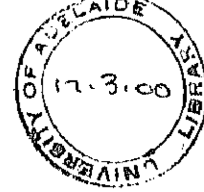
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BACTERIAL CELL SURFACES AND PATHOGENESIS

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SUMMARY OF WORK

The work presented in this thesis encompasses two major inter-related themes, namely the study of Bacterial Cell Surfaces and the study of Pathogenesis of a number of bacterial infections. In order to put each of the papers into perspective, they will be grouped according to the organism or class thereof.

I. Studies on *Escherichia coli* K-12.

Recipient ability in Bacterial Conjugation

At the commencement of my PhD candidature the state of both functional and genetic knowledge of the outer membrane proteins of *Escherichia coli* was limited. However, a collection of bacteriophage- and colicin-resistant mutants of *Escherichia coli* K-12 was available within the laboratory of Dr. (now Professor) Peter Reeves where I was undertaking my research, but the degree of characterization of these mutants was quite variable. These strains provided a resource to begin examining the nature of the mutations, in particular, their effect on the ability of the mutant bacterium to act as a recipient in bacterial conjugation, the process by which multiple antibiotic resistance is transferred from cell to cell in Gram negative bacteria. Examination of these mutants with a variety of conjugal donor strains (F'*lac*, Hfr, R100-1 and R64-11) enabled the differentiation of the cell surface interactions with the various donors (1,6). The data also highlighted that *con* mutants had the most significant defect as recipients in conjugation with F-plasmid donors. These mutations were subsequently shown to be affecting the same gene as *tolG* and *tut* mutations described in other laboratories. This gene was shown to be the structural gene for a major outer membrane protein of *E. coli* K-12 (3,4,5). Consequently, the gene was designated *ompA* (3). These

studies also characterized the OmpA protein (also referred to as protein 3A, protein II or II*) electrophoretically providing evidence to differentiate it from other outer membrane proteins. It is worth noting, that at this time there was no generally accepted system for examining the outer membrane proteins and it was only with the advent of slab polyacrylamide gels that it became possible to unify the nomenclature of the proteins described in different laboratories (7).

Studies of proteins from the various bacteriophage-resistant mutants under different electrophoretic conditions also revealed a correlation between the presence of a particular outer membrane protein and receptor activity for bacteriophage T6 and colicin K (2). The structural gene for this protein was designated *tsx*. The Tsx protein could be purified and shown to have the receptor or neutralizing capacity for both agents (9).

As a consequence of this work, it became possible to start analyzing the actual physiological role of the major outer membrane proteins of *E. coli* K-12 (8). In particular, the panel of *ompA* mutants, which had been isolated (4), could be used to demonstrate that the presence of OmpA was critical for a variety of growth functions.

My initial work as a post-doctoral fellow in Dr. Mark Achtman's laboratory was a continuation of the studies of different classes of conjugation-defective mutants of *E. coli* K-12 (10). It was concluded that *ompA* mutants were defective in conjugation and could not form stable mating aggregates and could be used to define one of the stages of the conjugation process (151).

Donor-associated functions in conjugation

As a result of experience with outer membrane functions of *E. coli*, it was decided to initially focus on the conjugal phenomenon of surface exclusion, which is responsible for the

poor recipient-ability of conjugal donors with donors harbouring the same or related sex factor. This also prevents unnecessary conjugation between donor cells in a pure culture.

At that time, the *tra* region of the F plasmid encoding the genes involved in conjugal DNA transfer, F-pilus biosynthesis, surface exclusion and other donor functions required for conjugation, had only recently been cloned. Analysis of the cloned genes in *E. coli* mini-cells provided a means of identifying most of the proteins encoded within the *tra* region and also procedures were developed to fractionate the mini-cells so that the actual cellular location of the proteins could be determined (12).

Complementation analysis of F'*lac* point mutants and subclones of the *tra* region of the F plasmid defined the genes, *traS* and *traT*, to be solely responsible for the phenomenon of surface exclusion (13). However, F plasmids with mutations in *traJ*, a *tra* region positive transcriptional regulator, were also defective indicating that TraJ plays a role in expression of the promoter distal cistrons *traS* and *traT*. This posed somewhat of a quandary when RNA polymerase binding studies and promoter analyses were performed. Electron microscopic visualization of *E. coli* RNA polymerase complexed to *tra* region DNA identified several strong binding sites internal to the *tra* operon (24). This implied the existence of promoters in addition to that regulated by *traJ* at the beginning of *tra* region. In particular, both *traS* and *traT* appeared to have their own promoters. Nucleotide sequence analysis of *traS* and *traT* together with analysis of this region in promoter detection vectors helped clarify matters (see below; 26). Both were shown to have constitutive promoters as well as being co-regulated by TraJ. The properties of various strains, including F-minus phenocopies, donor strains grown under conditions such that they behave like recipients, led to the conclusion that TraS is probably rapidly turned over in the cell. In addition, for TraT to function in surface exclusion, it may need to be newly synthesized, and presumably associated with zones of adhesion between the cytoplasmic and outer membranes.

Two new chromosomal genes also were shown to affect surface exclusion. The products of *sfrA* and *sfrB* are both required for surface exclusion, but function at the level of regulation (18). The product of *sfrA* is needed for efficient transcription of *traJ* whereas the *sfrB* product has a novel regulatory mechanism affecting the synthesis of a variety of different cell surface-associated components including outer membrane proteins, lipopolysaccharide and flagella besides the F pilus.

The promoter-distal region of the *tra* region was subjected to extensive genetic analysis by isolating both deletion- and insertion-derivatives of pRS31 containing this region cloned in pSC101. These were mapped by both restriction analysis and by DNA heteroduplex formation using the electron microscope. Together with identifying the plasmid-encoded proteins, this resulted in a detailed physical and functional map of the last third of the F sex factor *tra* region (11,19). Cloned regions of the closely-related conjugative plasmid R100 became available and so it was of particular interest to perform a similar analysis in order to make comparisons because they belong to different surface exclusion classes, that is, they do not exclude one another. At the same time DNA homology of the *tra* regions of both F and R100 was examined electron microscopically by DNA-DNA heteroduplexes. These studies demonstrated a high degree of conservation with the exception of the *traS*, which was shown to be responsible for the plasmid specificity of surface exclusion (20,21).

In line with predictions from the earlier studies (12,14) nucleotide sequence analysis revealed that TraS and TraT were inner and outer membrane proteins, respectively (26). TraT was also predicted to be a lipoprotein, which together with its association with peptidoglycan and high degree of surface exposure, as determined by its ability to be readily iodinated on intact cells by lactoperoxidase (14), indicates that it is a trans-outer membrane protein. The

reduced ability of OmpA to be labeled in the presence of TraT also inferred that the mechanism of action of TraT in surface exclusion was to occlude access to OmpA.

At the same time as these studies were being conducted, colleagues in Dr. (now Professor) Ken Timmis' laboratory had begun an analysis of plasmid R6-5-encoded serum resistance. Plasmid R6-5 belongs to the same incompatibility group as plasmids F and R100, and so I provided Dr. Timmis with a variety of subclones and mutants which had been constructed in the above studies (19,20). The results these studies implied that these latter plasmids also encoded serum resistance and that it was TraT that was responsible for the phenomenon. Consequently, this directed the experimental approach to R6-5 and led to the demonstration that the R6-5 TraT protein was responsible for plasmid-determined resistance to serum bactericidal activity (15,17). A series of mutants in R6-5 *traT* were isolated by hydroxylamine mutagenesis in order to begin a structure-function analysis of TraT (22). Surface-exposed TraT was shown to be essential for mediating both serum resistance and surface exclusion.

In conjunction with these studies several other plasmid-encoded functions were analyzed. It was possible to identify the F plasmid TraG protein and establish its role in conjugation (16). TraG (and TraN) was shown to have a comparable role for the donor to that of OmpA in the recipient. In particular, the cytoplasmic membrane-associated TraG protein was required for the formation of stable mating aggregates; however, it was also involved in F pilus biosynthesis. It was suggested that TraG may be part of a basal organelle essential for such functions as F pilus retraction that is necessary for converting the initial F pilus contacts into stable donor-recipient wall to wall contacts. A subsequent stage in conjugation is the DNA transfer, which has been shown to involve TraD (151). Nucleotide sequence analysis of *traD* predicted that TraD was a cytoplasmic membrane protein with features that imply it functions as the actual channel for conjugal DNA transfer (27).

Incidental to the studies on plasmid R6-5, another plasmid-encoded function was identified and characterized (25). It was apparent that R6-5 inhibited the induction of the SOS response and the induction of prophage λ. The responsible genes, designated *psiA* and *psiB*, were characterized and the sizes of their products determined.

Finally, collaborative studies with Dr. Reeves when I returned to Adelaide led to the characterization of another *E. coli* K-12 outer membrane protein associated with sensitivity to colicins (23). The TolC protein was found to form aggregates in the outer membrane, however, unlike the porin proteins, OmpC and OmpF, it did not appear to be peptidoglycan-associated.