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PROTEINS AND TRANSCRIPTS
INVOLVED IN
MINI-F MAINTENANCE

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June, 1984

Thesis submitted to the University of Auckland
for the degree of Doctor of Philosophy.

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ACKNOWLEDGEMENTS

I wish to thank my supervisors, Dr H.E.D. Lane and Prof. P.L. Bergquist, for their advice and enthusiasm during the course of this work. I am grateful for their critical reading of the manuscript, and for the care and concern they have shown me.

My thanks to Prof. P.L. Bergquist and Ms L. Malcolm for construction of the transposon (Tn5) insertion mutants of mini-F which form a major part of this thesis.

I am indebted to the staff and students of the Cell Biology Department for their general assistance, constant encouragement and friendship, and for tolerating my after-hours activities.

My appreciation to Mrs L. Howey and Mrs M. Tapene for typing the thesis, particularly to Mrs L. Howey for the long nights she put in, in order to meet the deadline.

To my wife Suzie and son Yun, who have waited patiently through the many months of thesis preparation, I convey my love. They have shared in the costs, but not in the rewards, of experimental science. Without their unfailing support, both morally and financially, this work would not have been completed.

ABSTRACT

The aim of the work described in this thesis was to understand the maintenance systems of the F plasmid of Escherichia coli. The work was centered on the biochemical studies of the primary replicative fragment of F, f5.

1. From an analysis of the protein species produced by miniF-vector hybrid plasmids in E. coli minicells, seven proteins were found to be encoded by f5. Similarly, the secondary F replicon, f7, was found to code for at least three proteins.
2. The f5-specific proteins were further characterized by determining their isoelectric points and molecular weights in two-dimensional gels.
3. Using transposon-induced and deletion mutants of miniF, the structural genes of the seven f5 proteins were mapped.
4. Analysis of the amounts of proteins made by transposon-insertion mutants revealed that some miniF proteins control the expression of miniF genes. These results suggested possible mechanisms of coordination between the three f5 maintenance systems (ccd, rep and par).
5. The polarities of the f5 transcripts synthesized in minicells were determined by hybridization with f5 DNA cloned into single-stranded phage M13 vectors. The results were consistent with the gene mapping data, in that the directions of transcription of the seven f5 structural genes are uniformly rightwards.
6. In addition, the RNA-DNA hybridization experiments revealed the existence of untranslated leftward transcripts produced from the regions involved in f5 replication and partition.