Poster Presentations

[MS9-P02] Structural analysis of the bacterial RM controller protein, C.Esp1396I, in complex with DNA.

Richard N.A. Martin[1], John E. McGeehan[1], G. Geoff Kneale[1].

[1]. *Institute of Biomedical and Biomolecular Sciences, University of Portsmouth, Portsmouth, UK, P01 2DY*
E-mail: richard.martin@port.ac.uk

Bacterial Restriction-Modification (RM) systems act as a form of primitive immune system to prevent the establishment of bacteriophage genomes within the bacterium. RM systems achieve this through the use of two complementary enzymes; a DNA methyltransferase and a restriction endonuclease. The DNA methyltransferase labels "self" DNA and the restriction endonuclease degrades unlabelled "non- self" DNA. In order to prevent auto-restriction (the endonucleolytic cleavage of the bacterial genome), a temporal control mechanism is required to delay endonuclease production. In the majority of type II RM systems, a third (controller, or C) protein regulates the expression of the two enzymes. All known C-proteins are homodimeric helix-turn-helix DNA binding proteins. In the type II RM system Esp1396I, the C-protein, C.Esp1396I, can act as both a transcriptional enhancer and a repressor by interacting with three distinct operator sites; O_M (methyltransferase repressor site, the highest affinity operator), O_L (C- transcriptional activator) & O_R (C-protein/endonuclease repressor site, the weakest affinity operator). These three operator sites are composed of palindromic trinucleotide recognition sequences and highly conserved TATA sequences between the recognition sites. Despite the similarities between the operator sites, C.Esp1396I binds to each site with a very different affinity, thus creating the temporal delay in endonuclease production. The affinity for the weakest operator site (O_R) is increased 100-fold when the directly adjacent O_L site is occupied by a C-protein. X-ray crystallography revealed the structure of the adjacent O_L and O_R sites occupied by a pair of C.Esp1396I dimers forming the repression complex [1] as well as the C-protein-O_M repression complex [2] and the transcriptional activation C-protein-O_L complex [3]. These complexes revealed a combination of both direct and indirect readout between the C-protein and the DNA, as well as significant DNA distortion and protein-protein cooperativity. Using site-directed mutagenesis, DNA binding assays and high resolution X-ray, we have elucidated the role of individual amino acids that interact with the DNA. In addition, further DNA-protein co-crystal complexes have revealed the nature of the DNA distortion both pre- and post- C-protein binding, suggesting that the DNA is highly susceptible to bending. Evidence is also presented that the binding of the first C-protein dimer to O_L prepares the DNA for binding of the second C-protein dimer (to O_R) by distorting the downstream DNA.

[1] J.E. McGeehan *et al.* (2008). Nucleic Acids Res. 36, 4778-4787.
[2] N.J. Ball *et al.* (2012) Nucleic Acids Res. 40, 10532-10542.
[3] J.E. McGeehan *et al.* (2012) Nucleic Acids Res. 40,
4158-4167

Keywords: molecular recognition, DNA distortion, DNA-protein complexes.