Feedback Control Architecture and the Bacterial Chemotaxis Network

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Abstract

Bacteria move towards favourable and away from toxic environments by changing their swimming pattern. This response is regulated by the chemotaxis signalling pathway, which has an important feature: it uses feedback to ‘reset’ (adapt) the bacterial sensing ability, which allows the bacteria to sense a range of background environmental changes. The role of this feedback has been studied extensively in the simple chemotaxis pathway of Escherichia coli. However, it has been recently found that the majority of bacteria have multiple chemotaxis homologues of the E. coli proteins, resulting in more complex pathways. In this paper we investigate the configuration and role of feedback in Rhodobacter sphaeroides, a bacterium containing multiple homologues of the chemotaxis proteins found in E. coli. Multiple proteins could produce different possible feedback configurations, each having different chemotactic performance qualities and levels of robustness to variations and uncertainties in biological parameters and to intracellular noise. We develop four models corresponding to different feedback configurations. Using a series of carefully designed experiments we discriminate between these models and invalidate three of them. When these models are examined in terms of robustness to noise and parametric uncertainties, we find that the non-invalidated model is superior to the others. Moreover, it has a ‘cascade control’ feedback architecture which is used extensively in engineering to improve system performance, including robustness. Given that the majority of bacteria are known to have multiple chemotaxis pathways, in this paper we show that some feedback architectures allow them to have better performance than others. In particular, cascade control may be an important feature in achieving robust functionality in more complex signalling pathways and in improving their performance.

Introduction

Living organisms respond to changes in their internal and external environment in order to survive. The sensing, signalling and response mechanisms often consist of complicated pathways the dynamical behaviour of which is often difficult to understand without mathematical models [1]. Considering the structure and dynamics of these signalling pathways as integrated dynamical systems can help us understand how the pathway architecture and parameter values result in the performance and robustness in the response dynamics [2].

One extensively studied sensory pathway is bacterial chemotaxis. This pathway controls changes in bacterial motion in response to environmental stimuli, biasing movement towards regions of higher concentration of beneficial or lower concentration of toxic chemicals. The chemotaxis signalling pathway in the bacterium Escherichia coli is a simple network with one feedback loop [3] which has been extensively studied and used as a paradigm for the mechanism of chemotaxis signalling networks [4]. In E. coli, chemical ligands bind to methyl-accepting chemotaxis protein (MCP) receptors that span the cell membrane and alter the activity of a cytoplasmic histidine kinase called CheA.

When attractant ligands stimulate the chemotaxis pathway by binding to MCP, there is a decrease in the autophosphorylation rate of CheA; conversely, repellent binding or lack of attractant binding increase CheA autophosphorylation activity. CheA, when phosphorylated, can transfer the phosphoryl group to two possible response regulators: CheY and CheB. CheY-P (where ‘-P’ denotes phosphorylation) interacts with FliM in the multiple E. coli flagellar motors resulting in a change in the direction of rotation of the motor. At the same time, a negative feedback loop allows the system to sense temporal gradients and react to a wide ligand concentration range: the MCP receptors, which are constantly methylated by CheR, are demethylated by CheB-P. This negative feedback loop restores the CheA autophosphorylation rate and the flagellar activity to the pre-stimulus equilibrium state [5,6].

Describing this pathway mathematically as a dynamical system can be facilitated by using tools from control theory. For example, it has been shown that the adaptation mechanism in the E. coli model [7,8] is a particular example of integral control, a feedback system design principle used in control engineering to ensure the elimination of offset errors between a system’s desired and actual signals, irrespective of the levels of other signals [9].
Author Summary

Bacteria move towards favorable environments by changing their swimming pattern. An important feature of this response, which is called bacterial chemotaxis, is that their sensing ability remains independent of the background environment in which they find themselves. This feature has been studied extensively in the bacterium *E. coli*, which has a simple chemotaxis decision mechanism. However, it has been recently found that most bacteria could potentially have a more complicated decision mechanism for this response. In this paper, we look at the chemotaxis behaviour of one such bacterium, *R. sphaeroides*. We develop mathematical models of possible decision mechanisms and undertake an experimental procedure to investigate their validity. We find that only one of four such models can explain the chemotaxis response in *R. sphaeroides*. Compared to the other models, this model corresponds to a decision mechanism that provides the bacterium with improved swimming performance over the others. Moreover, this decision mechanism has been used extensively to improve performance in several engineering systems. We suggest that this mechanism may play an important role in improving chemotactic performance in other bacteria and in other signalling pathways.

Many species have chemotaxis pathways that are much more complicated than that of *E. coli* [10,11], either containing chemotaxis proteins not found in *E. coli*, e.g. in the case of *Bacillus subtilis* [12]; or containing multiple homologues of the proteins found in the *E. coli* pathway, as in the case of *Rhodobacter sphaeroides* [11,13]. Furthermore, in *R. sphaeroides* there are two receptor clusters containing sensory proteins which localize to different parts of the cell, one located at the cell pole and the other in the cytoplasm [14]. Although the purpose of the two clusters is unclear, *in vitro* phosphotransfer experiments [15,16] show that the CheA homologues located at the two clusters can phosphotransfer to different CheY and CheB homologues: at the cell pole CheA2-P transfers to CheY3 and CheY4, while at the cytoplasm CheA3A4-P transfers to CheY6 and CheB2. The two methyl-esterase proteins, CheB1 and CheB2, which are homologues of CheB in *E. coli*, are responsible for the adaptation mechanism in *R. sphaeroides* [13,17]. Past localization studies have shown that CheB1 and CheB2 are found diffuse throughout the cytoplasm [14]. This is different to *E. coli* where the CheB protein is localized at the cell pole, and could potentially mean that the two proteins de-methylate either receptor cluster [14].

As a system featuring an adaptation mechanism similar to that in *E. coli*, but with multiple homologues of the *E. coli* chemotaxis proteins, it is useful to examine the *R. sphaeroides* chemotaxis pathway from a control engineering perspective. In this way, we can suggest structures for the *R. sphaeroides* chemotaxis pathway that integrate the control mechanisms thought to be responsible for adaptation in *E. coli* along with the possible feedback architectures that arise from the dual sensory modules present in *R. sphaeroides*. The relative evolutionary advantages of the different architectures can then be compared from both control engineering and biological points of view. The fact that there are two endogenous 'measurements' available to the feedback mechanism (CheB1-P and CheB2-P) which can be used to regulate two signals (CheA2 and CheA3A4) makes the whole chemotaxis feedback pathway a multi-input, multi-output control system (as opposed to possessing only one CheB and one CheA as in the *E. coli* models [7,18]). This introduces extra degrees of freedom in the feedback control mechanism of the system and, thus, the potential for better regulation.

However, the different conceivable connectivity configurations between the two CheB-P proteins and the two receptor clusters actually correspond to different feedback control architectures, each with different properties. Some of these configurations, as will be demonstrated, could allow the bacterium to integrate information from both internal and external sources and to function more efficiently, e.g., by varying how strongly it reacts to external attractants depending on its internal state. At the same time, the additional receptor cluster not found in *E. coli* has the potential of introducing extra sources of performance degradation such as noise (both intrinsic and extrinsic) and variations in quantities internal to the cell such as protein copy numbers and phosphorylation rates: the feedback signalling pathway may be required to remedy this, and in this regard, some of these feedback architectures perform better than others.

One of the different pathway configurations that is possible in this system has similarities to a feedback architecture commonly found in engineering control systems termed cascade control [19], which is usually employed when the process to be controlled can be split into a slow 'primary' sub-process (shown dashed) and a faster, secondary sub-process (shown in Figure 1). Without the internal feedback shown dashed in Figure 1 the primary module maintains a set-point for the secondary module to follow and the output of the secondary module is fed back to the primary. A cascade control design places an additional feedback loop around the fast secondary process (shown dashed). This has been known to improve system performance in several ways: it reduces the sensitivity of the output of the secondary module to changes in the parameters (thus improving robustness), it attenuates the effects of disturbance signals, it makes the step response of the control system to inputs and disturbances less oscillatory and, since the secondary process is relatively fast, the effects of unwanted disturbances are corrected before they affect the system output. Including this additional internal feedback also allows the control system designer more flexibility in increasing the feedback gain to achieve higher bandwidth and faster system responses without losing stability. In fact, cascade control is employed as a design principle in several engineering systems such as aircraft pitch control and industrial heat exchangers (see Text S1 for further details).

In our previous work [20], we used a model invalidation technique to arrive at a possible pathway architecture that allows the *R. sphaeroides* chemotaxis system to convey, via a signalling cascade, sensed changes in ligand concentration outside the cell to the flagellar motor. In that model, proteins CheY5-P and CheY4-P act together to promote autophosphorylation of CheA2A4 (schematically illustrated in Figure 2(A)) whilst CheY6-P binds...
with the FilM rotor switch to increase the frequency of motor switching (and hence reduce the motor rotation frequency). This stimulation of CheA₃A₄ need not be a direct interaction [20].

In this paper, we assume that the chemotaxis pathway has the same forward signalling pathway of [20] and then suggest four plausible interconnection structures for the feedback pathway between the two CheB-P proteins and the two receptor clusters. Following this, we present the results of experiments that are used to invalidate all but one of these structures. We then discuss the results of in silico experiments that highlight the differences in chemotactic performance between the different models with particular focus on the robustness of chemotaxis to parametric variations in the chemotaxis pathway and noise [21,22]. Using analytical techniques from control theory, we demonstrate that the model not invalidated by our experiments is structurally similar to the cascade control architecture, and we use the structural properties of this interconnection, which are commonly used to reduce the effects of uncertainty and disturbances in various engineering applications, to explain the robustness features of the suggested model.

Results

Chemotaxis model creation

Given the structure of the forward path of the chemotaxis pathway from [20], illustrated in Figure 2(A), and given the rates previously measured in [15,16] for the phosphotransfer reactions also shown in Figure 2(A), we constructed a generic ordinary differential equation model of the R. sphaeroides chemotaxis pathway, detailed in Materials and Methods. With this forward signalling pathway, the model makes the following assumptions:
Polar and cytoplasmic cluster receptors are either methylated or un-methylated.

- Only a subset of methylated receptors is active, as in [23].
- CheR2/CheR3 act to methylate inactive receptors whilst proteins CheB1-P/CheB2-P de-methylate active polar and cytoplasmic receptors with unknown connectivity, as in [23].
- A sensed increase in ligand concentration causes a reduction in the number of active receptors.
- Active polar and cytoplasmic receptors promote the auto-phosphorylation of CheA2 and CheA3A4 respectively.
- CheY3-P and CheY4-P act together to promote autophosphorylation of CheA3A4 (Figure 3) whilst CheY6-P binds the FIM rotor switch to increase the frequency of motor switching.

Through the phosphotransfer network, a decrease in the number of active receptors due to a sensed increase in ligand concentration results in a subsequent decrease in the amount of CheY3-P, CheY4-P, CheY6-P, CheB1-P and CheB2-P.

One effect of a sensed increase in ligand concentration is a decrease in the flagellar switching frequency due to decreased amounts of CheY3-P binding with Fim. Figure 3 shows the result of a simulation of the signalling pathway that demonstrates the fall in the concentration of CheY3-P in response to a step decrease in the number of active receptors at the polar or at the cytoplasmic clusters. The reaction rates of the phosphotransfer network are such that a change in the number of active receptors at the cytoplasmic cluster causes a faster fall in CheY3-P concentration than does a similar change in the number of active receptors at the polar cluster.

Quantitatively, the adaptation mechanism in the generic ODE model presented in Materials and Methods functions as follows: CheB1-P and CheB2-P are assumed to de-methylate active receptors, and the phosphotransfer network responds to a sensed increase in ligand concentration by reducing the concentration of CheB1-P, CheB2-P, CheY3-P, CheY4-P and CheY6-P. This results in a reduction in the de-methylation rate of active receptors in the two receptor clusters, and also results in a decrease in the flagellar stopping frequency (which corresponds to an increase in the flagellar rotation rate). The constant methylation of inactive receptors by CheR2 and CheR3 then causes the number of methylated receptors, and, it is assumed, of active receptors, to increase. Thus, the number of active receptors is eventually restored to its pre-stimulus equilibrium level. In turn, the phosphotransfer network then restores the amount of CheY3-P, and hence the flagellar switching frequency, back to its original level. According to the model of the forward signalling pathway, the proteins CheB1-P and CheB2-P therefore act as feedback signals that restore the chemotaxis pathway to its original state. However, the exact connectivity between CheB1-P/CheB2-P and the two receptor clusters is unknown.

To determine the most likely interconnection structure and to provide a rationale of how such a structure may be advantageous in terms of chemotactic performance, we created four variants of the generic ODE model with the forward pathway, each having a different interconnection structure between the proteins CheB1-P/CheB2-P and the two receptor clusters (Figure 2(B)). All models were able to produce wild type response data and behaved as expected for the response data generated with gene deletions available at the time. The unknown parameters in the models \( K_{1}, K_{2}, K_{21}, K_{3} \) and \( K_{1}, K_{2}, K_{3}, K_{21}, K_{22} \) were fitted to wild type data for each model. The significance of these parameters is as follows:

- \( K_{1}, K_{1} \) : Along with the sensed ligand concentration, these parameters determine the proportion of methylated receptors that are active at the polar and cytoplasmic clusters respectively.
- \( K_{2} \), \( K_{3} \) : These parameters determine the strength of the CheB1-P, CheB2-P feedbacks to the polar cluster respectively.
- \( K_{21} \), \( K_{22} \) : These parameters determine the strength of the CheB1-P, CheB2-P feedbacks to the cytoplasmic cluster respectively.
- \( K_{3} \), \( K_{3} \) : These parameters represent the activity of CheR2/CheR3 respectively.

For notational convenience, it is useful to group the CheB1-P/CheB2-P feedback gains \( K_{2}, K_{21}, K_{22}, K_{3} \) into a feedback matrix \( K_{2} = \begin{bmatrix} K_{2} & K_{21} \\ K_{22} & K_{3} \end{bmatrix} \). The four CheB1-P and CheB2-P feedback connectivities (and their associated \( K_{2} \)) for which models were constructed are as follows:

I. CheB1-P regulates the methylation state of the polar receptor cluster and CheB2-P of the cytoplasmic cluster only (shown in solid de-methylation reactions in Figure 2 (B)): \( K_{2} = \begin{bmatrix} K_{2} & 0 \\ 0 & K_{3} \end{bmatrix} \).

II. CheB1-P regulates the methylation state of both the polar cluster and the cytoplasmic cluster while CheB2-P de-methylates only cytoplasmic cluster receptors (solid de-methylation reactions and the dotted de-methylation reaction in Figure 2 (B)): \( K_{2} = \begin{bmatrix} K_{2} & 0 \\ K_{2} & K_{3} \end{bmatrix} \).

III. CheB1-P and CheB2-P both regulate the methylation state of the polar receptor cluster and CheB2-P of the cytoplasmic receptor cluster only (solid de-methylation reactions and the dashed de-methylation reaction in Figure 2 (B)): \( K_{2} = \begin{bmatrix} K_{2} & 0 \\ 0 & K_{22} \end{bmatrix} \).
IV. CheB₁-P and CheB₂-P both regulate the methylation state of both receptor clusters (solid de-methylation reactions, the dashed de-methylation reaction and the dotted de-methylation reaction in Figure 2 (B)): \( K_2 = \begin{bmatrix} K_{21} & K_{22} \\ K_{21} & K_{22} \end{bmatrix} \).

After constructing these four models, we carried out experiments to differentiate between them, by finding the optimal initial conditions of the cells in the assay so as to maximize the difference between the outputs of the different models [20,24]. The conditions searched were limited to what could be implemented experimentally and included deletions, over-expression of proteins and combinations of these. To confirm these conditions allow for invalidation, simulations were run of the four models I–IV testing the possible initial conditions and inputs. The simulations showed that the initial conditions that allow for the best model invalidation were the deletion of CheR₃ and, in a separate experiment, the deletion of CheB₁ (Figure 4).

The experiments were then implemented in *R. sphaeroides*, subjecting a population of cells to a step increase in ligand concentration (propionate) and then measuring the resulting flagellar activity through a tethered cell assay (Figure 4). Experimentally the deletion of either CheB₁ or CheR₃ resulted in cells with a rotation frequency of \(-8\) Hz that showed no noticeable response to the addition or removal of ligand. In the simulations, only Models I and III displayed this behaviour upon deletion of CheR₃ (Figure 4, top row) and only Model III displayed this behaviour upon deletion of CheB₁ (Figure 4, bottom row). Models I, II and IV were thus invalidated and only Model III was able to replicate the experimental data. As a test of this model invalidation, a further experiment wherein CheB₂ was deleted was performed. The result of this experiment and the outputs of the four models under the CheB₂ deletion (overlaid) are shown in Figure 5. Models I and III were once again able to replicate the deletion data whilst Models II and IV produced outputs that differed from the experimental outcome.

![Figure 4. Model invalidation.](doi:10.1371/journal.pcbi.1001130.g004)
Dynamic properties of chemotaxis models

The experiments described above demonstrated that the proposed Models I, II and IV are invalid, being unable to explain experimental data. To compare the four models further, in silico experiments were performed on the data-fitted Models I–IV that compared how the different feedback configurations affect chemotactic performance in terms of the sensitivity of the flagellar stopping frequency in response to variations in the values of the models’ biochemical parameters and in response to noise. Following these results, we use linear models with structures that represent the different connectivities of Models I–IV to analyze these structures’ relative sensitivities to parametric variations and noise.

Chemotactic performance. The performance of the different chemotaxis models was compared by simulating the efficiency of each model in ascending an attractant gradient, as illustrated in Figure 6 (left). For each chemotaxis model, Figure 6 shows the average distance travelled up the attractant gradient by ten bacteria during a simulation lasting 80 seconds. As shown in Figure 6 (right), the chemotactic performances of the different models according to this measure were nearly identical (see Materials and Methods for more details).

Response to noisy ligand variations. The bacterium’s environment is typically composed of regions of high and low chemoattractant or chemorepellant concentrations. Additionally, the bacterium will sense small, fast fluctuations in the detected level of ligand due to molecular noise. To test how sensitive the chemotaxis Models I–IV are to such ligand fluctuations, an in silico experiment was performed on each model in which the ligand concentration sensed by the polar cluster, \( L \), was modelled as the noisy signal \( L = \max(0, 1 + \eta) \), where \( \eta \) is a white noise signal with a zero-mean, unit variance Gaussian distribution. The resulting rotation frequencies were then recorded and are shown in Figure 7. As can be seen in Figure 7, ligand level fluctuations sensed at the polar cluster of receptors resulted in larger variance of the rotation frequency in Models I, II and IV than in Model III.

The sensitivity of the chemotaxis Models I–IV to ligand inputs was then tested in two in silico experiments which were performed on each model and in which the flagellar rotation frequency was recorded in response to sinusoidal variations in the ligand signals \( L \) and \( \tilde{L} \) (the latter of which corresponds to ligand inputs acting on the cytoplasmic cluster). As can be seen in Figure 8, ligand level fluctuations sensed at the polar cluster of receptors resulted in larger changes in the rotation frequency in Models II and IV than in I and III. When the ligand concentration variations were sensed at the cytoplasmic cluster the result was a greater variation in the rotation frequency in Models I and III than in the other two models. Once more, these simulations suggest that CheB1-P demethylating the cytoplasmic cluster differentiates the performance of Models II and IV from Models I and III.

Parametric sensitivity analysis of the chemotaxis models. To investigate the sensitivity of the models to parameter variations, we performed an in silico experiment in which, for each of the different chemotaxis models, the variation of the steady-state of the chemotaxis system was measured under randomly chosen values of the copy numbers of chemotaxis proteins.
Figure 7. Response to external ligand variations. Standard deviations of the flagellar rotation frequencies for each of the four chemotaxis models in response to a noisy ligand input sensed at the polar cluster given by $L = \max(0, 1 + \eta)$ (where $\eta$ is a white noise signal with a zero-mean, unity variance Gaussian distribution).

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Figure 8. Input-output gains of the two sensing clusters. Frequency response magnitude plots showing the response of the different models to sinusoidally-varying ligand concentrations modeling noisy ligand input signals. Top: Constant ligand to cytoplasmic cluster and variable ligand to polar cluster ($L = L^* + \sin(\omega t)$, $L^* = 3.5L^* + 0.035$, where $L^* = 2$). Bottom: Constant ligand to polar cluster, sinusoidal to cytoplasmic cluster ($L = L^*, L = 3.5(L^* + \sin(\omega t)) + 0.035$, where $L^* = 2$).

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(see Materials and Methods). For each chemotaxis protein, the resulting coefficient of variation of the steady-state is shown in Figure 9. Once more, there was a similarity in the sensitivity of each model to these parametric variations between Models I and III and between Models II and IV, with the latter pair showing slightly higher sensitivity to copy numbers of the chemotaxis protein CheY6 among others. In addition, Model III showed considerably lower sensitivity with respect to CheB1 copy numbers than the other models.

Linear model analysis. Further insight to the differences in performance between the models can be obtained by analyzing the interconnection structure of these models using control theory. In particular, the way in which such feedback arrangements can affect the performance of control systems like the R. sphaeroides chemotaxis pathway can be studied by comparing the behaviour of different linear systems that are structurally similar to Models I–IV. The block diagram in Figure 10 depicts a system composed of two modules representing the polar and cytoplasmic clusters. The CheB1-P/CheB2-P outputs of the two modules exhibit exact adaptation through integral control in response to step changes in the input ligand concentration level, as in E. coli [8].

Depending on the values of feedback gains $\kappa_2$ and $\kappa_2$ (which correspond to $K_2$ and $K_2$, respectively in the chemotaxis models described above), the system can represent one of the four chemotaxis models:

- Model I: $\kappa_2 = 0$, $\kappa_2 = 0$
- Model II: $\kappa_2 > 0$, $\kappa_2 = 0$
- Model III: $\kappa_2 = 0$, $\kappa_2 > 0$
- Model IV: $\kappa_2 > 0$, $\kappa_2 > 0$.

The gains $a$, $b$ in Figure 10 are such that $b > a > 0$, representing the fact that the cytoplasmic receptor cluster can, as a result of the measured reaction rates, relay a sensed ligand input signal to the flagellar motor faster than the polar receptors cluster (see Figure 3). For the examples we shall consider we set $a = 1$ and $b = 10$. Gains $\kappa_2$ and $\kappa_2$ correspond to $K_2$ and $K_2$ in the chemotaxis model respectively. The frequency domain transfer function of the system in Figure 10 from the ligand inputs $L$ and $\hat{L}$ to the output $Y$ is then

$$Y = \frac{bg_1G_1G_2}{s + abqK_2G_1G_2}L + \frac{G_2}{s + abqK_2G_1G_2}\hat{L} \quad (1)$$

where $G_1 = \frac{s}{s + K_2}$, $G_2 = \frac{s}{s + bK_2}$, $q = 1 - K_2$, $K_2 > 0$, $K_2 > 0$.

This function is a frequency-domain map from signals $L$ and $\hat{L}$ to the output $Y$, which corresponds to the flagellar rotation frequency. In the following, we shall use this frequency domain...
representation of the chemotaxis system to demonstrate how the feedback of linear systems with structures similar to the chemotaxis Models I–IV affects system performance.

The Bode magnitude diagrams (Materials and Methods) in Figure 11(A) illustrate the effect of increasing $k_{22}$ in reducing the sensitivity function of the system (1) over most excitation frequencies (see the Discussion and Text S1 for a brief introduction to sensitivity functions). At the same time, Figure 11(B) shows that strengthening the feedback $\tilde{k}_{22}$, which corresponds to increasing the demethylation of the cytoplasmic cluster by CheB$_2$-P, decreases the sensitivity of the polar cluster over low frequencies.

Figure 12 presents a Bode magnitude plot showing the gain of the linear system (1) to inputs $L$ and $\tilde{L}$ which represent sensed ligand at the polar and cytoplasmic receptor clusters respectively. The figures show that, similar to the simulations of Models I and III, the linear model with a gain $k_{22}=0$ (similar in structure to Model I) and $k_{22}=10$ (similar in structure to Model III) also shows a relatively low sensitivity to high frequency (noisy) inputs at the polar receptor cluster and a relatively high sensitivity to noise detected at the cytoplasmic receptor cluster.

**Discussion**

From the designed experiments performed, it was possible to invalidate all models but Model III. This suggests that the feedback in the chemotaxis system could occur in an asymmetric fashion. That is, CheB$_1$-P may only interact with the membrane signalling cluster whilst CheB$_2$-P interacts with both clusters. It is likely that the two chemotaxis pathways initially evolved independently and then became part of the same organism by horizontal gene transfer.
Thus one would possibly expect either full connectivity or complete isolation of the two pathways until a further mutation occurs.

Understanding the outputs of the designed experiments

*R. sphaeroides* has a more complex chemotaxis network than *E. coli* and the multiple receptor clusters and multiple feedback pathways mean that mutants will not always have an intuitive phenotype. For example the ΔcheB1 mutant does not have the loss of response phenotype one would expect from a direct comparison with the *E. coli* system. We can try to understand why ΔcheB1 has a steady state at −8 Hz by looking at the structure of the model we have been unable to invalidate, and the reason is as follows:

CheB1, CheB2 and CheY6 (along with CheY3 and CheY4) each compete for phosphoryl groups from CheA2-P. CheB1 is present in relatively large copy numbers and CheB1-P has negligible degradation rate (see Table 1). When present, CheB1 ‘stores’ a large proportion of phosphoryl groups. When absent, the competition for phosphoryl groups from CheA2-P remains between CheB2, CheY6, CheY3 and CheY4. The rate of phosphorylation of CheY6 by CheA2-P is relatively small, CheY6-P receiving most of its phosphorylation from the CheA3A4-P complex. Therefore deleting cheB1 shifts the equilibrium of the system so that a higher proportion of the phosphoryl groups from CheA2-P go to CheY3, CheY4 or CheB2. The increase in CheY3-P and CheY4-P results in a stronger negative feedback to the cytoplasmic cluster, and the steady-state amount of active receptors at the cytoplasmic cluster is therefore less in the case of ΔcheB1. The consequence of this is that the main source of...
phosphorylation for CheY6-P, which is CheA3A4-P, is reduced, and hence the level of CheY6-P is reduced. The stopping frequency is consequently reduced. Therefore, rather than a shift in the steady state to a high rotation frequency, leading to a loss of response to stimulus, the result of this deletion is an incremental change in the overall system’s transfer function in response to an incremental change in the transfer function of the system’s submodules G1 and G2. If the sensitivity of the chemotaxis system is low, then the bacterium would be able to maintain its chemotactic response despite changes in the system’s biological parameters. The Bode plots (Materials and Methods) in Figure 11(A) illustrate the effect of increasing $k_{22}$, in reducing the sensitivity function of the system (1) over most excitation frequencies. This effect can be observed in the chemotaxis models in Figure 9 and Figure 13, where it is shown that strengthening the CheB2 feedback to the polar cluster reduces the sensitivity of the steady state rotation frequency decreases in the copy numbers of CheB1 and CheA2 (see Materials and Methods).

Simulation results in Figure 7 show that the switching frequency in Model III has a low sensitivity to noisy variations in ligand signals detected at the polar receptor cluster relative to the other models. Figure 8 shows the result of a further set of simulations of the four chemotaxis models in which the gain of each chemotaxis model in response to sinusoidal ligand variation detected at the two clusters is given as a function of ligand fluctuation frequency (see Materials and Methods). The figure shows that the switching frequency in Models I and III has a relatively low gain with respect to varying ligand signals detected at the polar receptor cluster and a relatively high gain with respect to ligand variations detected at the cytoplasmic cluster. The Bode magnitude plots in Figure 12 show the frequency-dependent gain of the linear system (1) to sinusoidal ligand inputs in the case $K_3 = 0$, which is structurally similar to Models I and III. These plots parallel the results of the frequency response magnitude plots of Figure 8 which, for Models I and III, show low gain in response to high frequency inputs at the polar receptor cluster and high gain in response to high frequency signals at the cytoplasmic receptor cluster. The rejection of high frequency inputs at the cell pole may be advantageous in that the flagellar switching rate is then only varied when the polar cluster senses a relatively significant ligand concentration gradient that is large in spatial extent, and remains relatively unchanged when the receptors are subject to rapid fluctuations in sensed ligand due, for example, to molecular noise at the receptor such as that simulated in Figure 7.

Although the chemotaxis model assumes that the cytoplasmic cluster input depends on the sensed ligand, it is unknown what the cytoplasmic cluster senses. In addition to the possibility that this input is a function of the sensed ligand concentration, this cluster may potentially also integrate information about the metabolic state of the cell. In this case, this signalling may well be important to chemotactic performance and the relatively high gain of Model III to inputs at the cytoplasmic cluster may suggest that this
configuration would favour internal signals over external signals in terms of output. However, if chemotaxis is sensitive to such signals, it would be important that: (i) these signals are tightly controlled and relatively free of the influence of noise and (ii) the cytoplasmic cluster be insensitive to variations in its biological parameters, as sensitivity to such variations would diminish the system’s ability to correctly respond to inputs to the cytoplasmic cluster. In Model III, the CheB2-P feedback loop around the cytoplasmic cluster could offer this reduction in the sensitivity function of this cluster to such parametric variations. This reduction in sensitivity to variations of cytoplasmic cluster parameters is illustrated in Figure 11(B) using the linear model (1) of the chemotaxis system. The figure shows that increasing the feedback gain $K_{2,2}$, which corresponds to the gain of the CheB2-P feedback to the cytoplasmic cluster in Model III, achieves a reduction in the sensitivity of the cytoplasmic cluster. In this way, the cytoplasmic cluster remains sensitive to its inputs, as shown by the large gain at high frequency in Figure 12(B), whilst its sensitivity to parametric variation is reduced due to the internal CheB2-P feedback. This effect can be observed in the chemotaxis models in Figure 14, where it is shown that strengthening the CheB2 feedback to the cytoplasmic cluster reduces the sensitivity of the steady state rotation frequency to changes in the copy numbers of CheA3A4 and CheY6 (see Materials and Methods).

Figure 8 also shows that for Model I and III, high frequency variations in the ligand concentration sensed at the polar cluster are largely filtered out before causing flagellar switching. This may suggest that the relatively slow dynamics of the polar receptor cluster enable it to function as a low pass filter, preventing any high-frequency noisy variations in the sensed concentration of ligand from being signalled through to the flagellar motor. Figure 12(A) illustrates this attenuation of high frequency polar cluster ligand inputs for the linear model (1).

Chemotaxis as a cascade controlled system

When combined with the forward signalling pathway which was not invalidated previously [20], Model III has a feedback structure that corresponds to a control scheme termed cascade control. This term is used to denote a modular system that includes two feedback loops, one
nested within the other. The nested loop is used to regulate a sub-process of the system whilst the ‘external’ negative feedback loop from the system output to the input is used to regulate the entire system.

The measured reaction rates of the two clusters [15,16] are also such that the cytoplasmic cluster is faster than the polar cluster in responding to inputs, which would be required for the chemotaxis pathway to function as a cascade controlled system [19]. This modularization of the chemotaxis system into fast and slow parts mirrors the division of the cascade controlled system in Figure 1 into the slow and fast subsystems $G_1$ and $G_2$ respectively. The cascade control architecture enables the slow (primary) subsystem to fix a set-point for the fast (secondary) system and for the feedback around the secondary system to quickly regulate the secondary output in response to disturbances and variations in the secondary process [19]. This difference in speed is represented by having $b > a$, $\kappa_2 = 0$ and $\kappa_2 > 0$ in the linear model (1). Model III also features both an ‘internal’ feedback loop nested within an ‘external’ one corresponding to the dashed and solid feedbacks in Figure 1, respectively. These two feedbacks are manifested by the CheB2-P feedback that de-methylates the cytoplasmic and the polar clusters respectively.

Interestingly this architecture mirrors the ability of the system to phosphotransfer, with the membrane cluster being able to phosphotransfer to and be de-methylated by both CheB proteins and the cytoplasmic cluster only phosphotransferring to CheB2, the protein that is able to de-methylate it. It does however raise an interesting question. Whereas CheB in *E. coli* is localised to the polar signalling cluster, in *R. sphaeroides* both expressed CheB’s are found to be delocalised. Yet, only one of the CheB proteins interacts with both signalling clusters. Thus the advantage of having delocalised CheB1 is unclear.

We have shown that if the *R. sphaeroides* chemotaxis pathway has a cascade control architecture, this would enable robust chemotaxis in an uncertain, noisy environment, conferring a selective advantage. In *E. coli*, one feedback loop is used to achieve perfect adaptation and sensing of temporal gradients and because there is only one signalling cluster all signal integration occurs there. Unlike *E. coli*, the *R. sphaeroides* chemotaxis pathway with cascade

![Figure 14. Sensitivity to copy number with varying internal feedback.](https://doi.org/10.1371/journal.pcbi.1001130.g014)
control feedback provides the bacterium with two feedback loops, one embedded within the other, to adapt and to reduce its sensitivity to parameter variations and noise. The other advantage to this architecture is demonstrated by the simulations shown in Figure 12, which illustrate that with this structure the system would be strongly sensitive to fast-changing inputs to the cytoplasmic cluster, perhaps from the metabolic state of the cell.

Understanding how biological networks achieve robust functionality in the face of disturbances and noise in their internal and external environment is a key question in systems biology. Such networks can be seen as control engineering feedback systems and can be analyzed using system engineering tools in order to understand the advantages of particular internal connectivities over others. In line with this methodology, this paper first utilized a network discrimination approach [20] to construct a model of the feedback connectivity within the R. sphaeroides chemotaxis pathway, and then explained the robustness properties of that model by re-interpreting the theoretical advantages of its cascade control structure in a biological framework and comparing it to the other possible models. This suggests a mechanism by which the bacterium can achieve robust chemotactic performance despite biochemical parameter variations and noise. Given that many chemotactic systems have multiple homologues [10] it would appear that using more complex feedback architectures to improve performance may be common in chemotaxis and in other signalling pathways, raising the possibility that this methodology can be used to analyze a wide set of biological systems.

Materials and Methods

Modelling the chemotaxis pathway in R. sphaeroides

In the next three subsections, we present the three different modules of the chemotaxis signalling pathway: sensing, transduction and actuation.

Sensing. We assume the same underlying mechanisms for the polar (MCP) and the cytoplasmic (Tlp) receptors. The parameters of the Tlp cluster are labelled with a tilde superscript. We also make the same assumptions of our model as those in [20], which are adopted from the E. coli chemotaxis literature [23].

With the notation defined in Table 2, the model for the sensing mechanism is as follows:

\[
[K_n] = \frac{1}{(K_1 + \varepsilon[L])} [R_m]
\]

\[
[R_m] = K_3 ([R_T] - [R_n]) - [R_n] (K_1 [B_{1p}] + K_2 [B_{2p}])
\]

\[
[R_n] = \frac{[Y_{1p}] + [Y_{2p}]}{[K_1 ([Y_{1p}] + [Y_{2p}]) + [L]]} [R_m]
\]

\[
[R_m] = K_5 ([R_T] - [R_n]) - [R_n] (K_1 [B_{1p}] + K_2 [B_{2p}])
\]

We assume that the cytoplasmic receptor cluster senses extracellular ligand concentrations indirectly; for example, \(L\) could be internalized attractants, a by-product of the internalization process or a metabolic response to it. For simplicity, we assume the following affine relationship between \(L\) and \(L\):

\[
[L] = 3.5[L] + 0.035
\]

We let \(\varepsilon = 1\) (\(\mu\text{M}^{-1}\)) and \([R_T] = [R_T] = 1\mu\text{M}\). The remaining unknown parameters in this model are the dimensionless quantities \(K_1, K_1, K_3\), the feedback matrix \(K_3 = \begin{bmatrix} K_{11} & K_{12} \\ K_{21} & K_{22} \end{bmatrix}\) which have units of \((\mu\text{M} s)^{-1}\) and \(K_3, K_3\) (which have units of \(s^{-1}\)). The significance of these parameters was detailed in the Results section. We obtain the following values for these unknown parameters for the different models by fitting them to data from tethered cell assays:

\[
K_1 = K_3 = 20, \ K_3 = \begin{bmatrix} 33.75 & 0 \\ 0 & 33.75 \end{bmatrix}, \ K_3 = K_3 = 0.0612
\]

\[
K_1 = K_1 = 1, \ K_3 = \begin{bmatrix} 0.0022 & 0 \\ 0.0022 & 0.0022 \end{bmatrix}, \ K_3 = K_3 = 0.002
\]

\[
K_1 = K_1 = 20, \ K_3 = \begin{bmatrix} 33.75 & 33.75 \\ 0 & 33.75 \end{bmatrix}, \ K_3 = K_3 = 0.0612
\]

\[
K_1 = K_1 = 1, \ K_3 = \begin{bmatrix} 0.0022 & 0.0022 \\ 0.0022 & 0.0022 \end{bmatrix}, \ K_3 = K_3 = 0.002
\]

The difference between models I–IV lies in the structure of the CheB1-P, CheB2-P feedback.

| Table 2. Model notation. |
|--------------------------|
| Species | Definition |
| --- | --- |
| \(R_T\) | Total polar cluster receptors |
| \(R_T\) | Total cytoplasmic cluster receptors |
| \(R_m\) | Methylated polar cluster receptors |
| \(R_m\) | Methylated cytoplasmic cluster receptors |
| \(R_n\) | Active polar cluster receptors |
| \(R_n\) | Active cytoplasmic cluster receptors |
| \(A_2\) | Un-phosphorylated CheA2 |
| \(A_2\) | Phosphorylated CheA2 |
| \(\{A_4\}_1\) | Un-phosphorylated CheA1 - CheA4 |
| \(\{A_4\}_2\) | Phosphorylated CheA1 - CheA4 |
| \(B_1\) | Un-phosphorylated CheB1 |
| \(B_2\) | Phosphorylated CheB1 |
| \(B_3\) | Un-phosphorylated CheB2 |
| \(B_3\) | Phosphorylated CheB2 |
| \(Y_3\) | Un-phosphorylated CheY3 |
| \(Y_3\) | Phosphorylated CheY3 |
| \(Y_4\) | Un-phosphorylated CheY4 |
| \(Y_4\) | Phosphorylated CheY4 |
| \(Y_5\) | Un-phosphorylated CheY5 |
| \(Y_5\) | Phosphorylated CheY5 |
| \(Y_6\) | Un-phosphorylated CheY6 |
| \(Y_6\) | Phosphorylated CheY6 |
| \(L\) | Ligand acting on polar cluster receptors |
| \(L\) | Ligand acting on cytoplasmic cluster receptors |
| \(M\) | Motor activity |
| \(\gamma\) | Average bacterium body rotation rate |

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Transduction. We assume that the structure of the phosphotransfer network is the same as that of the models presented previously in [20], with the modification that when polar and cytoplasmic receptors are in their active state the respective auto-phosphorylation rates of CheA2 and CheA3, $k_1$ and $k_1^*$, are accelerated to $k_1 = 100R_kk_1$ and $k_1^* = 100R_kk_1^*$ where $k_1$ and $k_1^*$ are the reaction constants of the auto-phosphorylation of CheA2 and CheA3 obtained from in vitro experiments in the absence of the influence of receptors, as given in Table 1 and in [20]. Biologically, it would be expected that the ligand concentration (where distance along these two directions is unit-less), as observed in tethered cell assays. We use the following heuristic description to convert motor activity into a low-pass filter that attenuates fast frequency effectively constitutes a low-pass filter that attenuates fast stopping frequency decreases at a constant rate in the absence of CheY6-P. This relationship between the CheY6-P and the stopping frequencies with a mean given by the nominal value of the parameter. If the system is the so-called state space representation of the model exists to analyze and compare the properties of linear systems in the so-called frequency domain using their associated transfer functions [25]. Using such tools, it is possible to study the effects of excitation frequency on systems’ gains and sensitivities as was done in this paper. As an example of how this method works, consider a linear dynamical system $\dot{x} = Ax + Bu, \quad y = Cx$ (6)\[14\] where $A$, $B$ and $C$ are matrices of appropriate dimension, whose entries depend on the model parameters, and $u(t) = r \sin(\omega t)$ is a sinusoidal input with angular frequency $\omega$ and fixed amplitude $r$. System (6) is the so called state space representation of the model in this paper. As an example of how this method works, consider a linear dynamical system $\dot{x} = Ax + Bu, \quad y = Cx$ (6) where $A$, $B$ and $C$ are matrices of appropriate dimension, whose entries depend on the model parameters, and $u(t) = r \sin(\omega t)$ is a sinusoidal input with angular frequency $\omega$ and fixed amplitude $r$. System (6) is the so called state space representation of the model in the time domain. It is common in control systems engineering to investigate the behaviour of such a system’s dependency on excitation frequency $\omega$. This requires transforming the system to the frequency domain via the Laplace transform. We denote the Laplace transform of $u$ and by $U(s)$ and $Y(s)$ respectively, where $s$ is a complex independent variable. Then, $Y(s) = G(s)U(s)$ where $G(s)$ is the transfer function in the frequency domain and is given by [25]:

By evaluating this function for values of $s$ on the imaginary axis (by setting $s = jo$ where $j = \sqrt{-1}$) we obtain a frequency domain relationship between the system’s input and output. If the system is stable (the eigenvalues of matrix $A$ have negative real parts) and is excited with a periodic input signal $u$ of frequency $\omega$, then after some transient behaviour the output $y$ is given by a sinusoidal wave that is phase shifted and amplified with respect to $u$ by amounts dependent on $\omega$. The amplification factor is given in decibels by

$$|G(j\omega)|_{dB} = 20\log_{10}\sqrt{\text{Re}(G(j\omega))^2 + \text{Im}(G(j\omega))^2},$$

whilst the phase shift is given by

$$\angle G(j\omega) = \arctan\left(\frac{\text{Im}(G(j\omega))}{\text{Re}(G(j\omega))}\right).$$
The Bode magnitude plot shows the variation of $|G(j\omega)|_p$ in decibels with frequency of excitation $\omega$. The Bode phase plot shows the variation of $\angle G(j\omega)$ in radians with frequency of excitation $\omega$.

**Model parameters**

Model parameters were obtained by performing least squares fitting on previously obtained experimental data [20]. These are listed in Table 1. Protein concentrations were obtained via quantitative western blotting as described in [20].

**Response to noisy ligand input**

We tested the gain of each model to sinusoidally varying ligand input signals, applied separately at the polar and at the cytoplasmic clusters. In the first case we applied the constant ligand input $L = 3.5L_0^* + 0.035$, with $L_0^* = 2$ to the cytoplasmic cluster whilst simultaneously applying to the polar cluster sinusoidally varying ligand signals given by $L = L_0^* + \sin(\omega t)$, with frequency $\omega$ in the range 0.01 to 1 rads$^{-1}$.

In the second case we applied the constant ligand input $L = L_0^*$ to the polar cluster whilst simultaneously applying to the cytoplasmic cluster sinusoidally varying ligand signals given by $L = 3.5(L_0^* + \sin(\omega t)) + 0.035$, with frequency $\omega$ in the range 0.01 to 1 rads$^{-1}$.

The frequency response magnitude plots of Figure 8 show the magnitude of the fundamental frequency of the sinusoidal variation in the flagellar rotation frequency in response to these sinusoidal ligand input signals.

**Plasmids and strains**

The strains used in this study were shown in Table 3. *R. sphaeroides* strains were grown in succinate medium at 30°C under aerobic conditions with shaking. Where required, nalidixic acid was used at concentrations of 25 g ml$^{-1}$.

**Tethered cell analysis**

Tethered cell responses to propionate of the *R. sphaeroides* strains were characterized as described previously [20]. For each strain and wild type 4 slides were analyzed each containing 10 cells.

**Supporting Information**

Text S1 Supporting information text. Found at: doi:10.1371/journal.pcbi.1001130.s001 (0.95 MB DOC)

**Author Contributions**

Conceived and designed the experiments: AH MAJR EA PKM JPA AP. Performed the experiments: AH MAJR EA. Analyzed the data: AH MAJR EA PKM JPA AP. Contributed reagents/materials/analysis tools: AH MAJR EA PKM JPA AP. Wrote the paper: AH MAJR EA PKM JPA AP.

**Table 3. Strains used in this study.**

| Strain/Plasmid | Characteristics | Source |
|----------------|----------------|--------|
| *R. sphaeroides* WS8N | Spontaneous nalidixic acid resistant mutant of wild type WS8 | [26] |
| *R. sphaeroides* JPAS17 | WS8N with the cheR, gene deleted by genomic replacement | [17] |
| *R. sphaeroides* JPA 1320 | WS8N with the cheR, gene deleted by genomic replacement | [15] |

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