Modelling of Simplified Approach in R. Spaeroids–through Signalling of Chemotaxis

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Abstract

Here we investigate the chemotaxis signalling pathways of R. sphaeroides cells. We begin with a summary of the experimental and theoretical literature that represents the current understanding of chemotaxis in R. sphaeroides cells. A recent mathematical model of this system is then presented alongside a discussion of the main assumptions and simplifications used in its formulation. In particular, we consider a non-dimensional re-scaling. This is then used to demonstrate that there exists just one biologically feasible equilibrium state. Stability analysis then shows this equilibrium state to be asymptotically stable and the resulting eigenvalues are examined in order to assess the stiffness of the model system. Finally, we identify the need for new ideas by showing that this model fails to capture behaviour observed in recent experimental work.

DOI Number: 10.48047/NQ.2022.20.13.NQ88508 Neuroquantology 2022; 20(13): 4189-4193

1. Introduction

Biology Underlying Chemotaxis in R. sphaeroides Here we summarise the key biological processes allowing R. sphaeroides cells to exhibit bacterial chemotaxis. Within the experimental literature a number of features of this system have been studied in depth. However, some other areas are less well understood. We do not therefore possess a full understanding of the system from start to finish. As such, within this section we begin by explaining what is known both about the chemotactic swimming behaviour of R. sphaeroides cells and how this is controlled by the cells' intracellular signalling pathways. In particular, here we focus on explaining well understood features of the system and note areas where further work is required.

The first of these chemosensory pathways is that beginning at the polar receptor cluster. Similar to those found in E. coli, receptors span the cytoplasmic membrane of the cell with the external part able to bind molecules of certain attractants/repellents. A signal is then passed into the cell. Here the intracellular domain of the chemoreceptors bind to the protein CheA2 via the linker proteins CheW2 and CheW3. Similar to the HPK (histidine protein kinase) in E. coli, CheA2 autophosphorylates (forming CheA2-P) at a rate dependent upon the signal received.

2. Mathematical Modelling

In addition to the experimental work discussed, there are also a number of examples within the literature whereby mathematical modelling has sought to further understanding of chemotaxis in R. sphaeroides cells. Each of the examples discussed here utilise ODE models to investigate various aspects of the R. sphaeroides chemotaxis signalling network. As such, here we focus on the main findings of each paper and provide just a brief summary of the key differences between the theoretical approaches considered. Early examples of theoretical work on this system sought to

elucidate the network of reactions responsible for chemotaxis in R. sphaeroides.

In particular, the works of Roberts et al. and Hamadeh et al. each constructed a number of mathematical models containing different combinations of possible network connections (i.e. phosphorylation and methylation reactions). In each case the models were compared against experimental data in order to assess which was the most effective model of R. sphaeroides chemotaxis. However, whilst these models produce a reasonable fit to some experimental data, they have subsequently been shown to contain reactions that cannot occur biologically. Tindall et al. sought to utilise mathematical modelling to elucidate the roles of various components of the R. sphaeroides chemotaxis signalling pathways. This work was able to demonstrate that CheA3 functions as a bi-functional kinase-phosphatase protein.

Biologically, this means that CheA3-P phosphorylates CheY6 and CheB2 (kinase action), however both CheA3 and CheA3-P act to dephosphorylate CheY6-P (phosphatase action). This work also showed that a number of reverse phosphorylation reactions occur, i.e. phosphorylated response regulator proteins (CheB and CheY homologues) may transfer their phosphoryl groups on to non-phosphorylated CheA proteins. In particular, a reverse phosphorylation reaction was found to exist for each phosphotransfer reaction except for one - CheY6-P does not transfer phosphoryl groups onto CheA2. The work of Amin et al.modelled only reactions occurring at the cytoplasmic cluster of R. sphaeroides cells. As such, their model included proteins CheA3, CheA4 and CheY6 in their phosphorylated and non-phosphorylated states as well as the transient complexes formed during each reaction.

Using this model it was shown that the atypical HPK present in R. sphaeroides cells is capable of producing both ultrasensitivity and bistability under certain parameter regimes. Martin et al. investigated the mechanisms behind adaptation in R. sphaeroides cells by studying reactions involving CheR and CheB proteins. Whilst no mechanisms were proposed in this work, it was concluded that CheR and CheB homologues are likely to be responsible 108 for adaptation. They also went on to state that in spite of utilising homologues of the same proteins, the mechanism for adaptation in R. sphaeroides does not appear to be the same as that of B. subtilis or E. coli. Recently, the work of Hamadeh et al. and Kojadinovic et al.demonstrated the existence of fold-change detection (FCD) within chemotactic R. sphaeroides cells. The theoretical work of Kojadinovic et al. is of particular interest here since it extends the work of Tindall et al.and couples this with an MWC-based adaptation mechanism similar to those considered for E. coli chemotaxis modelling for both the polar and cytoplasmic receptor clusters. This model therefore represents the most complete description of the chemotaxis signalling pathways of R. sphaeroides within the literature.

Simplified Model 1: The Cytoplasmic Cluster

Here we consider a reduced model that contains only reactions occurring atthe cytoplasmic cluster. This is then used to answer a number of outstanding questions on theworkings of the cytoplasmic cluster.

In order to formulate this reduced model we consider only the set of reactions occurring atthe cytoplasmic receptor.

- CheA3 phosphorylation by CheA4 (simplified here to CheA3 autophosphorylation);
- phosphotransfer from CheA3-P onto CheY6 and CheB2;
- reverse phosphotransfer from CheY6-P and CheB2-P onto CheA3;
- autodephosphorylation of CheY6-P and CheB2-P; and
- CheA3 and CheA3-P phosphatase action on CheY6-P.

Considering steady-state and dynamic results together, we may propose a number of roles for the chemotaxis signalling proteins studied here.

- **Group A:** These terms are required to ensure functioning of the signalling pathway.
- **Group B:**Autodephosphorylation acts to reduce the phosphorylated CheY6 concentration slightly. The small degree to

which this occurs is likely to suggest that this process represents an additional level of robustness, allowing the cell to produce a limited response even when the phosphatase action fails.

• **Group C:** Phosphatase action of CheA3 and CheA3-P on CheY6-P is important in controlling the basal CheY6-P concentration. It therefore prevents the basal CheY6-P concentration from becoming too close to saturation as to hinder normal function.

• **Group D:** It is clear that CheB2 acts to create a sharing of phosphoryl groups between itself and CheY6. However, the effect on the CheY6-P concentration is very small due to the very large CheY6 concentration in R. sphaeroides cells. How then may CheB2 be involved in adaptation? In Chapter 7 it was shown that adaptation via an E. coli MWC mechanism leads to unrealistic CheY6 phosphorylation levels in protein deletion mutant strains. In particular, the deletion of CheB2 led to large increases in the phosphorylation level of CheY6. Considering a model of the form given in equations (8.4)-(8.6) appears to limit this issue. We could perhaps then suggest that the adaptation mechanism at the cytoplasmic cluster of R. sphaeroides cells is much weaker or slower than in the case of E. coli, the mechanism of which was previously assumed to hold for R. sphaeroides. This would likely lead to a much smaller difference in the steady-state CheY6-P concentration for a CheB2 deletion mutant cell since the effect of CheB2 on the system would be much more subtle than in the E. coli mechanism. It is worth noting that this very simple system lacking in an adaptation mechanism appears to solve the problem associated with the ΔCheB2 mutant cell type, since the CheY6-P concentration remains almost unaffected. However, the experimental data of de Beyer describes the behaviour of the ΔCheB2 deletion mutant as being inhibited, with much longer adaptation times. A system with no adaptation mechanism such as that considered here cannot produce a response of this type under a CheB2 deletion and thus another mechanism must be sought in future work. It is perhaps possible that the connection between the polar and cytoplasmic clusters via CheB2, may act to produce an appropriate response however the exact mechanism for this is as yet unknown. 140 The results presented within this section clearly demonstrate the roles of each cytoplasmic cluster process. Whilst useful in its own right, this model considers only the cytoplasmic cluster. Thus, in order to improve our understanding of other cell features it is necessary to consider the two chemosensory pathways together in the same model.

3. Validation of the Signalling Pathways

A simplified mathematical model of processes occurring at the cytoplasmic cluster in R. sphaeroides cells was investigated. Studying the steady-state and dynamic properties of this simplified ODE model with various processes systematically removed allowed us to assign likely roles to each of the relevant signalling proteins acting at the cytoplasmic cluster. Within this section we utilise Simplified Model 2 in order to investigate a key outstanding question relating to the polar cluster. This is as follows.

• A reverse phosphotransfer from CheB2-P onto CheA2 has been observed in in vitro experimental work. However, to date, this has not been shown to occur in vivo. Would we expect to see this reaction occurring? Within the previous literature, all phosphorylation reactions included within the Kojadinovic et al. model have been shown to occur in vitro. In fact, all apart from the reverse phosphotransfer from CheB2-P onto CheA2 have also been demonstrated to exist in vivo. An investigation into the existence of this reaction allows us to create a full, validated set of phosphorylation/dephosphorylation reactions occurring within R. sphaeroides cells. This is explored in-depth within the next section. Is Reverse Phosphotransfer from CheB2-P onto CheA2 Expected in vitro? As discussed, all phosphorylation/dephosphorylation reactions in the signalling pathways of R. sphaeroides cells have been validated except for one. This is the reverse phosphotransfer from CheB2-P onto CheA2 which has been shown to be

possible in vitro but not demonstrated in vivo. As such, here we utilise mathematical modelling to ascertain whether or not this reaction will be expected to occur in vivo. This will then result in a full set of validated phosphorylation/dephosphorylation reactions for the two chemosensory pathways of R. sphaeroides cells. In order to answer this question, the wild-type system described in Section 8.2.2 is compared with a system in which the reverse phosphotransfer in question has been eliminated (i.e. k−7 = 0).

Analysis of each of these two models shows the existence of just one biologically feasible steady-state, each of which is asymptotically stable. 141 Using a steady-state analysis of the two systems discussed here, the effects of a reverse phosphotransfer from CheB2-P onto CheA2 can be investigated. In particular, the differences between the steady-states of each signalling protein can be studied which should allow a prediction to be made as to whether this reverse phosphotransfer will be present in vivo.

We provided a summary of the past literature most relevant to the work contained within this paper. In particular, we began by summarising the biological work that sought to identify key components of chemotaxis signalling cascades and their respective roles. Additionally,we focused on how experimental results inspired mathematical modelling and vice versa, leading to the modern understanding of bacterial chemotaxis. Due to their importance throughout this paper, aspects of chemotaxis signalling cascades including the roles of various signalling proteins, chemoreceptors, methylation and phosphorylation were 160 explored in addition to their relevance to signal transduction, adaptation and the flagellar motor response. There have been a number of mathematical models formulated that have sought to elucidate key features of chemotactic responses of single E. coli cells.

The results we could observe that the cytoplasmic cluster produces the greatest variation from the experimental data. It is clear therefore that this model has been invalidated and as such we required alternative approaches in order to gain further knowledge of this species. These were utilised in order to gain further understanding of the R. sphaeroides chemotaxis signalling system. The first of these simplified models was a three ODE model of the cytoplasmic cluster. This allowed us to identify the role of each reaction. In particular, it was found that in order to regulate the phosphorylated concentration of CheY6-P, autodephosphorylation and phosphatase action are required. This phosphatase action was shown to be far more effective than autodephosphorylation at regulating CheY6-P levels, although this is likely to represent an additional layer of regulation should the phosphatase action fail. In addition to this it was shown that CheB2 cannot act as a phosphate sink due to the small concentration of this relative to CheY6. Further to the cytoplasmic cluster model, we also considered a non-adapting version in order to investigate two further questions. Similar to earlier work within this thesis, a number of variants of this model were considered representing mutant cell types 162 used within a recent experimental study (neglecting those relating to CheR and CheB proteins associated with adaptation). Within this work it was possible to show that a model without the reverse phosphotransfer reaction from CheB2-P onto CheA2 that has been demonstrated in vitro leads to near saturation of CheB2-P. It is clear therefore that some regulatory process is required and due to the in vitro evidence for this reverse phosphotransfer, it would seem likely that this is indeed the regulatory mechanism acting in vitro. The same model was then used in order to prove that CheY3 and CheY4 must play some role in setting flagellar rotation. In addition to this it is shown that the ratio of CheY3-P and CheY4-P to CheY6-P produced a correlation with the proportion of time flagellar motors spend in a stopped state. Using the work in Chapters 7 and 8 we postulate a new signalling model for R. sphaeroides and show that it helps remove some issues associated with previous models.

Results obtained from this model revealed two distinct response types. In particular, for

an extracellular ligand stimulus, the cell displays a response similar to that observed in E. coli. In contrast to this, intracellular metabolic signals acted to tune the steady-state phosphorylation levels of CheB2 and CheY6. Each of these responses appears in line with experimental data. The main improvement of this model however is that it removes the issue of near saturation of CheY6-P upon the deletion of CheB2. Whilst it is clear that we have identified a number of features associated with R. sphaeroides chemotaxis signalling pathways, there is still much work to do. In particular, it is necessary to produce both extra experimental and theoretical work in order to identify the exact mechanisms associated with adaptation at the polar and cytoplasmic clusters of chemotactic R. sphaeroides cells.

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