

P051 Negative regulation in the *Escherichia coli mel* operon: evidence for DNA wrapping by regulator protein MelR
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The *Escherichia coli* MelR protein is a melibiose-triggered transcription factor responsible for the induction of expression of the *melAB* genes, essential for melibiose metabolism. In the absence of melibiose, MelR autoregulates its own expression by repressing its own promoter (*pmelR*). This repression requires MelR binding to two operator sites. One site (Site R) overlaps the *pmelR* transcription start, whilst the second site (Site 2) is located 176 bp upstream. It has been suggested that repression requires loop formation between the two sites. We have studied the effects of single base substitutions at positions 5 and 13 of the 18 bp Site R sequence. Our results confirm that MelR binding to Site R is essential for repression of *pmelR*. Experiments with nested deletions of *pmelR* show that the, upstream MelR binding site, Site 2 is essential for optimal MelR-dependent repression. Addition or deletion of half a helical turn between the two operator sites relieves repression, whilst insertion of integral numbers of helical turns restores repression. This suggests that repression requires two MelR binding sites are in the same face of the *melR* promoter.

Studies of MelR-*pmelR* interactions using in-vitro transcription and atomic force microscopy (AFM) will be reported. Differences between MelR and the related *E. coli* AraC protein, that controls the expression of genes required for arabinose metabolism, are discussed.