Systems Biology
Doctoral Training Centre

Fluorescence imaging and image analysis of chemotaxis proteins in *Rhodobacter sphaeroides*

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BACKGROUND TO PRACTICAL

Bacterial chemotaxis

Most bacteria are motile and able to move around in their environment. The primary form of propulsion for bacteria in a liquid environment involves the bacterial flagellar motor[1]. Recently GFP fusions combined with highly specialised microscopy and analysis have been used to study the stoichiometry, dynamics and turnover of components of the flagellar motor[5]. Motility is used to ensure that the bacteria keep themselves in environments which are optimal for growth. Motility is controlled by the chemotaxis pathway, a two component signaling pathway, which has been extensively studied in *Escherichia coli*[11]. *E. coli* contains a single linear signaling pathway, the components of which have been shown by fluorescent protein fusions to localise predominantly to a large cluster at the poles of the cell[8]. This clustering has been proposed to be important for the process of signal generation, adaptation and gain[2,3,7,9].

Rhodobacter sphaeroides is a purple non-sulphur bacterium which is widely found in the environment. The chemotaxis pathway in *R. sphaeroides* is more complex than that found in *E. coli*. Namely, *R. sphaeroides* encodes multiple homologues of most of the chemotaxis proteins found in *E. coli*. There are three major chemotaxis operons of which *che*Op2 and *che*Op3 are essential for chemotaxis under laboratory conditions[6]. Most of the components of these chemotaxis operons have been tagged with GFP or its derivatives in order to determine their localisation within the bacterial cell. The fluorescently tagged genes have been introduced into the genome in place of the wild type gene, meaning that the fluorescent fusion protein should be produced at the same level as the wild type protein, allowing the fluorescence intensity to be used as a measure of protein concentration. Interestingly, whilst *E. coli* chemotaxis proteins predominantly localise to the cell pole, *R. sphaeroides* chemotaxis proteins localise either to the cell pole or to a discrete cluster of proteins within the cytoplasm of the bacterium. In general, components of *che*Op2 seem to localise to the polar cluster whilst components of *che*Op3 localise to the cytoplasmic cluster[12,13]. Therefore *R. sphaeroides* seems to have two complete chemotaxis signalling pathways which are physically separated in space within the cell.

The presence of a large cytoplasmic cluster of proteins presents the cell with an interesting problem on cell division. Bacterial cells elongate during growth until they reach a certain length at which point they septate in the

middle resulting in two daughter cells. The polar clusters of chemotaxis proteins will therefore be segregated so that each daughter cell obtains a cluster of these proteins. The cytoplasmic clusters of chemotaxis proteins however may require an active mechanism to ensure that they are replicated and positioned within a cell such that on division each daughter cell receives a copy of this cluster of proteins in much the same way that DNA has to be replicated and segregated. Interestingly, there is a protein encoded within *che*Op3 (the operon which encodes most of the components of the cytoplasmic cluster) denoted PpfA, which has homology to ParA[10]. ParA is a protein which in conjunction with another protein, ParB, is involved in the positioning and segregation of plasmids on cell division[4].

Work leading up to this practical

Recently our lab has deleted the *parA* homologue *ppfA* from the genome of a *R. sphaeroides* strain which contains a fluorescent protein fusion to an essential component of the cytoplasmic cluster, TlpT. This has allowed us to investigate the role of this protein in the positioning and segregation of the cytoplasmic cluster on cell division. The data showed that in the presence of PpfA, the cytoplasmic clusters seem to be specifically positioned and their numbers regulated so as to ensure that on cell division each daughter cell obtains a copy of the cytoplasmic chemotaxis protein cluster. In the absence of PpfA, cells only seem to contain a single cytoplasmic cluster and its position within the cell is not constrained[10].

We have also succeeded in tagging two homologous proteins from *che*Op2 and *che*Op3 with CFP and YFP. This has allowed us to monitor the position of both the polar and cytoplasmic chemtotaxis clusters in the same bacterial cell[13]. However, little information exists on the number, size and positioning of the two clusters within the same cell. We have also introduced the *ppfA* deletion into this strain with a view to determining the effect of this protein on the number, positioning and size of both the polar and cytoplasmic clusters in the same cell.

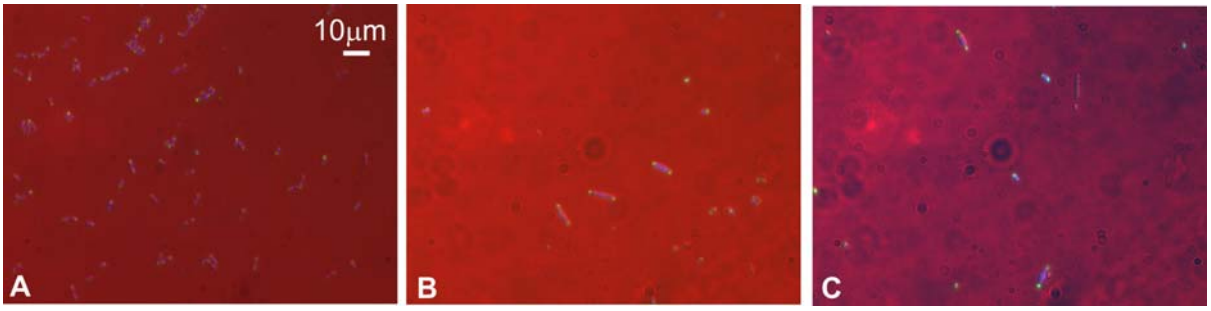

Figure 1. (A) CFP-CheW3, YFP-CheW4 cells, (B) the same, but treated with cephalexin, (C) cephalexin-treate cells which have *ppfa* gene deleted.

THE PRACTICAL

This practical will be split into two days. On day one you will acquire images of bacteria using brightfield illumination to determine the position of the bacterial cells and CFP and YFP fluorescence imaging to monitor the localisation of the polar and cytoplasmic chemotaxis protein clusters. You will be expected to acquire images which you will be able to analyse to answer questions about the size, position, number and dynamics of the two cluster types. For example, static images of cells taken with sub-saturating excitation may allow determination of the relative levels of protein in different protein clusters across a population of cells and within individual cells. Saturating excitation may be required to resolve all clusters within a cell and allow determination of the position of the clusters as a function of the length of the cell. Short timecourse imaging of the same field of view may allow the dynamics of the individual clusters to be determined. You may decide to do these experiments on cells

which contain *ppfA* or to compare cells containing *ppfA* with those in which it has been deleted. It is important to consider how you intend to analyse your data prior to collecting images to ensure that the acquisition parameters allow you to extract meaningful information from the images.

On day two you will take the images which you have obtained on day one and extract information from the imaging data. You may use available on-line software if it appears suitable for your needs (e.g. ImageJ) or write your own scripts to assist with the analysis.

Detailed Timetable

Day 1: 7th, 10th, 14th, 16th of April

9.00 – 9.15am: Meet in the reception area of the Clarendon Laboratory, Dept. of Physics (for directions see http://www.physics.ox.ac.uk/users/leake/group/contact.html). At 9.15am at the *very latest* your morning demonstrator will meet you and accompany you to the physics teaching laboratory located in the basement of the Denys Wilkinson Building, Keble Road. Note that entrance to this building is via swipe card for which your University Cards will not be authorised, therefore you will need to be accompanied by your demonstrator to gain access to the building, so please do not be late.

9.30am – 10.30am: Introduction to slide preparation, use of the microscopes and software

10.30am – 1.00pm: Phase I of the practical using dual-label CFP-CheW4/YFP-CheW3 cells

1.00pm – 2.00pm: Lunch. Your demonstrator will accompany you out of the building and will show you where to meet at the front entrance to the physics teaching labs after lunch.

2.00pm – 2.15pm: Meet at front entrance to physics teaching labs. Your afternoon demonstrator will let you into the building at 2.15pm at the *very latest*. Please do not be late.

2.15pm – 5.00pm: Phase II of the practical using cephalexin-treated cells with and without a *ppfA* mutation.

Day 2: 8th, 11th, 15th, 17th of April

Meet in the DTC offices at 9.00 am

9.00 – 10.00am: Introduction to ImageJ software

10.00 – 3.00pm: Quantitative analysis of microscopy experiments (including finding suitable on-line software)

3.00pm onwards: Writing up of practical

Practical Write up

Your write up should be written as a mini research article – with Introduction, Materials and Methods, Results and Discussion sections. In the results, include images, extracted data and assess the accuracy with which you were able to extract data from your images and where potential sources of error occurred.

Reference List:

[1] R. M. Berry and J. P. Armitage, "The bacterial flagella motor," Adv. Microb. Physiol **41**(1), 291 (1999).

[2] D. Bray, "Bacterial chemotaxis and the question of gain," Proc. Natl. Acad. Sci. USA **99**(1), 7 (2002).

[3] D. Bray, M. D. Levin, and Firth CJ Morton, "Receptor clustering as a cellular mechanism to control sensitivity," Nature **393**(6680), 85 (1998).

[4] Gitte Ebersbach and Kenn Gerdes, "PLASMID SEGREGATION MECHANISMS," Ann. Rev. Genet **39**(1), 453 (2005).

[5] Mark C. Leake, *et al.*, "Stoichiometry and turnover in single, functioning membrane protein complexes," Nature **443**(7109), 355 (2006).

[6] S. L. Porter, *et al.*, "The third chemotaxis locus of *Rhodobacter sphaeroides* is essential for chemotaxis," Mol. Microbiol. **46**(4), 1081 (2002).

[7] T. S. Shimizu, *et al.*, "Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis," **2**(11), 792 (2000).

[8] V. Sourjik and H. C. Berg, "Localization of components of the chemotaxis machinery of *Escherichia coli* using fluorescent protein fusions," Mol. Microbiol. **37**(4), 740 (2000).

[9] V. Sourjik and H. C. Berg, "Functional interactions between receptors in bacterial chemotaxis," Nature **428**(6981), 437 (2004).

[10] Stephen R. Thompson, George H. Wadhams, and Judith P. Armitage, "The positioning of cytoplasmic protein clusters in bacteria," Proc. Natl. Acad. Sci. USA **103**(21), 8209 (2006).

[11] G. H. Wadhams and J. P. Armitage, "Making sense of it all: Bacterial chemotaxis," Nat. Rev. Mol. Cell. Bio. **5**(12), 1024 (2004).

[12] G. H. Wadhams, *et al.*, "TlpC, a novel chemotaxis protein in *Rhodobacter sphaeroides*, localizes to a discrete region in the cytoplasm," Mol. Microbiol. **46**(5), 1211 (2002).

[13] G. H. Wadhams, *et al.*, "Targeting of two signal transduction pathways to different regions of the bacterial cell," Mol. Microbiol. **50**(3), 763 (2003).

Useful websites:

1. http://www.olympusmicro.com/primer/techniques/fluorescence/.html
2. http://www.physics.ox.ac.uk/users/leake