

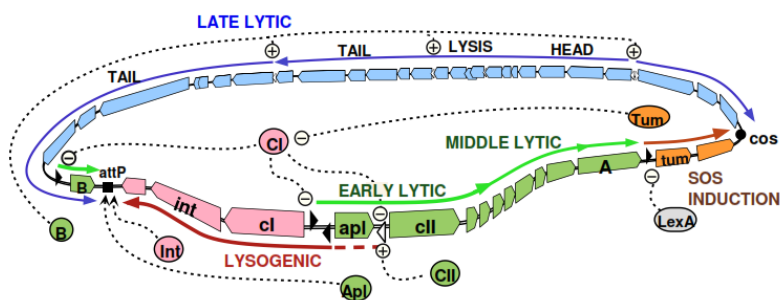
# Honours Projects available in the Shearwin Laboratory in 2012



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Our research integrates **biochemistry, genetics and mathematical modelling** to characterise fundamental mechanisms of gene control and how these elements are combined to create gene regulatory circuits with complex functions. Having a toolbox of well characterised genetic components allows us to ‘rewire’ them in a rational way in order to construct new genetic circuits with predictable behaviour for use in synthetic biology applications. The lessons learnt in the construction of artificial genetic circuits in turn give us a deeper understanding of how natural biological systems work.

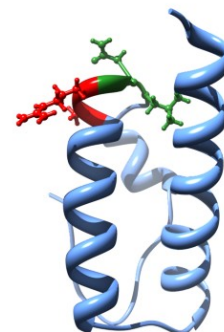
Our primary experimental systems are two *E. coli* bacteriophages, lambda and 186. These temperate phages can replicate their genomes using alternative developmental pathways, lysis and lysogeny, and are some of the simplest organisms to make developmental decisions. Despite their relative simplicity, the phage systems combine a wide range of gene control mechanisms in complex ways and have many lessons to teach us. Bacteriophage lambda continues to be a key model system for many molecular biological processes; phage 186 is less well characterised but provides a powerful comparison with lambda, as it achieves similar outcomes using different regulatory circuits. The fundamental biochemistry shared by all living things means that the study of any organism, from phages to humans, continues to illuminate universal principles that apply to all organisms.

**Projects will be offered in three areas:**

## 1. Understanding the Tumor anti-repressor protein of bacteriophage 186

*Co-supervised by Keith Shearwin and Senior Research Fellow Ian Dodd*

The Tumor protein of bacteriophage 186 is required to allow the phage to switch from the lysogenic state into lytic development – the process of prophage induction. It does this by interacting with, and rendering ineffective, the lysogenic repressor CI. While we have predicted *ab initio* the structure of an active N-terminal region of Tumor (*at right*), the molecular mechanism by which Tumor inactivates CI is unknown.



This project will use *in vitro* and *in vivo* approaches in order to address several key questions about the role of Tumor in prophage induction. For example, we may use our site specific chromosomal integration system to provide controlled levels of CI and Tumor to a pR-lacZ reporter, and ask how much Tumor is required to derepress pR. What are the strength and repression characteristics of the promoter (p95) which drives Tumor expression in the phage? Does Tumor work against mutants of CI which are only able to form dimers, rather than the native 14-mer wheel structure of CI?

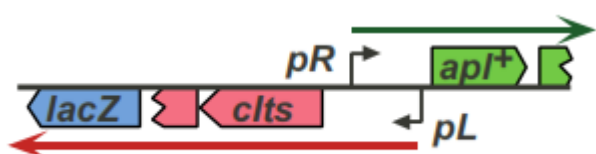
To probe the protein-protein interaction *in vitro*, we will make various Tum-CI chimeras and test for intramolecular interaction by cross linking, gel filtration and other biophysical approaches.

#### Techniques include:

- common molecular biology techniques, such as DNA cloning, PCR and sequencing
- microtiter plate based lacZ reporter gene assays
- bacterial protein expression and purification
- SDS polyacrylamide gel electrophoresis and western blotting
- recombineering to produce phage carrying Tum variants

## 2. The role of the Apl protein in the genetic switch of phage 186.

Co-supervised by Keith Shearwin and Senior Research Fellow Ian Dodd



The Apl protein has two distinct roles in bacteriophage 186. Apart from its role in the lytic/lysogenic decision switch, Apl is also the excisionase and binds at the phage *att* site. The properties of a 186  $\Delta apl$  phage showed that Apl

plays no role in the lysis-lysogeny decision after infection, but whether Apl's activity in the switch is important in prophage induction can not be deduced from study of this phage, because of the loss of excisionase activity. The Apl protein binds cooperatively to seven sites located between *pR* and *pL* and has repressive effects on both promoters. The presence of Apl also influences the way that these promoters respond to changes in the level of CI repressor. To examine the role of Apl in the switch, we will employ mutations that specifically eliminate the binding of Apl at the switch region, without affecting its action in excision. These Apl operator mutations will then be tested in transcription- and translation reporter assays, in self contained CI-Apl switch constructs, and in the context of the whole phage.

#### Techniques include:

- common molecular biology techniques, such as DNA cloning, PCR and sequencing
- microtiter plate based lacZ reporter gene assays

## 3. The molecular basis for differences in tail spike specificity of phages 186 and P2

Co-supervised by Keith Shearwin and Ian Dodd

Bacteriophages 186 and P2 are closely related at the level of DNA sequence; however recent work in our laboratory has shown that these two phage recognise distinct forms of receptor on the surface of the bacterial cells that they infect. 186 and P2 both attach to the bacterial lipopolysaccharide (LPS) complex on the outside of *E. coli*, but each phage binds a different subset of sugar groups within the LPS. The phage proteins responsible for LPS recognition are the tail fibres and/or short tail spikes. The crystal structure of the P2 tail spike protein has recently been determined (*PDB file 3AQJ*), revealing a novel trimeric, metal binding knitted beta sheet structure. Alignment of the amino acid sequences of the 186 and P2 tailspike proteins reveals a small number of key sequence differences, which may be responsible for their altered binding specificity.

This project would involve the use of recombineering to test whether we can change 186 receptor specificity to that of P2, simply by changing a few key residues in the tail proteins. This would initially be

done at the level of the domain structure of these proteins, followed by increasingly specific changes to particular amino acids.

We would also clone, express and purify GFP-tail spike fusion proteins, to use as tools to directly observe tail spike/fibre protein binding to cells by fluorescence microscopy.

#### Techniques include:

- recombineering to make precise sequence changes to lysogens of 186 and P2
- common molecular biology techniques, such as DNA cloning, PCR and sequencing
- phage plaque forming assays
- bacterial protein expression and purification
- SDS polyacrylamide gel electrophoresis and western blotting
- fluorescence microscopy to observe protein binding to bacterial cells

*Crystal structure of the C-terminal domain of the tail spike protein from bacteriophage P2. The LPS binding region is at the bottom of the structure, which also contains the metal binding motif.*



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