A Thesis on

GENETIC AND FUNCTIONAL ASPECTS
OF THE OUTER MEMBRANE PROTEINS OF
ESCHERICHIA COLI K12

by

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ABSTRACT

This thesis contributes towards the elucidation of the biological functions of an abundant protein in the outer membrane of *Escherichia coli* K12, designated protein 1, and the mapping of genes on the chromosome concerned with its expression. Protein 1 exists on the outer membrane as two closely-related forms of differing electrophoretic mobilities, 1a and 1b, which can be visualized by sodium dodecyl sulphate polyacrylamide gel electrophoresis on slab gels.

A search for novel bacteriophage from sewage which exploit outer membrane protein as receptors, uncovered a phage group to which resistant mutants could be selected. This mutant class, Aer, together with some previously isolated phage resistant and colicin tolerant mutant classes, were found to lack either protein 1a or 1b, or both on their outer membranes. The absence of protein in these mutants was correlated with phage resistance and colicin tolerance, in an attempt to define the role of these proteins.

The mutations in three of the colicin tolerant phenotypic classes, found to lack both proteins 1a and 1b, were all shown to map in the 74 minute region of the *Escherichia coli* chromosome. The locus is designated *omp B* (for outer membrane protein). Three-point crosses established the linear sequence of genes in this region.
to be **aro** **B** - **omp** **B** - **mal** **QP** - **glp** **D**.

The **omp** **B** mutants were investigated for physiological defects in order to determine the primary function of protein 1. **omp** **B** mutant strains were found to be disadvantaged in growth on solid and liquid media with low concentrations of carbon source, as well as defective in chemotaxis towards sugars and amino acids. These observations are interpreted in the light of the "porin" hypothesis (Nakae, 1975).

Mutations occurring at high frequency result in the reversion of the unstable **omp** **B** phenotype. Such reversions were shown to be in secondary loci and fall into two groups which restore either protein 1a or 1b, together with the expected colicin tolerance and/or phage resistance. The revertant mutation, designated **omp** **G**, mapped at 0 minutes on the **E. coli** chromosome.

A fine structure analysis of the **omp** **B** locus was attempted by transducing **omp** **B** mutations into **omp** **B** **aro** **B** mutant strains with phage P1 lysates. A suitable selection which ensured the survival of only wild type recombinants arising from intragenic crossovers, was devised. Opposing configurations of pairs of alleles in such crosses determined the sequence of alleles and enabled an allelic map to be constructed. Finally, the construction of merozygotes with P' prime factors carrying various **omp** **B** mutations showed that there were two complementation groups in the **omp** **B** locus.