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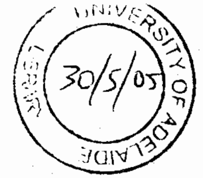

REGULATION OF EXPRESSION AND ACTIVITY OF THE LATE GENE ACTIVATOR, B, OF BACTERIOPHAGE 186.

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the School of Molecular and Biomedical Sciences (Biochemistry Discipline) at the University of Adelaide.

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THESIS SUMMARY

Structural gene expression in the temperate double-stranded DNA coliphage 186 shows similarities with other phage – transcription from four promoters late in infection follows phage replication and is activated by B, a small zinc-finger protein in the P2 Ogr family. However, *B* and late gene regulation in 186 shows some novel aspects which were investigated here.

Unusually, the *B* gene is transcribed from early in infection, well before B activates the late promoters. It was shown here that multiple copies of the *B* gene, as provided by phage replication, are necessary to express sufficient protein to stimulate late transcription and complement a 186 B^- phage burst. This was due to non-linear (presumably cooperative) activation of the 186 late promoters by low levels of B protein. However, premature late promoter stimulation by early expression of high B levels, had little effect on the phage infection, consistent with phage replication also being required to amplify 186 late gene dosage.

In a 186 lysogen, *B* transcription is repressed by the CI protein, however, I found that the level of protein produced from a single-copy prophage is insufficient to activate the late promoters and harm the cell. Instead, unrepressed B expression from the phage (or a plasmid) reduced the frequency of lysogen formation – presumably due to B enhancing transcription of lethal 186 late function/s from a partly replicated genome, and killing cells which would otherwise enter lysogeny.

Finally, I characterized four bacterial mutants which it was hoped would reveal novel host proteins required for B activity. Unfortunately in these cells, B function and 186 infection were apparently reduced non-specifically by mutations affecting the copy number of plasmids used and phage adsorption respectively. Re-examination of the *rpoA*109 mutation showed that B interacts with the RNA polymerase α-subunit to enhance transcription, like several related proteins.

Thus, B cooperatively activates the 186 late promoters to allow rapid stimulation of transcription after phage replication increases the *B* and late gene dosage. This sensitivity of the late promoters to B protein means that its expression must be repressed during the establishment of 186 lysogens when some phage replication may occur.