array for differing methylation levels. Stronger coupling leads to a steeper response versus attractant curve. In order to obtain the experimentally observed range of sensitivity across four orders of magnitude, Mello et al. (2004) noted that the receptor-attractant dissociation constant must depend upon the methylation level of the receptors, although they do not provide explicit model examples of this. The authors also addressed the issue of overshoot following adaptation and showed that it may be a result of signal amplification; a change in methylation for one receptor is amplified by coupling amongst the receptors. It was further shown that model estimates using the mean-field analysis compared well with experimental data, and that numerical solutions of the full model agreed with those of the mean-field analysis.

The recent work of Albert et al. (2004) has focused on an ODE model of receptor "team" formation to explain the observed gain within *E. coli*. The authors argue that the Ising model approach to receptor clustering does not allow the form of the interaction between receptors to be specified, and thus experimental justification is difficult. Citing the experimental work of Ames et al. (2002) and Kim et al. (2002), Albert et al. (2004) assume that attractant binding destabilizes teams of receptor dimers within the receptor clusters. The groups consist of either individual receptor units (homodimers), two dimers (two-fold dimers) or three dimers (trimers of dimers). The trimers of dimers are assumed to form stable complexes when coupled with CheA and CheW and are assumed to be the model output which drives autophosphorylation of CheA. Albert et al. (2004) construct a network diagram of the respective pathways which form the different possible attractant-bound and unbound receptor dimer teams. The model is only valid for the short timescale of attractant binding, and thus does not include a description of methylation or the phosphorylation cascade. Model results show good qualitative agreement with the experimental work of Li and Weis (2000), Bornhorst and Falke (2001), Levit and Stock (2002) and Sourjik and Berg (2002b). Importantly, the theoretically determined Hill coefficients for the description of kinase activity as a function of attractant concentration

are more closely aligned with the experimentally observed range. In order to explain the work of Sourjik and Berg (2002b), with respect to the receptor activity of Tar and Tsr, Albert et al. (2004) extend their model to differentiate between the two receptors, but allow receptor team interactions. They note that the analysis of a network of trimers of dimers is unduly complicated, and hence only consider up to two-fold receptor dimer formation. Model comparison with the experimentally observed sensitivity of CheR–CheB mutants and wild-type bacteria in Sourjik and Berg (2002b) shows good agreement.

Rao et al. (2004a) considered a two-state allosteric model of receptor clustering based on the work of Monod et al. (1965) and Shimizu et al. (2000). Their model focuses on describing the activity of receptor complexes (trimers of dimers) and the activity between them. The receptors are assumed to exist in either a relaxed or tense (active) state, the more stable state assisted by the association of CheW and CheA with the receptor clusters. Receptor methylation increases the level of receptor activity whilst attractant binding increases the likelihood of destabilizing the complexes, returning the complexes to the relaxed state. Rao et al. (2004a) note that attractant sensitivity is determined by the cooperative interaction between the receptor complexes and shows that the lowest methylation state has the greatest effect on increasing the number of active receptors. They model receptor clustering on a hexagonal lattice by using the Metropolis algorithm approach of Bray and Duke (2004) and assume that the receptor–receptor signaling is localized. Rao et al. (2004a) compared their model results with those of Sourjik and Berg (2002b) for mixed receptor types: two Tar and one Tsr receptor; and one Tar and two Tsr receptors. They found that both configurations fit well with the experimental data and further noted that their results agreed well with those of Mello and Tu (2003b). Other findings were that increased receptor methylation stabilizes the complexes (a result of the reduced attractant affinity for higher methylated receptors), and receptor cluster size is determined by the local concentration of receptor type, CheW and CheA.

A number of recent models (Mello and Tu, 2005; Skoge et al., 2006; Endres and Wingreen, 2006) have used the Monod–Wyman–Changeux (MWC) (Monod et al., 1965) model to understand receptor–receptor interactions. The MWC model accounts for clusters of receptor types rather than the interaction between individual receptors, thus details on the interaction between individual receptors is not required. The MWC model requires defining an energy Hamiltonian, where each receptor state, e.g., active or inactive, has a different corresponding energy level. Mello and Tu (2005) used the MWC model to reproduce the response of mutant and wild-type bacteria to serine and methyl-aspartate as reported by Sourjik and Berg (2004). Their model included a description of Tar and Tsr receptors and they found it could account for each of the fourteen response curves reported by Sourjik and Berg (2004). The authors also explored the response when a mixture of serine and methyl-aspartate is applied to the receptor cluster. They found that when both attractants are present one suppresses the response of the receptor cluster to the other attractant. Furthermore, the presence of one attractant increases the sensitivity of the receptor cluster to the other attractant. The receptor response to the two attractants is not additive.

Recent work by Skoge et al. (2006) has compared model predictions of one- and two-dimensional Ising models and those of the MWC model with the experimental results of Sourjik and Berg (2002b, 2004). They show that the MWC model provides a better prediction of the differing responses of wild-type, CheR mutant and CheR–CheB mutant strains to attractant concentration than do Ising models. Given the dependency of the

MWC on the number of receptors in either an on- or off-state, the authors infer that clustering of each type of receptor is required in order to reproduce the observed experimental differences in the mutant and wild-type bacteria. The importance of differing attractant dissociation constants for receptors in either state is discussed as is the sensitivity at low attractant concentrations.

Endres and Wingreen (2006) have also applied the MWC model to receptor clustering. The experimental basis for their work is that of Li and Hazelbauer (2005) who showed that CheR and CheB_P act on groups of five to seven receptors, thus forming "assistance neighborhoods." Endres and Wingreen (2006) adopt the adaptation model of Barkai and Leibler (1997) in allowing CheR to only methylate inactive receptors and CheB_P to demethylate active ones (see Section 6) and model receptor groups consisting of six receptors. Both Tar and Tsr receptors, and their response to asparate and serine, respectively, are included in the model. Endres and Wingreen (2006) show that the resultant mixed cluster types are both highly sensitive and exhibit the experimentally observed adaptation response, without the need for changing attractant-binding affinities. They note two different adaptation responses at high attractant concentrations: (i) cessation of receptor response due to receptor saturation by the attractant; or (ii) full receptor methylation which causes them to stop adapting. By modeling separate clusters of both Tar and Tsr receptors, they argue that even at high aspartate concentrations when the Tar receptors are fully methylated, and thus unable to sense changes in attractant concentration, the low-affinity binding of asparate to Tsr means there are still enough free receptor sites to initiate receptor signaling. Thus, the bacterium can still respond to attractant concentration changes.

6. The role of methylation in intracellular signaling

The models which have included a biochemical description of methylation, i.e., involving CheR and/or CheB_P, will be described briefly give its importance in adaptation. The model descriptions of Barkai and Leibler (1997), Spiro et al. (1997), Morton-Firth and Bray (1998) and Mello and Tu (2003a) have made assumptions regarding whether CheR and CheB_P affect active or inactive receptors as detailed in Table 1. Experiments have not been done to determine whether either protein is solely targeting receptors in either state or receptors in both states. While other models have included general descriptions of methylation or covalent modification (Asakura and Honda, 1984; Hauri and Ross, 1995), they have not explicitly included descriptions of the methylating and demethylating proteins and their effect on differing receptor activity.

Table 1 A comparison of models which have made different assumptions regarding the methylation and demethylation of active or inactive receptors. "All" here indicates that all receptors, irrespective of whether they are active or inactive, are affected by either CheR or CheB_P as indicated

Reference	CheR		CheB_P	
	Inactive	All	Active	All
Barkai and Leibler (1997)		√	√	
Spiro et al. (1997)		√		√
Mello and Tu (2003a)	√		√	
Morton-Firth and Bray (1998)	√		√	

Table 1 shows that the model of Barkai and Leibler (1997) is the most general and places the least restrictions on the mechanism of adaptation required for robustness. The more specific models of Mello and Tu (2003a) and Spiro et al. (1997) specify that methylation and demethylation occur in a sequential order which, as noted by Asakura and Honda (1984), is required for adaptation. It is further noted that Morton-Firth et al. (1999), Yi et al. (2000) and Mello and Tu (2003a) show that perfect adaptation is only possible if the methylation and demethylation rates depend upon receptor activity.

Each of the papers discussed here has used different models of the methylation pathway, encompassing various modeling approaches and has focused on slightly different aspects of methylation and demethylation. The analysis and reported outcome of each model differ, making a detailed and meaningful comparison difficult.

7. Spatial modeling of the intracellular signaling cascade

Apart from the work of Lipkow et al. (2005), no other mathematical modeling work on intracellular signaling has considered the importance of spatial localization and diffusion of the cytoplasmic proteins on network performance. Lipkow et al. (2005) focused on the distribution and diffusion of CheY, $CheY_P$, and CheZ in a single *E. coli* cell. Three-dimensional stochastic simulations of individual protein molecules were undertaken using the Smoldyn program based on Smoluchowski dynamics (Andrews and Bray, 2004). In respect of CheZ localization, Lipkow et al. (2005) showed that when the protein is restricted to the ends of the cell, where the receptors and high concentrations of $CheA_P$ are found, the concentration of $CheY_P$ is constant throughout the cytoplasm. However, when CheZ is allowed to diffuse freely through the cytoplasm an exponential gradient in $CheY_P$, highest at the anterior end, is seen across the length of the cell. Simulations show that the average lifetime of a $CheY_P$ protein is 0.1 s, but this may vary depending on the spatial position of CheZ, i.e., whether at the poles or evenly distributed throughout the cytoplasm. Lipkow et al. (2005) also analyzed the effects of receptor-to-motor separation and macromolecular crowding. Analysis of motor position revealed that when CheA is phosphorylated at the anterior end, a wave of $CheY_P$ travels between the receptors and the motors. To reproduce the effects of macromolecular crowding (anomalous diffusion), Lipkow et al. (2005) introduced arrays of impenetrable blocks within the cytoplasm. This increased the steepness in the $CheY_P$ concentration gradient when CheZ is distributed evenly throughout the cytoplasm. Lipkow et al. (2005) hypothesize that this is due to retardation of diffusion of $CheY_P$ away from the poles of the cell.

Lipkow has recently extended her previous model to introduce the novel concept of dynamic protein localization (Lipkow, 2006), demonstrated with CheZ and $CheY_P$. The work notes that a variant of CheA ($CheA_s$) is important for the localization of CheZ near the receptor poles. The model assumes that CheZ location is dependent upon the concentration of attractant or repellent. In the case of an attractant gradient, where a decrease in $CheY_P$ results, CheZ molecules are free to diffuse in the cytoplasm of the cell. When a repellent is detected, leading to increased levels of $CheY_P$, CheZ, and $CheY_P$ oligomerize with CheAs at the poles. The clustering of CheZ leads to enhanced dephosphorylation of $CheY_P$, thus introducing a negative feedback upon the concentration of $CheY_P$ within the cell. Lipkow notes that this leads to a robust "second tier" of adaptation; this feedback system can explain the overshoot of $CheY_P$ levels in CheR/CheB mutant bacteria,

i.e., adaptation which is methylation independent as has been observed experimentally by Stock et al. (1985).

8. The physics of individual cell chemotaxis

Since the work of Berg and Purcell (1977), which was based on physical arguments to describe how bacteria sensed their external environment and responded to it, a number of papers have focused on similar issues. We detail the work of these papers in this section.

The early work of Berg and Purcell (1977) focused on the physics of attractant particles interacting with receptors on the bacterial surface. Berg and Purcell obtained estimates of the effective absorption by specific receptors, the effect of initial deflection from the surface followed by secondary absorption, bacterial movement on uptake of attractant, the effects of possible local stirring by bacterial movement and the benefits which swimming imparts on the interactions between a bacterium and its environment. Their work showed that swimming (using flagella) is highly useful in non-uniform environments of attractant. If the attractant is spatially non-uniform, stirring of the bacterium's local medium as a method of increasing the likelihood of detecting attractant is too energy inefficient except if the medium viscosity is high. Energy expenditure is highly dependent upon the bacterial radius and calculations showed that receptors may only necessarily cover a small region of the membrane surface.

Strong et al. (1998) and Bialek and Setayeshgar (2005) extended the work of Berg and Purcell (1977). Strong et al. (1998) considered the optimal bacterial strategy for *E. coli* when the signal-to-noise ratio (SNR) is low or high. A high SNR means a bacterium can evaluate the chemotactic gradient in the direction it is swimming in a shorter time than any other processes it is undertaking. A low SNR means the bacterium needs to filter out background noise in order to determine the signal and also requires a threshold attractant level at which to respond. Strong et al. (1998) note that although the high SNR problem may not necessarily be realistic it is more mathematically tractable in terms of possible solutions. Estimates of the time spent tumbling and the tumble angle as a function of the tumbling time are derived. The low SNR problem is more difficult mathematically and the authors note that by assuming: (i) completely disorientating tumbles are of finite duration and; (ii) instantaneous tumbles do not completely disorientate the bacteria, asymptotic solutions to the filter behavior can be found for various timescales. The authors discuss ways of comparing their theoretical predictions with experiment.

The work of Bialek and Setayeshgar (2005) focused on deriving an accurate limit to which bacterial receptors are able to respond to a change in concentration of signaling molecules. Their work was motivated by the claim of Berg and Purcell (1977) that the bacterial ability to sense a change in the attractant gradient is independent of the size of the receptor cluster detecting the change and the assumptions underlying this finding. Bialek and Setayeshgar (2005) show that a noise level can be defined which is independent of kinetic parameters and that there exists a minimum noise "floor" due to diffusion of attractant molecules. Comparison with experiment shows that bacterial systems operate near this theoretically derived limit of diffusive noise.

9. Discussion and future directions

Mathematical modeling is a useful tool which can provide insight into often complex biological problems. The case of bacterial chemotaxis is a good example. This review has highlighted the development of mathematical models over the past thirty years that have aided in our understanding of intracellular signaling within bacteria. This has included understanding the mechanisms responsible for providing the initial excitatory response, adaptation, sensitivity, and gain. Of particular note is the elucidation that receptor–receptor interactions can explain the observed sensitivity and gain of the system. Mathematical modeling in this area has allowed a number of different hypotheses to be tested, using various mathematical techniques, and where necessary, the model outcomes to be examined experimentally. Such methodology is not uncommon in applied mathematical modeling of real world phenomena: models based on current knowledge can be used to test theoretical hypotheses without the need for often expensive experiments.

This overview has highlighted a number of characteristics which are inherent to a number of these models, in particular those which have focused on adaptation and modeling the phosphorylation cascade. Detailed notes on each model discussed are given in Appendix A and an overview of model properties is provided in Appendix B.

General assumptions which have been made by a number of modelers include the following.

- Attractant is assumed to be in excess and binding is assumed to be rapid.
- One of the most common receptor types to be modeled is the Tar receptor. This is not unexpected given that a large degree of the experimental literature has focused on the response of *E. coli* to asparate which is detected by this receptor. Tar is also one of the most abundant receptors within the cytoplasmic membrane.
- In models which have focused on adaptation and/or the phosphorylation cascade, receptors are commonly modeled as complexes consisting of MCP, CheW, and CheA. This is justified by the tight association of CheW and CheA to the MCP receptors.
- Descriptions of motor bias. Given the limited detail available on the interaction of both CheY_P and CheY with the cytoplasmic FliM end of the flagellar motors and that this interaction may be quite complex, it has been a common, simplifying assumption of a number of authors that the fraction of time a motor spends spinning counter-clockwise can be expressed in terms of CheY_P by a Hill function (see Eq. (2)). Such relationships are based upon experimental observations (Segall et al., 1986; Kuo and Koshland, 1989).

While these assumptions hold for a number of models, we note that the modeling work highlighted here has in each case generally only focused on one aspect of the bacterial system, for instance receptor clustering, the phosphorylation cascade, sensitivity, etc. A range of different mathematical approaches have been used to tackle such issues. These differences have meant comparison between all of the models presented in this review, and particularly a difference in reported outcomes, has not been straightforward, e.g., modeling methylation as discussed in Section 6. As such, we have attempted to draw as many general conclusions from the work reviewed here as is plausible.

What is the future of mathematical modeling in helping to understand bacterial chemotaxis? In order to answer this question, we reflect upon the exact goal of understanding

Table 2 Known biological attributes of bacterial chemotactic systems. For more information on all these, the reader should consult the website (http://www.pdn.cam.ac.uk/groups/comp-cell/Chemotaxis.html)

Parameter	Extent of experimental information
Chemotaxis proteins:	Identified, with roles assigned to all of them.
Methyl accepting chemotaxis proteins (MCPs)	Detect changes in chemoeffector concentration.
CheA	Histidine protein kinase. Autophosphorylation rate is controlled by the MCPs.
CheW	Scaffold protein helping to link CheA to MCPs.
CheY	Response regulator—controls motor switching.
CheB	Response regulator—MCP methylesterase. Mediates adaptation.
CheR	MCP methyltransferase. Mediates adaptation.
CheZ	CheY$_P$ phosphatase. Mediates signal termination.
Chemotaxis protein structure	High resolution structures are available for all of the soluble signal transduction proteins and for soluble domains of the chemoreceptors.
Chemotaxis protein copy number	Determined for all proteins.
Chemotaxis protein localization	Determined for all proteins.
Interaction kinetics between the chemotaxis proteins.	Rate constants and equilibrium constants have been determined *in vitro* for an extensive range of chemotaxis proteins and complexes. Some *in vivo* data on these interactions is available.

bacterial chemotaxis systems. Is it not to elucidate the mechanisms of sensing and moving in order that we may be able to predict the behavior of bacterial chemotactic systems in the environment? In order to meet this overall aim, the recent use of multiscale approaches (Emonet et al., 2005; Erban and Othmer, 2004, 2005; Kreft et al., 1998) appears a plausible way of integrating and understanding the effects of individual cell behavior on the bacterial population macroscale. However, in order to do this, we note that no single mathematical model yet exists on the single cell scale to explain the full behavior exhibited by an individual cell. This is not a failure of the modeling. Rather it is telling us that we do not yet fully understand the system—either there are key biological components still to be discovered, or we are not correctly integrating the processes we are including. A fully comprehensive model would include receptor sensitivity, phosphotransfer, adaptation, motor response, and appropriate gain and either the full single cell model or an appropriately reduced form would be used to inform the macroscale behavior.

It is also worthy of note that there exists a range of unanswered questions regarding single cell behavior. The spatial organization of proteins within the cytoplasmic domain plays an important, as yet unconfirmed, role in the signaling cascade. Protein diffusion and localization, in particular that of CheY, CheY$_P$, and CheZ, as recently noted by the work of Lipkow (Lipkow et al., 2005; Lipkow, 2006), can have important consequences on the overall behavior of the signaling system. Further investigation of protein localization within the cell is thus required.

Furthermore, prokaryotes and eukaroytes are not simply well-stirred “bags of fluid” as is often assumed in modeling their intracellular signaling pathway. Instead, they may be densely packed with, e.g., membrane, protein, or cytoskeletal filaments which may hinder protein diffusion. Such delays may have important consequences for protein interactions,

and thus affect the overall network response. In this case other modeling alternatives, for instance anomalous diffusion (Metzler and Klafter, 2000), are worthy of investigation.

A list of further issues include:

1. modeling individual receptor types, apart from Tar (e.g., Tsr) on their own;
2. modeling repellents and the bacterial response;
3. modeling mixed receptor types, including Tar and Tsr, Tar and Trg and Tsr and Aer;
4. modeling mixed concentrations of receptors and repellents with mixed families of receptor types;
5. modeling attractants that do not use MCPs for signaling, e.g., the action of transporting PTS sugars causes an MCP independent metabolic change altering CheA activity; and
6. does metabolic activity modify the bacterial response to attractant and repellent gradients?

For a more detailed list of outstanding issues in bacterial chemotaxis, the reader should consult http://www.pdn.cam.ac.uk/groups/comp-cell/Questions.html.

With the current, and ever growing biological knowledge of bacterial species and systems in general, as detailed in Table 2, the likelihood that many of these questions will be answered, with the assistance of appropriately formulated mathematical models, is an ever increasing one.

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Appendix A: Model overview—assumptions & outcomes

A.1 *Adaptation*

Model by	Main assumptions & observations	Main outcomes
Block et al. (1983)	Considered model of Koshland (1977) and the two-state model proposed in their earlier paper (Block et al., 1982). Early ODE model incorporating adaptation and methylation.	Showed transition between run and tumble was dependent upon the sensory input.
Goldbeter and Koshland (1982)	Two-state model to explain adaptation via covalent modification (methylation).	Showed receptor modification could give adaptation. Best experimental/model agreement when transition rates depend on receptor occupancy. Ratio of rate of modification/de-modification is important for ensuring adaptation.
Asakura and Honda (1984)	Two-state model of attractant-bound and unbound receptors. Methylation/de-methylation (*n* states) occurs in a set order. Bound and unbound receptors can undergo methylation. Effect of attractant and repellent can be considered.	Multiple methylation allows bacteria to adapt to changes in attractant/repellent at both high and low concentrations. Constant ratio of methylation/de-methylation rates required for steady-state to be reached.
Segel and Goldbeter (1986)	Four state model of receptor modification. Adaptation is defined as a linear sum of each receptor state.	Exhibits adaptation for certain parameter values. Parameter requirements for exact and inexact adaptation are calculated.
Barkai and Leibler (1997)	Includes description of receptor methylation and of $CheB_P$ and CheR on active and inactive receptors respectively. Receptor complex assumed to consist of MCP, CheA and CheW.	Model exhibits perfect adaptation and is robust for a wide range of parameter values.
Hauri and Ross (1995)	Extension of (Segel and Goldbeter, 1986) model to include phosphorylation. Receptor complex assumed to consist of MCP, CheA and CheW. Number of receptor states extended from four to ten. Rate of CheA phosphorylation dependent on methylation level. Methylation and de-methylation governed by Michaelis–Menten kinetics.	Exhibits exact adaptation to attractants and repellents. Agrees well with experiments in respect of excitation and adaptation timescales. Large changes in $CheB_P$ not essential for adaptation. Model could not account for observed experimental sensitivity and gain.

A.1 contd.

Model by	Main assumptions & observations	Main outcomes
Almogy et al. (2001)	Receptor modification-free adaptation. De-phosphorylation of $CheY_P$ by both CheZ and complex of $CheA_s$ and CheZ ($CheA_s$–CheZ).	Only perfect adaptation when both CheZ and $CheA_s$–CheZ de-phosphorylate $CheY_P$.
Mello and Tu (2003a)	Model of receptor complex (MCP+CheA+CheW) modification by methylation (CheR and $CheB_P$) and role of dephosphorylation. Active and inactive receptors can be demethylated/methylated.	Statement of six assumptions for obtaining perfect adaptation. Model results show good agreement with stochastic model of (Morton-Firth and Bray, 1998). Model does not exhibit experimentally observed gain.
Arocena and Acerenza (2004)	Extends work of Segel and Goldbeter (1986) and Hauri and Ross (1995). Compares difference in bacterium range of response when receptor modification is via attractant binding or methylation/phosphorylation.	Covalent modification via methylation or phosphotransfer gives wider (five orders of magnitude) response than attractant binding. Number of modification reactions governs the order of attractant concentration response, e.g. five reactions give approximately five orders of magnitude response.

A.2 *Modeling the phosphorylation cascade*

Model by	Main assumptions & observations	Main outcomes
Bray et al. (1993)	Extension of Block et al. (1982, 1983). Model bias as a function of CheY$_P$ concentration. Does not include adaptation.	Model results agree well with those for mutants. Model can not give reported sensitivity.
Spiro et al. (1997)	Incorporates attractant binding, phosphorylation and methylation. Total of three methylation states. Phosphorylation increases with methylation state. Methylation rate greater for attractant-bound than unbound receptors. De-methylation rate independent of receptor binding. CheR assumed to be in excess (de-methylation described by Michaelis–Menten kinetics).	Shows adaptation, but no sensitivity. Attempts to obtain appropriate gain by changing rate constants, but not successful.
Levin et al. (1998)	Model of phosphorylation cascade which includes receptor formation as detailed in Bray and Bourret (1995). Two models—fine tuned model includes adaptation mechanism similar to Segel and Goldbeter (1986), robustness model based upon Barkai and Leibner (1997). Work investigates changes in protein concentrations and the effect this has on the concentration of CheY$_P$ given experimentally observed differences in bacterial response of cloned populations.	Increasing Tar, CheW or CheA gives maximum CheY$_P$ levels. Increasing CheR or CheB gives negative CheY$_P$ gradients. Increasing CheR and CheY gives positive gradients. Robustness of CheR and CheB based on Barkai and Leibner (1997). Variance in CheY$_P$ concentration increases with increase in individual protein concentrations.
Morton-Firth and Bray (1998)	Stochastic model of intracellular signaling pathway (StochSim). Accounts for interactions between individual molecules which occur with a given probability.	Fluctuations in CheY$_P$ not sufficiently long to explain run-tumble behavior. Filtering of CheY$_P$ gives run-tumble distributions which are in good agreement with experiment.
Rao et al. (2004b)	Comparison of intracellular pathways of *E. coli* and *B. subtilis*. Combined models of Barkai and Leibler (1997) and Sourjik and Berg (2002a) and applied to both bacterial species, although the model of *B. subtilis* differentiated between active, inactive, weakly active and weakly inactive receptors. Considered effect of CheY dependent differential methylation of *B. subtilis*.	Both pathways shown to be adaptive and robust. *B. subtilis* pathway more robust than *E. coli*. *B. subtilis* can perform methylation independent chemotaxis due to presence of CheV–CheY pathway.

A.3 *Sensitivity and gain—the role of receptors*

Model by	Main assumptions	Main outcomes
Bray and Bourret (1995)	Considered formation of Tar–CheW–CheA complexes. Networks of seven, ten and twelve binding steps considered. Combined model with that of Bray et al. (1993) to consider role of complex formation on motor bias.	All networks gave binding constants three to four orders of magnitude smaller than experimental values. Fixing rates at experimental values did not give the correct motor bias. Increasing over-expression of proteins 30-fold showed the discrepancy between experimental and theoretical values of the dissociation constants were reduced by a factor of 10.
Bray et al. (1998)	Discrete model of receptor interactions to explain sensitivity. Receptors assumed to work both individually and in clusters. Unbound receptors are more active than bound receptors. Considers varying ratios of clustered and free receptors.	Ability of receptors to respond to attractant decreases as number of inactive receptors increases. Best response when clustering is maximized at low attractant concentrations and minimized at high concentrations.
Shi and Duke (1998)	Ising model of receptor clustering. Array activity corresponds to average magnetization of a lattice of particles, attractant binding is represented by local magnetic field. Model includes methylation and adaptation (equivalent to applying a magnetic field opposite to that induced by attractant binding).	Mean-field analysis of model. Strength of receptor coupling affects response more than attractant binding.
Duke and Bray (1999)	Monte Carlo simulations of model by Shi and Duke (1998).	Model responds to change in attractant concentration over five orders of magnitude. Coupled system gives better response than uncoupled system.
Morton-Firth et al. (1999)	Extension of Barkai and Leibler (1997) to model Tar receptor and phosphotransfer using StochSim (Morton-Firth and Bray, 1998) Like Barkai and Leibler (1997) only active receptors affected by $CheB_P$. Looks at local (receptor) versus global (cytoplasm) effects. Considers ordered versus random methylation.	Model reproduces experimentally observed adaptation, but 7% error in post- & pre-stimulus $CheY_P$ concentration. If CheR is at saturation then adaptation is not exact. Agrees with experimental data on duration of response.

A.3 contd.

Model by	Main assumptions & observations	Main outcomes
Shi (2000)	Extended model of Shi and Duke (1998) using adaptive Ising model (AIM). AIM incorporates negative feedback, essentially the effects of CheR and $CheB_P$ on receptor activity. Coarse-grained model to account for timescale differences of attractant binding, phosphorylation and adaptation.	Negative feedback, i.e. effect of CheR and $CheB_P$ causes model to give perfect adaptation. Feedback also gives more robust model than Shi and Duke (1998) allowing relaxation of assumptions regarding the field strength. Model also shows sensitivity to changes in attractant concentration.
Shi (2001)	Comparison of Shi and Duke (1998) and Shi (2000) models with experiment.	Comparison allows attractant binding and receptor–receptor ratio as well as adaptation time to be quantified. Shows good agreement with experiment. Ratio of pre-stimulus and post-stimulus CheA shows 165-fold increase. Agrees with experimental prediction of over 100-fold increase.
Shi (2002)	Ising model of floating receptors.	Expressions of receptor activity and average number of receptors. Monte Carlo analysis shows correlation strong for nearby receptors, but decreases exponentially with increasing distance between receptors. Receptor interaction more dependent on receptor state than on whether receptor is attractant-bound.
Levin et al. (2002)	Stochastic simulation of binding and diffusion of CheR within receptor cluster using “dumbbell” or brachiation model. Model uses StochSim and considers effect of CheR binding strength on ability to diffuse through the receptor cluster.	If binding is within physiological limits then model shows CheR molecule can visit and also methylate large number of receptors within the cluster. If binding is too strong then CheR binds for longer periods and does not wander freely over the receptor cluster.

A.3 contd.

Model by	Main assumptions & observations	Main outcomes
Shimizu et al. (2003)	Model of receptor–receptor interactions including role of CheB$_P$, CheR, CheY$_P$ using StochSim and Ising model. Adaptation and methylation (up to five levels) model based upon work of (Barkai and Leibler, 1997). Considers effect of receptor array size and geometry (hexagonal, trigonal or square) on sensitivity, gain and signal-to-noise ratio.	Sensitivity increases with receptor array size. Gain is largest for trigonal lattice array. Increasing receptor–receptor coupling decreases the signal-to-noise ratio, but increases gain. Signal-to-noise ratio and gain insensitive to attractant concentration. Coupled receptors reproduce overshoot whereas uncoupled do not. Receptor coupling improves model comparison with experimental data, but can not reproduce reported gain.
Mello and Tu (2003b)	Extension of Ising model to include methylation and coupling amongst different receptor species (Tar & Tsr) to explain experimental work of Sourjik and Berg (2002b). Like Barkai and Leibler (1997) assume only inactive receptors methylated and active ones demethylated. Mean-field analysis of the model.	Model is very good at explaining mutants (CheR/CheB deleted), but variation in parameter values required to explain mutant and wild-type data. Model explains Tsr activity at low concentrations by strong Tar–Tsr coupling. Only active receptors can bind attractant. Attractant unbinds from inactive receptors. This leads to result that attractant binding is dependent upon receptor activity.
Albert et al. (2004)	ODE model of receptor team formation to explain experimentally observed sensitivity and gain. Model considers network of various receptor dimer formations, both attractant-bound and unbound. Model makes a number of assumptions of which some include attractant binding is independent of dimer state, model is over attractant binding timescale so descriptions of methylation and phosphotransfer are not included, gain is expressed as a the ratio of active attractant-bound receptors and free receptors.	Concentration of unbound receptor trimers of dimers is taken to be model output. Good qualitative agreement with experimental curves of activity versus attractant binding from various literature sources. Differences in activity versus attractant binding can be explained by altering dissociation constants, assuming different levels of methylation given different rates of receptor team formation. Model also shows good agreement with Sourjik and Berg (2002b) on interaction between Tar and Tsr receptors.

A.3 contd.

Model by	Main assumptions & observations	Main outcomes
Goldman et al. (2004)	Monte Carlo simulation of a lattice gas model of protein association on 2-D lattice which can have trigonal, square or hexagonal geometry. Applies to receptor array interactions. Proteins have defined bond strengths and bond angles with neighboring proteins. Importance of array geometry considered.	Analysis considers steady-state solutions to the simulations. Protein cells clustered together after given number of steps. Local binding causes protein association to diffuse across 2-D lattice. Single protein species and systems with two protein species considered. Proteins of different types could form bonds if contact angle between them is the same. Changing initial ratio of the two proteins affects number of bonds formed and protein aggregation.
Mello et al. (2004)	Extension of Mello and Tu (2003b) to include multiple receptor types and methylation levels. Mean-field analysis (MFA) and Monte Carlo (MC) simulations. Inactive receptors methylated and active demethylated. Check to see if MFA results agree with MC results.	Model has two steady-states, one of which is oscillatory. Oscillatory steady-state ignored as not observed experimentally. Attractant binding dissociation constant needs to depend on methylation level to obtain observed sensitivity. MFA and MC results both agree with experimental results. Receptor coupling has large effect on receptor activity for differing methylation levels.
Rao et al. (2004a)	Two-state allosteric model of receptor clustering, based on models by Monod et al. (1965) and Shimizu et al. (2000). Receptors exist in sets of trimers of dimers, which can adopt a relaxed or active state. Stability is assisted by concentration of CheA and CheW. Methylation increases receptor activity, receptor-attractant sensitivity decreases with activity. Receptor clustering based on model of Bray and Duke (2004) considered on a hexagonal array.	Model compares well with experimental results of Sourjik and Berg (2002a) for mixed receptor types. Increased methylation stabilizes receptor complexes. Receptor cluster size is determined by the local concentration of receptor type.

A.3 contd.

Model by	Main assumptions & observations	Main outcomes
Skoge et al. (2006)	Compares one- and two-dimensional Ising models and Monod–Wyman–Changeux (MWC) model with experimental results of Sourjik and Berg (2002b, 2004). Consider different responses of wild-type and CheR and CheR–CheB mutant bacteria.	MWC shows most favorable agreement with experiment. MWC also able to explain differences between wild-type and mutant responses.
Endres and Wingreen (2006)	MWC model of mixed receptor clusters of Tar and Tsr receptors. One receptor cluster contains 18 receptor dimers. Based upon “assistant-neighborhoods” (Li and Hazelbauer, 2005). Incorporates adaptation model of Barkai and Leibler (1997): only inactive receptors methylated by CheR and active ones demethylated by $CheB_P$.	Model gives required sensitivity and adaptation response for mixed receptors. Two different types of response at high attractant concentration: (i) no receptor response due to attractant saturation; or (ii) no adaptation because all receptors become methylated.

A.4 Spatial modeling of intracellular signaling

Model by	Main assumptions & observations	Main outcomes
Lipkow et al. (2005)	Stochastic 3-D simulations of spatial diffusion of cytoplasmic proteins CheY, CheY$_P$ and CheZ as well as phosphotransfer by CheA$_P$. Simulations based on theory developed by Andrews and Bray (2004). Considers effect of CheZ spatial localization, position of the motors and macromolecular crowding, on spatial variation in CheY$_P$ concentrations and motor response.	When CheZ restricted to anterior (receptor end) CheY$_P$ concentration is constant throughout cytoplasm. Spatially invariant concentration of CheZ leads to spatially varying exponential concentration of CheY$_P$ with maximum at receptors. Macromolecular crowding leads to steeper exponential curve due to retarded diffusion of CheY$_P$ away from the receptors.
Lipkow (2006)	Looks at importance of CheZ location, either membrane-bound or free to move in the cytoplasm. Attractant detection means CheZ is free to move in the cytoplasm of the cell. Repellent leads to binding at the poles to CheA$_S$.	CheZ clustering causes enhanced dephosphorylation of CheY$_P$. Forms a “second tier” of adaptation which explains adaptation in methylation-free cells.

Appendix B: Overview of model properties

Modeling assumption	Block et al. (1983)	Goldbeter and Koshland (1982)	Asakura and Honda (1984)	Segel and Goldbeter (1986)	Barkai and Leibler (1997)	Hauri and Ross (1995)	Almogy et al. (2001)	Mello and Tu (2003a)	Arocena and Acerenza (2004)	Bray et al. (1993)	Spiro et al. (1997)	Morton-Firth and Bray (1998)	Rao et al. (2004a)	Bray and Bourret (1995)	Bray et al. (1998)	Shi and Duke (1998)	Duke and Bray (1999)	Morton-Firth et al. (1999)	Shi (2000)	Shi (2001)	Shi (2002)
Temporal-deterministic-continuous	√	√	√	√	√	√	√	√	√	√	√		√	√							
Temporal-discrete															√	√	√		√	√	√
Temporal-stochastic												√						√			
Spatiotemporal-continuous																					
Spatiotemporal-discrete															√						
Spatiotemporal-stochastic																					
Receptor complex (MCP+CheA+CheW)[a]	√				√	√		√	√	√	√	√	√	√							
Receptor–receptor interactions															√	√	√		√	√	√
Methylation and/or demethylation[b]	√	√	√	√	√	√		√	√		√	√						√			
Cytoplasmic phosphorylation[c]						√	√			√	√	√	√	√				√			
Motor dynamics[d]										√											
Adaptation		√	√	√	√	√	√	√	√	√	√	√	√				√	√			
Description of gain										√							√				

Appendix B contd.

Modeling assumption	Levin et al. (2002)	Bornhorst and Falke (2003)	Shimizu et al. (2003)	Mello and Tu (2003b)	Albert et al. (2004)	Goldman et al. (2004)	Mello et al. (2004)	Rao et al. (2004b)	Mello and Tu (2005)	Skoge et al. (2006)	Endres and Wingreen (2006)	Lipkow et al. (2005)	Lipkow (2006)
Temporal-deterministic-continuous	√	√			√								
Temporal-discrete				√		√	√	√	√	√	√		
Temporal-stochastic													
Spatiotemporal-continuous													
Spatiotemporal-discrete													
Spatiotemporal-stochastic			√									√	√
Receptor complex (MCP+CheA+CheW)[a]	√		√		√								
Receptor–receptor interactions		√	√	√	√	√		√	√	√	√		
Methylation and/or demethylation[b]	√		√	√			√		√		√		
Cytoplasmic phosphorylation[c]	√		√									√	√
Motor dynamics[d]													
Adaptation	√		√				√		√		√		√
Description of gain			√		√		√						

[a]Refers to models which consider the receptor complex as a single entity, i.e. assume it comprises MCP, CheA and CheW.

[b]This includes any model which has focused explicitly or implicitly on the role of CheR, CheB or $CheB_P$.

[c]Any model which has included a description of CheY, $CheY_P$ and CheZ.

[d]Includes a detailed description of motor response and dynamics.

References

Adler, J., 1966. Chemotaxis in bacteria. Science 153, 708–716.
Albert, R., Chiu, Y., Othmer, H., 2004. Dynamic receptor team formation can explain the high signal transduction gain in *Escherichia coli*. Biophys. J. 86, 2650–2659.
Almogy, G., Stone, L., Ben-Tal, N., 2001. Multi-stage regulation, a key to reliable adaptive biochemical pathways. Biophys. J. 81, 3016–3028.
Alon, U., Surette, M., Barkai, N., Leibler, S., 1999. Robustness in bacterial chemotaxis. Nature 397, 168–171.
Ames, P., Studert, C., Reiser, R., Parkinson, J., 2002. Collaborative signalling by mixed chemoreceptor teams in *Escherichia coli*. Proc. Natl. Acad. Sci. 99, 7060–7065.
Andrews, S., Bray, D., 2004. Stochastic simulation of chemical reactions with spatial resolution and singled molecule detail. Phys. Biol. 1, 137–151.
Armitage, J., 1999. Bacterial tactic response. Adv. Microb. Physiol. 41, 229–289.
Arocena, M., Acerenza, L., 2004. Necessary conditions for a minimal model of receptor to show adaptive response over a wide range of levels of stimulus. J. Theor. Biol. 229, 45–57.
Asakura, S., Honda, H., 1984. Two-state model for bacterial chemoreceptor proteins: The role of multiple methylation. J. Math. Biol. 176, 349–367.
Barkai, N., Leibler, S., 1997. Robustness in simple biochemical networks. Nature 387, 913–917.
Berg, H., 2000. Constraints on models for the flagellar rotary motor. Philos. Trans. R. Soc. Lond. B 355, 491–501.
Berg, H., 2003. The rotary motor of bacterial flagella. Annu. Rev. Biochem. 72, 19–54.
Berg, H., Purcell, E., 1977. Physics of chemoreception. Biophys. J. 20, 193–219.
Berry, R., Armitage, J., 1999. The bacterial flagellar motor. Adv. Microb. Physiol. 41, 291–337.
Beyerinck, M., 1895. Ueber *Spirillum desulfuricans* als ursache von sulfatreduction. Zentralbl. Bakteriol. Parasitenkd. 1, 1–9, 49–59, 104–14.
Bialek, W., Setayeshgar, S., 2005. Physical limits to biochemical signalling. Proc. Natl. Acad. Sci. 102(29), 10040–10045.
Block, S., Segall, J., Berg, H., 1982. Impulse response in bacterial chemotaxis. Cell 31, 215–226.
Block, S., Segall, J., Berg, H., 1983. Adaptation kinetics in bacterial chemotaxis. J. Bacteriol. 154, 312–323.
Bornhorst, J., Falke, J., 2001. Evidence that both ligand binding and covalent adaptation drive a two-state model equilibrium in the aspartate receptor signalling complex. J. Gen. Phys. 118, 693–710.
Bornhorst, J., Falke, J., 2003. Quantitative analysis of aspartate receptor signalling complex reveals that the homogenous two-state model is inadequate: Development of a heterogenous two-state model. J. Mol. Biol. 326, 1597–1614.
Bray, D., 2002. Bacterial chemotaxis and the question of gain. Proc. Natl. Acad. Sci. 99(1), 7–9.
Bray, D., Bourret, R., 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.
Bray, D., Duke, T., 2004. Conformational spread: The propagation of allosteric states in large multiprotein complexes. Annu. Rev. Biophys. Biomol. Struct. 33(1), 53–73.
Bray, D., Bourret, R., Simon, M., 1993. Computer simulation of the phosphorylation cascade controlling bacterial chemotaxis. Mol. Biol. Cell 4, 469–482.
Bray, D., Levin, M., Morton-Firth, C., 1998. Receptor clustering as a cellular mechanism to control sensitivity. Nature 393(7), 85–88.
Bren, A., Eisenbach, M., 2000. How signals are heard during bacterial chemotaxis: Protein–protein interactions in sensory signal propagation. J. Bacteriol. 182(24), 6865–6873.
Crissman, H., Darzynkiewicz, Z., Tobey, R., Steinkamp, J., 1985. Correlated measurements of DNA, RNA, and protein in individual cells by flow cytometry. Science 228, 1321–1324.
Darzynkiewicz, Z., Crissman, H., Traganos, F., Steinkamp, J., 1982. Cell heterogeneity during the cell cycle. J. Cell Physiol. 113, 465–474.
Delbrück, M., Reichardt, W., 1956. System analysis for the light growth reactions of *Phycomyces*. In D. Rudnick (Ed.), Cellular Mechanisms in Differentation and Growth, pp. 3–44. Princeton University Press, Princeton.
Duke, T., Bray, D., 1999. Heightened sensitivity of a lattice of membrane receptors. Proc. Natl. Acad. Sci. 96, 10104–10108.
Duke, T., Novére, N.L., Bray, D., 2001. Conformational spread in a ring of proteins: A stochastic approach to allostery. J. Mol. Biol. 308, 541–553.

Eisenbach, M., 1990. Control of bacterial chemotaxis. Mol. Microbiol. 20, 903–910.
Eisenbach, M., Lengeler, J., Varon, M., Gutnick, D., Meili, R., Firtel, R., Segall, J., Omann, G., Tamada, A., Murakami, F., 2004. Chemotaxis. Imperial College Press, London.
Emonet, T., Macal, C., North, M., Wickersham, C., Cluzel, P., 2005. Agentcell: A digital single-cell assay for bacterial chemotaxis. Bioinformatics 21(11), 2714–2721.
Endres, R., Wingreen, N., 2006. Precise adaptation in bacterial chemotaxis through "assistance neighbourhoods". Proc. Natl. Acad. Sci. 103(35), 13040–13044.
Engelmann, T., 1881a. Neue methode zur untersuchung der sauerstoffaussheidung pflanzlicher und thierischer organismen. Pflugers Arch. Gesamte Physiol. Menschen Tiere 25, 285–292.
Engelmann, T., 1881b. Zur biologie der schizomyceten. Pflugers Arch. Gesamte Physiol. 26, 537.
Erban, R., Othmer, H., 2004. From individual to collective behaviour in bacterial chemotaxis. SIAM J. Appl. Math. 65, 361–391.
Erban, R., Othmer, H., 2005. From signal transduction to spatial pattern formation in *E. coli*: A paradigm for multiscale modelling in biology. Multiscale Model. Simul. 3(2), 362–394.
Garrity, L., Ordal, G., 1995. Chemotaxis in *Bacillus subtilis*: How bacteria monitor environmental signals. Pharmacol. Ther. 68(1), 87–104.
Goldbeter, A., Koshland, D., 1982. Simple molecular model for sensing and adaptation based on receptor modification with application to bacterial chemotaxis. J. Mol. Biol. 161, 395–416.
Goldman, J., Andrews, S., Bray, D., 2004. Size and composition of membrane protein clusters prediced by Monte Carlo analysis. Eur. Biophys. J. 33, 506–512.
Guo, C., Levine, H., 1999. A thermodynamic model for receptor clustering. Biophys. J. 77(5), 2358–2365.
Guo, C., Levine, H., 2000. A statistical mechanics model for receptor clustering. J. Biol. Phys. 26(3), 219–234.
Hauri, D., Ross, J., 1995. A model of excitation and adaptation in bacterial chemotaxis. Biophys. J. 68, 708–722.
Kim, S., Wang, W., Kim, K., 2002. Dynamic and clustering model of bacterial chemotaxis receptors: Structural basis for signalling and high sensitivity. Proc. Natl. Acad. Sci. 99(18), 11611–11615.
Koshland, D., 1977. A response regulator model in a simple sensory system. Science 196, 1055–1063.
Kreft, J., Booth, G., Wimpenny, J., 1998. Bacsim, a simulator for individual-based modelling of bacterial colony growth. Microbiology 144, 3275–3287.
Kuo, S., Koshland, D., 1989. Multiple kinetic states for the flagellar motor switch. J. Bacteriol. 171(11), 6279–6287.
Levin, M., Morton-Firth, C., Abouhamad, W., Bourret, R., Bray, D., 1998. Origins of individual swimming behavior in bacteria. Biophys. J. 74, 175–181.
Levin, M., Shimizu, T., Bray, D., 2002. Binding and diffusion of CheR molecules within a cluster of membrane receptors. Biophys. J. 82, 1809–1817.
Levit, M., Stock, J., 2002. Receptor methylation controls the magnitude of stimulus-response coupling in bacterial chemotaxis. J. Biol. Chem. 277(39), 36760–36765.
Li, G., Weis, R., 2000. Covalent modification regulates ligand binding to receptor complexes in the chemosensory system of *Escherichia coli*. Cell 100, 357–365.
Li, M., Hazelbauer, G.L., 2005. Adaptational assistance in clusters of bacterial chemoreceptors. Mol. Microbiol. 56(6), 1617–1626.
Lipkow, K., 2006. Changing cellular location of CheZ predicted by molecular simulations. PLoS Comput. Biol. 2(4), 301–310.
Lipkow, K., Andrews, S., Bray, D., 2005. Simulated diffusion of phosphorylated CheY through the cytoplasm of *Escherichia coli*. J. Bacteriol. 187(1), 45–53.
Lybarger, S., Maddock, J., 2001. Polarity in action: Asymmetric protein localization in bacteria. J. Bacteriol. 183(11), 3261–3267.
Macnab, R., Koshland, D., 1972. The gradient-sensing mechanism in bacterial chemotaxis. Proc. Natl. Acad. Sci. 69(9), 2509–2512.
Maddock, J., Shapiro, L., 1993. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. Science 259(9), 1717–1723.
Mello, B., Tu, Y., 2003a. Perfect and near-perfect adaptation in a model of bacterial chemotaxis. Biophys. J. 84, 2943–2956.
Mello, B., Tu, Y., 2003b. Quantitative modeling of sensitivity in bacterial chemotaxis: The role of coupling among different chemoreceptor species. Proc. Natl. Acad. Sci. 100(14), 8223–8228.
Mello, B., Tu, Y., 2005. An allosteric model for heterogeneous receptor complexes: Understanding bacterial chemotaxis responses to multiple stimuli. Proc. Natl. Acad. Sci. 102(48), 17354–17359.

Mello, B., Shaw, L., Tu, Y., 2004. Effects of receptor interaction in bacterial chemotaxis. Biophys. J. 87, 1578–1595.

Metzler, R., Klafter, J., 2000. The random walk's guide to anomalous diffusion: A fractional dynamics approach. Phys. Rep. 339, 1–77.

Monod, J., Wyman, J., Changeux, J., 1965. On the nature of allosteric transitions: A plausible model. J. Mol. Biol. 12, 88–118.

Morton-Firth, C., Bray, D., 1998. Predicting temporal fluctuations in an intracellular signalling pathway. J. Theor. Biol. 192, 117–128.

Morton-Firth, C., Shimizu, T., Bray, D., 1999. A free-energy based stochastic simulation of the Tar receptor complex. J. Mol. Biol. 286, 1059–1074.

Murray, J., 1993. Mathematical Biology, 2nd edn. Springer, New York.

Novère, N.L., Shimizu, T., 2001. Stochsim: Modelling of stochastic biomolecular processes. Bioinformatics 17, 575–576.

Pfeffer, W., 1888. Uber chemotaktische bewegungen von bacterien, flagellaten and volvocineen. Unters. Bot. Inst. Tübingen 2, 582.

Rao, C., Frenklach, M., Arkin, A., 2004a. An allosteric model for transmembrane signalling in bacterial chemotaxis. J. Mol. Biol. 343, 291–303.

Rao, C., Kirby, J., Arkin, A., 2004b. Design and diversity in bacterial chemotaxis: A comparative study in *Eschericia coli* and *Bacillus subtilis*. PLoS Biol. 2(2), 239–252.

Segall, J., Block, S., Berg, H., 1986. Temporal comparisons in bacterial chemotaxis. Proc. Natl. Acad. Sci. 83(23), 8987–8991.

Segel, L., 1976. Incorporation of receptor kinetics into a model for bacterial chemotaxis. J. Theor. Biol. 57, 23–42.

Segel, L., 1977. A theoretical study of receptor mechanisms in bacterial chemotaxis. SIAM J. Appl. Math. 32(3), 653–665.

Segel, L., Goldbeter, A., 1986. A mechanism for exact sensory adaptation based on receptor modification. J. Theor. Biol. 120, 151–179.

Shi, Y., 2000. Adaptive Ising model and bacterial chemotactic receptor network. Eur. Lett. 50(1), 113–119.

Shi, Y., 2001. Effects of thermal fluctuation and the receptor–receptor interaction in bacterial chemotactic signalling and adaptation. Phys. Rev. E 64, 1–8.

Shi, Y., 2002. Clustering and signalling of cell receptors. Physica A 311, 199–212.

Shi, Y., Duke, T., 1998. Cooperative model of bacteria sensing. Phys. Rev. E 58(5), 6399–6406.

Shimizu, T., Aksenov, S., Bray, D., 2003. A spatially extended stochastic model of the bacterial chemotaxis signalling pathway. J. Mol. Biol. 329, 291–309.

Shimizu, T., Novère, N.L., Levin, M., Beavil, A., Sutton, B., Bray, D., 2000. Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis. Nat. Cell Biol. 2, 792–796.

Skoge, M., Endres, R., Wingreen, N., 2006. Receptor-receptor coupling in bacterial chemotaxis: Evidence for strongly coupled receptors. Biophys. J. 90, 4317–4326.

Sourjik, V., Berg, H., 2002a. Binding of the *Escherichia coli* response regulator CheY to its target measured *in vivo* by fluorescence resonance energy transfer. Proc. Natl. Acad. Sci. 99, 12669–12674.

Sourjik, V., Berg, H., 2002b. Receptor sensitivity in bacterial chemotaxis. Proc. Natl. Acad. Sci. 99(1), 123–127.

Sourjik, V., Berg, H., 2004. Functional interactions between receptors in bacterial chemotaxis. Nature 428, 437–441.

Spiro, P., 1997. Mathematical studies of cell signal transduction. Ph.D. thesis, The University of Utah.

Spiro, P., Parkinson, J., Othmer, H., 1997. A model of excitation and adaptation in bacterial chemotaxis. Proc. Natl. Acad. Sci. 94, 7263–7268.

Spudich, J., Koshland, D., 1976. Non-genetic individuality: Changed in the single cell. Nature 262, 467–471.

Stock, J., Kersulis, G., Koshland, D., 1985. Neither methylating of demethylating enzymes are required for chemotaxis. Cell 42, 683–690.

Strong, S., Freedman, B., Bialek, W., Koberle, R., 1998. Adaptation and optimal chemotactic strategy for *E. coli*. Phys. Rev. E 57(4), 4604–4617.

Toda, M., Kubo, R., Saito, N., 1983. Statistical Physics I. Springer, Berlin.

Wadhams, G., Armitage, J., 2004. Making sense of it all: Bacterial chemotaxis. Nat. Rev. Mol. Cell Biol. 5(12), 1024–1037.

Wang, H., Matsumura, P., 1997. Phosphorylating and dephosphorylting protein complexes in bacterial chemotaxis. J. Bacteriol. 179, 287–289.

Windisch, B., Bray, D., Duke, T., 2006. Balls and chains—a mesoscopic approach. Biophys. J. 91, 2383–2392.

Yi, T., Huang, Y., Simon, M., Doyle, J., 2000. Robust perfect adaptation in bacterial chemotaxis through integral feedback control. Proc. Natl. Acad. Sci. 97(9), 4649–4653.