MECHANISMS CONTROLLING TRANSFER OF THE
E. COLI K12 SEX FACTOR F

by

MICHAEL JOHN GASSON

A thesis presented for the degree of Doctor of Philosophy

Department of Molecular Biology, University of Edinburgh. November 1978
The fertility inhibition systems of 28 Fin plasmids have been characterized using Flac* mutants insensitive to inhibition by R100 or R62. All F-like plasmids tested (except R455) and one N group plasmid determined systems analogous to R100; this is designated the FinOP system. None of the plasmids tested could supply a FinP component of the transfer inhibitor able to replace that of F itself. In addition to the FinOP and R62 (FinQ) transfer inhibition systems that were already described, new systems were encoded by the F-like plasmid R455, the In plasmid JR66a and the X group plasmid R465. Besides inhibiting F transfer, JR66a also inhibited F pilus formation and surface exclusion, whereas R465 inhibited only pilus formation and R455 inhibited neither. All three R factors inhibited transfer of J-independent Flac* elements, suggesting that they act directly on one or more genes (or products) of the transfer operon. These properties and the isolation of Flac* mutants insensitive to fertility inhibition by JR66a (traU mutants) and by R465 (traV mutants), were used to demonstrate that R455, R465 and JR66a encode systems for fertility inhibition that are different both from each other and from the FinOP and FinQ systems. These new systems, encoded by JR66a, R465 and R455 have been designated FinU, FinV and FinW respectively.

Results of a genetic assay for the presence of transfer gene products during fertility inhibition by R62 suggest that FinQ fertility inhibition causes incomplete termination of transcription (or translation) of the transfer operon between traC and traF.
In an attempt to provide a technology for biochemical studies of transfer gene expression, four systems for generating fusions of the transfer genes of F to well studied bacterial operons have been devised. During this work an Flac$^+$ (\$ptp^+$ BG2) co-integrate, in which the prophage was located within the transfer operon, was isolated. The properties of this plasmid, EDFL362, have been investigated and an interpretation of them suggested.

In addition a selection for tra$^+$ transducing phages that had been generated both in vivo and in vitro has been used. The properties of two phages (JB$^7$ and JB$^5$), which carry Ecor R I generated fragments of F DNA, are described and an explanation for these properties suggested.
ACKNOWLEDGEMENTS

This work has relied on bacterial strains, bacteriophages and plasmids kindly supplied by many people, to all these, too numerous to name, I am indebted. I am grateful to all members of this Department who have given help and advice. In particular, I wish to mention Jean Beggs, Bill Brammar, John Davison, John Haule, Sarah McIntyre, Noreen Murray, John Scaife and Russell Thompson.

Especially, I wish to thank Neil Willetts for his advice, guidance and encouragement throughout the past three years, for having read and constructively criticized this thesis, and for introducing me to a fascinating area of scientific research.

I have been supported by an M.R.C. scholarship for training in research methods.
SUMMARY

The fertility inhibition systems of 28 Fin plasmids have been characterized using Flac mutants insensitive to inhibition by R100 or R62. All F-like plasmids tested (except R455) and one N group plasmid determined systems analogous to R100; this is designated the FinOP system. None of the plasmids tested could supply a FinP component of the transfer inhibitor able to replace that of F itself. In addition to the FinOP and R62 (FinQ) transfer inhibition systems that were already described, new systems were encoded by the F-like plasmid R455, the Ia plasmid JR66a and the X group plasmid R485. Besides inhibiting F transfer, JR66a also inhibited F pilus formation and surface exclusion, whereas R485 inhibited only pilus formation and R455 inhibited neither. All three R factors inhibited transfer of J-independent Flac elements, suggesting that they act directly on one or more genes (or products) of the transfer operon. These properties and the isolation of Flac mutants insensitive to fertility inhibition by JR66a (traU mutants) and by R485 (traV mutants), were used to demonstrate that R455, R485 and JR66a encode systems for fertility inhibition that are different both from each other and from the FinOP and FinQ systems. These new systems, encoded by JR66a, R485 and R455 have been designated FinU, FinV and FinW respectively.

Results of a genetic assay for the presence of transfer gene products during fertility inhibition by R62 suggest that FinQ fertility inhibition causes incomplete termination of transcription (or translation) of the transfer operon between traC and traF.
In an attempt to provide a technology for biochemical studies of transfer gene expression, four systems for generating fusions of the transfer genes of F to well studied bacterial operons have been devised. During this work an Plac$^+$ (λptrp$^+$ B2) co-integrate, in which the prophage was located within the transfer operon, was isolated. The properties of this plasmid, EDF262, have been investigated and an interpretation of these suggested.

In addition a selection for λtra$^+$ transducing phages that had been generated both in vivo and in vitro has been used. The properties of two phages (JBλ 5 and JBλ 7), which carry Ecm R0 generated fragments of F DNA, are described and an explanation for these properties suggested.
## CONTENTS

### CHAPTER 1. INTRODUCTION

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Plasmid determined characters</td>
<td>1</td>
</tr>
<tr>
<td>The variety and importance of plasmids</td>
<td>1</td>
</tr>
<tr>
<td>The sex factor F</td>
<td>2</td>
</tr>
<tr>
<td>R factors</td>
<td>4</td>
</tr>
<tr>
<td>Colicins</td>
<td>5</td>
</tr>
<tr>
<td>1.2 General properties of plasmids</td>
<td>6</td>
</tr>
<tr>
<td>The sex pilus</td>
<td>6</td>
</tr>
<tr>
<td>Surface exclusion</td>
<td>9</td>
</tr>
<tr>
<td>Transfer of the host chromosome</td>
<td>10</td>
</tr>
<tr>
<td>Plasmid replication and incompatibility</td>
<td>11</td>
</tr>
<tr>
<td>1.3 The mechanism of conjugation</td>
<td>14</td>
</tr>
<tr>
<td>The role of sex pilis</td>
<td>14</td>
</tr>
<tr>
<td>DNA transfer</td>
<td>17</td>
</tr>
<tr>
<td>1.4 Physical study of plasmids</td>
<td>20</td>
</tr>
<tr>
<td>Plasmid molecules</td>
<td>20</td>
</tr>
<tr>
<td>DNA:DNA hybridisation</td>
<td>21</td>
</tr>
<tr>
<td>Heteroduplex mapping</td>
<td>22</td>
</tr>
<tr>
<td>Use of restriction enzymes</td>
<td>24</td>
</tr>
<tr>
<td>1.5 Genetic analysis of conjugation</td>
<td>24</td>
</tr>
<tr>
<td>Transfer systems</td>
<td>24</td>
</tr>
<tr>
<td>Transfer defective mutants</td>
<td>25</td>
</tr>
<tr>
<td>Mapping of the transfer genes</td>
<td>28</td>
</tr>
<tr>
<td>Operon structure</td>
<td>29</td>
</tr>
<tr>
<td>Plasmid specificity</td>
<td>30</td>
</tr>
</tbody>
</table>
1.6 Control of transfer

Fertility inhibition 32

Mutations in fertility inhibition 32

Two component transfer inhibitor 34

Positive control and the mechanism of R100 fertility inhibition 35

Kinetics of fertility inhibition 36

Fertility inhibition by non F-like plasmids 37

CHAPTER 2. MATERIALS AND METHODS

2.1 Media 39

2.2 Reagents 42

2.3 Bacterial strains 44

2.4 Plasmids 49

2.5 Phage strains 53

2.6 Techniques used to construct strains 54

2.7 Transfer of plasmids 58

2.8 Mating conditions 59

2.9 Complementation tests 61

2.10 Surface exclusion 62

2.11 Acridine orange curing 62

2.12 Phage techniques 62

2.13 Penicillin enrichment for homozygotes 65

2.14 Tryptophan operon expression 65
CHAPTER 3. THE MECHANISM OF F FERTILITY INHIBITION BY VARIOUS PLASMIDS

3.1 Introduction

67

3.2 Inhibition of Flac\textsuperscript{+} transfer by various Fin\textsuperscript{+} plasmids

68

3.3 Inhibition of Flac\textsuperscript{+} pilus formation by Fin\textsuperscript{+} plasmids

72

3.4 Discussion

75

CHAPTER 4. TRANSFER OF FinOP PLASMIDS IN THE PRESENCE of Flac\textsuperscript{+}

4.1 Introduction

78

4.2 Relative transfer levels of Flac\textsuperscript{+} and FinOP plasmids

79

4.3 Transfer levels of ColE2 and R124

81

CHAPTER 5. FURTHER CHARACTERISATION OF TRANSFER INHIBITION BY JR66a, R435 and R455

5.1 Introduction

85

5.2 Surface exclusion

86

5.3 J-independent mutants

87

5.4 Transfer inhibition by R435

88

5.5 Discussion

69

CHAPTER 6. Flac\textsuperscript{+} MUTANTS INSENSITIVE TO FERTILITY INHIBITION BY JR66a and R455

6.1 Introduction

93

6.2 Selection procedure

93

6.3 Mutants insensitive to fertility inhibition by JR66a

94

6.4 Mutants insensitive to fertility inhibition by R455

96
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity of the fertility inhibition systems</td>
<td>98</td>
</tr>
<tr>
<td>Discussion</td>
<td>101</td>
</tr>
<tr>
<td><strong>CHAPTER 7. THE MECHANISM OF ACTION OF THE R62 TRANSFER INHIBITOR</strong></td>
<td></td>
</tr>
<tr>
<td>7.1 Introduction</td>
<td>102</td>
</tr>
<tr>
<td>7.2 Construction of <em>Fli</em>&lt;sup&gt;+&lt;/sup&gt; <em>traG12</em></td>
<td>102</td>
</tr>
<tr>
<td>7.3 Construction of <em>Fmac</em>&lt;sup&gt;+&lt;/sup&gt; <em>traX</em> <em>traC</em> double mutants</td>
<td>103</td>
</tr>
<tr>
<td>7.4 Isolation of <em>Fmac</em>&lt;sup&gt;+&lt;/sup&gt; <em>traC</em> <em>traO</em></td>
<td>107</td>
</tr>
<tr>
<td>7.5 Assay of <em>traX</em> products during R62 fertility inhibition</td>
<td>113</td>
</tr>
<tr>
<td>7.6 Interpretation of the genetic assay for transfer gene products</td>
<td>113</td>
</tr>
<tr>
<td><strong>CHAPTER 8. OPERON FUSION INVOLVING THE TRANSFER REGION:</strong></td>
<td></td>
</tr>
<tr>
<td>8.1 Introduction</td>
<td>119</td>
</tr>
<tr>
<td>8.2 Insertion of <em>λ</em>&lt;sup&gt;+&lt;/sup&gt; <em>ptrp</em>&lt;sup&gt;+&lt;/sup&gt; <em>R62</em> into the <em>Fmac</em>&lt;sup&gt;+&lt;/sup&gt; plasmid</td>
<td>121</td>
</tr>
<tr>
<td>8.3 Properties of EDFL262</td>
<td>127</td>
</tr>
<tr>
<td>8.4 Model for the structure of EDFL262</td>
<td>134</td>
</tr>
<tr>
<td><strong>CHAPTER 9. FUSION OF THE TRANSFER GENES TO OTHER BACTERIAL OPERONS BY DELETIONS</strong></td>
<td></td>
</tr>
<tr>
<td>9.1 Introduction</td>
<td>136</td>
</tr>
<tr>
<td>9.2 Fusion to the lactose operon</td>
<td>139</td>
</tr>
<tr>
<td>9.3 Fusion to the galactose operon</td>
<td>142</td>
</tr>
<tr>
<td>9.4 Fusion to the arginine operon</td>
<td>144</td>
</tr>
<tr>
<td>CHAPTER 10.</td>
<td>THE ISOLATION OF LAMBDAL TRANSUDING PHAGES CARRYING TRANSFER GENES</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>10.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>10.2</td>
<td>Transducing phages generated \textit{in vivo}</td>
</tr>
<tr>
<td>10.3</td>
<td>Transducing phages generated \textit{in vitro}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER 11.</th>
<th>CONCLUSIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>165</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>167</td>
</tr>
</tbody>
</table>
1.1 Plasmid determined characters.

The variety and importance of plasmids

Bacterial plasmids are non-essential genetic elements that are capable of autonomous replication. Many plasmids encode genes that produce a transfer system. As a result, such "conjugative" plasmids are able to transfer themselves, and under some circumstances host DNA, to a recipient cell.

In addition, genes carried by plasmids confer other properties on host cells, such as resistance to drugs and the production of colicins. Plasmids from *Escherichia coli* encode genes for enterotoxin and haemolysin production (Williams-Smith and Halls, 1967; 1968; Williams-Smith and Linggood, 1970), production of the K88 antigen (Leth Bak et al., 1972), fermentation of raffinose and production of hydrogen sulphide (Árakov and Árakov, 1973). Plasmid controlled sucrose fermentation has been demonstrated in *Salmonella* strains (Volheiter et al., 1975), and an extrachromosomal element MP10, commonly encountered in *Salmonella*, inhibits F transfer, but has no other apparent function. Plasmids in *Pseudomonads* have been found to control degradation of naphthalene (Dunn and Ganasalu, 1973), salicylate (Chakrabarty, 1972), camphor (Rheinwald et al., 1973), benzoate, and m-toluic (Wong and Dunn, 1974).

Furthermore, plasmids are not restricted to Gram--ve bacteria. Perhaps the best known plasmids of Gram +ve species are the penicillinase plasmids of *Staphylococcus aureus*. Plasmid encoded resistance to neomycin, kanamycin, tetracycline and erythromycin is also found in this species (Lacey, 1975). R factors from
Haemophilus influenzae (Deng Van, 1975), Streptococcus faecalis (Clew, et al., 1975), and Clostridium perfringens A (Sebold and Bouanchaud, 1975), have been reported.

Resistance and sensitivity of host bacteria to some bacterial viruses, and the production of some restriction enzymes, are also controlled by plasmids.

Hence plasmids are of interest in their own right, as an important component of the genetic resource of bacteria, and because sex factor activity allows their exploitation in studies of the chromosome genetics of important bacteria, such as E. coli (J. Heringer, personal communication), Streptococcus (Hopwood et al., 1973), Pseudomonas (Pamberton and Holloway, 1972), and Salmonella (Sanderson et al., 1972), in addition to the well documented case of F in Escherichia coli.

**The sex factor F.**

Transfer of chromosomal genes was first demonstrated between multiply marked strains of Escherichia coli (Hayes, 1953a; 1952; Lederberg and Tatum, 1946; Lederberg et al., 1952), and Davis (1953) showed that cell to cell contact was required for this conjugation to occur. The discovery of distinct donor (F+) and recipient (F−) strains, and that the donor character could be transferred independently of chromosomal genes, led to a hypothesis that an extrachromosomal element (the sex factor, F) encodes genes responsible for the donor character (Cavalli et al., 1953; Hayes, 1953a; 1953b; Lederberg et al., 1952).

The autonomous sex factor F is transferred at high frequency, and promotes transfer of the bacterial chromosome at a much lower frequency. Variant strains were described, from which chromosomal
transfer occurred at an unusually high frequency (Hayes, 1953a; Cavalli et al., 1953). Such strains were denoted Hfr, for high frequency of recombination. The discovery that an Hfr strain no longer transferred the male character alone, although it did revert to F\(^+\), together with the results of an extensive analysis of Hfr x F\(^-\) matings (Jacob and Wellman, 1961), revealed that F could insert into the chromosome. The linear time of entry maps for various Hfr strains, obtained by Jacob and Wellman, could be explained on the basis of a circular bacterial chromosome, a structure later confirmed by autoradiography (Cairns, 1963). Insertion of F at different points on the chromosome generated Hfr strains, initiating transfer of chromosomal genes from various points, and in both directions. The Campbell model explained attachment of \(\lambda\) to the bacterial chromosome on the basis of the fusion of two circular DNA molecules into a larger circle, by a single reciprocal cross-over event (Campbell, 1962). The model explains Hfr formation from an F\(^+\) strain.

This ability of the F factor to insert into the bacterial chromosome has led to its classification, with \(\lambda\) and other temperate bacteriophages, as an episome. However, the usefulness of this term is now doubted (Hayes, 1969).

Excision of an episome from its integrated state on the bacterial chromosome would occur by a second, single reciprocal cross-over event, leading to regeneration of F\(^+\) strains in the case of an Hfr. Although this event may normally be an accurate reversal of the insertion process, as is known for \(\lambda\), inaccurate excision provides an explanation for F-prime formation. F-prime elements are autonomous derivatives of F, in which bacterial chromosome DNA forms part of the replicon. Their ability to promote high level transfer of the host chromosome was observed by Adelberg and Burm (1960). An F-prime element carrying genes for lactose fermentation was described by Jacob and Adelberg (1958).
and the term F-prime was introduced by Hirota and Suzuki (1961).

A large number of F-prime elements, carrying genes from many parts of
the Escherichia coli chromosome, have been described, and recently
they have been collectively reviewed (Low, 1972). Senice (1966) has
distinguished two types of F-prime element according to the position
of the cross-over event which led to their formation. Type I F-prime
formation involves a cross-over event between F DNA and chromosomal DNA,
whereas type II F-prime formation involves a cross-over event between
the chromosomal DNA on either side of the inserted P factor.

The P factor controls sensitivity of host cells to sex specific
bacteriophages. Male specific phages adsorb to the sex pili, surface
appendages encoded by genes of the F factor, and involved in
conjugation (see sections 1.2 and 1.3). Brakck and Brakck (1960)
demonstrated the presence of a surface antigen on F+ cells that was
absent from F− cells. This has subsequently been equated with the
sex pilus (Ishibashi et al., 1967). Female specific phages such as
&1, T3, T7, &II, W31, and T2, plate less efficiently on F+ than on
F− cells (Nakura et al., 1964; Maynell et al., 1968; Morrison and
Nalosy, 1971). Morrison and Nalosy (1971) showed that P encoded
inhibition of the female specific phages T7 and &II operated at the
level of mRNA translation.

P factors

P factors are the largest class of plasmids characterised, and
their relevance to medicine makes understanding of them particularly
important. Multiply antibiotic resistant strains of Shigella
avesenterica were first isolated in Japan. The resistances could be
transferred together to antibiotic sensitive strains of Shigella
avesenterica and, more remarkably, to other bacterial species (Watanabe,
1963). A vast number of drug resistance factors have now been isolated
from various bacterial species, and from many parts of the world. Although probably originating to counteract natural antibiotics, the current prominence of drug resistance factors may reflect the evolution of bacteria in response to the extensive use of antibiotics in human and veterinary medicine (Helinski, 1973).

Three properties, autonomous replication, antibiotic resistance, and the ability to transfer, are commonly associated with R factors. Differences in the arrangement of genes for these properties has led to the identification of two classes of R factor. A class 1 R factor consists of physically linked components; R, which encodes genes for antibiotic resistance, and RTF, which encodes genes for transfer and sometimes tetracycline resistance. In contrast, a class 2 R factor consists of physically separate replicons encoding resistance to antibiotics and transfer. The replicon encoding drug resistance is not self transmissible, but achieves transfer by its association with another replicon that carries genes for transfer (Anderson, 1968; Watanabe and Fukasawa, 1961). It has been suggested that class 2 R factors represent an earlier stage in the evolution of class 1 R factors with physically linked r and RTF components (Helinski, 1973).

**Colicin**

Colicins, first reported by Gratia (1932), are diffusible substances produced by some strains of *Escherichia coli* that are able to kill other *E. coli* strains. They are encoded by genes carried on plasmids (Frederiq, 1957; Lederberg, 1953), and these plasmids are often "conjugative." Although they usually exist as autonomous replicons, colicin factors are known to integrate into the bacterial chromosome, to form strains analogous to Hfr strains (Kahn, 1968). Aberrant excision of colicin factors from the bacterial chromosome also occurs, to yield Col factors carrying chromosomal genes, such as ColVSty" (Frederiq, 1969).
1.2 General properties of plasmids

The sex pilus

Sex pili are filaments projecting from the surface of male bacteria. They were first observed on $F^+$ and $Hfr$ cells, as the site to which a male specific bacteriophage, $R17$, adsorbed (Crawford and Gesteland, 1966). They were named pili by Brinton et al., (1964), and the term sex pilus was introduced to distinguish them from the common pili, present on bacteria regardless of sex (Maynoll and Laxm, 1967).

Many male specific bacteriophages have been isolated from sewage. Of those adsorbing to pili produced by $F^+$ bacteria, the $F$ specific bacteriophages, two types have been distinguished. The first type are isometric RNA phages, which adsorb to the sides of the pilus. They form three sub-groups, based on their densities in a caesium chloride gradient. $152$, 2H, NY, $R17$, f2 and $G1$ form one group, $A1$, SD, $E1$, $CH$, $KJ$ a second, and $Q$, $V$, $NK$, $ST$ and $IH$ a third (Hoffmann-Berling et al., 1966; Nishihara and Watanabe, 1969). The groups also differ in the RNA base ratios and coat protein composition of their members (Maynoll, 1973). The second type are filamentous DNA containing phages that adsorb tip to tip with the pili, examples being fd, f1, $M13$, 21/2, $Le$, $A2$ and $Hr$ (Harvin and Hohn, 1969).

Both DNA and RNA containing $F$ specific bacteriophage have been observed, adsorbed to the pili of cells carrying ColV-126 (Caro and Schnuss, 1965), and propagation of 152 has been demonstrated for cells carrying $R$ factors (Maynoll and Datta, 1965) and the colicin factors ColB-K77 and ColB-K166 (Maynoll and Datta, 1966). The pili to which $F$ specific bacteriophages adsorb are closely related and form a group known as $F$-like pili. Similarly the plasmids responsible for their synthesis have closely related transfer systems and form a group called $F$-like plasmids, members of which belong to five $F$ incompatibility groups.
Other plasmids do not synthesize pili susceptible to F specific bacteriophages, and of these, ColIb-P9 produces pili that are morphologically and serologically distinct from F pili. Two bacteriophages have been isolated which adsorb to ColIb-P9 pili, but not to F-like pili. These I-like bacteriophages, If1 and If2, are filamentous, contain single stranded DNA, are serologically identical, and are related to M9 but not to fd or E09 (Waynell and Laum, 1966). They are 50% longer and contain 50% more DNA than the filamentous bacteriophages (Marvin and Hohn, 1969). Despite numerous attempts no I specific, isometric, RNA containing bacteriophages have been found (Waynell, 1973). A series of plasmids produce pili to which the I specific bacteriophages adsorb, and these are known as I-like plasmids and pili.

Further classes of plasmid specific bacteriophage have been isolated which adsorb to cells carrying plasmids of the N, P, and T incompatibility groups.

Bacteriophage M13 adsorbs to cells carrying N group plasmids (Khatom et al., 1972), but no sex pilus has yet been definitely demonstrated on the surface of such cells.

A group of E factors originally isolated from Pseudomonas aeruginosa strains, but also found in Proteus and Providencia, form the P group plasmids. These plasmids produce a short pilus (Bradley, 1974), and render their host cell sensitive to a group of male specific phages. The RNA phage F11 (Olsen and Shipley, 1973; Olsen and Thomas, 1973) adsorbs to the pilus, whereas the phages F3, FR3 and FR4 do not, perhaps requiring another plasmid specified surface structure for adsorption (Bradley, 1974). F3 is a filamentous single stranded DNA phage, whilst FR3 and FR4 are hexagonal double stranded DNA phages (Stanisich, 1974). A further icosahedral bacteriophage, F24 (Olsen et al., 1974) is of interest because, with FR3 and FR4, it also infects
cells carrying an N group plasmid (Bradley, 1974., Olsen et al., 1974), suggesting that the N and P group transfer systems are related.

A bacteriophage pilE/R1 has been isolated which adsorbs specifically to cells carrying T incompatibility group plasmids, such as Rts1 (To, C.K., To, A., and Brinton, C.C. unpublished).

Although no W specific bacteriophages have yet been described, the pili produced by W incompatibility group plasmids, S-s, R388, and R7K, have been observed in the electron microscope. They were relatively long, and had the interesting feature of being pointed at the tip (Bradley, 1975).

Using immunological techniques the pili of F-like and I-like plasmids were shown to be different, whereas only slight differences could be found between the pili of F-like plasmids R1-19 and F (Lawn et al., 1967). Four serological sub-groups amongst F-like pili, and two sub-groups amongst I-like pili were subsequently defined (Lawn and Maynell, 1970). Furthermore, the pili produced by a cell carrying F-like plasmids of more than one sub-group, produced mixed pili, with sub-units specified by plasmids of both sub-groups. In contrast, cells carrying an F-like plasmid and an I-like plasmid did not produce mixed pili (Lawn, et al., 1971).

Structural studies of pili have tended to centre on F-like pili, produced by the sex factor F. The F pilus is a rod of 90 Å (Brinton, 1965; Lawn, 1966). It appears to have an axial groove about 25 Å across (Brinton, 1965; Lawn 1966), and probably consists of two parallel protein fibres (Brinton, 1971).

Brinton has extensively analysed the biochemical nature of F pili (Brinton, 1971). The pilus consists of sub-units of a single protein, called pilin. F pilin has a molecular weight of 12,000 daltons and carries two phosphate residues and one glucose residue per molecule.
The F pilin has a hydrophobic composition, lacking arginine, cysteine, histidine and proline (Brinton, 1971). The cell has a pool of pre-existing pilin molecules located in the outer membrane (Beard and Connolly, 1975) and this allows pilus regeneration to occur after their removal by shearing, even in the presence of chloramphenicol (Brinton, 1965).

The F transfer gene *traI* has been shown to code for F pilin (section 1.5) and the outer membrane protein, detected by Beard and Connolly (1975), is absent from cells carrying Flac⁺ *traI*.

**Surface exclusion**

Male cells (P⁺, F-prime or Hfr) in exponential phase are poor recipients when mixed with other donor cells. This F determined property is called surface exclusion. Efficient male recipients, referred to as F⁻ phenocopies, can be generated by reduced protein synthesis as in stationary phase (Lederberg, et al., 1952), growth at 27⁰ (Bonhoeffer, 1966), or amino acid starvation (Curtiss et al., 1969).

Surface exclusion has also been demonstrated for the I-like plasmid ColI (Maynall, 1969) and P group plasmids (Hedges and Jacob, 1974). Amongst F-like plasmids, four specificity groups for surface exclusion have been defined (Willett and Haule, 1974; section 1.5).

Surface exclusion is a phenomenon independent both of incompatibility and the ability to transfer. Separate genetic determinants for the three properties have been defined and mapped (Willett, 1974a). Point mutations of Flac⁺ in genes for surface exclusion (*traS*) have been isolated. Although these were transfer proficient, the efficiency of transfer was reduced, probably because mating pair formation between donor F⁺ cells reduced mating with recipient F⁻ cells (Achtman and Helmuth, 1975).
Mating pairs are formed after the pilus tip attaches to a receptor site on the recipient cell surface (Ou, 1973; Ou and Anderson, 1972; see section 1.3). Under conditions where surface exclusion can be demonstrated genetically to be occurring, the number of mating pairs observed in the mating mixture is reduced (Achtman et al., 1971). It is possible that surface exclusion prevents mating pair formation by inactivating the pilus receptor sites (Achtman, 1973; Achtman and Helmuth, 1975). This conclusion is consistent with the nature of surface exclusion expressed by cells carrying pairs of F-like plasmids with different surface exclusion specificities (Villette and Haule, 1976).

Beard and Bishop (1975) used two derivatives of R3-12, carrying mutually exclusive resistance determinants, to study the role of the mucoprotein in the expression of conjugal functions. The use of spheroplasts in R⁺ x R⁻ matings had no effect on donor or recipient abilities. However, in R⁺ x R⁺ matings surface exclusion was totally abolished if the donor cell (carrying the excluded plasmid) was a spheroplast. Surface exclusion occurred normally when the recipient (carrying the excluding plasmid) was a spheroplast. Hence surface exclusion is dependent on an intact mucoprotein layer in the donor cell (carrying the excluded plasmid). Beard and Bishop (1975) suggest that, in addition to the traG product encoded by the excluding plasmid in the recipient cell, the excluded plasmid specifies a donor component, called the ML factor, which interacts with the traG product to cause surface exclusion. The ML component is dependent on an intact mucoprotein layer for its action, and surface exclusion appears to prevent the exit of DNA from the donor cell.

**Transfer of the host chromosome**

Transfer of the chromosome occurs when F or ColV2 (Kahn, 1968)
are integrated into the host chromosome to form Hfr strains. Also, in a RecA+ host, an F-prime element mobilizes the chromosome from the area of morphoploid homology (Curtiss, 1969; Winters, 1969). In addition to these instances of chromosome transfer by covalent linkage to a sex factor, autonomous plasmids promote a much lower frequency chromosome mobilisation which, with the exception of R1-12, lacks polarity. The process may, however, be to some extent RecA dependent, because it is reduced in a RecA− host (Clover and Moody, 1965), and it is stimulated by ultraviolet irradiation (Evanish and al., 1969). Chromosomal transfer promoted in a RecA− host may rely on another plasmid or host encoded recombination system, not involving homologous DNA (Willett, 1972a). Alternatively, the plasmid transfer system may recognize DNA sequences on the host chromosome that resemble the original transfer, and so initiate transfer without physical association. The high frequency RecA independent, polarized transfer of the E. coli chromosome by R1-12 might be due to a chromosomal sequence closely related to the R1-12 origin of transfer (Cooke and Haynott, 1969; Willott, 1972a).

**Plasmid replication and incompatibility**

The number of copies of plasmid DNA per bacterial chromosome is regulated and has been estimated, by a hybridization assay, to be 1-4 for Escherichia coli carrying Fgal+ (Frame and Bishop, 1971). Collins and Pritchard (1973) found the number of Flac+ copies per chromosome varied with growth rate, but was consistent with there being 2 Flac+ copies per chromosome terminus regardless of growth rate. The isolation of CCC plasmid DNA (section 1.4) has also been used to estimate the number of copies of F, R64, R15, R100, R28K, R538 and RP1 present in Escherichia coli, values between 0.7 and 4.0 copies per chromosome being reported (Kontomichalou et al., 1970; Misicoka
et al., 1970; Vapnek et al., 1971; Grinstead et al., 1972; Milliken and Clowes, 1973). This type of replication, where plasmid to chromosome ratio is approximately 1:1, is said to be "stringent" (Round, 1969). In contrast situations occur where there are a greater number of plasmid copies per chromosome, and here replication is under "relaxed" control (Round, 1969). Examples are R6K present in 13-38 copies per E.coli chromosome (Kontomichalou et al., 1970), and the various small non-conjugative plasmids such as ColEl (Clewell and Helinski, 1970), and multiple copy drug resistance determinants (Smith et al., 1974).

Incompatibility was recognised as the inability of an F-prime element to be established in an Hfr strain, or of a cell to maintain two, separately recognisable, autonomous F factors (Schles, 1963; De Haan and Stouthamer, 1963; Maas, 1963; Maas and Maas, 1962; Scaife and Gross, 1962). The property is distinct from surface exclusion and separate genetic determinants for these independent properties have been mapped (Willett, 1974a).

Although stable derivatives carrying more than one autonomous factor cannot be isolated, temporary heterozygotes carrying two F-prime elements can be generated. These have been used extensively in a genetic analysis of the F transfer process by Willett, Achtman and their associates (section 1.5), and are used in this thesis (chapters 7 and 8).

Cells stably maintaining more than one F factor can be generated if all are integrated in the bacterial chromosome, as in double male strains (Clark, 1963) and triple male strains (Bastarrachea and Clark, 1968).

The many bacterial plasmids now isolated have been classified by their incompatibility relationships. Plasmids from the same
incompatibility group cannot co-exist, whereas plasmids from different groups can. The assumption that members of an incompatibility group are closely related is supported by DNA hybridisation studies (Guerry and Falkow, 1971; Grindley et al., 1973; Ingram, 1973). Furthermore, plasmids of a given pilus type never belong to an incompatibility group including plasmids of a different pilus type.

The F-like and I-like plasmids have been sub-divided on the basis of incompatibility. Five incompatibility groups constitute the F incompatibility complex. Most F-like plasmids belong to group F11 and there are four other minor groups. F1 includes the sex factor F, FIV contains R124 and ColVHmp+; whilst F1II and FV each contain only one plasmid. The I incompatibility complex contains two groups; I∞ (which includes plasmids previously assigned to groups Iβ and Iω) and Iγ (Hedges and Datta, 1973; N.Datta, personal communication).

In addition, incompatibility groups, which are not sub-divisions of a group classified on the basis of male specific phage sensitivity, have been defined. An ever growing number of such incompatibility groups exists, examples are A, C, H, J, K, M, N, O, T, W and X.

When two plasmids of the same incompatibility group form an unstable bi-plasmid derivative, plasmid segregation is sometimes non-random. F and ColV3 are preferentially eliminated from \((\text{ColV2, } F)^+\) and \((\text{ColV3, } F)^+\) cells respectively (MacFarren and Clowes, 1967).

Furthermore, which of a pair of incompatible plasmids is resident when a bi-plasmid derivative is constructed may affect the stability of the trans-conjugant. When R683 is transferred to a strain carrying another I∞ group plasmid segregation results, but
when the cross is performed in reverse stable cells carrying Rc63 and the second \( I^{ac} \) group plasmid are derived (Hedges and Datta, 1973).

Two models to explain the control of plasmid replication have been proposed. Limitation of membrane attachment sites, essential for plasmid replication, formed the basis of a positive control model, proposed by Jacob et al., (1963). Incompatibility is explained by different plasmids utilising the same attachment site. A major limitation of this model is its failure to account for incompatibility by an Hfr, where the F factor is replicated as part of the bacterial chromosome.

Pritchard et al., (1969) proposed a negative control model, based on the dilution of a plasmid specific inhibitor of replication. Replication of the gene encoding the postulated inhibitor would cause its concentration to be doubled, so preventing the initiation of further rounds of replication until the inhibitor concentration was halved by cell growth. Here incompatibility would reflect the specificity of the inhibitor protein. One replication of two incompatible plasmids would cause a fourfold increase in inhibitor concentration, preventing further plasmid replication for two cell divisions. During this time segregation of the incompatible plasmids could occur.

1.3 The mechanism of conjugation.

The role of sex pili

Sex pili have been implicated in the generation of cell to cell contacts, recognised as a requirement for conjugation soon after its discovery (Tatum and Lederberg, 1947; Davis, 1950).

Removal of sex pili, either temporarily by blending (Ehrlich, 1965; Novotny et al., 1969) and cultural conditions such as amino acid starvation (Curtiss et al., 1969), or permanently by sex factor mutation (Achtman et al., 1971; Ohmuro, 1970), prevented
conjugation and sensitivity to male specific bacteriophages which adsorb to the pilus. Using the Coulter counter, transfer defective mutants of Flac which lacked the pilus proved unable to form mating pairs, even though transfer defective mutants which retained the pilus did so normally (Achtman et al., 1971). Cells carrying F-like plasmids, which normally transfer at low frequency, do not have sex pili visible in the electron microscope. However, when such plasmids enter an uninfected population of cells, to form an HFT culture (section 1.6) from which transfer occurs at high frequency, sex pili become visible in the electron microscope (Datta et al., 1966; Maynell and Lawn, 1967).

When the pilus was present, mating pair formation could be prevented by specifically blocking the pilus with antibody (Harden and Maynell, 1972). Similarly, the pilus tip could be inactivated by adsorption of filamentous, DNA containing bacteriophages (Ippen and Valentine, 1967; Novotny et al., 1968; Qi, 1973) and by zinc ions (Qi and Anderson, 1972a).

Zinc ions were implicated in pilus tip inactivation, since they prevented adsorption of DNA containing male specific bacteriophages but not RNA containing male specific bacteriophages, which adhere to the tip and sides of the pilus respectively (Qi and Anderson, 1972b).

Hence the initial step in conjugation is the formation of a mating pair by an interaction between the pilus tip and a receptor site on the recipient cell surface. Mutants of recipient cells which lack this receptor site have recently been described (Reiner, 1974; Moumer et al., 1971; Skurray et al., 1974). Observation of mating mixtures in the electron microscope have revealed mating pairs apparently linked by F pili (Brinton et al., 1964), and pairs
of cells intimately linked by a conjugation bridge (Anderson, 1958). The latter structures have, however, been reported to occur between female cells (Brinton, 1965).

The function of the pilus once mating pairs have formed is the subject of much discussion and a definite answer has yet to be found. The controversial point is whether DNA is transferred to the recipient along the pilus, or from the donor cell directly to the recipient after formation of close mating pairs. The former model is favoured by Brinton (Brinton et al., 1964) and the latter by Curtiss (1969).

A pertinent study involved observation of individual mating pairs in the light microscope, followed by their separation and growth to determine ex-conjugant genotypes (Ou and Anderson, 1970).

The major limitation of this work was that pili and conjugation bridges were invisible in the light microscope, making interpretation of the observations speculative. Mating pairs either achieved close cell to cell contact, or remained loosely connected, presumably by the invisible sex pili. Analysis of the ex-conjugants after a thirty minute mating revealed that loose mating pairs produced recombinants, although these were more frequent when close cell to cell contact had been achieved. Ou and Anderson (1970) suggested that, in addition to initiation of contacts between mating cells, the sex pili served to transfer genetic material, and to draw mating pairs close together, increasing the fertility of the union.

Recent investigation of F pili has produced evidence that they retract, perhaps by sequential depolymerisation of pilin sub-units into the cell membrane. Curtiss (1969) incorporated the concept of pilus retraction into a model for conjugation where pili retract
after making contact with a female cell. This facilitates close contact between cells prior to the transfer of DNA.

F pili disappear in the presence of NaN₃ (Novotny et al., 1972) or arsenate (O'Callaghan et al., 1973). This loss, when energy production is inhibited, might have been due to pilus retraction, or the pili may fall off the cell.

Novotny and Fives-Taylor (1974) failed to demonstrate free pili in the culture supernatant after pili were lost in the presence of NaN₃. This suggested that F pili were able to retract. Novotny and Fives-Taylor also showed that pilus retraction was inhibited by the attachment of either A17 phage or F pili antibody to the sides of pili, and by temperatures below 25°C.

Furthermore, studies of the early stages of F1 infection involving observation in the electron microscope, led to the conclusion that F pili retract to allow penetration of this filamentous bacteriophage (Jacobson, 1972).

In conclusion, there is some evidence that pili act as conjugation tubes, allowing DNA transfer without close mating pair formation. However, pili also appear able to retract, perhaps facilitating more efficient transfer of DNA after the formation of close mating pairs.

**DNA transfer**

Jacob, Brenner and Cuzin (1963) proposed that transfer of the Hfr chromosome to an F⁻ recipient during conjugation was a consequence of DNA replication triggered by mating. Gross and Caro (1966) and Ptashne (1965) designed experiments to investigate the process, and their results indicated that the transferred DNA found in the recipient after mating, consisted of one pre-existing
donor strand and one strand synthesized during mating. Cohen et al., (1968a; 1968b) studied DNA transferred from F+, F-prime, and Hfr donors to minicell recipients. The transferred DNA appeared to be single stranded initially, but was later converted to partially double stranded DNA in the case of F+ and short F-prime donors, suggesting that the new strand was synthesized in the recipient cell.

Rupp and Ihler (1968) and Ohki and Tomizawa (1968) found that only one particular pre-existing strand of labelled donor DNA could be recovered from the recipient. Rupp and Ihler used 32p labelled donor DNA, and recovered the transferred DNA after mating. They used donors that were lysogenic for λ and recipients that were non-lysogenic. Upon transfer, lyogenic induction occurred and the transferred DNA was recovered as lambda phage after recipient cell lysis. The transferred lambda DNA was separated into its constituent single strand on the basis of differential binding of polyU,G. The 32p label incorporated into the donor DNA before mating was found predominantly in one strand of the progeny phage. By isolating the DNA transferred by donor strains which promoted transfer in opposite directions, Rupp and Ihler demonstrated that the particular labelled strand depended on the polarity of chromosome transfer. From the known orientation of the λ prophage in the host chromosome, the polarity of Hfr transfer, and knowledge of which recovered strand was labelled, it was concluded that the single stranded DNA was transferred with the 5' terminus leading.

Similar experiments were performed by Ohki and Tomizawa (1968), but the transferred DNA was assayed by hybridisation with separated λ strands instead of recovering intact λ phage.
A 5-bromouracil resistant mutant was used as recipient in their experiments and it was therefore possible to demonstrate that DNA synthesis occurred in the recipient during transfer.

Vapnek and Rupp (1970) studied F DNA transfer directly. They also prevented recipient cells from incorporating exogenous labelled thymidine by using thymidine kinase deficient mutants (tdk). Hence they were able to confirm that a new strand of DNA was synthesized in the recipient cell. Vapnek and Rupp recovered both donor and recipient DNA after mating by lysing the other parent with T6. F DNA was then isolated as covalently closed circles (section 1.4). The F DNA was separated into its constituent single strands in CsCl density gradients in the presence of poly U,G. The distribution of the labelled F DNA after transfer was then determined. Only one strand of pre-existing F DNA was recovered from the recipient, and this was the denser strand in the CsCl-poly U,G gradient. A complement to this strand was synthesized in the recipient, to form the isolated CCC DNA. The labelled strand of F DNA that was not transferred remained in the donor, and was isolated as a less dense strand in the gradient. A complement to the pre-existing strand was synthesized in the donor cell during mating, to form the isolated CCC DNA.

The generally accepted model for conjugal replication and DNA transfer, which takes account of this experimental evidence, is based on the rolling circle proposed by Gilbert and Dressler (1966). A specific endonuclease (perhaps encoded by gene traI, section 1.5) generates a single stranded nick at a specific site in the covalently closed circular DNA of the sex factor. Transfer of single stranded DNA from the nick occurs with the 5' terminus.
leading, whilst the 3' terminus remains hydrogen bonded to the
other circular DNA strand to prime transfer replication.

Jacob et al., (1963) suggested that the energy released by
the hydrolysis of ATP, during polynucleotide synthesis, provided
the "driving force" required for transfer. The implication,
that chromosome transfer cannot occur in the absence of replication
in the donor, has been an unsettled question (Curtiss, 1969).
However, a pertinent study has been reported by Sarathy and
Siddiqi (1973). Transfer replication by F was studied in a
thymine requiring Hfr containing a temperature sensitive dnaB
mutation. It was found that ordinarily, DNA synthesis in the
Hfr accompanied chromosome transfer at the non-permissive
temperature. However, when the donors were deprived of thymine
transfer occurred without DNA synthesis. It therefore seems
that transfer replication in the donor is not essential for
transfer of the chromosome. This finding is not compatible with
any hypothesis demanding DNA synthesis in the donor to "drive"
transfer of the chromosome to the recipient.

1.4 Physical study of plasmids

Plasmid molecules

The first method for isolating plasmid DNA was evolved in
studies on intergeneric transfer and buoyant density determination
of chromosomal DNA. DNA from Serratia marcescens (G+C=58%)
carrying Flac or Proteus mirabilis (G+C=38%) carrying Flac
was subjected to equilibrium centrifugation in a cesium chloride
gradient. The Flac DNA, with a buoyant density similar to
that of Escherichia coli (G+C=50%), appeared respectively as a
shoulder or peak separate from the chromosomal DNA.
Subsequently plasmids have been isolated directly from *Escherichia coli* cells. Separation from chromosomal DNA can be achieved by using minicells, or by gentle lysis with non ionic detergent, such as Brij 58, followed by low speed centrifugation to remove chromosomal DNA, still attached to the cell membrane.

Based on studies of the double stranded replicative form of ϕX174 DNA (Burton and Sinhaheimer, 1965; Piers and Sinhaheimer, 1962) and of polyoma virus (Vinograd *et al.*, 1965), the configurations taken up by plasmid DNA molecules have been determined. Plasmid DNA commonly occurs as a covalently closed circular duplex, often called CCC DNA. A single stranded nick in CCC DNA generates an open, nicked, or relaxed circle, conveniently denoted CC DNA, whilst a double stranded break in CCC DNA generates a linear duplex.

CCC DNA takes up a supercoiled structure when extracted, and the resistance of this configuration to shearing has allowed further techniques for plasmid isolation to be developed. These include sucrose gradients using "cleared lysates", and ethidium bromide-CsCl density gradient centrifugation (Clowes, 1972). The isolation of plasmid DNA has enabled the structure of various plasmids and the relationships between them to be studied.

**DNA : DNA hybridisation**

DNA : DNA hybridisation involves shearing of the plasmid DNA into small fragments and their denaturation. Binding of radioactively labelled DNA of one plasmid to cold DNA of a second, immobilised on an agar gel, provides an estimate of genetic homology (Falkow and Citarella, 1965). Hydroxylapatite has been used to separate homologous and heterologous DNA fragments (Guerry and Falkow, 1971). In this way R1 was found to have 74% homology.
with R100; 38% with F; 56% with an F-like enterotoxin plasmid; but only 15 to 18% with the L-like plasmids R-3, R144 and ColIB (Quarry and Falkow, 1971). Similarly the relationships between various L-like plasmids, and their partial homology with plasmids of incompatibility group 0, have been described (Falkow et al., 1974).

**Heteroduplex mapping**

Plasmid DNA in the GC configuration is used for heteroduplex mapping. DNA of the two plasmids to be compared is denatured and a mixture allowed to renature. If homology exists, some of the reconstituted DNA molecules will be heteroduplexes, with one strand from each plasmid. In the electron microscope the double stranded regions of homology can be distinguished from the regions of non-homology, represented by single stranded loops. As with DNA : DNA hybridisation the extent of homology is discovered, but also the arrangement of the homologous DNA can be determined. For instance, the homologous region of R6 and F comprised 44% of the F factor and was in a single contiguous segment (Cohen et al., 1971).

Observation of heteroduplexes between various F and F-prime elements has provided information for a physical map of F DNA. This map uses the anti-clockwise junction of F DNA with bacterial chromosome DNA, in the F-prime elements R100 and F152, as a reference point. Mapping begins from this reference point which is denoted 94-5/0. The 94-5 kilobases of F DNA are recorded anti-clockwise from this point. The region 50 to 94-5/0 of F exhibits considerable homology with the F-like plasmids R1, R100, R6, and ColV, and the genes for F transfer lie clockwise of co-ordinate C2. This is interesting because F-like plasmids complement any
transfer defective mutants of F (Hillette, 1971). The position, where known, of the individual transfer genes of this physical map is recorded in figure 14. The genes for autonomous replication lie between co-ordinates 43 and 53, and the genes for T3, T7, and dIII resistance between co-ordinates 33 and 43. The interval 0 to 17.6 contains several special DNA sequences at which integration of F into the bacterial chromosome occurs.

Three different sequences have been recognised as important in this respect. They are $\delta$ (the $\alpha/\beta$ sequence) occurring twice, at co-ordinates 13.7 - 15.0 and 93.2 - 94.5/0; $\gamma^\prime$ at 2.0 - 3.5; and $\gamma$ (the $\epsilon/\delta$ sequence) at 16.3 - 17.6 (Davidson et al., 1974; Hu et al., 1975b; 1975c).

The importance of insertion sequences and related $F$ sequences, such as $\delta$, in plasmid related recombination events may account for the non-random distribution of Hfr origins on E. coli chromosome. The observation of $\gamma$ sequences at both $F$ DNA - chromosomal DNA boundaries of the type II $F$-prime element $F13$ supports this theory (Hu et al., 1975b). Furthermore, reversion of an Hfr or $F$-prime to $F^+$ may involve these sequences. Indeed, the two $\gamma^\prime$ sequences of $F14$ could account for the 0.11 per generation rate of $F^+$ segregation from this $F$-prime (Ohmomo et al., 1974). This segregation, and that of $FLP12$ used in chapter 9 of this thesis, are $recA$ independent, perhaps indicating that a special form of recombination is associated with $\delta$ and similar sequences.

Partial denaturation of $F$ DNA has also been studied in the electron microscope. Conditions were used in which only DNA with a low guanine + cytosine content would denature, and these regions were mapped by analysing partially denatured heteroduplexes.
between F and various F-prime elements (Hsu, 1974). Host adenine + thymine rich sequences (i.e., those with low guanine + cytosine content) were clustered between co-ordinates 0 and 30 on the physical map of F. This is the region of F important for insertion into the E.coli chromosome. An additional adenine + thymine rich sequence was at co-ordinate 63, the area in which the origin of transfer has been located (Hsu, 1974).

Use of restriction Enzymes

Digestion of plasmid DNA with specific endonucleases, and separation of the resulting fragments by agarose gel electrophoresis, provides a technique for identifying plasmids. The resolution of this method is high, even the closely related R factors R100-1 and R6 being clearly distinguished (Thompson et al., 1974).

Restriction enzymes, such as EcoRI, which generate staggered breaks or "sticky ends", facilitate the isolation of the different fragments on receptor molecules; pSC101, ColE1, and bacteriophage λ, commonly being used. Study of the isolated fragments provides an auxiliary to heteroduplex mapping, allowing more precise physical location of plasmid genes (Skurray and Clark, 1975).

1.5 Genetic analysis of conjugation

Transfer systems

The four groups of plasmids F-like, I-like, P and N, distinguished by male specific phage sensitivity and incompatibility, produce four distinct transfer systems, which can be divided into
three interlocking parts:

1. The sex pilus and/or other surface structures, which facilitate mating pair formation in order that DNA can be transferred.

2. The enzymes involved in DNA metabolism at transfer, together with whatever plasmid DNA sequences they may recognise.

3. The surface exclusion products.

Transfer defective mutants

Mutants with defective transfer systems have been described for the I-like plasmid R64-II, but have not been studied in detail (Nillette, 1970). IIE resistant mutants of an N group plasmid were isolated by Dennison and Baumberg (1973), and amongst transfer deficient mutants of the P group plasmid RP1, many were simultaneously resistant to the bacteriophages PR1, Pf3, PR3 and PR4, whilst two retained sensitivity to all four phages (Stanisich, 1974).

In contrast to these cursory studies of I-like, N, and P plasmid transfer, the F-like transfer system has been the subject of several sophisticated genetic analyses, although much biochemical information remains undisclosed. Several mutations of Hfr strains and F-prime elements in which transfer was impaired were reviewed by Curtiss (1969). Performing complementation tests with F-prime elements is complicated by the inability to form stable F/F diploids because of incompatibility. The problem was overcome by Ohtsubo and co-workers (Ohtsubo et al., 1970) and by Nillette, Achtman and their colleagues (Achtman et al., 1971; 1972; Nillette and Achtman, 1972).
The first group exploited observations that F could complement transfer defects in some F-like R factor and Col factor mutants (Clowes, 1963; Hirota et al., 1966; Oseki et al., 1962), and conversely a de-repressed R factor mutant R100-1 could complement transfer defective mutants of F (Nishimura et al., 1967). Mutants of R100-1 and the Fgal+ element F6 were selected on the basis of male specific phage resistance or loss of transfer ability. Because these plasmids, although both F-like, belong to different incompatibility groups, stable bi-plasmid cells could be constructed, so facilitating complementation testing. P1 transduction was used to transfer the Tra- R100-1 mutants to cells carrying Tra- F6 mutants and complementation groups determined. In this way six genes common to both plasmids were identified (Ohtsubo et al., 1970).

The transfer defective mutants of Flac+ isolated by Achtman, Willetts and Clark (1971) were assigned to complementation groups by two procedures that generated F/F heterozygotes; conjugation and P1 transduction.

In the first procedure, Tra- Flac+ elements carrying suppressible point mutations were transferred from a Su+ donor strain to a Su- recipient strain carrying a different Tra- Flac+ element. The recipient culture was used in stationary phase to reduce surface exclusion (phenocopied culture, section 1.2). Mating was interrupted by killing the donor cells with T6 (the donor strain was T6S whilst the recipient strain was T6R) and the retransfer of lac+ from the temporary F/F heterozygotes was measured. From the frequency of retransfer the occurrence or not of complementation was established. In this way nine transfer cistrons, traA through traH and traJ, were identified. (Achtman
In the normal procedure, strains carrying the $^{32}$P-labeled mutants were transformed with $P_1$ from an strain carrying different $^{32}$P-labeled mutants, and the resultant transformational $P_1/ P_1$ heteroepigens were immediately tested for $lcr^+$ transfer to a $lcr^-$ $P_1^+$ recipient strain. Eleven cistrons, $lcr^+$ through $lcr^+$, were defined confirming the assignments made by conjugal transfer analysis (Millette and Ackman, 1970). The further cistron $lcr^-$ has subsequently been described (Millette, 1973).

All mutants in $lcr^+$, $lcr^-$, $lcr^+$, $lcr^+$, $lcr^+$, $lcr^+$, $lcr^+$, and none in $lcr^+$, were resistant to $P$ specific bacteriophages (Ackman et al., 1971; Millette, 1973). Recently, the $lcr^+$ gene has been shown to encode the pilus sub-unit protein, pilin. Pilin produced by an other $lcr^+$ mutant suppressed by $lcr^+$ (which inserts tyrosine) contained three tyrosine residues per molecule, whereas that suppressed by $lcr^+$ (which inserts serine) the pilin contained only two tyrosine residues, as the pilin produced by wild type $P_1$ (Haddow et al., 1975). Presumably the other eight gene products chemically modify the pilin protein (which is known to carry one glucose and two phosphate residues per molecule, section 1.2) and assemble the pilus.

Intact $lcr^+$, $lcr^-$, and some in $lcr^+$ continued to synthesize pilis. These appeared normal conferring motility to male specific bacteriophages, although $lcr^+$ mutants were resistant to $P$ (Millette and Ackman, 1972). They continued to form abortive pilis (Ackman et al., 1971) and retracted upon treatment withynonoid ions (Boyett and Rives-Taylor, 1976).

The $lcr^+$ product may be a specific endonuclease, which acts at the origin of transfer and it is further discussed below (section 1.5, plasmid specificity).
Mutants in *traD* allowed RNA phage adsorption and ejection (Achtman *et al.*, 1971; Paranchych, 1975; Paranchych *et al.*, 1971). Their resistance to phages such as f2 implies that the *traD* product is required for penetration of the cell by RNA. By analogy, it could also be necessary for DNA penetration during conjugation.

Some *traD* mutations prevent pilus formation whilst others do not. This may be because the *traD* product is bifunctional, perhaps serving to link pilus function and DNA metabolism at transfer.

The *traD* product is a positive control protein and its function is discussed below (section 1.6, positive control).

**Mapping of the transfer genes**

The transfer cistrons were arranged into a sequence by deletion mapping. Ohtsubo (1970) isolated deletions ending at various points within the transfer region by P1 transduction of F8 to *traD* strains, a process referred to as transductional shortening. Complementation of these deletions by the Tra- R100-1 point mutants gave the gene order (Ohtsubo, 1970). Ippen-Ihler *et al.*, (1972) made use of a transposition Hfr strain that has Flac+ inserted near the λ attachment site. Co-deletion of λ ci857 and part of F from a lysogen of this strain provided a series of deletions ending at various points within the transfer region. These were used in complementation tests with Flac+ *traD* point mutants to give the gene order shown in figure 1.1 (Ippen-Ihler *et al.*, 1972; Willetts, 1973). In addition to genes for transfer, a gene(s) *traS* which was essential for surface exclusion was located between *traQ* and *traD* (Willetts, 1974a).
The relative positions of genes for female specific phage resistance and incompatibility, and a site which is essential for transfer, known as the origin of transfer, have also been determined (Ippen-Ihler et al., 1972; Willetts, 1972b; 1974a).

Operon structure

The operon structure of the transfer genes has been determined by analysis of polar mutations. Amber and frameshift mutations, notably \( \text{tra}M \), showed that \( \text{tra}K, \text{tra}E, \text{tra}C, \text{tra}F, \text{tra}G, \text{tra}H \) and the postulated gene(s) for surface exclusion, \( \text{tra}S \), form a single operon (Achtman et al., 1972; Willetts and Achtman, 1972).

Helmuth and Achtman (1975) used the bacteriophage Mu-1 to study the operon structure of the transfer genes systematically. Mu-1 forms lysogen by insertion at random points within the bacterial genome. The prophage causes an absolute polar mutation at the site of insertion (Bukhari and Zipser, 1972; Jordon et al., 1968). Transfer deficient mutations of Flac\(^+\) were isolated by Mu-1 insertion at various points, to inactivate all the defined transfer cistrons. With the exception of \( \text{tra}J \), the Mu-1 induced mutants gave poor complementation in tests with point mutants in a particular cistron and all rightward (clockwise in figure 1.1) cistrons. The results were interpreted to mean that Mu-1 was inserted in, or to the left of, the leftmost cistron that gave poor complementation. The demonstrated polarity of the Mu-1 insertions mean that all the cistrons \( \text{tra}A \) through \( \text{tra}I \) are in one operon which is transcribed in a clockwise direction from \( \text{tra}A \) through \( \text{tra}I \). All Mu-1 insertion mutations in, or to the left of, \( \text{tra}G \) were also defective in the surface exclusion property. This confirms that \( \text{tra}G \) lies between \( \text{tra}G \) and \( \text{tra}D \) and is part of the
operon. From the data concerning the physical location of the transfer cistrons (Sharp et al., 1972), it has been calculated that the operon, which will be referred to as the transfer operon, is more than 15 megadaltons in size (Holmuth and Achtman, 1975).

**Plasmid specificity**

The transfer systems of F-like plasmids are closely related. Heteroduplex mapping has revealed that 90\% of the transfer region of F and R100 or R6 is homologous (Sharp et al., 1973). Pili produced by two different plasmids can be assembled into mixed pili which demonstrates similarity at the gene product level (Leam et al., 1971). Complementation of a transfer defect by a different F-like plasmid occurs, and six genes common to F6 and R100-1 have been defined on this basis (Ohtsubo et al., 1970).

Indeed, mutations in all pili producing genes of the F transfer operon are complemented by ColV2, ColVetrp, R100-1, R1-19 and R538-1 fin\textsuperscript{−} (Alfaro and Willetts, 1972; Willetts, 1971; 1975). Furthermore, R1-19 and R both have one glucose and two phosphate residues per pilin molecule (Beard, J.P., unpublished; Brinton, 1971).

In contrast genes \textit{traL}, which may encode an endonuclease acting at the origin of transfer, and the positive control gene \textit{traJ}, exhibit plasmid specificity. It is interesting that in both cases their function probably involves recognition of a specific DNA sequence: the origin of transfer and an operator for the transfer operon respectively. Flac\textsuperscript{+} and R100-1 failed to complement \textit{traL} or \textit{traJ} mutants of each other (Willetts; 1971, 1975), and R1-19 did not complement Flac\textsuperscript{+} \textit{traL} or Flac\textsuperscript{+} \textit{traJ}, although ColV2 and ColVetrp\textsuperscript{+} did (Alfaro and Willetts, 1971).
The related specificity of the origin of transfer has been tested using Hfr strains, deleted for the transfer genes, but retaining the origin of transfer. Ability to mobilise the Hfr host chromosome indicates that a plasmid recognises the F origin of transfer and suggests that its own origin of transfer and that of F are closely related. ColV2 and ColVBstr' mobilised the Hfr chromosome but R100-1, R1-19 and R538-1 fin' did not (Reeves and Willetts, 1974). It is clear that, in these cases, the specificity of the tral gene parallels that of the origin of transfer. This is indirect evidence that the tral product recognises the origin of transfer (Reeves and Willetts, 1974).

The tral gene, although not plasmid specific for transfer, does exhibit some variation in that the pili of various F-like plasmids differ in their sensitivity to male specific bacteriophages. Although, by definition, sensitive to F-specific bacteriophages, efficiency of plating estimates reveal that some F-like plasmids are more sensitive than others. This variation is concerned with the pilin protein gene tral (Willetts, 1971). Also, by direct observation of the attachment of antibody molecules to sex pili, four serotypes amongst F-like plasmids have been distinguished (Leman and Maynell, 1970).

Surface exclusion is also plasmid specific, four groups having been defined. A plasmid excludes itself and members of its own specificity group, but not members of other groups (Willetts and Maule, 1974).

Further specificity in the components of fertility inhibition are discussed below (section 1.6).
Figure 1.1 Genetic and heteroduplex mapping of F.

[Diagram of genetic and heteroduplex mapping of F, showing various genetic markers and their relative positions.]
1.6 Control of transfer

**Fertility inhibition**

Most F-like plasmids transfer at a frequency considerably lower than the F sex factor. Furthermore, the donor ability of the F factor is inhibited when it co-exists with such low transferring F-like plasmids. This property is denoted Fin'.

Despite the normally low transfer level of Fin' F-like plasmids they have the potential to transfer as efficiently as the sex factor F. Highly efficient transfer occurs when a bacterial cell is newly infected (Osaki et al., 1962; Watanabe, 1963). Such infected cells are said to form HFT (high frequency transfer) cultures.

**Mutations in fertility inhibition**

Egawa and Hirota (1962) isolated a mutant of R100, called R100-1, which failed to inhibit the transfer of F. The mutant was interpreted as no longer producing a cytoplasmic inhibitor acting on the F transfer system. Maynell and Datta (1965) suggested that transfer inhibition of F indicates a close relationship between the transfer control systems of the Fin' F-like plasmid and F, both being inhibited by the same mechanism. This notion was supported by the later discovery that R100-1 transfer occurred at high frequency (Nishimura et al., 1967). It was further concluded that the normally high transfer levels of F and ColV-K94 were due to their inability to produce an active inhibitor of transfer.

The inhibitor model predicted that high level transferring mutants would be of two types, inhibitor deficient, i−, and inhibitor insensitive, o0 (Maynell and Cooke, 1969; Hoar, 1970). Distinguishing between these two types is complicated by
incapability, which prevents the use of stable diploids to test dominance. Hence mutants of III group plasmids have been tested for their ability to inhibit chromosomal transfer by HfrC (O° inhibits; i° does not), and for their susceptibility to the ColE4 transfer inhibitor (i° is susceptible, O° is not; Haynall and Cooke, 1969; Frydman and Haynall, 1969). These tests do not, however, unequivocally test dominance, since the different plasmids may have non-interchangeable inhibitor components. For example Hausman and Clowes (1971) isolated mutants of ColE2, which did not inhibit the transfer of Flac+, but surprisingly were not susceptible to transfer inhibition by a Fin+ plasmid, 222/R4. Similarly ambiguous results have been reported for high level transfer mutants of R124 (Haynall and Lawm, 1973). These instances are discussed in detail and an explanation for the properties suggested in chapter 4 of this thesis.

Hoar (1970) was able to test male specific phage sensitivity of R100 / mutant R100 diploids by selecting for the maintenance of different antibiotic sensitive R100 derivatives in a recA− host. In this way 40 recessive and 14 dominant mutants were defined. Furthermore, 4 recessive mutants proved to be suppressible nonsense mutants, supporting the view that a cytoplasmic inhibitor was involved.

Mutants of F, which are no longer inhibited by co-existing Fin+ plasmids, have also been isolated (Frydman et al., 1970; Grindley et al., 1971; Negrotti and Magal de Zwaig, 1972). In these cases the mutants were considered to be of the inhibitor insensitive, O°, type.
Two component transfer inhibitor

A better understanding of the genetics of fertility inhibition resulted from a study of Fin+ mutants no longer inhibited by R100 (Finnegan, 1972; Finnegan and Willett, 1971). Dominance of a series of insensitive mutants were tested by measuring their retransfer frequency from transient heterozygotes (section 1.5) also carrying Fin+ and R100. Cis-dominant, inhibitor insensitive mutants defined the site of inhibitor action, traO. Surprisingly however, recessive mutants were also isolated. This implied that the transfer inhibitor consisted of an F encoded component defined by these recessive mutants (finR, originally called traP), in addition to the component naturally absent from F, but supplied by co-existing Fin+ plasmids (finO, originally called i, fi, or fin, Finnegan and Willett, 1971). These three types of mutant (finO, finP and traO) were found amongst high level transferring mutants of the R factor R136 to confirm this hypothesis (Grindley et al., 1973).

The finO products of most F-like plasmids are interchangeable (Finnegan and Willett, 1972) accounting for fertility inhibition of F by other F-like plasmids. In contrast, the finP products are not always interchangeable, and five specificity groups for the finP products of various F-like plasmids have been defined (Finnegan and Willett, 1972; Willett, unpublished data). Mutations of finP have been isolated for the Fin+ plasmids ColE2 (Hausmann and Cloves, 1971), R124 (Haynall and Lann, 1973), R136 (Grindley et al., 1973) and R6 (Silver and Cohen, 1972). These finP mutants transfer at high frequency, carry recessive mutations, but unlike finO mutants, continue to inhibit transfer of F.
Positive control and the mechanism of R100 fertility inhibition

All mutations in *traJ*, and a Mu-1 insertion mutation in *traJ*, were defective in transfer, pilus formation, and surface exclusion (Achtman et al., 1972; Helmsuch and Achtman, 1975). The Mu-1 insertion mutation in *traJ* was able to complement point mutations in all transfer cistrons except *traJ*, demonstrating that *traJ* is not part of the transfer operon (Helmsuch and Achtman, 1975). The pleiotropic phenotype of *traJ* mutations, and the location of the gene outside the transfer operon, led to the proposal that *traJ* is a positive control gene, its product required for the expression of the other tra genes.

The R100 transfer inhibitor prevents transfer, pilus formation, and surface exclusion by F (Willetts and Finnegan, 1970). Since the properties are encoded by genes of the transfer operon, it was suggested that inhibition may act on the proposed positive control gene *traJ*, so indirectly preventing expression of the transfer operon (Finnegan and Willetts, 1972). Two experiments have provided evidence that *traJ* is a positive control gene, and that R100 transfer inhibition is by initial inactivation of *traJ*.

In the first experiment, Achtman (1973b) isolated transfer proficient mutants of a Tra- Flac+ element, carrying a suppressible *traJ* mutation. The derivatives isolated transferred inefficiently from a Su- host but transferred with normal efficiency from a Su+ host. The suppressible *traJ* mutant was still present but a second "J-independent" mutation, allowing partial expression of the transfer operon in the absence of *traJ* product (in a Su- host), had been isolated. The ability to isolate mutations of this type would be predicted if *traJ* was a positive control gene.
R100 did not inhibit transfer of J-independent mutations (Achtman, 1973b; Willetts and Paranchych, 1974). Again this would be expected if the mechanism of R100 fertility inhibition was eliminating positive control, the function of gene traJ.

In the second experiment, Finnegan and Willett (1973) used a genetic assay for the presence of individual F transfer gene products during fertility inhibition by R100. The technique involved measurement of the retransfer frequency of Flac\textsuperscript{+} traK\textsuperscript{-} traA\textsuperscript{-} double mutants from cells also carrying Fhis\textsuperscript{+} and R100. The frequency of retransfer indicated whether or not the traK gene was directly inhibited. It was found that the only directly inhibited gene was traJ, proving that R100 fertility inhibition prevents expression of the transfer operon indirectly, by inhibiting traJ. An additional feature of interest, was that traK, the site of action of R100 transfer inhibition, was tightly linked to traJ.

The low transfer abilities of cells carrying R100 and an Flac\textsuperscript{+} traK\textsuperscript{-} traA\textsuperscript{-} double mutant, indicated that these cells contain only inhibited levels of the non-plasmid-specific R100 traK products. This showed (assuming fertility inhibition of F and R100 to be by the same mechanism, via traJ) that the R100 traJ product was needed for synthesis of the other R100 transfer (traK) gene products. By analogy this showed that for F, traJ is a positive control gene.

**Kinetics of fertility inhibition**

Willetts (1974b) studied the kinetics of Flac\textsuperscript{+} transfer inhibition by R100. When Flac\textsuperscript{+} entered exponentially growing cells carrying R100, the establishment of transfer inhibition was
delayed for approximately six hours. This was not due to the absence of the transfer inhibitor, but was probably caused by transient synthesis of \textit{traJ} product. As a result, transfer inhibition was established only after dilution out, firstly of the \textit{traJ} product, and secondly of the transfer gene products whose synthesis depends on \textit{traJ} during growth. By extrapolating to transfer control of \textit{Fin}^+ F-like plasmids, such as \textit{R100}, \textit{Hfr} formation, which occurs when such plasmids encounter a population of uninfected cells, can be explained. It seems that the dual control of transfer (negative on \textit{traJ}, and positive by \textit{traJ} on the transfer operon) causes a time lag before the establishment of inhibition, fundamental to the infectious spread of \textit{Fin}^+ F-like \textit{R} factors.

\textbf{Fertility inhibition by non F-like plasmids}

Although \textit{F} fertility inhibition is a common characteristic of plasmids that encode \textit{F-like} transfer systems, plasmids which are not \textit{F-like} have recently been shown to be \textit{Fin}^+. These include the \textit{I-like} plasmids \textit{R62}, \textit{JR66a}, \textit{TP101} = \textit{TP109} and \textit{R805a} (Datta and Hedges, 1973; Datta and Clarke, 1974; Grindley and Anderson, 1971; Meynell, 1973), and plasmids belonging to the \textit{N} and \textit{X} incompatibility groups (Grindley at al., 1972; Hedges, at al., 1973).

Meynell (1973) isolated mutants of two \textit{F-like} plasmids \textit{R538-1drd1} and \textit{F/R1drd9}, which produced pili in the presence of \textit{R62}. The mutants overproduced pili but were still susceptible to the \textit{R62} inhibitor. Willett and Parenchych (1974) studied the inhibition of \textit{F} by \textit{R62} and isolated \textit{F} inactivated mutants in which the site of action of \textit{R62} was defective. The mutations (\textit{tra}^-) were cis-dominant and have been used to show that \textit{R62}-encoded transfer
inhibition is different from transfer inhibition by the F\textsuperscript{+}
F-like plasmid R100 (chapter 3, section 3.1).

As discussed above the transfer of J-independent mutants of
\textit{F\textsuperscript{+} tra\textsuperscript{+}} from a Su\textsuperscript{-} host is not affected by R100. In
contrast R62-encoded fertility inhibition does inhibit transfer
of the J-independent mutants (Willetts and Paranchych, 1974).
This has led to the conclusion that, unlike R100, R62 transfer
inhibition acts directly on the transfer operon to prevent transfer,
pilus formation and surface exclusion by \textit{F\textsuperscript{+}}.

This thesis includes a comparison of transfer inhibition by
various F\textsuperscript{+} plasmids; five types of control have been
discovered and partially characterised, including a more detailed
study of F transfer inhibition by R62.
CHAPTER 2  MATERIALS AND METHODS

2.1 Media.

All quantities are given per litre of distilled water unless stated otherwise.

Amino acids: made up as stock solutions at 8mg/ml and used at a final concentration of 20µg/ml.

Thyamine: made up as a stock solution at 1mg/ml and used at a final concentration of 50µg/ml.

1xA medium

\[ \text{K}_2\text{HPO}_4 \, 10.5g; \, \text{KH}_2\text{PO}_4 \, 4.5g; \, (\text{Mg})_2\text{SO}_4 \, 1g; \, \text{Na citrate H}_2\text{O} \, 0.5g. \]

Acetate buffer 0.1M

25ml solution A added to 24.5ml solution B and 50ml distilled H\textsubscript{2}O, pH adjusted to 4.6, autoclaved and pH checked.

solution A: 11.55 ml concentrated glacial acetic acid made up to 1 litre with distilled H\textsubscript{2}O.

solution B: Na acetate .3H\textsubscript{2}O 27.29g.

EBL bottom agar

NaCl 5g; EBL tryptone 10g; Difco agar 10g.

EBL top agar

NaCl 5g; EBL tryptone 10g; Difco agar 6.5g.

Citrate buffer pH5.5

100ml citric acid monohydrate 21g; added to 300ml trisodium citrate dihydrate 29.5g.

EMB (agar) agar

water agar 300ml; EMB peptone base 75ml; Eosin yellow solution (4%) 4ml; Methylene blue solution (0.65%) 4ml;
sugar solution (20%) 20ml.

**EMB peptone base**
- Difco bacto casamino acids 42.5g
- Difco bacto yeast extract 5.2g
- NaCl 27g
- KH₂PO₄ 10.4g

**Giemsa bottom agar**
- Tryptone 10g
- yeast extract 10g
- NaCl 8g
- Difco agar 15g

After autoclaving the following were added to 500ml of this agar: 0.5M CaCl₂ 4ml; glucose (20%) 50ml; Giemsa "R66" 12.5ml.

**L broth nutrient agar**
- nutrient L broth solidified with 15g/l of Davis New Zealand agar.

**Lactose tetrascilium agar**
- L broth nutrient agar 500ml
- 2,3,5-triphenyl-2H-tetrascilium chloride solution (1%) 1ml
- lactose solution (20%) 25ml.

**LC top agar**
- Tryptone 5g
- NaCl 5g
- yeast extract 5g
- Difco agar 7g

After autoclaving 0.5ml of 0.5M CaCl₂ was added to 100ml LC top agar.

**LC bottom agar**
- Tryptone 10g
- NaCl 5g
- yeast extract 5g
- Difco agar 10g

After autoclaving the following were added to 500ml of this agar: 0.5M CaCl₂ 2ml; glucose solution (20%) 2.5ml; thymidine solution (0.25%) 2ml.

**M9 minimal salts x 4**
- Na₂HPO₄ 20g
- KH₂PO₄ 12g
- NaCl 2g
- NH₄Cl 4g

**M9 minimal salts**
- 100 ml M9 salts (x4) diluted 1 in 4 with distilled water with the following additions:
sugar solution (20%) 4ml; \( \frac{1}{2} \) \( \text{M} \) \( \text{MgSO}_4 \) solution 0.5ml; thiamine hydrochloride (0.8mg/ml) 0.1ml.

**Minimal agar**

K salts (x4) diluted 1 in 4 with water agar; sugar added to 0.2%; \( \frac{1}{2} \) \( \text{M} \) \( \text{MgSO}_4 \) solution 0.5ml; thiamine hydrochloride (0.8mg/ml) 0.1ml.

**Nutrient broth**

Difco tryptone 10g; yeast extract 5g; NaCl 10g.

**Oxoid nutrient agar**

Oxoid No.2 nutrient broth powder 25g; Davis New Zealand agar 15g.

**Phosphate buffer**

\( \text{KH}_2\text{PO}_4 \) 3g; \( \text{Na}_2\text{HPO}_4 \) 7g; NaCl 4g; \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \) 0.2g.

**Phosphate (phage) buffer**

\( \text{KH}_2\text{PO}_4 \) 3g; \( \text{Na}_2\text{HPO}_4 \) 7g; NaCl 5g; \( \text{MgSO}_4 \cdot 0.1\text{H}_2\text{O} \) solution 10ml; CaCl\(_2\) 0.01M solution 10ml; 1% gelatin solution 1ml.

**Spizizen minimal salts**

\((\text{NH}_4)_2\text{SO}_4\) 10g; \( \text{K}_2\text{HPO}_4 \) 70g; \( \text{KH}_2\text{PO}_4 \) 30g; sodium citrate 5g; \( \text{MgSO}_4 \) 1g.

**Spizizen minimal agar**

Spizizen salts (x5) diluted 1 in 5 with water agar; sugar added to 0.2%; thiamine hydrochloride (0.8mg/ml) 0.1ml.

**Vogel Bonner salts 50x**

\( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \) 10g; citric acid.\( \text{H}_2\text{O} \) 100g; \( \text{K}_2\text{HPO}_4 \) 500g; Na\((\text{NH}_4)_2\text{HPO}_4 \cdot 4\text{H}_2\text{O} \) 175g; distilled water 670ml.2ml chloroform was added as preservative and the solution autoclaved before use.
Voel Bonner nutrient medium

2ml Vogel Bonner salts (50x) diluted 1 in 30 with distilled water with the following additions:
- glucose solution (20%) 0.25ml; uridine (2mg/ml) 1ml; L tryptophan was added to a final concentration of 4µg/ml or 100 µg/ml.

Water agar

Davis New Zealand agar 20g; pH adjusted to 7.2.

Water top agar

Difco agar 7g.

2.2 Reagente

N-acetyl-arginine, N-acetyl-histidine, sulphonamide, tetracycline and trimethoprim solutions were stored at -20°C; all other stock solutions were stored at 4°C.

Acridine orange

EDH Chemicals Ltd., Poole, England, purified in this laboratory. Stock solution 1mg/ml.

Amoxicillin

'Penbritin', Beecham Laboratories, Brentford, England. Stock solution 10mg/ml, used at a final concentration of 25µg/ml.

Chloramphenicol sodium succinate


Ethyl methane sulphonate

Kodak Chemicals Ltd., London.

Kanamycin sulphate

'Kantrex', Bristol Laboratories Ltd., Stamford House
Lungley, Bucks, England. Stock solution 10mg/ml, used at a final concentration of 50µg/ml.

**N-acetyl-L-arginine**

Sigma Chemical Co., St. Louis, U.S.A. Stock solution 6mg/ml used at a final concentration of 20µg/ml or 100µg/ml.

**N-acetyl-L-histidine monohydrate**

Calbiochem, San Diego, Calif., U.S.A. Stock solution 8mg/ml used at a final concentration of 20µg/ml.

**Malidixic Acid**

Sodium malidixate, Winthrop Laboratories, Edgefield Ave., Pawtuck, Newcastle-upon-Tyne. Stock solution 8mg/ml in 0.3M NaH₂PO₄ used at a final concentration of 40µg/ml.

**N-methyl-N-nitro-N-nitrosoguanidine (MN)***

Boehringer-Light Laboratories, Colnbrook, Bucks, England.

**Spectinomycin sulphate**

*Trobicin*, a gift of the Upjohn Co., Kalamazoo, Michigan, U.S.A. Stock solution 40mg/ml used at a final concentration of 100µg/ml.

**Streptomycin sulphate**

Glaxo Laboratories Ltd., Greenford, England. Stock solution 50mg/ml, normally used at a final concentration of 200µg/ml. In matings in which the donor strain carried an R factor coding for Str² and Str² was the counterselecting marker, streptomycin was used at 2mg/ml. This reduced the donor background growth due to frequent R factor mutants carrying resistance to greater than 200µg/ml streptomycin (Pearce and Haynall, 1960).

**Sulphonamide**

Sulphadimidine, I.C.I. Ltd., Macclesfield, Cheshire. Stock solution 333mg/ml, used at a final concentration of 100µg/ml.
Tetracycline hydrochloride

'Achromycin', Cyanamid, Great Britain. Stock solution 500 μg/ml, used at a final concentration of 10 μg/ml in minimal agar and 20 μg/ml in nutrient agar.

Tetrazolium chloride

2,3,5-Triphenyl-2H-tetrazolium chloride. Eastman Organic Chemicals, Rochester, N.Y., U.S.A. Stock solution 1% (w/v), used at a final concentration of 0.005%.

Trimethoprim

Burroughs Wellcome and Co., Tuckahoe, N.Y., U.S.A.

Stock solution 100 μg/ml.

2.3. Bacterial strains

The strains used are listed in tables 2.1-2.4. Where these have been constructed during this study details of their derivation are given. The nomenclature used is that of Taylor and Trotter (1972).
Table 2.1  Strains used in chapters 3 - 7

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>other markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lac</td>
<td>Gal</td>
</tr>
<tr>
<td>ED24</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ED26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ED57</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ED1686</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ED1799</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ED2149</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ED2195</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ED2197</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ED3537</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ED4354</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ED4467</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JC3272</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JC5455</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>JC6255</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>JC5266</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JC6455</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KL98</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M174</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RC711</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>W1607</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2.2  Additional strains used in chapter 8

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid carried</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED4410</td>
<td>Hfr KL98</td>
<td>topA</td>
</tr>
<tr>
<td>KD4438</td>
<td></td>
<td>( \text{lac}<em>{X74} (\text{trp , tonB})</em>{\Delta \text{AE12}} , \text{his}_{\text{am}} , \text{str , tax} )</td>
</tr>
<tr>
<td>ED4430</td>
<td></td>
<td>( \text{lac}<em>{X74} , \text{trp}</em>{\text{am}} , \text{str , spo , tax , topA} )</td>
</tr>
<tr>
<td>ED4431</td>
<td>JCP100</td>
<td>( \text{lac}<em>{X74} (\text{trp , tonB})</em>{\Delta \text{AE12}} , \text{his}_{\text{am}} , \text{str , tax} )</td>
</tr>
<tr>
<td>ED4434</td>
<td>JCP100</td>
<td>( \text{lac}<em>{X74} (\text{trp , tonB})</em>{\Delta \text{AE12}} , \text{str , tax , supF} )</td>
</tr>
<tr>
<td>ED4438</td>
<td></td>
<td>( \text{lac}<em>{X74} , \text{trp}</em>{\text{am}} , \text{str , spo , tax , topA} , \text{\lambda , imm} , \text{434} )</td>
</tr>
<tr>
<td>ED4444</td>
<td></td>
<td>( \text{lac}<em>{X74} (\text{trp , tonB})</em>{\Delta \text{AE12}} , \text{spo , tax , recA86} )</td>
</tr>
<tr>
<td>ED4481</td>
<td>ED1263</td>
<td>( \text{lac}<em>{X74} (\text{trp , tonB})</em>{\Delta \text{AE12}} , \text{his}_{\text{am}} , \text{str , tax} )</td>
</tr>
</tbody>
</table>

Derivations are given in figure 2.1 which includes a group of strains constructed for use with \( \lambda \, p \, \text{trp}^+ \, \text{BG2} \).
Figure 2.1 Strains constructed for use with ptrp^+BG2 (chapter 8)

```
(lac pro)ΔX11  his^am  trp^am  str  tsx
Hfr CA77
ED2024

(lacΔX74  his^am  trp^am  str  tsx)
NTG mutagenesis
ED4427

(lacΔX74  his^am  trp^am  str  tsx)
P1  his^+
ED4428

(lacΔX74  trp^am  str  tsx)
spontaneous T5
ED4430

(lacΔX74  trp^am  str  tsx  tonA)
AE12  str  tsx
trimethoprim selection
ED4443

(lacΔX74  his^am  (trp tonB))
AE12  spc  tsx  thy
Hfr JC088
ED4444

(lacΔX74  his^am  (trp tonB))
AE12  spc  tsx  recA

ED4423

P1  trp^+  cys^-
ED4424

P1  cys^−(trp tonB)
AE12
ED4425

P1  supF
ED4433
```

Table 2.3 Additional strains used in chapter 9

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Derivation or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED3542</td>
<td>$\text{lac}_{\Delta X74}$ his trp leu str tax recA</td>
<td>Dr. E. Thompson</td>
</tr>
<tr>
<td>ED3667</td>
<td>$\text{lacZ}$ leu metE proC trp str tax recA</td>
<td>Dr. P. Broda</td>
</tr>
<tr>
<td>ED4407</td>
<td>$\text{lac}_{\Delta X74}$ his trp metB (ppc arg ECRH)$\Delta MN42$&lt;br&gt;str tax recA</td>
<td>Fig. 2.3</td>
</tr>
<tr>
<td>ED4500</td>
<td>$\text{lac}_{\Delta X74}$ his trp metB (ppc arg ECRH)$\Delta MN42$&lt;br&gt;str spo tax recA</td>
<td>Fig. 2.3</td>
</tr>
<tr>
<td>AS2</td>
<td>his metB arg$\Delta$sup102 purD str</td>
<td>Dr. J. Scaife</td>
</tr>
<tr>
<td>CA8001</td>
<td>HfrH lac$\Delta L1$</td>
<td>Dr. J. Scaife</td>
</tr>
<tr>
<td>KL351</td>
<td>$\text{lacZ}$ leu metE proC purE trp str tax recA</td>
<td>Dr. B. Bachmann</td>
</tr>
<tr>
<td>XA8001</td>
<td>lac$\Delta L1$ str</td>
<td>Dr. J. Scaife</td>
</tr>
</tbody>
</table>

Some derivations are given in figure 2.2, which includes a group of strains constructed for use in argF fusion experiments
Figure 2.2  Strains constructed for selection of argB fusions
(chapter 9)

ED3529
lac\textsubscript{ΔX74} his trp lys leu str tsx

Hfr P4X (ppc arg ECBH)\textsubscript{ΔMN42} metB\textsuperscript{-}

ED4451
lac\textsubscript{ΔX74} his trp lys (ppc arg ECBH)\textsubscript{ΔMN42} metB str tsx

spontaneous
BF23\textsuperscript{R}

ED4452
lac\textsubscript{ΔX74} his trp lys (ppc arg ECBH)\textsubscript{ΔMN42} metB str tsx bfe

trimethoprim selection

ED4496
lac\textsubscript{ΔX74} his trp lys (ppc arg ECBH)\textsubscript{ΔMN42}
metB str tsx thy

ED4500

lac\textsubscript{ΔX74} his trp lys (ppc arg ECBH)\textsubscript{ΔMN42}
metB str spc tax

EMS mutagenesis

Hfr JC5088

ED4497
lac\textsubscript{ΔX74} his trp lys (ppc arg ECBH)\textsubscript{ΔMN42} metB str tsx recA
<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid carried</th>
<th>Genotype</th>
<th>Derivation or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED395</td>
<td>EDFG160</td>
<td>( \text{lac}^{\Delta} \text{U}124 )</td>
<td>Dr. N. S. Willetts</td>
</tr>
<tr>
<td>ED3213</td>
<td>EDFG163</td>
<td>( \text{lac}^{\Delta} \text{U}124 (\text{nadA gal att bio}) \text{ tax} )</td>
<td>Dr. N. S. Willetts</td>
</tr>
<tr>
<td>ED2310</td>
<td>EDFG163</td>
<td>( \text{lac}^{\Delta} \text{U}124 (\text{nadA gal att bio}) \text{ tax} )</td>
<td>Dr. N. S. Willetts</td>
</tr>
<tr>
<td>ED2244</td>
<td></td>
<td>( \text{lac}^{\Delta} \text{X}74 \text{ his sup } \text{trp } \text{ str } \text{ spo tax} )</td>
<td>Dr. N. S. Willetts</td>
</tr>
<tr>
<td>ED2245</td>
<td></td>
<td>( \text{lac}^{\Delta} \text{X}74 \text{ his sup } \text{trp } \text{ str } \text{ spo tax} )</td>
<td>Dr. N. S. Willetts</td>
</tr>
<tr>
<td>ED3794</td>
<td>EDYL223</td>
<td>( \text{lac}^{\Delta} \text{U}124 (\text{nadA gal att bio}) \text{ tax} )</td>
<td>Dr. S. McIntyre</td>
</tr>
<tr>
<td>ED3795</td>
<td>EDYL224</td>
<td>( \text{lac}^{\Delta} \text{U}124 (\text{nadA gal att bio}) \text{ tax} )</td>
<td>Dr. S. McIntyre</td>
</tr>
<tr>
<td>ED4471</td>
<td></td>
<td>( \text{lac}^{\Delta} \text{X}74 \text{ gal his sup } \text{trp sup } \text{ str tax} ) (( \text{ins}^{\Delta} \text{21} ))</td>
<td>( \text{ins}^{\Delta} \text{21} ) lysogen ex ED3736</td>
</tr>
<tr>
<td>ED4472</td>
<td></td>
<td>( \text{lac}^{\Delta} \text{X}74 \text{ gal his sup } \text{trp sup } \text{ str spo tax} ) (( \text{ins}^{\Delta} \text{21} ))</td>
<td>EMS mutagenesis ex ED4471</td>
</tr>
<tr>
<td>C500</td>
<td></td>
<td>\multicolumn{2}{c</td>
<td>}{\text{recA sup}^+}</td>
</tr>
<tr>
<td>QR48</td>
<td></td>
<td>\multicolumn{2}{c</td>
<td>}{Dr. R. Murray}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\multicolumn{2}{c</td>
<td>}{Dr. M. Bork}</td>
</tr>
</tbody>
</table>
2.4 Plasmids

The plasmids used, together with sources and references, are given in tables 2.5 and 2.6. Various derivatives of JCVLO, F57, F100 and KLP12, isolated during this study, are included in table 2.7. JCVLO is lacZ+ a derivative of F42 carrying lacZ and 1ac13 (Achtman et al., 1971).

Table 2.5a. F-prime elements used. I with wild type F factor

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Reference</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCVLO</td>
<td>Achtman et al., 1971</td>
<td>Dr N.S. Willette</td>
</tr>
<tr>
<td>F57</td>
<td>Achtman et al., 1972</td>
<td>Dr N.S. Willette</td>
</tr>
<tr>
<td>FI00</td>
<td>Low, 1972</td>
<td>Dr N.S. Willette</td>
</tr>
<tr>
<td>KLP8</td>
<td>Low, 1972</td>
<td>Dr J. Sasife</td>
</tr>
<tr>
<td>KLP12</td>
<td>Low, 1972</td>
<td>Dr J. Sasife</td>
</tr>
<tr>
<td>KLP44</td>
<td>Low, 1972</td>
<td>Dr B. Bachmann</td>
</tr>
<tr>
<td>KLP47</td>
<td>Low, 1972</td>
<td>Dr B. Bachmann</td>
</tr>
<tr>
<td>Plasmid</td>
<td>Mutation carried</td>
<td>Chromosomal markers</td>
</tr>
<tr>
<td>------------</td>
<td>------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>EDG160</td>
<td>traJ80</td>
<td>gal att, gal att</td>
</tr>
<tr>
<td>EDG163</td>
<td>traA1</td>
<td>gal att, gal att</td>
</tr>
<tr>
<td>JCFL90</td>
<td>traJ90</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL11</td>
<td>traA1</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL27</td>
<td>traJ31.1</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL13</td>
<td>traE18.1</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL24</td>
<td>traK4</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL2</td>
<td>traB2</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL5</td>
<td>traG5</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL16</td>
<td>traC16</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL13</td>
<td>traJ13</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL50</td>
<td>traH50</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL71</td>
<td>traJ71</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL83</td>
<td>traD83</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL40</td>
<td>traA40</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL65</td>
<td>traE65</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL119</td>
<td>traJ90; J-independent</td>
<td>leu</td>
</tr>
<tr>
<td>JCFL130</td>
<td>traJ90; J-independent</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL67</td>
<td>traA304</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL68</td>
<td>traA305</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL63</td>
<td>finP301</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL66</td>
<td>finP303</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL55</td>
<td>traK316</td>
<td>leu</td>
</tr>
<tr>
<td>WPFL39</td>
<td>traQ312</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL223</td>
<td>traK or traK (ED4)</td>
<td>leu</td>
</tr>
<tr>
<td>EDFL224</td>
<td>traA (c1857 cro -)</td>
<td>leu</td>
</tr>
</tbody>
</table>

Plasmids EDFL223 and EDFL224 were supplied by Dr S. McIntyre
and all other plasmids in this table by Dr N.S. Willetts.
<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Reference</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Meynell &amp; Datta, 1967</td>
<td>Dr.E.E.M.Moody</td>
</tr>
<tr>
<td>R1-16</td>
<td>Meynell &amp; Datta, 1967</td>
<td>Dr.E.E.M.Moody</td>
</tr>
<tr>
<td>R1-18</td>
<td>Meynell &amp; Datta, 1967</td>
<td>Dr.E.E.M.Moody</td>
</tr>
<tr>
<td>R6</td>
<td>Watamabe et al., 1964</td>
<td>Dr.R. Thompson</td>
</tr>
<tr>
<td>R62</td>
<td>Meynell, 1973</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>JB66a</td>
<td>Datta &amp; Hedges, 1973</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R100</td>
<td>Egawa &amp; Hirota, 1962</td>
<td>Dr.E.Meynell</td>
</tr>
<tr>
<td>R100-1</td>
<td>Egawa &amp; Hirota, 1962</td>
<td>Dr.E.Meynell</td>
</tr>
<tr>
<td>R124</td>
<td>Hedges &amp; Datta, 1972</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R124 dmd</td>
<td>Meynell &amp; Law, 1973</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R138</td>
<td>Grindley et al., 1972</td>
<td>Dr.Datta</td>
</tr>
<tr>
<td>R130</td>
<td>Meynell &amp; Cooke, 1969</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R366</td>
<td>Datta &amp; Hedges, 1972</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R404</td>
<td>Datta &amp; Hedges, 1972</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R444</td>
<td>Hedges &amp; Datta, 1973</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R455</td>
<td>Hedges &amp; Datta, 1973</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R465</td>
<td>Hedges &amp; Datta, 1973</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>R38-1</td>
<td>Romero &amp; Meynell, 1969</td>
<td>Dr.E.Meynell</td>
</tr>
<tr>
<td>R605a</td>
<td>Datta &amp; Clarke, 1974</td>
<td>Dr.N.Datta</td>
</tr>
<tr>
<td>ColB1(-CA1β)</td>
<td>Clowes et al., 1969</td>
<td>Dr.E.E.M.Moody</td>
</tr>
<tr>
<td>ColB2(-K77)</td>
<td>Frydman &amp; Meynell, 1969</td>
<td>Dr.E.E.M.Moody</td>
</tr>
<tr>
<td>ColB3 for</td>
<td>Hausmann &amp; Clowes, 1971</td>
<td>Dr.E.E.M.Moody</td>
</tr>
<tr>
<td>ColB4(-K98)</td>
<td>Clowes et al., 1969</td>
<td>Dr.E.E.M.Moody</td>
</tr>
<tr>
<td>Ent-H10 ( )</td>
<td>Williams-Smith &amp; Halls, 1968</td>
<td>Dr.H.Williams-Smith</td>
</tr>
<tr>
<td>Ent-P3 ( )</td>
<td>Williams-Smith &amp; Linggood, 1970</td>
<td>Dr.H.Williams-Smith</td>
</tr>
<tr>
<td>Hly-P233 ( )</td>
<td>Williams-Smith &amp; Halls, 1967</td>
<td>Dr.F.Ørakov</td>
</tr>
<tr>
<td>H.S-D1146</td>
<td>Williams-Smith &amp; Linggood, 1970</td>
<td>Dr.F.Ørakov</td>
</tr>
<tr>
<td>SSP</td>
<td>Šrakov &amp; Šrakov, 1973</td>
<td>Dr.E.S.Anderson</td>
</tr>
<tr>
<td>HP10-36</td>
<td>Smith et al., 1973</td>
<td>Dr.F.Ørakov</td>
</tr>
<tr>
<td>Tra-A14</td>
<td>Williams-Smith, 1974</td>
<td>Dr.H.Williams-Smith</td>
</tr>
<tr>
<td>O-D1122</td>
<td>Šrakov &amp; Šrakov, 1973</td>
<td>Dr.H.Williams-Smith</td>
</tr>
<tr>
<td>Vir-SS</td>
<td>Williams-Smith, 1974</td>
<td>Dr.H.Williams-Smith</td>
</tr>
<tr>
<td>Plasmid</td>
<td>Chromosomal marker</td>
<td>Mutations carried</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>EDFP268</td>
<td>motB arg</td>
<td>argΔsup102</td>
</tr>
<tr>
<td>EDFQ168</td>
<td>gal Δtt</td>
<td>traC16</td>
</tr>
<tr>
<td>EDFH161</td>
<td>his</td>
<td>traQ312</td>
</tr>
<tr>
<td>EDFJ207</td>
<td>lac</td>
<td>traJ98; traQ312</td>
</tr>
<tr>
<td>EDFK208</td>
<td>lac</td>
<td>traA15; traQ312</td>
</tr>
<tr>
<td>EDFJ207</td>
<td>lac</td>
<td>traE15; traQ312</td>
</tr>
<tr>
<td>EDFJ208</td>
<td>lac</td>
<td>traH80; traQ312</td>
</tr>
<tr>
<td>EDFJ209</td>
<td>lac</td>
<td>traG71; traQ312</td>
</tr>
<tr>
<td>EDFJ210</td>
<td>lac</td>
<td>traD33; traQ312</td>
</tr>
<tr>
<td>EDFJ250</td>
<td>lac</td>
<td>traU321</td>
</tr>
<tr>
<td>EDFJ251</td>
<td>lac</td>
<td>traU322</td>
</tr>
<tr>
<td>EDFJ252</td>
<td>lac</td>
<td>traU323</td>
</tr>
<tr>
<td>EDFJ253</td>
<td>lac</td>
<td>traU324</td>
</tr>
<tr>
<td>EDFJ254</td>
<td>lac</td>
<td>traU325</td>
</tr>
<tr>
<td>EDFJ255</td>
<td>lac</td>
<td>traU326</td>
</tr>
<tr>
<td>EDFJ256</td>
<td>lac</td>
<td>traU327</td>
</tr>
<tr>
<td>EDFJ257</td>
<td>lac</td>
<td>traU328</td>
</tr>
<tr>
<td>EDFJ259</td>
<td>lac</td>
<td>traV329</td>
</tr>
<tr>
<td>EDFJ260</td>
<td>lac</td>
<td>traV330</td>
</tr>
<tr>
<td>EDFJ261</td>
<td>lac</td>
<td>traV331</td>
</tr>
<tr>
<td>EDFJ262</td>
<td>lac</td>
<td>traK or traB polar</td>
</tr>
<tr>
<td>EDFJ263</td>
<td>lac</td>
<td>traC15; traQ333</td>
</tr>
<tr>
<td>EDFJ264</td>
<td>lac</td>
<td>traC15; traQ334</td>
</tr>
</tbody>
</table>
2.5 Phage strains

The phage strains used, together with references and sources, are listed in table 2.8.

<table>
<thead>
<tr>
<th>Phage</th>
<th>Reference</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>f1, f2</td>
<td>Losb, 1960</td>
<td>Dr. N. Achtmann</td>
</tr>
<tr>
<td>μ3</td>
<td>Davis et al., 1961</td>
<td>Dr. N.D.F. Grindley</td>
</tr>
<tr>
<td>Q5</td>
<td>Watanabe, 1964</td>
<td>Dr. N. Achtmann</td>
</tr>
<tr>
<td>λp1857</td>
<td></td>
<td>Dr. N.S. Willetts</td>
</tr>
<tr>
<td>λpam21</td>
<td></td>
<td>Dr. N. Murray</td>
</tr>
<tr>
<td>λpam434</td>
<td></td>
<td>Dr. N. Murray</td>
</tr>
<tr>
<td>λp bio1um434 via R</td>
<td></td>
<td>Dr. N. Murray</td>
</tr>
<tr>
<td>λsus0</td>
<td></td>
<td>Dr. N. Murray</td>
</tr>
<tr>
<td>λsusP</td>
<td></td>
<td>Dr. N. Murray</td>
</tr>
<tr>
<td>λint-</td>
<td></td>
<td>Dr. N. Murray</td>
</tr>
<tr>
<td>λp try&lt;sup&gt;+&lt;/sup&gt; BG2</td>
<td>Davison et al., 1974</td>
<td>Dr. W. Brammar</td>
</tr>
<tr>
<td>Δ 80 vir</td>
<td></td>
<td>Dr. N.S. Willetts</td>
</tr>
<tr>
<td>λNM 461, carrying EcoRI generated KIF1 fragments</td>
<td></td>
<td>Dr. J. Beggs</td>
</tr>
<tr>
<td>λNM 540, carrying HindIII generated KIF1 fragments</td>
<td></td>
<td>Dr. J. Beggs</td>
</tr>
<tr>
<td>RF23</td>
<td></td>
<td>Dr. J. Scaife</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;82</td>
<td></td>
<td>Dr. N.S. Willetts</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td></td>
<td>Dr. J. Scaife</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td></td>
<td>Dr. N. Achtmann</td>
</tr>
</tbody>
</table>
2.6 Techniques used to construct strains

Selection of $\text{top}^-$ and $\text{topA}^-$ derivatives

0.1ml of an overnight culture of the parent strain and 0.1ml of a $\text{T}_6$ (for $\text{top}^-$) or $\text{T}_6$ (for $\text{topA}^-$) phage lysates ($10^{10}$ p.f.u.) were plated in water top agar on Oxoid nutrient plates. Surviving colonies were cross streaked against the relevant phage to confirm resistance. Derivatives were purified by three rounds of single colony isolation to remove contaminating phage.

**Lysogens of $\lambda$**

Low titre ($10^7$ p.f.u/ml) $\lambda$ suspension was spotted onto L plates overlayed with the non-lysogenic parent strain. After overnight incubation (37° or 30° for $\lambda$ c1857) cells from the area of turbid lysis were purified and spot tested for resistance to the relevant homoimmune $\lambda$ phage and sensitivity to a heteroimmune $\lambda$ phage (to eliminate $\lambda$ resistant mutants).

**Nalidixic acid resistant mutants**

Spontaneous nalidixic acid resistant mutants ($\text{Nal}^R$) were isolated by spreading a concentrated shaken overnight culture of the parent strain on nutrient agar plates containing $40\mu$g/ml nalidixic acid. The surviving colonies were tested for the $\text{Nal}^R$ phenotype.

**Thymine requiring strains**

Spontaneous Thy$^-$ mutants were selected by a technique adapted from that of Stacey and Simson (1965). 0.04ml of an overnight culture of the Thy$^+$ parent strain, 0.1ml of thymine solution (1mg/ml) and 0.2ml trimethoprim solution (100µg/ml) were added to 2ml of appropriately supplemented minimal medium, and
incubated at 37°. After 1-2 days the culture was streaked on
nutrient agar with added thymine. The resulting colonies were
patched onto the same medium and Thy− clones identified by replica-
ting the master plates onto minimal medium plates with and without
thymine.

**Phase P1 lysates**

P1ko (Yanofsky and Lemoen, 1959) and a clear plaque mutant,
P1vir (Wolf et al., 1968) were used for P1 transduction.

Transducing lysates were prepared by confluent plate lysis.

0.1ml P1 phage lysate containing 10⁷ pfu/ml and 0.5ml of a donor
strain grown to exponential phase, were overlayed in LC top agar
onto LCTG plates. After overnight incubation at 37°, the top
agar layer was scraped into a screw cap bottle containing 0.5ml
chloroform. The plate was washed with 2ml L broth and the
washings added to the bottle, which was centrifuged after vigorous
shaking. The supernatant was collected and titred for P1 phage
on LCTG plates using ABL157 as an indicator strain.

**Phase P1 transduction**

(a) For P1vir 0.5ml exponential recipient culture (2 x 10⁸
cells/ml), concentrated X10 in tryptone, 0.5ml P1 lysate
(2 x 10⁷ pfu/ml) in L broth and 0.5ml of 3 x Ca/Lig (0.01M CaCl₂ +
0.03M MgSO₄) were incubated for 15 minutes at 37°. Cells were
washed in an equal volume of phosphate buffer and resuspended in
0.1ml buffer before spreading on minimal medium selective for
transductants.
For $P_{1ko}$ the procedure as for $P_{1\text{vir}}$ was followed, but to eliminate $P_1$ lysogens the transducing phage was U.V. irradiated (2000 ergs/$\text{cm}^2$) before use and resuspended prior to plating in 0.1ml 0.1M citrate buffer pH7. Transductants were tested for $\lambda_{ol}$ sensitivity, $P_1$ lysogens being resistant.

**Mutagenesis**

**Ethyl methane sulphonate (EMS):** 10ml of an exponential culture at $2 \times 10^8$ cells/ml of the strain to be mutagenised was centrifuged, the cells resuspended in phosphate buffer, centrifuged and resuspended for a second time in 5ml phosphate buffer. 0.05ml EMS was added and the mixture incubated at 37°C for 30 minutes. 0.05ml of these mutagenised cells were diluted into 5ml of L broth and grown overnight to allow mutant expression.

**N-methyl-N'-nitro-N-nitrosoguanidine (NTG):** 10ml of an exponential culture at $2 \times 10^8$ cells/ml of the strain to be mutagenised was centrifuged, washed and resuspended in 4.5ml 0.1M citrate buffer pH5.5. 0.5ml of a fresh solution of NTG (2mg/ml in 0.1M citrate buffer pH 5.5) was added and the mixture incubated at 37°C for 30 minutes. 0.05ml of those mutagenised cells were diluted into 5ml L broth and grown overnight to allow mutant expression.

**Ultra violet irradiation**

U.V. mutagenesis is used in chapter 9 in an attempt to isolate deletions. Exponential phase cells were centrifuged and resuspended in a half volume of phosphate buffer. After exposure to 300 ergs/$\text{cm}^2$ or 3000 ergs/$\text{cm}^2$ ultra violet irradiation,
cells were diluted into M9 minimal medium and grown overnight (in the dark to prevent photoreactivation) to allow mutant expression and segregation. (Minimal medium was used in the experiments with KLF12 because this plasmid is unstable; see chapter 9.)

**Nitrous acid**

Overnight cultures of the cells to be mutagenised were centrifuged, washed in an equal volume of acetate buffer and resuspended in 0.5 ml freshly prepared nitrous acid (NaNO₃ dissolved in 0.1M acetate buffer pH 4.6 to a final concentration of 0.05M). After 10 minutes incubation, 5 ml of 1 x A medium was added and the cells centrifuged and resuspended in of minimal medium. Cells were grown overnight to allow mutant expression and segregation.

**Conjugation: isolation of RecA⁻ derivatives**

Exponential cultures of the parent strain and an Hfr which transfers a required marker early were mated (1.0 ml + 1.0 ml). After sufficient time to allow entry of the marker into the parent strain dilutions in water top agar were subjected to violent agitation in a mechanical interrupter (Low & Wood, 1965) to break mating pairs. The dilutions were plated on minimal medium to select recombinants carrying the relevant marker.

Recombination deficient derivatives were isolated by mating a Thy⁻ parental strain with JC5088, a RecA⁻ Thy⁺ Hfr, which transfers both Thy⁺ and RecA⁻ early. Str⁺ or Spc⁺ was constitutively selected and RecA⁻ recombinants among the Thy⁺, Str⁺ or Spc⁺
progeny were detected by their increased UV-sensitivity using the replica plate method (Clark & Margulies, 1965).

3.7 Transfer of plasmids

Transfer proficient plasmids

0.1ml of donor and 0.1ml of recipient overnight cultures and 0.6ml L broth were incubated for 45 minutes and a loopful of the mating mixture streaked out on minimal plates to select the required trans-conjugant.

Transfer deficient plasmids

F-prime factors carrying suppressible transfer mutations were transferred to a recipient strain by using a F\textsuperscript{+} donor strain.

Non-suppressible transfer-deficient F-prime factors were transferred by complementation of their \textit{tra} \textsuperscript{-} mutation with a wild type \textit{Fhis} \textsuperscript{+} element. The experimental procedure was as described for complementation tests (section 2.9) with selection for the required derivatives of the final recipient. Colonies growing on selective plates were purified and tested to confirm the presence of a transfer mutation.

Transfer of plasmids carrying a \textit{\lambda} prophage to non-lyogenic recipients

To avoid zygotic induction when a plasmid carrying a \textit{\lambda} prophage was transferred, recipient cultures with temporary immunity to the phage were prepared. The method was adapted from Hohn and Korn (1969). The recipient strain was grown overnight in L broth with 0.2% maltose, centrifuged, and resuspended at a concentration of approximately 10\textsuperscript{9} cells/ml. The cells were infected with a
with a \( \lambda \) phage, of appropriate immunity and carrying a mutation (see below) to prevent lysogenisation, at a multiplicity of infection approximately 10. After 15 minutes at room temperature to allow phage adsorption, the cell suspension was diluted into L broth and regrown to exponential phase. Although the cells carried phage unable to insert as a prophage, the \( \lambda \) product produced rendered cells temporarily immune to homimmune \( \lambda \) phages.

The \( F^{+} \) elements carrying a \( \lambda pt\) \( \text{BC2} \) prophage (discussed in chapter 8) were transferred to recipients treated with \( \lambda \text{phio} \text{imm}^{+} \text{R} \) (which has a defective \text{att} site). \( F^{+} \) elements carrying putative \( \lambda \text{tra}^{+} \) prophage (chapter 10) were transferred to a recipient treated with \( \lambda \text{int}^{-} \).

2.8 Mating conditions

Matings in test tubes

Matings were performed in 18mm x 160mm test tubes at \( 37^\circ \) unless a temperature sensitive lysogen of \( \lambda \) was involved, when \( 33^\circ \) was used. Mating mixtures were of a total volume of 2ml. Matings designed to measure frequencies of plasmid transfer were performed by mixing 0.2ml of the donor culture with 1.8ml of the recipient culture, and incubating for 30 min. at \( 37^\circ \) (for the large plasmids KLF44 and KLF12 a 60 min. mating time was also used) or for 60 min. at \( 33^\circ \).

In all cases the parental cultures were checked to ensure that \( >96\% \) of the cells carried the appropriate plasmids. Instances of segregation of the plasmids used are mentioned in the relevant places.
In experiments where mating was interrupted with T₈, 0.1ml each of donor and recipient cultures were used and 0.2ml of T₈ (concentrated to approximately 10⁵ pfu/ml and irradiated with 2500 ergs/mm² U.V.) was added.

Donor and recipient cultures were normally grown to exponential phase and used when they had reached 2 x 10⁸ cells/ml. F⁻ phenocopy cultures (referred to as stationary phase cultures in the text) of F-prime carrying strains, to be used as recipients, were made by inoculating 1ml L broth and shaking at 37°C for approximately 16 hours. These cultures were diluted to 10ml with fresh L broth immediately before mating.

Plate matings

These were performed by replica-plating as described by Clark and Margulies (1985).

Selection of progeny

Throughout this thesis squared brackets will be used to denote the phenotype used for contraselecting the donor strain.

Cells which had received an F-prime factor during conjugation were selected by means of one or more of the chromosomal markers which it carried. Progeny carrying R-factors were selected by means of their resistance markers. Resistance to tetracycline was used to select R6, R62, R100, R124, R128, R136, R389, R404, R444 and R455; resistance to kanamycin was used for R1, JR66a and R805a; resistance to chloramphenicol for R538-1; and resistance to sulphadimidine for R485.
Clones to be tested for the production of colicin by the ColB factors were stabbed into nutrient plates and after overnight growth the cells were killed with chloroform vapour. 0.2ml of a colicin sensitive indicator strain was poured over the clones in 2.5ml LC top agar. Col^+ clones could be distinguished by a clear area surrounding them after 8-10 hr. incubation.

The frequency of ColB transfer during a mating was measured by plating 10^4 and 10^8 dilutions of the mating mixture on nutrient agar selective for the recipient strain, and overlaying this with a second layer of top agar to prevent surface growth of the colonies. After 24-36 hr growth a third layer of top agar containing 0.2ml of an indicator strain, was poured over the plate. The Col^+ clones could be detected by a clear area around them after 8-12 hr. at 37°C.

2.9 Complementation tests

Exponential phase donor cultures, JC6355(Su^+, T^R, Str^R) carrying Flac^+ tra^- suppressible mutants, were mated with stationary phase cultures (Su^- T^O Str^O) carrying the transfer defective mutation to be tested (0.1ml + 0.1ml). After 40 minutes the donor cells were killed by the addition of concentrated (10^{11} pfu/ml), UV irradiated T (0.2ml) and incubation continued for 20 minutes. L broth (0.6ml) was then added and after 40 minutes incubation exponential phase recipient cells (T^R Str^R; 1.0ml) were added and mating for 40 minutes allowed before plating on minimal medium to select Lac^+ (Str^R) progeny.
2.10 Surface exclusion

Surface exclusion measurements were made using JC3272 as the host strain, except for EDFL263 when ED4426 was used. An exponential culture of the appropriate JC3272 derivative was mixed with an exponential culture of the Hfr KL88 (lml:1ml for 60 min.). ED4410 as Tn5 derivative of KL88 was used to measure surface exclusion of EDFL263 which carried a \( \lambda^\text{ptrp}^+ \) BG3 prophage.

The KL88 Hfr transfers his as an early marker. Dilutions of the mating mixture were plated on minimal medium selective for His\(^{+}\)[Str\(^R\)] progeny. The surface exclusion index of a strain is defined as the number of His\(^{+}\)[Str\(^R\)] recombinants with JC3272, divided by the number of His\(^{+}\)[Str\(^R\)] recombinants with that strain.

2.11 Acridine orange curing

5 x 10\(^2\) - 5 x 10\(^3\) cells/ml were inoculated into L broth (pH adjusted to 7.6 with NaOH) containing acridine orange. Acridine orange was used at 60\(\mu\)g/ml for Rec\(^+\) strains and 25\(\mu\)g/ml for Rec\(^-\) strains. The cultures were shaken at 37\(^\circ\) in the dark for 16-20h.

Single colonies were recovered and tested by replica plating, or by the use of indicator media, for loss of plasmid markers.

2.12 Phage techniques

Preparation of phage lysates

Male specific phage lysates were prepared by infecting an exponential phase culture of JC3272 carrying \( \text{F}\) with the relevant phage at an m.o.i. of 10. The infected culture, growing in L broth with 0.005M Ca\(^{2+}\), was shaken for 5 hours, sterilized...
with chloroform (or by millipore filtration in the case of \( \phi 1 \)), centrifuged, and stored at 4\(^\circ\). Phage titration was performed using Oxoid nutrient agar plates and LC top agar.

Other phage stocks were prepared by a confluent plate lysis technique, sterilized with chloroform, and stored at 4\(^\circ\). LC top agar and nutrient agar plates were used, except for \( \lambda \) lysates which were prepared on L agar plates in L top agar.

\( \lambda \) lysates were titred on BBL plates using BBL top agar.

\( \lambda \) lysates were prepared as described by Achtman et al. (1971).

**Testing phage sensitivity**

Strains were tested for their sensitivity to phages by one of the following three techniques.

**Plating for plaques**

0.2ml of a culture of the indicator strain and 0.1ml of a dilution of the phage to be tested were added to 2.5ml of top agar and poured on nutrient agar plates. Plaques were counted after 16-18 hours incubation at 37\(^\circ\).

For \( \lambda \) BBL top and bottom agar were used. LC top agar and Oxoid nutrient agar plates were used for male specific and other phages. Male specific phages were plated on cells previously grown to exponential phase. In other cases fresh overnight cultures of the indicator strain were used.

**Spot tests**

0.2ml of an overnight culture of the strain to be tested was added to 2.5ml of LC top agar and poured onto nutrient agar plates.
0.01ml of the phage suspension (approximately $10^7$ pfu/ml) was spotted onto these and the plates incubated at $37^\circ$ for 10-18 hr. BBL top and bottom agars were used for $\lambda$ spot tests.

**Replica plating**

A convenient way to test male-specific phage sensitivity of a large number of clones was to patch them on nutrient or minimal plates, incubate overnight at $37^\circ$, replicate onto Oxoid nutrient agar plates and incubate these for 6-8 hours. The exponential patches were replicated onto Giemsa plates spread with 0.1ml of a high titre lysate of $\lambda$E2 or $\lambda$2. Sensitive clones grew poorly and cell lysis led to a colour change in the dye.

**Induction of $\lambda$ lysogens**

Propagates carrying the c1857 mutation were induced by growth at $42^\circ$; other propagates were induced by exposure to ultraviolet light.

For temperature induction, lysogens were grown to exponential phase at $33^\circ$, transferred to a $42^\circ$ water bath and shaken for 2 hours. Chloroform was added and the lysates centrifuged and stored at $4^\circ$.

For U.V. induction, exponential cultures were centrifuged and resuspended in a half volume of phosphate buffer. Cells were subjected to 300 erg/mm$^2$ UV irradiation and added to an equal volume of L broth. The cells were shaken for 2 hours (in the dark to prevent photoreactivation), chloroform added, the lysate centrifuged and stored at $4^\circ$.
2.13 Penicillin enrichment for homoygotes

This technique was used to isolate EDYA265, a derivative of KLF12 carrying the arg sup103 deletion. AE2 (arg sup103, metB) carrying KLF12 (arg sup103, metB) was grown overnight in M9 minimal medium supplemented with arginine, but lacking methionine (to ensure plasmid maintenance). The culture was diluted into fresh minimal medium and grown to exponential phase. The cells were washed and resuspended in the same volume of buffer and 0.1ml of this suspension added to 6ml of M9 minimal medium lacking both arginine and methionine. The culture was shaken for 120 minutes at 37° before ampicillin was added to give a final concentration of 600 µg/ml, and shaking continued overnight. The cultures were streaked out on minimal medium plates selective for clones still carrying KLF12. These were tested by replica plating for the presence of the arg sup103 mutation (allows growth on ornithine, but not without arginine or ornithine supplement) and the fertility of the KLF12 plasmid.

2.14 Tryptophan operon expression

Measurement of anthranilate synthase activity (Ito et al., 1969)

Anthranilate synthase (Asase) catalyzes the conversion of chorismic acid to anthranilic acid, both intermediates in the pathway of tryptophan biosynthesis.

A substrate mixture was prepared immediately before use. It contained 2 x 10^{-4}M chorismic acid (a 10-fold dilution of lag dissolved in 4.4ml water), 4 x 10^{-3}M MgSO_4, 10^{-2}M glutamine, 10^{-2}M potassium phosphate buffer pH 7.6 and 10^{-3}M 2-mercaptoethanol.

65.
Between 0.05ml and 0.2ml of cell extract was added to 1.6ml of substrate mixture, prewarmed to 37° in a quartz cuvette, mixed, and the reaction allowed to proceed after transfer of the cuvette to a recording Locarte fluorimeter Mk4. The fluorimeter was fitted with a water-heated cuvette holder to maintain the temperature of the cuvette contents at 37°.

The machine was calibrated with standard concentrations of anthranilic acid. During reactions the synthesis of anthranilate was followed as the increase in fluorescence at an emission wavelength of 380nm, using an excitation wavelength of 313nm.

The specific activity of anthranilate synthase is expressed as units of activity per mg protein, where one unit of activity catalyses the synthesis of 100nmol of anthranilate in 20 minutes at 37°.

**Measurement of protein concentration**

Protein concentration was measured by the method of Lowry et al., (1951).

**Inhibition by 5-methyl tryptophan**

The method described by Allen and Yanofsky (1963) was used. 10⁶ cells were added to 2.5ml top water agar and poured onto a minimal plate. A sterile filter paper disc was placed in the centre of the plate. A sterile solution of 5-methyl tryptophan (60μg/ml) was pipetted onto the disc. After 24 hours incubation at 37° the radius of the zone of inhibition was measured.
3.1 Introduction

The nature of fertility inhibition by R100 has been studied (Willett and Finnegan, 1970) and two types of inhibitor-insensitive Flac‘ mutant isolated. Dominant traQ mutations affect the site of action of the R100 transfer inhibitor, and recessive finP mutations (formerly denoted traP) reveal the participation of an F encoded component in F transfer inhibition (Finnegan and Willett, 1971).

Although fertility inhibition of F, denoted Fin‘, is a frequent phenotype of F-like plasmids it is not exclusively associated with those plasmids (section 1.6). Flac‘ mutants insensitive to transfer inhibition by the I-like plasmid R63 have been isolated. These dominant traQ mutations affect the site of action of the R63 transfer inhibitor and have been used, in conjunction with traQ and finP mutants of Flac‘, to demonstrate that transfer inhibition by the F-like plasmid R100 is different from that by the I-like plasmid R63 (Willett and Paranichych, 1974).

Here wild type Flac‘ and three Flac‘ mutants Flac‘ traQ04, Flac‘ finP301 and Flac‘ traQ12, representative of the mutant types mentioned above, have been used in a survey of fertility inhibition by 28 Fin‘ plasmids with varied properties.
3.2 Inhibition of $\text{Flac}^+$ transfer by $\text{Fin}^+$ plasmids

$\text{Fin}^+$ plasmids already classified into incompatibility groups were tested for their abilities to inhibit transfer of wild type $\text{Flac}^+$ and the three mutant $\text{Flac}^+$ elements. Four derivatives of JC5455 were constructed carrying the $\text{Fin}^+$ plasmid to be tested and one of the $\text{Flac}^+$ factors, JCFL0 (wild type), EDPL67 (tra0304), EDPL51 (finP301) or WPPL36 (tra0312). Quantitative donor ability estimates were made for the derivatives, using JC3272 as a recipient and selecting $\text{Lac}^+$ [Str$^R$]. ED57 (Col$^V$ derivative of JC3272) and ED26 (Col$^R$ derivative of JC3272) were substituted as recipients for plasmids producing colicin B and for R62 (Col$^+$) respectively.

Results are presented in table 3.1; those in column 3 confirm that the plasmids tested were $\text{Fin}^+$ and indicate that the extent of $F$ transfer inhibition varies from 14 fold for R124 to 80,000 fold for R485.

Susceptibility of the three mutant $\text{Flac}^+$ elements to transfer inhibition by the $\text{Fin}^+$ plasmids investigated is shown in the last three columns of table 3.1. The results for no $\text{Fin}^+$ plasmid, R100 and R62 are taken from previously published work carried out in this laboratory (Willett and Parenchych, 1974) and illustrate (1) the wild type transfer level of the $\text{Flac}^+$ mutants when alone, (2) the insensitivity of $\text{Flac}^+$ and $\text{Flac}^+$ to R100 transfer inhibition, and of $\text{Flac}^+$ to R62-encoded transfer inhibition, (3) the continued susceptibility of $\text{Flac}^+$ to R100 transfer inhibition and of the two mutants $\text{Flac}^+$ to R62 transfer inhibition.
Footnotes to table 3.1

The % of Lac⁺ [Str⁺] progeny is given. Donor strains were JC5455 derivatives, and recipient strains were JC3272 or its ColV₀ or ColR₀ derivative, where appropriate. Matings were carried out as described in chapter 2.

a. R369 and R404 are composite plasmids, the Pin⁺ and Tet⁺ characters being carried by plasmids of unclassified incompatibility groups (N. Datta, personal communication; Datta and Hedges, 1973).

b. JR66a is a member of incompatibility group Ia which includes the group Iv to which JR66a was originally assigned (N. Datta, personal communication; Datta and Hedges, 1973).

c. These results are taken from Willett and Paranchych, (1974).
Table 3.1 Inhibition of transfer by Fin⁺ plasmids

<table>
<thead>
<tr>
<th>Fin⁺ Plasmid</th>
<th>Incompatibility Group</th>
<th>tra⁺</th>
<th>Flac⁺ mutation</th>
<th>FIA0P301</th>
<th>FIAQ312</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>C</td>
<td>135</td>
<td>120</td>
<td>160</td>
<td>125</td>
</tr>
<tr>
<td>R100</td>
<td>FII</td>
<td>0.06</td>
<td>135</td>
<td>160</td>
<td>0.2</td>
</tr>
<tr>
<td>R62</td>
<td>FII</td>
<td>1.1</td>
<td>0.3</td>
<td>0.9</td>
<td>95</td>
</tr>
<tr>
<td>R455</td>
<td>F₁</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>R1</td>
<td>FII</td>
<td>0.8</td>
<td>145</td>
<td>160</td>
<td>0.3</td>
</tr>
<tr>
<td>R6⁺</td>
<td>FII</td>
<td>0.2</td>
<td>100</td>
<td>110</td>
<td>0.1</td>
</tr>
<tr>
<td>R136</td>
<td>FII</td>
<td>0.06</td>
<td>86</td>
<td>47</td>
<td>0.01</td>
</tr>
<tr>
<td>R192</td>
<td>FII</td>
<td>0.3</td>
<td>81</td>
<td>81</td>
<td>0.1</td>
</tr>
<tr>
<td>R444</td>
<td>FII</td>
<td>0.2</td>
<td>105</td>
<td>97</td>
<td>0.1</td>
</tr>
<tr>
<td>R635-1</td>
<td>FII</td>
<td>0.3</td>
<td>120</td>
<td>110</td>
<td>0.1</td>
</tr>
<tr>
<td>ColB₂</td>
<td>FII</td>
<td>2.3</td>
<td>78</td>
<td>81</td>
<td>2.3</td>
</tr>
<tr>
<td>ColB₄</td>
<td>FIII</td>
<td>0.3</td>
<td>120</td>
<td>115</td>
<td>0.3</td>
</tr>
<tr>
<td>ColB₁</td>
<td>F</td>
<td>0.3</td>
<td>130</td>
<td>90</td>
<td>0.3</td>
</tr>
<tr>
<td>R124</td>
<td>FIV</td>
<td>9.4</td>
<td>91</td>
<td>125</td>
<td>11.3</td>
</tr>
<tr>
<td>JR66a</td>
<td>E₀</td>
<td>0.03</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>R128</td>
<td>N</td>
<td>0.2</td>
<td>80</td>
<td>97</td>
<td>0.1</td>
</tr>
<tr>
<td>R389</td>
<td>?</td>
<td>0.1</td>
<td>170</td>
<td>160</td>
<td>0.05</td>
</tr>
<tr>
<td>R404</td>
<td>?</td>
<td>0.08</td>
<td>120</td>
<td>105</td>
<td>0.04</td>
</tr>
<tr>
<td>R455</td>
<td>X</td>
<td>0.002</td>
<td>0.002</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>R485a</td>
<td>X</td>
<td>0.3</td>
<td>0.4</td>
<td>0.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

69.
The remaining results in table 3.1 show the relationship of the tested Fin\(^+\) plasmid transfer inhibition systems to those encoded by R100 and R62. All members of incompatibility groups F\(\text{II}\), F\(\text{III}\), F\(\text{IV}\), N and the two unclassified plasmids R389 and R404 (see footnote to table 3.1), inhibited transfer of Fin\(^+\) traO but not of Fin\(^+\) traO or of Fin\(^+\) finP. To express the Fin\(^+\) character these plasmids required both the site of action traO, and the F-encoded finP product. Hence their mechanism of F transfer inhibition is related to that of R100. For convenience this common system will be referred to FinO\(^+\).

The FinO\(^+\) plasmids R100, R1, R136 and R6 have been shown to encode a finP product involved in control of their own transfer (Finnegan and Willetts, 1972; Grindley et al., 1973; Silver and Cohen, 1972), and it is reasonable to suppose that other FinO plasmids may also produce a finP product. If true for the FinO plasmids tested, these finP products are unable to substitute for the FinO finP product, as in no case was the FinO finP mutant inhibited. This extends an earlier observation that the FinP component of the FinO transfer inhibitor is relatively plasmid specific (Finnegan and Willetts, 1972). In contrast, the FinO plasmids defined in this study attest the relatively non-specific nature of the FinO component of the transfer inhibitor; however fairly large quantitative differences were observed.
Table 3.2 Confirmation of transfer inhibition characteristics of JR66a, R485 and JR66a

<table>
<thead>
<tr>
<th>Fin&lt;sup&gt;+&lt;/sup&gt; plasmid</th>
<th>Flac&lt;sup&gt;+&lt;/sup&gt; traQ305</th>
<th>Flac&lt;sup&gt;+&lt;/sup&gt; finP303</th>
<th>Flac&lt;sup&gt;+&lt;/sup&gt; traQ316</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>60</td>
<td>111</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>JR66a</td>
<td>0.005</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R485a</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R455</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

Donor ability and male specific phage sensitivity are expressed and were measured as described in the footnotes to tables 3.1 and 3.3 respectively.

a These results are taken from Finnegan and Willetts (1972) and Willetts and Paranchych (1974).
The Fim* plasmids tested all inhibited Fim* strain to similar extent as wild type Fim*, leaving B20 as the sole plasmid determining what will be referred to as the Fim system of transfer inhibition. However, three plasmids, B20, B40, and B80, each inhibited the transfer of Fim* strain, Fim* B40, and Fim* strain to the same extent as wild type Fim*. Therefore, these plasmids cause fertility inhibition of 7 by neither the Fim* system, nor the Fim* system, and must determine new system(s). In order to confirm these results, second examples of the three mutant plasmids, Fim* muB20, Fim* muB40, and Fim* muB80 were tested and shown to be inhibited by B20a, B40, and B80 (table 2.1). Furthermore, the mutant Fim* plasmids were recovered from the bi-plasmid cells and shown to have retained the mutation originally carried.

A variant of B20, which is designated B40a, is a low efficient inhibitor of F transfer, but otherwise appears identical in its properties to B40. B40a was the original isolate from a B40 carrying strain supplied by Dr. N. Satta. The more efficiently inhibiting B20 was characterized subsequently, and 4 further isolates from the original clone were also efficient inhibitors. Since the original isolate B20 is considered to be atypical.

1.3 Inhibition of Fim* plasmid strain by Fim* plasmid

In addition to measurement of donor ability, the four J53400 derivatives carrying each Fim* plasmid together with Fim*, Fim* muB20, Fim* muB40, or Fim* muB80 were tested for sensitivity, by spot tests, to the 7 specific phages, 11, 12, and 17. Resistance was taken as an indication of the chances
of the 7 pilus.

Results in table 3.1 also show that for the 7

plasmid tested

plasmid formation paralleled the results for donor ability. 7

plasmid derivatives also carrying wild type 7 or 7 and

were resistant to mix specific bacteria whereas those having

carrying 7 or 7 were negative.

Of the three plasmids with transfer inhibition systems set

of the 7 or 7 type, 8388 and R46 (and R46) inhibited

plasmid production by wild type 7, 7 and 7 and 7 again paralleling donor ability estimates.

In contrast R45 did not inhibit plasmid formation nor by wild

type 7 despite its 600 fold reduction in the level of 7 transfer. R45 has been classified as a vector of the 7

incompatibility group (Bridge et al., 1973) to which F strain

belongs. However, although displacement of 7 by an incoming

plasmid was frequent (>50), the reverse transfer (7 into a cell carrying R45) generally resulted a bi-plasmid cell.

This segregated 7 colonies at a low frequency, comparable to

that by cells carrying 7 and the R4 plasmid R100, observed

during these experiments and by Bridge and East (1973). Experiments

with (R45, 7) cells were always initiated from single colonies, and

the forms of bacteria in spot test experiments were always to be

7 or 7, eliminating the possibility that 7 specific

plasmid mutability was due to segregation of 7 7 strain.

Because, with this exception of R45, inhibition of transfer

was always accompanied by inhibition of 7 formation (see

table 3.1 and 3.3), the simple spot test for 7 specific plasm
Footnote to table 3.3

Sensitivity or resistance to the F specific bacteriophages f1, f2 and Q5 was determined by spot tests (see chapter 2) and is indicated by the letters S and R.

Host strains were JC3458 for those plasmids included in table 3.1; *Salmonella typhimurium* for MP10; Nal\(^R\) or Sm\(^R\) derivatives of RC711 for Ent, Hly, Vir and Tra plasmids; and W1607 for To-D1122, H\(_3\)S-D1148 and K86.

a. RC, R136, R192, R444, R538-1, ColR\(_2\) and ColR\(_1\) were also tested and gave this result.

b. Cells carrying this plasmid were grown at 35\(^\circ\)C because Tra-A14 is temperature sensitive (Williams-Smith, personal communication).

c. Cells taken from the lawns on which these results were obtained were >95% Lac\(^+\) Tet\(^R\).

d. Some segregation of Lac\(^-\) clones from these strains was observed and is discussed in the text.
Table 3.3  Inhibition of Flag$^+$ pilus formation by Fin$^+$ plasmids

<table>
<thead>
<tr>
<th>Fin$^+$ Plasmid</th>
<th>tra$^+$</th>
<th>tra0304</th>
<th>finP301</th>
<th>traQ312</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>R100$^a$</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>R63$^c$</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>R485</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>R1</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Co194</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>R124</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>JR66</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R128</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>R389</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>R404</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>R486</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R486$^a$</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>MP10-36</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Ent-H19</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Ent-P3</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Ent-P307$^d$</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Hly-7233</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Vir-85$^d$</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>KB6</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Tra-A14$^b$</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>To-D1122</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>H$_2$-D1148</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>

74.
sensitivity was used to test a further group of Fin+ plasmids. For these four derivatives of the Fin+ plasmid bearing strain were constructed also carrying Flac+ wild type, Flac+ traQ, Flac+ finP or Flac+ traQ. Spot tests were performed with f1, f2 and Q3.

Although Fin+ plasmids were of widely diverse origins and phenotypes, all exhibited a pattern of phage sensitivity characteristics of the FinOP transfer inhibition system (table 3.3). Again none of the FinOP plasmids synthesized a finP component able to replace that of F itself.

None of these plasmids had been assigned to incompatibility groups and, with two exceptions, no significant segregation of Lac- clones from hi-plasmid cells was observed. The enterotoxin plasmid Ent-P307, which has now been assigned to the F1 incompatibility group (Go et al., 1975), showed limited Lac- segregation (15% Lac- clones during the experiment) as did the virulence enhancing factor Vir88 (30% Lac- clones during the experiment). It may be that Vir88 is also a member of the F1 incompatibility group, whereas the other plasmids tested are not. However the possibility that more than one plasmid are present in the hemolysin and enterotoxin producing cells (Williams-Smith and Linggood, 1970) complicates such inferences.

3.4 Discussion

The vast majority of F-like plasmids transfer at a much lower level than that observed for the sex factor F. The existence of a control mechanism inhibiting the full expression of their own
transfer genes is responsible for this; F transfers at a higher frequency because its system for transfer control is defective, in that it lacks finO. F-like, FinOP plasmids inhibit the transfer of F because a component of their transfer control system, FinO, is able to complement the defect in the F transfer control system. The prevalence of the FinOP system for F fertility inhibition amongst F-like plasmids, emphasizes the relatively non-plasmid-specific nature of the FinO component of the transfer inhibitor. The quantitative variations in the extent of transfer inhibition, more fully discussed in chapter 4, shows that a degree of plasmid specificity for finO does however exist.

In contrast a second component FinP, which is produced by wild type F, is markedly plasmid specific. Indeed none of the F-like, FinOP plasmids tested produced a finP product able to complement the Flac+ finP mutant, to cause inhibition of its transfer. For those FinOP plasmids lacking an F-like transfer system, R128 of incompatibility group N, and the otherwise cryptic plasmid MP10 present in many Salmonella strains (Smith et al., 1973), the Fin+ character may not be a by-product of a system for the inhibition of the plasmids own transfer system. Consequently a finP product may not be produced by such plasmids.

The finding of a Fin+ plasmid which does not inhibit F pilus formation, suggested that the initial test for the Fin+ phenotype should involve testing a plasmids effect on the level of F transfer, rather than solely on male specific phage sensitivity. This would avoid overlooking further examples of Fin+ plasmids like R455.
The natures of the fertility inhibition systems produced by R455, JR66a and R485, which are different from both the FinOP system and the FinQ system are further described in chapter 5.
4.1 Introduction

During conjugal transfer of the F sex factor a unique pre-existing single strand of DNA is transferred from the 5' end to the recipient cell. The initiation of this event demands the presence of a site on the F factor known as the origin of transfer ori. An endonuclease presumably generates a single strand nick on the covalently closed plasmid DNA at this site. Transfer replication by a rolling circle mechanism may then begin at the nick to allow single strand transfer to the recipient, whilst maintaining the double stranded molecule in the donor (Willetts, 1972). Plasmid specificity for the origin of transfer has been demonstrated by Reeves and Willetts (1974; see Chapter 1). Hence F-like plasmid transfer involves the recognition of a plasmid specific site. Similarly the positive control product of gene tral exhibits plasmid specificity perhaps recognising a plasmid specific site at the beginning of the transfer operon (see Chapter 1).

For the FinCP plasmids identified in table 3.1, the quantitative donor abilities of the Fin plasmids alone and in the presence of wild type Flac+, Flac+ tral and Flac+ finP was measured. This allows comparison of the extent of transfer inhibition of F with inhibition of the plasmid's own transfer system. Also if the plasmid specific interactions of the FinCP plasmids (see above) show an overlap in specificity with those interactions of F, this will be revealed.
### 4.2 Relative transfer levels of Flac\(^+\) and Fin\(\text{OP}\) plasmids

#### Table 4.1 Transfer of Fin\(\text{OP}\) plasmids in the presence of Flac\(^+\) elements

<table>
<thead>
<tr>
<th>Fin(^+) plasmid</th>
<th>none</th>
<th>Flac(^{+})tra(^+)</th>
<th>Flac(^{+})tra3004</th>
<th>Flac(^{+})fin301</th>
<th>Flac(^+) transfer% in presence of Fin(^+) plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.07</td>
<td>0.08</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>R6</td>
<td>0.2</td>
<td>0.3</td>
<td>0.9</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>R100</td>
<td>0.3</td>
<td>0.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.06</td>
</tr>
<tr>
<td>R136</td>
<td>0.005</td>
<td>0.006</td>
<td>0.3</td>
<td>0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>R192</td>
<td>0.4</td>
<td>0.2</td>
<td>0.9</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>R444</td>
<td>0.1</td>
<td>0.1</td>
<td>0.7</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>R538-1</td>
<td>4.9</td>
<td>2.2</td>
<td>3.8</td>
<td>2.9</td>
<td>0.3</td>
</tr>
<tr>
<td>ColB2</td>
<td>1.3</td>
<td>1.6</td>
<td>86</td>
<td>79</td>
<td>2.3</td>
</tr>
<tr>
<td>ColB4</td>
<td>10</td>
<td>10</td>
<td>31</td>
<td>19</td>
<td>0.3</td>
</tr>
<tr>
<td>ColB1</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.09</td>
<td>0.08</td>
<td>0.3</td>
</tr>
<tr>
<td>R124</td>
<td>0.6</td>
<td>13.3</td>
<td>150</td>
<td>125</td>
<td>9.4</td>
</tr>
<tr>
<td>R128</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.2</td>
</tr>
<tr>
<td>R389</td>
<td>0.07</td>
<td>0.01</td>
<td>0.3</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>R434</td>
<td>0.04</td>
<td>0.09</td>
<td>0.5</td>
<td>0.4</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Matings were performed as described in Chapter 2 using derivatives of JCB455 as donor and JCB273, or its ColV\(R\) derivative when testing ColB factors as recipient. Markers selected are the antibiotic resistances given in Chapter 2 or colicin production. Numbers represent plasmid carrying \([\text{Str}\(R\)\]) progeny per 100 donor cells.

(a) This column is taken from Table 3.1 and shows the extent of transfer inhibition of Flac\(^+\) by the Fin\(\text{OP}\) plasmids. Numbers represent Lac\(^+\) \([\text{Str}\(R\)\]) progeny per 100 donors and are included for ease of comparison.

The results in Table 4.1 show that the transfer level of various Fin\(\text{OP}\) plasmids range from 0.005% for R136 to 10% for ColB4. The extent to which the Fin\(\text{OP}\) plasmids inhibit F transfer also varies (from 0.06 for R136 and R100 to 9.4 for R124). However a particular Fin\(\text{OP}\) plasmid does not always inhibit its own and the F transfer...
system by the "two-enzyme" system. Particularly notable is the
mutant strain R1134, which inhibits its own transfer more efficiently, and
and 6310, a better inhibitor of 7 transfer. These different degrees of
inhibition may reflect a mild amount of plasmid specificity for
the 7 transfer product. Although the R1134 products, unlike the R1134
products, are active in the transfer system of 7-like plasmids,
the efficiency with which they act varies. These may arise from slight
variation in the efficiency of transfer inhibition may be caused
and: the interaction between the R1134 transfer inhibitor and its
site of action (turn); the unidentified interaction between the
R1134 product and the R1134 products of 7 and the R1134 plasmid; and
the positive control of the transfer operon by 7?A4, where some
plasmids may produce greater amounts of their R1134 product than others,
for efficient expression of the transfer operon.

In most cases the R1134 plasmid transfer level is unaffected by
the presence of wild type 7?A4. The presence of 7?A4 alone or
R1134 R1134 causes a small increase in R1134 plasmid transfer. This
increase reflects the fact that the transfer system of 7?A4 alone
and R1134 R1134 is fully active in the presence of the R1134 plasmid
and it is likely that the R1134 plasmid can transfer at a low frequency
by inefficient utilization of the 7 transfer system. This might
occur by recombination with the 7?A4 plasmid, or by new overlap in
the specificity of the 7 and/or R1134 products.

R122-1 and R128 did not show this characteristic increase in
donor ability when 7?A4 alone or 7?A4 R1134 was present. R122-1
already transfers at a fairly high level alone, so that
utilization of the 7 transfer system may not cause a measurable
increase. R128 is a plasmid of incompatibility group I, and its
7?A4.
transfer system may be distinct from that of the F-like plasmids precluding interaction with the F transfer system. Indeed, it may be that the finO product of R128 is not concerned with regulation of its own transfer. Alternatively or in addition, the homology between R128 and F may be insufficient to allow significant recombination to occur.

4.3 Transfer levels of ColB2 and R124

Table 4.2 Complementation of F lac⁺ traI by ColB2 fdr and R124 der-2

<table>
<thead>
<tr>
<th>Fin⁺ plasmid</th>
<th>Transfer of Fin⁺ plasmid</th>
<th>Transfer of F lac⁺ traI65</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>0.0001</td>
</tr>
<tr>
<td>ColB2 fdr</td>
<td>160</td>
<td>77</td>
</tr>
<tr>
<td>R124 der-2</td>
<td>75</td>
<td>63</td>
</tr>
</tbody>
</table>

Figures represent the number of progeny cells carrying the plasmid indicated per 100 donor cells. The donor strain was HD2149 carrying the plasmids indicated, and the recipient strain was HD2194, MalR being contraselected.

In contrast, ColB2 and R124 always transferred at the same level as F lac⁺, whether this was wild type, traO or finP. In the latter two cases these FinOP plasmids had donor ability levels of approximately 100%.

These results for R124 and ColB2 can be explained if their origin of transfer sequences are closely related to that of F. This would predict that the traI products of these plasmids and F have similar specificities (Reeves and Willette, 1974). Support for such an explanation has been obtained by demonstrating the ability of ColB2 fdr and R124 der-2 to complement an F lac⁺ traI mutant.
The ColE2 fdp and R124 dgd-2 plasmids transfer at high levels because they are finP mutants (see below). Despite the fact that they produce finO products, the Flac+ traI mutant is transferred at high level. This is because the Flac+ traI mutant is using the transfer system of ColE2 or R124 dgd-2 to achieve a high transfer level, regardless of inhibition of the F transfer system caused by the finO products (see below).

Therefore, because of related origin sequences, the fully active transfer system of Flac+ traO (or finP) is able to recognise and transfer R124 and ColE2 at high levels (table 4.1), even though transfer systems of the FinO plasmids are inhibited. Although the traI products of R124 and ColE2 have a similar specificity to that of F, a further overlap in specificity, for the traI products, may occur. This would cause the observed results (table 4.1) because the traI product of Flac+ traO (or finP) would activate the transfer operon of ColE2 or R124, to circumvent the absence of positive control for R124 and ColE2 during inhibition. Indeed, it has been established that the traI products of F and R124 are interchangeable (N. Willetts, unpublished data; N. Maynell unpublished data).

It was observed that the growth rate of cells carrying Flac+ traO (or finP) and ColE2 was markedly lower than that of equivalent strains carrying Flac+ wild type (or traO) and ColE2. A possible explanation is that overlap in specificity of traI products imposes a detrimental diversion of cellular energy into the production of two sets of transfer products in the former case.

By taking account of the specificity of the finP and traI (or traI) products of R124, ColE2 and F the unexplained properties of the derepressed mutants, R124 dgd-2 (Maynell and Lawn, 1973) and
ColB2 fdr (Hausmann and Cloves, 1971) can be clarified. In both instances the mutants appear to be $\text{fin}O^{+}$ by some criteria, but fail to inhibit $\text{Frac}^{+}$ transfer, suggesting that they are $\text{fin}O^{-}$.

Also R124 drd-2 transfer is inhibited by ColB2 but not by ColB4, further complicating interpretation.

The basis for understanding these results is that both mutants are $\text{fin}O^{+} \text{fin}P^{-}$, and that whereas the $\text{traJ}$ products (and transfer origins) of F, ColB2 and R124 are interchangeable, the $\text{fiaP}$ product of F differs from those of R124 and ColB2, which themselves have a similar specificity. As a result:

(i) F is transferred at a high frequency in the presence of ColB3 fdr (Hausmann and Cloves, 1971), and R124 drd-2 (Meynell and Lawn, 1973) because, although expression of the F transfer system is inhibited, the ColB2 fdr and R124 drd-2 transfer system can be efficiently used for F DNA transfer.

(ii) Cells carrying R124 drd-2 and $\text{Frac}^{+}$ produce both type II pili (characteristic of R124) and type I pili (characteristic of F; E. Meynell, unpublished). This is because the $\text{traJ}$ products of F and R124 are interchangeable (N. Willetts, unpublished). Positive control by the $\text{traJ}$ product of R124 drd-2 allows expression of the $\text{Frac}^{+}$ transfer operon. This also accounts for the failure of R124 drd-2 to inhibit $\text{Frac}^{+}$ transfer.

(iii) Transfer of $\text{fin}O^{-}$ mutant R factors is inhibited by R124 drd-2 (Meynell and Lawn, 1973). This is because R124 is $\text{fin}O^{+}$, and the $\text{fin}O^{-}$ mutant R factors require $\text{traJ}$ and $\text{traJ}$ products of different specificity, so preventing their utilization of the R124 drd-2 transfer system.
(iv) ColB2 fdr transfer is not inhibited by R100 (Hausmann and Clowes, 1971), or by the F1 group plasmid R388 (Gassen and Willetts, 1973), but is inhibited by R124 (Gassen and Willetts, 1973). This is because the fdp products R124 and ColB2 have the same specificity, which is different from that of R100 and of R388 (Willetts, unpublished data).

(v) R134 dfr transfer is not inhibited by ColB4 or R100, but is inhibited by ColB2 (Meynell and Lawn, 1973). This is because the fdp products of R124 and ColB2 have the same specificity, which is different from that of R100 and of ColB4 (Willetts, unpublished).
CHAPTER 5  FURTHER CHARACTERISATION OF TRANSFER INHIBITION

BY JR66a, R485 and R455

5.1 Introduction

Three plasmids, JR66a, R485 and R455, were shown in chapter 3 to have transfer inhibition systems different from both those of PlacGP plasmids and of R62 (FinQ).

R455 inhibited transfer, but not pilus formation by Plac+, whereas JR66a and R485 inhibited both properties. The nature of transfer inhibition by these plasmids was further studied by testing their abilities to inhibit surface exclusion by Plac+, and transfer of J-independent mutants of Plac+.

Surface exclusion was of interest because the gene(s) responsible for the property (traS), although part of the transfer operon, is not required for transfer per se (Willetts, 1974; Willetts and Achtman, personal communication). Inhibition of this property, as has been observed during fertility inhibition by R100 and R32, provides evidence that the mechanism of inhibitor action involves prevention of expression of all, or part of, the transfer operon (Willetts and Flynn, 1970; Willetts and Paranchych, 1974).

Expression of the transfer operon may be inhibited directly, or indirectly by elimination of positive control, the function of gene traJ. One way of deciding which alternative is correct is to use the Plac+ mutations called "J-independent" by Achtman (1973). These were isolated as Tra+ "revertants" of Plac+ traJ80, which
(footnote to table 8.1)

The figures are surface exclusion indices, defined as the ratio of the number of His\(^+\) recombinants obtained with \(F^-\) strain JC3272, to the numbers obtained with the JC3272 derivative, carrying the plasmid(s) indicated, in crosses with Hfr KL66.

(a) Results for R100 and R62 carrying strains are taken from Willetts and Paranchych (1974)

(b) R485a is a variant of R485 which inhibits F\(^{\text{flag}}\) transfer to a lesser extent (see chapter 3).
retained the original amber traJ00 mutation, but gained second
mutations permitting a low level of expression of the transfer
operon in the absence of positive control (Achtman, 1973). R100
did not inhibit transfer of J-independent \textit{flag}^+ mutants, whereas
R62 did, and these properties suggested that R100 causes indirect
inhibition of transfer operon expression through the positive control
gene \textit{traJ}, while R62 acts directly (Willette and Paranichych, 1974).
Such interpretations are supported by independent genetic evidence

3.2 Surface exclusion

Surface exclusion indices were determined for derivatives of
JC3272 carrying wild type \textit{flag}^+, \textit{flag}^+ \textit{traJ}, \textit{flag}^+ \textit{finP} or \textit{flag}^+ \textit{traJ},
together with one of the \textit{fin}^+ plasmids JR66a, R456 and R466. Results
are given in table 6.1 which, for comparison, includes the surface
exclusion indices of equivalent strains carrying R100 and R62 taken
from Willette and Paranichych (1974).

<table>
<thead>
<tr>
<th>Table 6.1</th>
<th>Inhibition of \textit{flag}^+ surface exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{fin}^+ plasmid</td>
<td>\textit{flag}^+ element</td>
</tr>
<tr>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>R100^a</td>
<td>2.6</td>
</tr>
<tr>
<td>R62^a</td>
<td>1.2</td>
</tr>
<tr>
<td>R456</td>
<td>13</td>
</tr>
<tr>
<td>JR66a</td>
<td>0.9</td>
</tr>
<tr>
<td>R466</td>
<td>0.9</td>
</tr>
<tr>
<td>R466 \textit{a}</td>
<td>0.3</td>
</tr>
</tbody>
</table>

86.
JR66a inhibited surface exclusion by Flac\(^+\), whereas R485 and R485 did not. As was found for donor ability and pilus formation (chapter 3) the presence of mutations traQ, finP or traQ had no effect on the surface exclusion index.

5.3 J-independent mutations

The J-independent Flac\(^+\) mutants JCFL119 and JCFL130 were used. In a su\(^-\) strain the traJ30 mutation carried by these plasmids is expressed and, as a result, JCFL119 and JCFL130 transfer at a low level, possible because of the presence of the J-independent mutations. This low level of transfer is not inhibited by R100 but is inhibited by R52 (Willett and Paranchych, 1974; table 5.2). JR66a, R485, and R485 inhibited transfer of both JCFL119 and JCFL130 from a su\(^-\) donor strain (table 5.2).

<table>
<thead>
<tr>
<th>Fin(^+) plasmid</th>
<th>Flac(^+) element</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JCFL119</td>
</tr>
<tr>
<td>none</td>
<td>1.3</td>
</tr>
<tr>
<td>R100(^a)</td>
<td>1.6</td>
</tr>
<tr>
<td>R52(^a)</td>
<td>0.038</td>
</tr>
<tr>
<td>R485</td>
<td>0.0004</td>
</tr>
<tr>
<td>JR66a</td>
<td>0.003</td>
</tr>
<tr>
<td>R485</td>
<td>&lt;0.00006</td>
</tr>
<tr>
<td>R485(^a)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Derivatives of JC6256 carrying either of the two J independent Flac\(^+\) elements JCFL119 or JCFL130, together with the Fin\(^+\) plasmid, were used as donors in crosses with ED2197. The figures represent the number of Lac\(^+\) [Mal\(^R\)] progeny per 100 donor cells.

(a) results for R100 and R52 carrying strains were taken from Willett and Paranchych (1974).
Donor ability and male specific phage sensitivity were measured as described in tables 3.1 and 3.2, respectively. Donor strains were derivatives of JC5455 and the recipient strain was JC3272, contraselecting [StrR] progeny, except where recovered R455 elements were tested. In these instances (R455 ex in the table) JC3272 was the donor strain, ED2194 was the recipient strain and [NalR] was contraselected.
This suggests that all three Fin$^+$ plasmids, JR36a, R436 and R455, act directly to inhibit F transfer. If their mechanisms of action were by inhibiting traJ, the transfer of JCFL119 and JCFL130, (allowed by their J-independent mutations) would not be inhibited, because by definition this transfer does not require the traJ product. Hence, in common with the FinQ system, but unlike the FinO system, the systems of transfer inhibition encoded by JR36a, R436 and R455 do not prevent expression of traJ. Indeed, since R436 and R455 did not inhibit surface exclusion and, in the latter case, pilus formation, this result was expected, because inhibition of traJ would indirectly prevent expression of the entire transfer operon (to eliminate transfer, pilus formation and surface exclusion by F).

5.4 Transfer inhibition by R455

<table>
<thead>
<tr>
<th>plasmid in donor</th>
<th>Donor ability selected plasmid transfer frequency</th>
<th>male specific phage sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flac$^+$ Flac$^+$; R455</td>
<td>Flac$^+$ Flac$^+$</td>
<td>138</td>
</tr>
<tr>
<td>R1-19; R455</td>
<td>R1-19 R1-19</td>
<td>95 S</td>
</tr>
<tr>
<td>R455</td>
<td>R455 R455</td>
<td>0.002 S</td>
</tr>
<tr>
<td>ex(R1-19,R455) Flac$^+$; R455 ex(R1-19,R455)</td>
<td>Flac$^+$ Flac$^+$</td>
<td>0.6 S</td>
</tr>
<tr>
<td>R1-19</td>
<td>R1-19 R1-19</td>
<td>99 S</td>
</tr>
<tr>
<td>R455</td>
<td>R455 R455</td>
<td>0.0006 R</td>
</tr>
<tr>
<td>ex(R1-19,R455) Flac$^+$; R455 ex(R1-19,R455)</td>
<td>Flac$^+$ Flac$^+$</td>
<td>0.4 S</td>
</tr>
</tbody>
</table>
Segregation of Flac\(^+\) from cells carrying R455 and Flac\(^+\) occurs because R455 and Flac\(^+\) both belong to incompatibility group F\(_I\).

Although during experiments using bi-plasmid cells of this type, segregation occurred at an insignificantly low frequency, it was desirable to study R455 inhibition in a completely stable situation.

The effect of R455 on the transfer of two high level transferring mutants of the F\(_II\) group plasmid R1 was tested. R1 carries genes for kanamycin resistance but not for tetracycline resistance, whereas the converse is true of R455. Hence, tetracycline and kanamycin were used to select R455 and R1, respectively.

The R1 mutants used were R1-19, a finO mutant susceptible to FinOP transfer inhibition, and R1-16, an \(x^\circ\) (equivalent to \(tra^0\) of Flac\(^+\)) or possibly \(fin^+\) (E. Raumberg, personal communication) mutant which is normally insensitive to the \(finO\) product of other FinOP plasmids.

Surprisingly, R455 did not inhibit transfer or pilus formation by either R1-16 or R1-19 (table 5.3). Furthermore R455 was separated from the R1 mutants used in this experiment and shown to be repressed for its own transfer and pilus formation, and still able to inhibit Flac\(^+\) transfer but not pilus formation (table 5.3).

Hence, there is no evidence for FinOP transfer inhibition by R455, despite the plasmid being F-like and inhibiting its own transfer and pilus formation. Furthermore the inhibition of Flac\(^+\) transfer (but not pilus formation) by R455 seems to be plasmid specific since all F-like plasmids are not susceptible to it.

5.5 Discussion

The properties of the transfer inhibition systems, FinOP and FinQ, together with those specified by JR66a, R485 and R455 are
ized in table 5.4. Since the properties of R485 and R455 are unique, and JR66a has been shown to inhibit $\text{Flag}^+$ by a system distinct from FinOP and FinQ, three different mechanisms of fertility inhibition have been demonstrated. These will be referred to as, FinU, FinV and FinW, for the systems specified by, JR66a, R485 and R455, respectively.

Table 5.4 Summary of the characteristics of the transfer inhibition systems

<table>
<thead>
<tr>
<th>plasmid</th>
<th>incompatibility group</th>
<th>inhibition system</th>
<th>pilus formation</th>
<th>inhibition of surface exclusion</th>
<th>J-independent $\text{Flag}^+$ transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>R100</td>
<td>$\text{F}_{1\text{II}}$</td>
<td>FinOP</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>R128</td>
<td>$\text{N}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R389, R404</td>
<td>$\text{P}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R62</td>
<td>$\text{I}_{\text{A}}$</td>
<td>FinQ</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>JR66a</td>
<td>$\text{I}_{\text{A}}^b$</td>
<td>FinU</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>R485</td>
<td>$\text{X}$</td>
<td>FinV</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>R455</td>
<td>$\text{F}_{1}$</td>
<td>FinW</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

(a) all those $\text{F}_{1\text{II}}$, $\text{F}_{1\text{III}}$ and $\text{F}_{1\text{IV}}$ group plasmids and those of as yet undetermined incompatibility groups tested in chapter 3, also specified transfer inhibition systems of the FinOP type.

(b) R389 and R404 are probably composite R factors, the Fin$^+$ character being carried by a plasmid of unidentified incompatibility group (Datta and Hedges, 1972; N. Datta personal communication).

JR66a belongs to incompatibility group $\text{I}_{\text{A}}^b$ which includes the group $\text{I}_{\text{A}}$ to which JRCCa was originally assigned (N. Datta, personal communication).

Conclusions drawn from the properties of the five inhibition systems, and the present understanding of their modes of action can be summarized as follows:

90.
(a) FinCP plasmids, such as R100, prevent the expression of the positive control gene $traJ$ and, in consequence, indirectly inhibit expression of the transfer operon (Finnegan & Willetts, 1973; Willetts, 1974).

(b) The FinQ system of R62 inhibits all properties encoded by the transfer operon, but does not act via $traJ$. It probably directly prevents expression of all or part of the transfer operon (see chapter 7).

(c) The FinU system of JR66a has similar properties to those of the FinQ system. It probably prevents expression of all or part of the transfer operon directly, but its site of action is different from that of the FinQ plasmid R62.

(d) The FinV system of R485 inhibits transfer and pilus formation by F but does not affect surface exclusion. Since such a phenotype would not be produced by inhibition of $traJ$, it must act directly, to inhibit one or more genes required for pilus formation.

The gene(s) for surface exclusion $traS$ are transcribed in the transfer operon distal to the last essential gene for pilus formation, $traD$ (some $traD$ mutants retain a functional pilus). Hence, R485 inhibition of transfer and pilus formation does not cause a polar effect on $traS$, making it unlikely that transcription of pilus forming genes is prevented. Perhaps the most plausible explanation for FinV transfer inhibition is prevention of the function of a gene product(s) required for pilus formation.

(e) Transfer inhibition by the $F_1$ group plasmid R485 is particularly interesting because, although R485 is a repressed $F$-like plasmid, no evidence of a FinCP transfer inhibitor was found. It is possible that inhibition of the R485 transfer system involves a plasmid specific $finQ$ product. However, it is not clear whether
FinW fertility inhibition of Flac+ is in any way connected with inhibition of the R456 transfer system. Derepressed mutants of R456 would clarify this point.

This apart, the mechanism of FinW inhibition of F clearly does not affect the whole transfer operon, since only transfer of Flac+ and not pilus formation or surface exclusion is prevented. Perhaps expression of a transfer gene not required for pilus formation is inhibited. A likely candidate would be traJ, which is thought to encode the endonuclease responsible for producing a single stranded nick at the origin of transfer. Alternatively, this transfer origin could be blocked by the FinW fertility inhibition system. Indeed, the plasmid specificity of this part of the transfer system (chapter 4) might explain the plasmid specificity of FinW inhibition.
CHAPTER 6  
FLAG+ MUTANTS INSENSITIVE TO FERTILITY  

INHIBITION BY JR66a AND R483

6.1 Introduction

Mutations of Flag+, insensitive to transfer inhibition by R483 or JR66a were selected. These mutations were of relevance for two reasons. Firstly they could be used to confirm the distinction of the transfer inhibition systems of these plasmids both from each other and from the other three systems, FinQ, FinQ and FinW, that have been defined (chapter 6). Secondly they could form, together with Flag+ traO, Flag+ fin and Flag+ traO mutants, the basis for a simple means of classifying further Fin+ plasmids by using sale specific phase spot tests.

6.2 Selection procedure

A two stage selection for inhibitor insensitive mutants was used. Flag+ was mutagenized and transferred from a donor strain carrying the Fin+ plasmid (JR66a or R483) to an intermediate recipient also carrying the Fin+ plasmid, before being re-transferred to the final recipient.

A culture of JCF256 (T6 G StrS) carrying JCFLO and either JR66a or R483 was mutagenized, using EMS or NTG. After overnight growth to allow mutant expression and segregation, the culture was diluted into L-broth and re-grown to exponential phase. This donor was mated for 40 minutes with a stationary phase intermediate recipient JCS455 (T6 R StrS) carrying JR66a or R483 (0.1 ml + 0.1 ml), and mating interrupted by the addition of U.V. irradiated T6 (concentrated to
approximately $10^{11}$ pfu/ml, 0.2ml). After incubation for 20 minutes, L broth (0.6ml) was added and incubation continued for 40 minutes to allow expression of the incoming plasmid's transfer genes. An equal volume (1.0ml) of the exponential phase final recipient JC3272 ($T^R_g S_{tr}^R$) carrying JR66a or R486 was added, and a 40 minute period of mating allowed. The mating mixture was plated to detect transfer of $\text{Flac}^+$ to JC3272 by selecting Lac$^+$ [Str$^R$] progeny. These colonies were patched and screened for clones transferring lac efficiently to ED24, selecting Lac$^+$ [His$^+$ Trp$^+$ Lys$^+$ Spc$^R$] in a plate mating. Alternatively, or in addition, clones sensitive to $F$ specific phages were sought by use of the Giomaa2 replica plating technique.

Clones transferring $\text{Flac}^+$ at a high level and sensitive to $F$ specific phages were purified, and the $\text{Flac}^+$ elements separated from JR66a or R486 by transfer to JC5455 selecting Lac$^+$ [Spc$^R$] progeny. The relevant $\text{Fin}^+$ plasmid was then re-introduced to JC5455 carrying the mutant $\text{Flac}^+$ element. The donor ability and male specific phage sensitivity of these JC5455 derivatives were determined (tables 6.1 and 6.2).

6.3 Mutants insensitive to fertility inhibition by JR66a

Mutants obtained by selection for insensitivity to JR66a inhibition had two unexpected and somewhat undesirable properties. Firstly, none of the mutants obtained was completely insensitive to FinU transfer inhibition, although susceptibility was convincingly reduced. Secondly, the transfer level of many of the mutant $\text{Flac}^+$ elements, even in the absence of JR66a was considerably less than 94.
Table 6.1  *Flac*<sup>+</sup> mutants insensitive to JR66s fertility inhibition

<table>
<thead>
<tr>
<th>Plasmid number</th>
<th>donor ability&lt;sup&gt;a&lt;/sup&gt;</th>
<th>male specific phage&lt;sup&gt;b&lt;/sup&gt; sensitivity</th>
<th>JC5458</th>
<th>JR66a/JC5458</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCFL0</td>
<td>133</td>
<td>0.02</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>EDFL250</td>
<td>37</td>
<td>4.1</td>
<td>S</td>
<td>SR</td>
</tr>
<tr>
<td>EDFL251</td>
<td>108</td>
<td>4.1</td>
<td>S</td>
<td>SR</td>
</tr>
<tr>
<td>EDFL252</td>
<td>68</td>
<td>5.7</td>
<td>S</td>
<td>SR</td>
</tr>
<tr>
<td>EDFL253</td>
<td>66</td>
<td>6.7</td>
<td>S</td>
<td>SR</td>
</tr>
<tr>
<td>EDFL254</td>
<td>8.4</td>
<td>2.7</td>
<td>SR</td>
<td>SRR</td>
</tr>
<tr>
<td>EDFL255</td>
<td>6.0</td>
<td>0.6</td>
<td>SR</td>
<td>SRR</td>
</tr>
<tr>
<td>EDFL256</td>
<td>8.0</td>
<td>1.2</td>
<td>SR</td>
<td>SRR</td>
</tr>
<tr>
<td>EDFL257</td>
<td>12.0</td>
<td>3.0</td>
<td>SR</td>
<td>SRR</td>
</tr>
</tbody>
</table>

a. The number of *Lac*<sup>+</sup> [Str<sup>R</sup>] progeny per 100 donors is given using strain JC9272 as recipient.

b. Sensitivity to the F specific bacteriophages f1, f2 and Qφ was determined by spot tests. The letters given indicate the degree of lysis observed as follows:-

- **S** clear lysis
- **SR** turbid lysis
- **SRR** extremely turbid lysis
- **R** no lysis

95.
that of wild type \textit{Flac}$. As can be seen from the results in table 6.1 the mutants fell into two groups. One, including \textit{EDFL230}, showed a small reduction in mutant transfer level compared to \textit{JCEF10}, whereas the other, including \textit{EDFL220}, showed a greater reduction.

Male specific phage sensitivity (table 6.1) paralleled these results for transfer level. Where donor ability was high cells were fully sensitive to $f_1$, $f_2$ and $Q_1$ giving clear areas of lysis in the spot test. However when donor ability was reduced, phage sensitivity was also reduced, so that areas of lysis in the spot test showed equivalent turbidity.

The mutants are probably in the site of action of the \textit{FinU} fertility inhibitor, and will be referred to as \textit{traU} mutants.

6.4 Mutants insensitive to fertility inhibition by \textit{R485}

Mutants of \textit{Flac} insensitive to the \textit{FinV} fertility inhibition system of \textit{R485} were obtained as described above. As with the \textit{FinU} insensitive mutants, the transfer level of these mutants was reduced compared to wild type \textit{Flac}, giving donor abilities of approximately 10% (table 6.2). Also the mutants were only poorly sensitive to $F$ specific phages, resulting in extremely turbid areas of lysis in the spot test (table 6.2).

However \textit{R485} did not further reduce transfer level or phage sensitivity of the mutants. Indeed, the presence of the \textit{FinV} fertility inhibition system slightly enhanced both the donor abilities and the $F$ specific phage sensitivities of the mutants (table 6.2).
These mutations probably represent the site of FinV action, and will be referred to as \textit{trav} mutations.

Table 6.2 \textit{Plac} $^+$ mutants insensitive to R485 fertility inhibition

<table>
<thead>
<tr>
<th>plasmid number</th>
<th>donor ability $^a$</th>
<th>male specific phage $^b$ sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JC5455</td>
<td>R485/JC5455</td>
</tr>
<tr>
<td>JCFL0</td>
<td>135</td>
<td>0.002</td>
</tr>
<tr>
<td>EDF1269</td>
<td>9.8</td>
<td>15.9</td>
</tr>
<tr>
<td>EDF1260</td>
<td>7.3</td>
<td>12.4</td>
</tr>
<tr>
<td>EDF1261</td>
<td>5.3</td>
<td>8.9</td>
</tr>
</tbody>
</table>

\textit{a.} The number of \textit{Lac} $^+$ [\textit{Str} $^R$] progeny per 100 donors is given using strain JC3272 as recipient.

\textit{b.} Sensitivity to the F specific bacteriophages f1, f3 and Q3 was determined by spot tests. The letters given indicate the degree of lysis observed as follows:

- \textit{S} clear lysis
- \textit{SR} turbid lysis
- \textit{SRR} extremely turbid lysis
- \textit{R} no lysis

97.
6.8 Specificity of the fertility inhibition systems

If, as has been suggested in chapter 5, the fertility inhibition systems of R485 and JR66a are distinct from each other, and from the other systems discussed, then the two classes of $\text{Flag}^+$ mutant described above should be susceptible to fertility inhibition by all other $\text{Fin}^+$ plasmids so far tested. This prediction was confirmed by measuring the donor abilities and male specific phage sensitivities of mutant $\text{Flag}^+$ derivatives also carrying $\text{FinQ}$, $\text{FinQ}$, $\text{FinU}$, $\text{FinV}$ or $\text{FinW}$ plasmids.

Transfer of the $\text{traU}$ mutants EDF1250 and EDF1256 was inhibited by R100, R62, R465 and R456 (table 6.3). Male specific phage sensitivity was also inhibited by all these plasmids except for R456. The $\text{FinW}$ transfer inhibition system of R456 did not prevent pilus formation by wild type $\text{Flag}^+$ (chapter 3).

Similarly transfer and pilus formation by the $\text{traV}$ mutant EDF1259 was inhibited by R100, R62 and JR66a, whilst R456 again prevented transfer but not pilus formation (tables 6.3 and 6.4).

R62 had a more pronounced effect on the $\text{traV}$ mutant than on either $\text{traU}$ mutant. In the case of EDF1250, male specific phage sensitivity was not completely inhibited by R62. It is of relevance that the R62 transfer inhibition system causes a relatively small inhibition even of wild type $\text{Flag}^+$ donor ability (chapter 3).

98.
### Table 6.3 Transfer inhibition of EDFL250, EDFL256 and EDFL259 by Fin<sup>+</sup> plasmids

<table>
<thead>
<tr>
<th>Fin&lt;sup&gt;+&lt;/sup&gt; plasmid</th>
<th>Fin system</th>
<th>EDFL250</th>
<th>EDFL256</th>
<th>EDFL259</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>-</td>
<td>37</td>
<td>4.6</td>
<td>9.8</td>
</tr>
<tr>
<td>JR66a</td>
<td>FinU</td>
<td>4.1</td>
<td>0.8</td>
<td>0.002</td>
</tr>
<tr>
<td>R485</td>
<td>FinV</td>
<td>0.0006</td>
<td>0.0002</td>
<td>15.9</td>
</tr>
<tr>
<td>R485a</td>
<td>FinV</td>
<td>0.06</td>
<td>0.006</td>
<td>16.2</td>
</tr>
<tr>
<td>R62</td>
<td>FinQ</td>
<td>0.5</td>
<td>0.05</td>
<td>0.004</td>
</tr>
<tr>
<td>R100</td>
<td>FinOP</td>
<td>0.06</td>
<td>0.007</td>
<td>0.008</td>
</tr>
<tr>
<td>R455</td>
<td>FinW</td>
<td>0.1</td>
<td>0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

The number of Lac<sup>+</sup> [Str<sup>R</sup>] progeny per 100 donors is given. Donor strains were JC5455, containing the plasmids indicated, and the recipient was JC3272 or its Coli<sup>R</sup> derivative ED26 when the donor strain contained R62 (Coli<sup>+</sup>).
Table 6.4 Male specific phage sensitivity of strains carrying a Fin⁺ plasmid and a FinU or FinV insensitive Flac⁺ mutant

<table>
<thead>
<tr>
<th>Fin⁺ plasmid</th>
<th>Fin system</th>
<th>mutant plasmid</th>
<th>EDF1260 (traU)</th>
<th>EDF1286 (traU)</th>
<th>EDF1260 (traV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n576</td>
<td>-</td>
<td>S</td>
<td>SR</td>
<td>SRR</td>
<td>SRR</td>
</tr>
<tr>
<td>JR66a</td>
<td>FinU</td>
<td>S</td>
<td>RR</td>
<td>SRR</td>
<td>R</td>
</tr>
<tr>
<td>R485</td>
<td>FinV</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>SRR</td>
</tr>
<tr>
<td>R485a</td>
<td>FinV</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>SRR</td>
</tr>
<tr>
<td>R32</td>
<td>FinQ</td>
<td>SRR</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>R100</td>
<td>FinQP</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R455</td>
<td>FinW</td>
<td>S</td>
<td>SR</td>
<td>S</td>
<td>SRR</td>
</tr>
</tbody>
</table>

Sensitivity to the F specific bacteriophages f1, f2 and Q3 was determined by spot tests. The letters given indicate the degree of lysis observed:

- S  clear lysis
- SR turbid lysis
- SRR extremely turbid lysis
- R  no lysis
0.6 Discussion

The mutants obtained by selecting for insensitivity to the transfer inhibitors of JR66a or R485 were defective in their transfer when alone. This is probably because mutation of the site of action of these transfer inhibitors impairs the expression or function of part of the F transfer system. The inability to obtain Flac+ mutants completely insensitive to FinU transfer inhibition by JR66a, may be because complete inactivation of the FinU inhibitor's site of action causes a transfer defective mutation.

These unexpected properties of the mutants obtained preclude the routine use of Flac+ mutants and simple F specific phage spot tests to assign further Fin+ plasmids to Fin groups. However, the traU and traV mutants have been used to confirm the difference of FinU and FinV inhibition from each other, and from the FinOP, FinQ and FinW systems of transfer inhibition.
TRAINER INHIBITOR

7.1 Introduction

The R62 transfer inhibitor prevents transfer, pilus formation and surface exclusion by Flac+ and because it inhibits transfer of J independent mutants of Flac+ (Willetta and Paranchych, 1974), probably directly prevents expression of all or part of the transfer operon.

Further understanding of how this inhibition is produced can be gained by a genetic assay for the presence of particular transfer gene products during R62 fertility inhibition. The technique requires construction of a series of Flac+ double mutants each carrying an amber suppressible mutation in a transfer gene together with an inhibitor insensitive mutation. Measuring the retransfer frequency of such an Flac+ element from a temporary heterozygote, also carrying R62 and wild type Fhis+ indicates the presence or absence of the gene product for which the Flac+ double mutant is transfer defective. By measuring retransfer frequencies of a series of Flac+ double mutants defective in each transfer gene, those genes which are directly inhibited can be determined. The method has been successfully used to define FisJ as the only transfer gene directly inhibited during FinOP inhibition of F by R100 (Finnegan and Willetta, 1973).

7.2 Construction of Fhis+ trqG12

In order to construct the double mutants of Flac+ needed for the genetic assay, an Fhis+ trqQ mutant was required. This was
obtained by recombination between wild type \textit{Fhis}^+ and the
\textit{Flac}^+ trac312 element WPPL39.

A 60 minute mating between an exponential phase culture of
JC6255 \( (\text{Str}^S \text{Spo}^S \text{T}_{6}^S) \) carrying \textit{Fhis}^+ and stationary phase
JC5455 \( (\text{Str}^S \text{Spo}^R \text{T}_{6}^R) \) carrying R62 and WPPL39 \( (0.1\text{ml} + 0.1\text{ml}) \) was
interrupted with UV irradiated \( T_{6} \) \( (0.2\text{ml}) \). After 30 minutes
incubation, 0.01L L broth was added and a further 60 minute
incubation allowed for recombination and segregation of recombin-
ants. The mating mixture was diluted x 10 into N9 minimal
medium selective for \textit{Fhis}^+ derivatives of JC5455 also carrying
R62. After overnight growth this culture was diluted x 10 into
L broth and regrown to exponential phase, before mating for 60
minutes with exponential phase JC3372 \( (\text{carrying R62} (1.0\text{ml} + 1.0\text{ml}) \).
The mating mixture was plated for \textit{Fhis}^+ derivatives of JC3372 \( (\text{carrying R62} \) and such \textit{His}^+\textit{[Str}^R\textit{]} colonies were screened for male
specific bacteriophage sensitivity, using the Giemsa-\mu3 replica
plating technique. Sensitive clones were putative \textit{Fhis}^+ \textit{trac7}
recombinants. A derivative \textit{Fhis}^+ \textit{trac312} element was isolated, and
its donor ability alone, and in the presence of R62 (51% and 73%
respectively), confirmed its insensitivity to R62 fertility
inhibition.

7.3 Construction of \textit{Flac}^+ traX traQ double mutants

The double mutants were constructed by recombination between
the \textit{Fhis}^+ \textit{tracQ} element and each of a series of \textit{Flac}^+ elements
carrying amber suppressible mutations in the transfer genes.
When referred to collectively these suppressible mutations will
be denoted \textit{traX}.
Exponential cultures of JC6355 (Su\(^+\), T\(_6\)\(^G\), Spc\(^R\), Str\(^R\)) carrying Flac\(^+\) traQ elements were mated for 60 minutes with stationary phase cultures of ED1799 (Su\(^+\), T\(_6\)\(^R\), Spc\(^R\), Str\(^R\)) carrying Flia\(^+\) traQ and R62 (0.1ml + 0.1ml). Mating was interrupted with concentrated (to 10\(^{11}\) pfu/ml), U.V. irradiated T\(_6\) (0.2ml) and after 20 minutes incubation, L broth (0.6ml) was added and a further 60 minutes incubation allowed. The mating mixtures were diluted 10 fold into liquid M9 minimal medium (2.0ml) selective for Lac\(^+\) [Spc\(^R\)] progeny (supplements were lactose, histidine and spectinomycin). After shaking overnight at 37\(^\circ\), the cultures were diluted into L broth and regrown to exponential phase. They were then used as donors in a mating with exponential phase ED1686 (Su\(^+\), Str\(^R\), Thy\(^-\)) carrying R62 (1.0ml + 1.0ml) and Lac\(^+\) [Str\(^R\)] progeny were selected.

The colonies obtained were patched on minimal plates and used in a plate mating with JC5455 (Su\(^-\), Thy\(^+\), Str\(^G\)) selecting Lac\(^+[\text{Thy}^+]\) progeny. High level transfer indicated clones carrying Flac\(^+\) traQ elements. The JC5455 derivatives of these plasmids were repatched from the mating plate and used in a second plate mating with JC3272 (Str\(^R\)) selecting Lac\(^+[\text{Str}^R]\) progeny. This mating distinguished clones carrying transfer proficient Flac\(^+\) traQ elements from those carrying transfer defective Flac\(^+\) traX traQ elements. The latter clones were purified from the master plate, and each Flac\(^+\) traX traQ double mutant was transferred to three strains, JC6256 (au\(^-\)), JC6255 (au\(^+\)), and JC6255 carrying R62. Donor abilities were determined for these derivatives, and results are included in table 7.1. It is clear that the derivatives are
Insensitive to R62 transfer inhibition (traQ), and have suppressible transfer defects (traX).

The traX defect was confirmed to be due to mutation in the given transfer gene, by performing complementation tests. In all cases, transfer of the Flac+ traX traQ double mutants was not complemented by the equivalent Flac+ traX single mutant.

The donor abilities recorded for Flac+ traX traQ double mutants, when the traX mutation was suppressed, were low (table 7.1). The values are lower than the donor abilities of equivalent suppressed Flac+ traX single mutants. Although the traQ mutation has little effect on the transfer level of Flac+, its presence impairs the transfer of suppressed Flac+ traX mutants, particularly severely for Flac+ traX50 and Flac+ traAl. It is possible that presence of the traQ mutation has a detrimental effect on transfer operon expression, but does not cause a measurable decrease in Flac+ donor ability. The transfer system of Flac+ traX, when traX is suppressed, is probably inefficient, relative to Flac+ wild type, because of the suppressed traX product. Combination of a suppressed traX mutation with the traQ mutation, may in itself cause the markedly lowered donor abilities of the Flac+ traX traQ double mutants (table 7.1).

An Flac+ traC traQ double mutant could not be constructed by this method. None were found amongst 362 Flac+ traQ elements isolated after the procedure described above. In order to increase recombination between Flac+ traC and Flac+ traQ, the recipient strain, carrying Flac+ traQ, was exposed to U.V. irradiation (600 ergs/cm²) prior to the first mating of this procedure.

105.
### Table 7.1 Characteristics of Flag<sup>+</sup> traK traQ double mutants

<table>
<thead>
<tr>
<th>Plasmid number</th>
<th>Mutations carried</th>
<th>JC6256</th>
<th>JC6255</th>
<th>JC6255 (R62)&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDF1201</td>
<td>traJ90; traQ312</td>
<td>0.002</td>
<td>4.8</td>
<td>4.4</td>
</tr>
<tr>
<td>EDF1202</td>
<td>traA1; traQ312</td>
<td>&lt;0.00005</td>
<td>5.6</td>
<td>5.5</td>
</tr>
<tr>
<td>EDF1203</td>
<td>traE16; traQ312</td>
<td>&lt;0.00005</td>
<td>13.0</td>
<td>18</td>
</tr>
<tr>
<td>EDF1204</td>
<td>traI14; traQ312</td>
<td>&lt;0.00005</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>EDF1205</td>
<td>traF2; traQ312</td>
<td>&lt;0.00005</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>EDF1263</td>
<td>traC15; traQ333</td>
<td>&lt;0.00005</td>
<td>8.9</td>
<td>9.8</td>
</tr>
<tr>
<td>EDF1264</td>
<td>traC15; traQ334</td>
<td>&lt;0.00005</td>
<td>9.8</td>
<td>23</td>
</tr>
<tr>
<td>EDF1207</td>
<td>traF13; traQ312</td>
<td>&lt;0.00005</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>EDF1208</td>
<td>traH80; traQ312</td>
<td>&lt;0.00005</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>EDF1209</td>
<td>traG71; traQ312</td>
<td>0.0001</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>EDF1210</td>
<td>traD83; traQ312</td>
<td>&lt;0.00005</td>
<td>61</td>
<td>30</td>
</tr>
</tbody>
</table>

Numbers given are Lac<sup>+</sup> [Str<sup>R</sup>] progeny per 100 donors recipients were JC3372 or EDF26 for donors carrying R62 (Col1)<sup>+</sup>
Amongst 1300 progeny colonies, 744 Flac⁺ traQ recombinants were transfer proficient; no Flac⁺ traC traQ double mutants were found.

7.4 Isolation of Flac⁺ traC traQ

It was concluded that the difficulty in obtaining recombination between traQ and traC was due to their being very close together. Two approaches were used to isolate the desired Flac⁺ traC traQ double mutant. One relied on the fact that the mutation traB60 is poorly suppressed by su₁₁₁ (Achtman et al., 1971), to provide a selection for the products of recombination between traQ and traC. This method was expected to be successful only if the traQ mutation was on the promoter proximal side of the traC15 mutation (see figure 7.1). Consequently, the alternative procedure of mutagenising Flac⁺ traC15 and selecting new traQ mutations was also employed.

Mutagenesis of Flac⁺ traC15

The two stage selection procedure for inhibitor insensitive mutations, as described in chapter 6, was used here. However, because an amber suppressible transfer deficient Flac⁺ element was involved, all strains used carried a suppressor mutation.

Six single colonies of JC5455 (Su₁, Str₃, T₆), carrying Flac⁺ traC15 and R62, were subjected to EMS mutagenesis. An exponential phase culture of each mutagenised clone was mated with exponential phase ED1799 (Su₁₁₁, Str₃, T₆), carrying R62 (0.1ml + 0.1ml). After 40 minutes, U.V. irradiated T₆ (concentrated to 10¹¹ pfu/ml, 0.2ml) was added, and 20 minutes further incubation allowed to kill the donor cells. L broth (0.6ml)
was added, and after 40 minutes incubation to allow expression of the incoming Lac⁺ traCl5 elements, exponential phase ED3537 (Su⁺₁ II, Str⁺, T₆⁺), carrying R62, was added (1.0 ml). After 40 minutes mating, the mixture was plated to select Lac⁺ [Str⁺] derivatives of ED3537 (R62)⁺.

Such colonies were screened, by plate mating, for high transferring clones which carried a new traQ mutation. They were found in all six experiments, and one colony each from two experiments was purified and further tested. Donor abilities for these two plasmids, EDFL263 and EDFL264, from host strains JC6255 and JC6255 (R62), to confirm the TraQ⁻ phenotype, and from JC6256, to confirm the TraC⁻ phenotype, were measured. Results are included in table 7.1.

The dominance of the traQ333 and traQ224 mutations was also tested, using the experimental procedure described by Willetts and Paranchych (1974). Because EDFL263 and EDFL264 carry the traCl5 mutation, host strains for the dominance test were Su⁺, as given in the footnote to table 7.2. The results in table 7.2 show that both new traQ mutations are dominant.
Table 7.3 Dominance tests for \textit{traG} mutations of \textit{EDFL263} and \textit{EDFL264}

<table>
<thead>
<tr>
<th>Donor plasmid</th>
<th>Mutation carried</th>
<th>Retransfer from intermediate strain carrying no plasmid</th>
<th>R62</th>
<th>\textit{F14} \textsuperscript{+}, R62</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{JCY10} \textsuperscript{a}</td>
<td></td>
<td>61</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>\textit{WPFL26}\textsuperscript{b}</td>
<td>\textit{traG322}</td>
<td>43</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>\textit{EDFL263}\textsuperscript{b}</td>
<td>\textit{traG333}</td>
<td>51</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>\textit{EDFL264}\textsuperscript{b}</td>
<td>\textit{traG394}</td>
<td>43</td>
<td>77</td>
<td>13</td>
</tr>
</tbody>
</table>

The donor plasmid was transferred from JC6255 to the intermediate strain (JC6455 for \textit{a}; ED1799 for \textit{b}) carrying the plasmids indicated. After killing the JC6255 donor with \textit{T}_{6}, the frequency of \textit{F14} \textsuperscript{+} retransfer to ED26 (per 100 intermediate cells which had received it) was measured.

From these results, taken from Willetts and Paranchych (1974), are included for comparison.
Recombination between \( \frac{1}{2} \), \( \frac{1}{3} \) and \( \frac{1}{4} \)

1. Isolation of \( \frac{1}{2} \), \( \frac{1}{3} \) and \( \frac{1}{4} \)

An attempt was made to select for recombination between \( \frac{1}{2} \) and \( \frac{1}{4} \). This exploited the fact that the \( \frac{1}{2} \) mutation is poorly suppressed by \( \frac{1}{4} \), whereas \( \frac{1}{3} \) is efficiently suppressed by \( \frac{1}{4} \). Since a \( \frac{1}{2} \), \( \frac{1}{3} \), \( \frac{1}{4} \) double mutant was available, this property of \( \frac{1}{3} \) could be used to select \( \frac{1}{2} \), \( \frac{1}{3} \), \( \frac{1}{4} \) recombinant. In order to do this, \( \frac{1}{2} \), \( \frac{1}{3} \), \( \frac{1}{4} \) were used, together with \( \frac{1}{2} \), \( \frac{1}{4} \), in a recombination experiment.

To construct an \( \frac{1}{2} \) recombinant, which was not already available, an exponential phase culture of K174 (\( \frac{1}{2} \), \( \frac{1}{4} \)) carrying \( \frac{1}{3} \) was mixed, for 40 minutes, with a stationary phase culture of B3367 (\( \frac{1}{3} \), \( \frac{1}{4} \)) carrying \( \frac{1}{2} \), \( \frac{1}{4} \) (0.1% + 0.1%). Incubation was interrupted by the addition of UV. Irradiated \( \frac{1}{4} \) (concentrated to \( 10^{11} \) phage/ml, 0.2%) and after 30 minutes, 2 broth (1.5ml) was added. Incubation was continued for 2 hours to allow recombination and recombinant segregation and expression to occur. The mating mixture was streaked out on naseo tetrazolium indicator plates containing streptomycin to select \( \frac{1}{3} \) and indicate \( \frac{1}{2} \) derivatives of B3367. The \( \frac{1}{2} \) colonies were patched, plate mixed to B3360 (\( \frac{1}{4} \)) selecting \( \frac{1}{2} \) (\( \frac{1}{3} \), \( \frac{1}{4} \)) progeny, and the \( \frac{1}{2} \), \( \frac{1}{4} \), \( \frac{1}{3} \) derivatives of B3360 re-patched to selective plates to remove background B3367 clones. The re-patched derivatives were plated to B3367 selecting \( \frac{1}{2} \) (\( \frac{1}{3} \), \( \frac{1}{4} \)) progeny. So transferring clones carried \( \frac{1}{2} \), \( \frac{1}{3} \), \( \frac{1}{4} \).
derivatives. One of these clones was purified from the master plate in host strain ED3537. The suppressible transfer mutation was confirmed by estimating donor abilities from JC6285 (su\(^{+}\)) and JC6256 (su\(^{-}\)), values of 83% and 0.001%, respectively, being found. The transfer defect was shown to be due to traC mutation by the absence of complementation with Flac\(^{+}\) traC16.

Recombination between Fgal\(^{+}\) traC16 and Flac\(^{+}\) traH0 traQ12

II. Recombination procedure

The traH0 mutation is poorly suppressed by su\(^{III}\), and this phenotype is maintained by the Flac\(^{+}\) traH0 traQ12 double mutant. This has a donor ability of 21% when transferred from JC6255 (su\(^{+}\)), and of 0.005% when ED3537 (su\(^{III}\)) is the donor host strain.

By selecting for Flac\(^{+}\) traH\(^{+}\) traQ12 recombinants resulting from recombination between the parent plasmids Fgal\(^{+}\) traC16 and Flac\(^{+}\) traH0 traQ12, the relative frequency of the required Flac\(^{+}\) traC16 traQ12 recombinants would be considerably increased compared to the original procedure described in section 7.3. Figure 7.3 is a diagram of the recombination event sought and clarifies this rather complex experiment.

The following experimental procedure was used. Exponential donor strain JC6255 (Su\(^{I}\), T\(_{g}\), Nal\(^{R}\)) carrying Flac\(^{+}\) traH0 traQ12 was U.V. irradiated (500 ergs/mm\(^2\)) and mated for 60 minutes with stationary phase ED4364 (Su\(^{III}\), T\(_{g}^{R}\), Nal\(^{S}\)) carrying R63 and Fgal\(^{+}\) traC16, which had also been U.V. irradiated (500 ergs/mm\(^2\); 0.1ml + 0.1ml). Mating was interrupted by addition of U.V. irradiated T\(_{g}\) (concentrated to \(10^{11}\) pfu/ml, 0.2ml) and, after

111.
The cross-over events needed to generate an Flac\textsuperscript{+} traC traQ recombinant of Flac\textsuperscript{+} traH\textsuperscript{80} traQ and Fgal\textsuperscript{+} traC are shown. Two cross-overs are required if traQ3I2 is to the promoter proximal side of traC15, but four cross-overs are required if traQ3I2 is to the promoter distal side of traC15.
30 minutes, L broth (0.6ml) was added and incubation continued for 60 minutes to allow recombination and plasmid segregation. The mating mixture was diluted x10 into W3 minimal medium, selective for Flac\(^+\) derivatives of ED4334 also carrying RC2. After overnight growth, this culture was diluted x10 into L broth, grown to exponential phase, and mated for 60 minutes with exponential phase ED4487 (Su\(_{III}\)\(^+\), Tc\(^R\), Nal\(^R\), Thy\(^-\)) carrying RC2 (1.0ml + 1.0ml). The mating mixture was plated to select Lac\(^+\) [Nal\(^R\)] trans-conjungants. Colonies growing on the selective plates were patched and used in a plate mating with JC6256 (Su\(^-\), Thy\(^+\), Str\(^5\)), selecting Lac\(^+\)[Thy\(^+\)] progeny. In this plate mating three types of transfer were expected. Transfer defective clones would represent background transfer of non-recombinant, Flac\(^+\) tra\(E\)c0 elements; low level transfer proficient clones would represent recombinant Flac\(^+\) tra\(^+\) tra\(Q\)\(^+\) and Flac\(^+\) tra\(C\)15 tra\(Q\)\(^+\) elements; whilst high level transfer proficient clones would include Flac\(^+\) tra\(^+\) tra\(Q\)12 recombinants and the required Flac tra\(C\)15 tra\(Q\)12 recombinants.

Therefore, clones transferring at high level to JC6256 (Su\(^-\), Str\(^5\)), were re-patched from the mating plate and these JC6256 derivatives were used in a second plate mating, with JC3272 (Str\(^R\)) as recipient and selecting Lac\(^+\) [Str\(^R\)] progeny. Amongst 200 Flac\(^+\) tra\(^+\) tra\(Q\)12 recombinants, screened for the presence of a suppressible transfer defect, none was found.

Since the experiments performed with the Flac\(^+\) tra\(C\) tra\(Q\) double mutants ED1263 and ED1264, derived by mutagenesis (see above and section 7.5 and 7.6) showed that tra\(Q\) was probably to the right of tra\(C\), and since this would render the selection procedure
suitable (see figure 7.1), this experiment was not continued.

7.6 Assay of zygote products during HO fertility inhibition

Exponential phase cultures of J54796 (\( \Delta \) \( \text{H}^+ \), \( \Delta \) \( \text{L}^+ \), \( \Delta \) \( \text{A}^+ \), \( \Delta \) \( \text{B}^+ \)) carrying \( \Delta \) \( \text{L}^+ \) wild type, \( \Delta \) \( \text{L}^+ \) \( \Delta \) \( \text{H}^+ \), or \( \Delta \) \( \text{L}^+ \) \( \Delta \) \( \text{B}^+ \) elements were used in 40 minute matings with a stationary phase culture of J54796 (\( \Delta \) \( \text{H}^+ \), \( \Delta \) \( \text{L}^+ \), \( \Delta \) \( \text{A}^+ \), \( \Delta \) \( \text{B}^+ \)) carrying HO and \( \Delta \) \( \text{L}^+ \) (0.1ml + 0.1ml). Matings were interrupted by adding 1% saturated \( \text{T} \) (concentrated to \( 10^{-1} \) pH/2%, 0.1ml), followed after 20 minutes incubation, by addition of 1 broth (0.2ml) and further incubation for 40 minutes. At this stage the mating mixture was divided.

Portion added to exponential phase final recipient J54773 (\( \Delta \) \( \text{B}^+ \)) and a 20 minute mating period allowed (0.2ml + 1.8ml). Dilutions were plated to select \( \Delta \) \( \text{L}^+ \) [\( \Delta \) \( \text{B}^+ \)], \( \Delta \) \( \text{H}^+ \) [\( \Delta \) \( \text{B}^+ \)] and \( \Delta \) \( \text{L}^+ \) [\( \Delta \) \( \text{B}^+ \)] progeny. The remaining culture was diluted \( 1 \times 10^{-5} \), and spread in duplicate on lactose tetrauronic plates containing spectinomycin. A proportion of the colonies growing on the plates were restreaked, due to segregation of \( \Delta \) \( \text{L}^+ \) \( \Delta \) \( \text{L}^+ \) and \( \Delta \) \( \text{L}^+ \) \( \Delta \) \( \text{L}^+ \) colonies from temporary intercrosses containing \( \Delta \) \( \text{L}^+ \) [\( \Delta \) \( \text{L}^+ \)] (or \( \Delta \) \( \text{L}^+ \) wild type or \( \Delta \) \( \text{L}^+ \) [\( \Delta \) \( \text{L}^+ \)]) and \( \Delta \) \( \text{L}^+ \) wild type. This proportion indicated the number of temporary intercrosses in the J54796 culture used for the final mating with J54773. The number of progeny resulting from this mating were related to the proportion of temporary intercrosses, as shown in the footnote to table 7.1. The retransformer frequencies obtained are presented in this table.

Interspecific \( \Delta \) \( \text{L}^+ \) elements were patched and used in a plate mating with HO, selecting \( \Delta \) \( \text{L}^+ \) [\( \Delta \) \( \text{H}^+ \), \( \Delta \) \( \text{L}^+ \), \( \Delta \) \( \text{B}^+ \), \( \Delta \) \( \text{A}^+ \)] progeny. The percentage of \( \Delta \) \( \text{H}^+ \) \( \Delta \) \( \text{L}^+ \) elements present, also included in
Table 7.3 Retransfer of Flac\(^*\) traX traQ312 elements from an
 
\((\Phi\text{his}^+ \text{R62})^+ \text{intermediate}\)

<table>
<thead>
<tr>
<th>Plasmid No.</th>
<th>Donor plasmid</th>
<th>Mutations carried</th>
<th>Retransfer frequency</th>
<th>% Flac(^*) traX recombines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lac(^+)</td>
<td>His(^+)</td>
</tr>
<tr>
<td>JCF10</td>
<td>NONE</td>
<td></td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>WPFL39</td>
<td>traQ312</td>
<td></td>
<td>33</td>
<td>31</td>
</tr>
<tr>
<td>EDFL301</td>
<td>traJ90; traQ312</td>
<td></td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>EDFL302</td>
<td>traA1; traQ312</td>
<td></td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>EDFL303</td>
<td>traE16; traQ312</td>
<td></td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>EDFL304</td>
<td>traK4; traQ312</td>
<td></td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>EDFL305</td>
<td>traE2; traQ312</td>
<td></td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>EDFL306</td>
<td>traC16; traQ333</td>
<td></td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>EDFL307</td>
<td>traF13; traQ312</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>EDFL308</td>
<td>traRE0; traQ312</td>
<td></td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>EDFL309</td>
<td>traC71; traQ312</td>
<td></td>
<td>3.4</td>
<td>3.3</td>
</tr>
<tr>
<td>EDFL310</td>
<td>traG53; traQ312</td>
<td></td>
<td>5.2</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Retransfer frequencies are the number of trans-conjugants per 100 intermediate donors carrying a plasmid. For \(\Phi\text{his}^+\), the number of trans-conjugants per 100 intermediate donors carrying Flac\(^*\) is presented. The intermediate donor strain was JC5455 carrying \(\Phi\text{his}^+\) and R62. Primary donor strains were derivatives of JC5255 carrying the Flac\(^*\) element indicated, and the final recipient strain was RD26. The experimental procedure is described in section 7.5.

114.
7.6 Interpretation of the genetic assay for transfer gene products

The results in table 7.3 suggest that R63 inhibits the completion of transcription of the transfer operon. In all cases the retransfer frequencies for F8ac" and Fhis" are similar, because both plasmids are transferred by the available F transfer system. Retransfer of R63 is low, since this plasmid encodes a separate repressed I-like transfer system. The retransfer frequencies for WPFL39 and JCF10 show the resolution of the genetic assay, representing maximum and minimum expression of the F transfer system respectively. It is clear, from the results in table 7.3, that during R63 fertility inhibition all transfer gene products from traJ through traC are present, whereas those genes to the promoter distal side of traC are inhibited. The further interpretation of this observation, and some qualifications of it are presented as a series of points.

1. R63-encoded transfer inhibition does not inhibit expression of gene traJ. This confirms the conclusion, based on its inhibition of J-independent F8ac" mutants (section 1.6), that R63 fertility inhibition acts directly on the transfer operon, and not indirectly via traJ (Willette and Paramchych, 1974).

2. In addition to traJ, genes traA, traE, traB and traC of the transfer operon are not inhibited by R63. Genes traF, traI, traG, and traD are inhibited by R63. This suggests that R63-encoded transfer inhibition prevents complete transcription (or translation) of the transfer operon, by causing termination at a site between traC and traF.

115.
3. The retransfer frequency recorded for the \textit{traK4} mutant is low. Although \textit{traK} lies in the uninhibited promoter proximal region of the transfer operon (\textit{traA} through \textit{traC}) the \textit{traK4} mutation is strongly polar causing inactivation of genes \textit{traK} through \textit{traG} and the surface exclusion gene(s) \textit{traB} (Achtman \textit{et al.}, 1972). The low value for \textit{traK4} is therefore due to its causing mutation of genes \textit{traF} through \textit{traG} by polarity. It is likely that a suppressible point mutation in \textit{traK}, which is not available, would give a high value in the genetic assay.

Similarly, \textit{traB2} is mildly polar on promoter distal genes of the transfer operon, causing a reduction in surface exclusion (N.W. Willetts, unpublished). This probably accounts for the slightly lower retransfer value for \textit{traB2} compared to other \textit{tra} genes not inhibited by R62.

4. Although the results convincingly demonstrate that \textit{traF} and \textit{traH} are inhibited during fertility inhibition by R62, the values for \textit{traG} and \textit{traD} are rather high. Since surface exclusion, encoded by gene(s) located between \textit{traG} and \textit{traD}, is inhibited by R62, it was expected that genes \textit{traG} and \textit{traD} would also be inhibited. It is likely, therefore, that the higher values for \textit{traG} and \textit{traD} are caused by processes other than relatively poor inhibition of these genes by R62. There are two factors which might cause the recorded values to be higher than expected.

Firstly, the inhibition of transfer operon expression by R62 is probably incomplete, this R factor causing only a 100 fold reduction in transfer of wild type \textit{Flic}$. As a result, small levels of the inhibited gene products will be produced during
transfer inhibition. Variation in the values recorded in the genetic assay will be caused if the various inhibited gene products are needed in different amounts for transfer to occur. Any gene product required in only catalytic amounts will exhibit a degree of complementation in the experiment, because its production is not completely prevented by R62 transfer inhibition. It is known that when transfer operon expression is reduced 10 to 20 fold, sufficient traD product is present to allow between 60% and 100% complementation of a complete traD mutant (Foster and Willette, unpublished data; Dempsey and Willette, manuscript in preparation). Hence, the higher value for traD may be because only low concentrations of traD product are required to allow transfer.

A second factor to be considered is recombination. During the genetic assay recombination events may occur to generate traX+ traQ- combinations in cis. This would cause a complete F transfer system to be produced, regardless of R62-encoded transfer inhibition of the traX gene. It was therefore desirable to assess the extent of recombination during the experiments. Production of recombinant plasmids could be estimated by screening the retransferred Flac+ or Phia+ elements, selected during the genetic assay experiments, for non-parental genotypes. Since screening for the traX mutation is simpler than screening for the traQ mutation, the retransferred Flac+ elements were tested for transfer ability. The percentages of transfer proficient, recombinant Flac+ elements were determined and they are presented in table 7.3. It was demonstrated that during the experiments recombination did
Cross-overs involved in the formation of $\text{traQ312 trax}^+$ combinations in cis are shown. Variation in the size of the areas in which these cross-overs occur is shown using $\text{Flac}^+\text{traQ312 trAF13}$ and $\text{Flac}^+\text{traQ312 trAD83}$ as examples. The implications of this variation in a and b are discussed in the text.
Figure 7.2. Cross-overs involved in the production of trao312 traX recombinants during the genetic assay for traX products.

Q312: F13

F DNA

bacterial DNA

region of non-homology

region of non-homology
occur. When performing the genetic assay for traF and traH gene products, 15% and 30% respectively of the retransferred Flac\(^+\) elements were transfer proficient. However for traG and traD few Tra\(^+\) Flac\(^+\) recombinants were found. The explanation for this is that for expression of the F transfer system a single cross-over between traX and traQ is required to generate a traX\(^+\) traQ\(^-\) combination, whereas in order to produce a Tra\(^+\) Flac\(^+\) recombinant two cross-overs are required. Figure 7.2 shows the cross-over events involved in recombination. The relevant cross-over event that leads to expression of traX by production of a traX\(^+\) traQ\(^-\) combination occurs in region a. As the size of region a increases, the probability of a cross-over event occurring in the region increases. Since traQ appears to be close to traC, the size of region a is greater when the more promoter distal genes are assayed (i.e., a\(_D\) > a\(_G\) > a\(_H\) > a\(_F\)). Recombination occurs during the assay for traF and traH (table 7.3) and therefore it almost certainly occurs during the assay for traG and traD. The reason that Tra\(^+\) Flac\(^+\) recombinants are not found during the traG and traD experiments is that formation of those recombinants requires a second crossover event in region b of figure 7.2. Region b becomes smaller when the promoter distal genes are assayed (i.e., b\(_F\) > b\(_H\) > b\(_G\) > b\(_D\)). Consequently although transfer due to recombination would be expected to occur in the traG and traD assay experiments, the proportion of Tra\(^+\) Flac\(^+\) elements isolated would be expected to be small.

In conclusion, R62 transfer inhibition probably causes incomplete termination of transcription (or translation) of the transfer operon between traC and traF.
CHAPTER 8  OPERON FUSION INVOLVING THE TRANSFER REGION

FUSION TO THE TRYPTOPHAN OPERON

8.1 Introduction

Technology required for study of transfer gene control

Although a detailed understanding of the genetic structure and control of the F transfer region has been gained from purely genetic experiments, further advances demand a biochemical approach. In order to reveal the details of gene control, it is necessary to study the expression of the genes involved in the transfer process. Little knowledge of individual transfer gene products has yet been gained, precluding their assay to study gene expression. However in this and the subsequent chapters two approaches are used in attempts to provide a technology for biochemical study of transfer gene expression.

In chapter 10 the isolation of λ transducing phages carrying transfer genes is discussed. These could be used to assay mRNA produced during transcription of the transfer region, giving a direct means for investigating transfer gene control.

Here and in chapter 9 are described attempts to fuse the transfer genes to other, well studied, bacterial operons, such that expression of the latter is subject to transfer gene control. This would enable the enzymes and mRNA of the well studied bacterial operon to be used as an indicator of transfer gene expression. The first example of this indirect approach involves the tryptophan operon, whilst further systems are discussed in chapter 9.

119.
Attempts to fuse the tryptophan operon to the transfer region of the E. coli sex factor P have exploited a novel lambdoid transducing phage $\lambda p trp^+BG2$, that was isolated in this department.

**Structure of $\lambda p trp^+BG2$**

$\lambda p trp^+ BG2$ is a plaque forming $trp^+$ transducing phage derived from a $\lambda$/φ80 hybrid. The bacteriophage has the left arm of φ80 and the right arm of $\lambda$ giving the structure shown in figure 8.1. The immunity region is that of phage 434, and the non-essential int-φIII region of $\lambda$ has been deleted and replaced by bacterial DNA containing genes tonB and trpA, B, C, D and E. The promoter for the tryptophan operon is not present. (Devison et al., 1974). For the experiments described an extended host range mutant, $h^+$ of $\lambda p trp^+ BG2$ was used.

**Control of tryptophan operon in $\lambda p trp^+ BG2$**

The expression of the promoterless tryptophan operon of $\lambda p trp^+ BG2$ is by transcription initiated at the early leftward promoter $P_L$ of $\lambda$, and is consequently subject to negative control by the $\lambda$ repressor, product of gene $cI$. The full expression of the tryptophan operon is also dependent upon the presence of the N product, although a lower level of tryptophan gene expression does occur in the absence of N product. It is likely that, either the site of N action ($t_1$) is partially deleted, or that one of two sites of N action is deleted in $\lambda p trp^+ BG2$, accounting for the only partial N dependence.

A trpE or trpB mutant bacterial host lysogenized by $\lambda p trp^+$ BG2 still requires tryptophan for growth because transcription
Figure 8.1. Structure of $\lambda$prtB$^+$BG2.

bacterial DNA

$\lambda$ DNA

$\phi$ 80 DNA

434 DNA

transcription
Figure 8.2. Tryptophan independent lysogens of λptrp BG2 formed by illegitimate recombination at site 1 (Figure 8.1).
from $P_\lambda$ of $\lambda$ is repressed by the $oI$ product (Davison et al., 1974).

**Tryptophan independent lysogens of $\lambda p^{trp\,+\,BG2}$**

Lysogens of $\lambda p^{trp\,+\,BG2}$ which express the tryptophan operon do occur at a very low frequency. When a bacterial host carrying a complete deletion of the tryptophan operon is infected with $\lambda p^{trp\,+\,BG2}$, tryptophan independent colonies appear at a frequency of approximately $10^{-9}$. These strains appear not to be normal lysogens, and their occurrence is explained by a "Campbell" type insertion of the phage into the host chromosome involving illegitimate recombination. (Franklin, 1971). This event is presumed to occur at a site between $P_\lambda$ and the tryptophan operon in $\lambda p^{trp\,+\,BG2}$, and a site on the bacterial chromosome, such that upon insertion of the tryptophan operon is placed under the control of a bacterial promoter (W. Brammar and H. Burdon, personal communication). The process is shown diagramatically in figures 8.1 and 8.2.

**8.2 Insertion of $\lambda p^{trp\,+\,BG2}$ into the Flac$^+$ plasmid**

Tryptophan independent lysogens were sought in which $\lambda p^{trp\,+\,BG2}$ had been inserted in the transfer region of Flac$^+$. In this way fusion of the tryptophan operon to the transfer genes was expected to occur, so providing a means of studying transfer gene expression by assay of tryptophan operon enzymes and mRNA.

A series of E. coli strains was constructed for the experiment, and their genotypes and derivations are presented in figure 2.1 (chapter 2).
The host strain used was ED4431, which carried the Flac\textsuperscript{+} plasmid and had chromosomal deletions of the lactose operon and of the entire tryptophan operon plus gene tonB. Tryptophan independent clones of ED4431 were sought after infection with $\lambda_{ttrp}^+ B\text{G2}$.

Initially a double selection for transfer defective, tryptophan independent clones was performed by growing $\lambda_{ttrp}^+ B\text{G2}$ infected cultures in liquid minimal medium lacking tryptophan, and subjecting these to killing with the male specific bacteriophages $\mu_2$ or $f_1$, which adsorb to the F pilus. However due to the inefficiency of the selection for male specific phage resistant mutants and the rarity of trp\textsuperscript{+} clones, the desired insertions were not found. Twenty trp\textsuperscript{+} tra\textsuperscript{-} clones were isolated, but all proved to be double mutants in which a spontaneous transfer gene mutation was accompanied by a separate lysogenization of $\lambda_{ttrp}^+ B\text{G2}$ at another site within the genome. Many of the isolates were unstable, spontaneously losing the $\lambda_{ttrp}^+ B\text{G2}$ prophage at a high frequency (see below).

Consequently, in subsequent experiments selection for tryptophan independent growth was followed by screening the trp\textsuperscript{+} clones isolated, for transfer defects.

For this, a stationary phase culture of ED4431, grown for 17-19 hours (500ml), was centrifuged and resuspended in 0.01M magnesium sulphate (250ml). This cell suspension was starved by shaking at 37\textdegree{} for 60 minutes and added to a lysate of $\lambda_{ttrp}^+ B\text{G2}$ (50ml at a concentration of $2 \times 10^{10}$ phage per ml),
giving a multiplicity of infection of approximately 1. After allowing 30 minutes for phage adsorption, the infected cells were centrifuged and resuspended in buffer at a concentration of approximately $10^{11}$ cells per ml. Aliquots (0.1 ml) of this concentrated cell suspension were spread onto minimal plates without tryptophan and incubated for 48 hours.

Tryptophan independent colonies were patched onto the same minimal medium and screened for transfer defective clones by plate mating, using KD4438 as recipient, and selecting Lac\textsuperscript{+}[Spc\textsuperscript{R} His\textsuperscript{+}] progeny. KD4438 was lysogenic for\textsuperscript{434}$\lambda$ int\textsubscript{434}, to prevent zygotic induction.

A total of 4200 trp\textsuperscript{+} clones were isolated in experiments of this kind, and of these, 8 failed to transfer lac\textsuperscript{+} in the plate mating test. These were purified by streaking on EMB lactose plates and studied further. Of the 8 clones, 3 were resistant to the male specific bacteriophages f1, f2 and QB, whereas 5 were sensitive. One of this latter group was an unstable lysogen of \textit{\lambda trp} trp\textsuperscript{+} B22, but the remaining 7 lysogens appeared to be stable (see below).

The seven stable lysogens were subjected to acridine orange curing, which proved successful for the 3 male specific phage resistant strains. However no Lac\textsuperscript{−} colonies were produced when the male specific phage sensitive clones were tested. It was concluded that in these latter strains Hfr formation had occurred by integration of the F\textit{lac}\textsuperscript{+} element into the host chromosome. This phenomenon is known to occur by insertion.
of Flac\(^+\) at the lactose deletion present in the host strain. Since this deletion is incomplete, sufficient homology with the bacterial DNA carried by the Flac\(^+\) element remains, to allow insertion of the plasmid by general recombination. The resultant Hfr strains transfer lac\(^+\) as a late marker, causing an apparent transfer defect that is detected during the screening procedure for clones not transferring lac\(^+\).

The three tryptophan independent lysogens of \(\lambda ptcrp^+\) BG3 that were cured of Flac\(^+\) by acridine orange, carried autonomous, transfer defective Flac\(^+\) elements. In order to determine whether the \(\lambda ptcrp^+\) BG3 prophage was located on the Flac\(^+\) element, the transfer defective plasmids were mobilized by complementation with Phiz\(^+\) as follows.

Exponential phase JC6256 (Spc\(^S\) \(T_6^S\)) carrying Phiz\(^+\) and stationary phase tryptophan independent lysogens of ED4431 (Spc\(^S\) \(T_6^R\)) were mated for 40 minutes (0.1ml + 0.1ml). U.V. irradiated \(T_6\) (concentrated to \(10^{11}\) pfu/ml, 0.2ml) was added, and incubation continued for 20 minutes, when L broth (0.2ml) was added. After 40 minutes dilutions were spread on lactose tetrasodium plates to reveal temporary heterozygotes as Lac\(^+\)/Lac\(^-\) sectored colonies. Simultaneously this mating mixture was used as donor in a further 30 minute mating (0.2ml + 1.6ml) with exponential phase ED4436 (Spc\(^R\) \(T_6^R\)). Dilutions were plated on spizizen salts minimal media to select Lac\(^+\)[Spc\(^R\)] progeny as well as Trp\(^+\)[Spc\(^R\)] progeny.
Table 8.1  Mobilization of transfer defective plasmids present
in tryptophan independent \( \lambda_{\text{ptrp}}^+ \) B2 lysogens of ED4431

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>( \text{lac}^+ ) mobilisation</th>
<th>( \text{trp}^+ ) mobilisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>16/47</td>
<td>24.3</td>
<td>0.05</td>
</tr>
<tr>
<td>33/37</td>
<td>19.7</td>
<td>0.05</td>
</tr>
<tr>
<td>41/34</td>
<td>20</td>
<td>18.6</td>
</tr>
</tbody>
</table>

Figures given are the number of \( \text{Lac}^+[\text{Spc}^R] \) or \( \text{Trp}^+[\text{Spc}^R] \)
progeny per 100 temporary heterozygote donors, in a
mating with ED4436. Temporary heterozygotes were
formed by transfer of \( \Phi \text{his}^+ \) into the tryptophan
independent lysogens of ED4431, as described in
section 8.2. Isolate number 41/34 has been
designated EDF1262.

The results in table 8.1 show that mobilization of \( \text{lac}^+ \)
occur for all 3 transfer defective plasmids, but \( \text{trp}^+ \) mobilization
occurs for only one lysogen. It was concluded that in the 3
instances when \( \text{trp}^+ \) was not mobilized, a double event led to the
phenotype observed. A spontaneous transfer gene mutation of
\( \Phi \text{lac}^+ \) presumably accompanied a tryptophan expressing lysogenisation
of the bacterial chromosome. In the third instance co-transfer
of \( \text{lac}^+ \) and \( \text{trp}^+ \) shows the tryptophan expressing lysogenization
to have occurred by insertion into the \( \Phi \text{lac}^+ \) plasmid. This
\( \Phi \text{lac}^+ (\lambda_{\text{ptrp}}^+ \text{B2}) \) plasmid, designated EDF1262, has been exten-
sively studied and will be discussed in section 8.3.
An equivalent \( \lambda^{ptrp +} \) BG2 infection experiment using ED4434, which carried Flac\(^+\) tra J90 in a Su\(^+\) background, produced 3,800 Trp\(^+\) colonies from which 11 Tra\(^-\) clones were isolated. All of these were sensitive to male specific bacteriophages and resisted curing. They were consequently not studied further, being interpreted as transfer proficient Hfr strains.

Unstable lysogens of \( \lambda^{ptrp +} \) BG2

As mentioned above, during the \( \lambda^{ptrp +} \) BG2 infection experiment, tryptophan independent clones were isolated that were unstable. This instability was detected because the TonB phenotype (the host strain is tonB and \( \lambda^{ptrp +} \) BG2 carried the tonB gene) was clearly indicated by growth on EMB lactose indicator plates. For Lac\(^+\) strains, TomB\(^-\) colonies growing on EMB lactose agar were small, very darkly pigmented and shiny, whereas TomB\(^+\) colonies growing on EMB lactose agar were larger, more lightly pigmented and were not shiny. For Lac\(^-\) strains growing on EMB lactose agar, TomB\(^+\) colonies were larger and less shiny than TomB\(^-\) colonies, but since neither take up pigment when growing on EMB lactose agar, no difference in this was observed.

A single colony of unstable TomB\(^+\) lysogens of \( \lambda^{ptrp +} \) BG2 produced 10-90% TomB\(^-\) colonies when streaked out a second time on EMB lactose agar. Simultaneous loss of the TomB\(^+\) phenotype, tryptophan independence, and phage 434 immunity proved the instability of the TomB\(^+\) phenotype to be due to the loss of the \( \lambda^{ptrp +} \) BG2 prophage. Further study of an unstable clone suggested that the \( \lambda^{ptrp +} \) BG2 prophage was located on the Flac\(^+\) element. Acridine orange curing of an unstable lysogen never
produced a Lac^{-} clone that had retained the $\lambda_{ptrp}^+$ B2 prophage, and transfer of the Flac$^+$ element to another host strain was frequently accompanied by transfer of the $\lambda_{ptrp}^+$ B2 prophage.

An unstable Flac$^+$ ($\lambda_{ptrp}^+$ B2) element was transferred to the recA$^{-}$ strain ED4444. The $\lambda_{ptrp}^+$ B2 prophage continued to exhibit instability in this host, suggesting that the instability was recA independent (transfer of Flac$^+$ ($\lambda_{ptrp}^+$ B2) ED4444 was achieved by using temporary immunity to prevent zygotic induction, chapter 2).

The unstable lysogens were not studied further since, regardless of their properties, the instability would cause their use in studies of gene expression to be very difficult.

8.3 Properties of EDFL1262

Insertion of $\lambda_{ptrp}^+$ B2 into the transfer region of Flac$^+$

As described above, one tryptophan independent clone carried a transfer defective Flac$^+$ element that was linked to a $\lambda_{ptrp}^+$ B2 prophage. The strain, ED4481, carried the Flac$^+$ element EDFL1262.

ED4481 was Trp$^+$ and TomB$^+$ (capable of normal growth on N9 salts minimal medium and sensitive to $\lambda B0 v_{ir}$). It was immune to phages, such as $\lambda ima 434$, which had the immunity region of phage 434.

EDFL1262 was transfer defective, its donor ability being $6 \times 10^{-5}$%, and was resistant to the male specific bacteriophages f1, f2 and Qφ.
Acridine orange curing of EDFL262 from ED4481 yielded lac^- clones, all of which were trp^-. One such lac^- trp^- clone was shown to be topB^- and sensitive to \( \lambda \text{inv} \), indicating the loss of all properties associated with \( \lambda \text{ptrp}^+ \) BC2.

Mobilization of EDFL262 selecting for lac^+ transfer has been described (section 8.2) and it was accompanied by mobilization of trp^+. 100 lac^+ progeny obtained upon transfer of EDFL262 proved to be trp^+.

Hence \( \lambda \text{ptrp}^+ \) BC2 and Flac^+ were lost and transferred from ED4481 together, proving that the bacteriophage had inserted into the Flac^+ DNA generating the plasmid co-integrate EDFL263.

The defective transfer and absence of pilus formation (indicated by male specific bacteriophage resistance) by EDFL262 could be explained by \( \lambda \text{ptrp}^+ \) BC2 insertion within the transfer region. The surface exclusion index of ED4481 was measured and found to be low (table 8.2), suggesting that the surface exclusion gene(s) of EDFL262 was not expressed. A surface exclusion defect would be caused by insertion of \( \lambda \text{ptrp}^+ \) BC2 into gene tral or any of genes trAA through trAG, due to lack of positive control or polarity, respectively.

Subsequently, complementation tests were performed to further define the nature of the EDFL262 transfer defect.
Table 8.3 Surface exclusion by EDF1262

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid carried</th>
<th>Surface exclusion index</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED4431</td>
<td>JCF10</td>
<td>61</td>
</tr>
<tr>
<td>ED4481</td>
<td>EDFL262</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Figures are surface exclusion indices, defined as the ratios of the number of His\(^+\) recombinants obtained with \(F^-\) strain ED4431, to the numbers obtained with the ED4481 derivates given, in crosses with Hfr ED4410 (a \(tonA^-\) derivative of KL88, which is resistant to \(\lambda p_{trp}^+\) BC2).

Complementation tests for EDF1262

Complementation tests were performed as described in chapter 2, and results obtained are presented in table 8.3. In addition to testing the Trp\(^+\) isolate EDFL262 (anti-immune form, see section 8.4), selected complementation tests were carried out for a Trp\(^-\) form of EDFL262 (in the immune state, see section 8.4). The state of tryptophan operon expression (see section 8.4) had no effect on complementation.

Complementation occurred for genes \(traJ\), \(traA\) and \(traE\) suggesting that \(\lambda p_{trp}^+\) BC2 had inserted to the promoter distal side of \(traE\). Complementation for the other genes of the transfer operon did not occur. The higher value for \(traD\) was probably caused by recombination and/or the fact that this gene product is required in only small amounts for complementation to occur (see chapter 7 for full discussion of this).
Table 8.3 Complementation tests for EDL262

<table>
<thead>
<tr>
<th>Donor plasmid</th>
<th>Mutation carried</th>
<th>Retransfer % when intermediate varies</th>
<th>% Tra&lt;sup&gt;+&lt;/sup&gt; recombinants for anti-Immune EDL262</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>anti-Immune</td>
<td>Immune</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDL262</td>
<td>EDL262</td>
</tr>
<tr>
<td>Fhis&lt;sup&gt;+&lt;/sup&gt;</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fhis&lt;sup&gt;+&lt;/sup&gt;</td>
<td>-</td>
<td>113&lt;sup&gt;*&lt;/sup&gt;</td>
<td>NT</td>
</tr>
<tr>
<td>JCF180</td>
<td>traJ90</td>
<td>94</td>
<td>NT</td>
</tr>
<tr>
<td>JCF11</td>
<td>traA1</td>
<td>67</td>
<td>NT</td>
</tr>
<tr>
<td>JCF118</td>
<td>traE18</td>
<td>80</td>
<td>65</td>
</tr>
<tr>
<td>JCF14</td>
<td>traK4</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>JCF12</td>
<td>traB2</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>JCF15</td>
<td>traC6</td>
<td>2.7</td>
<td>NT</td>
</tr>
<tr>
<td>JCF113</td>
<td>traF13</td>
<td>3.8</td>
<td>4.3</td>
</tr>
<tr>
<td>JCF180</td>
<td>traH50</td>
<td>6.6</td>
<td>NT</td>
</tr>
<tr>
<td>JCF171</td>
<td>traG71</td>
<td>7.6</td>
<td>3.4</td>
</tr>
<tr>
<td>JCF183</td>
<td>traD83</td>
<td>41.0</td>
<td>47.0</td>
</tr>
</tbody>
</table>

Figures represent the number of Lac<sup>+</sup> [His<sup>+</sup> Spc<sup>R</sup>] progeny obtained in complementation tests performed as described in chapter 2, and expressed as a percentage of the number obtained when Fhis<sup>+</sup> was the donor. ED4438 was recipient, except in line 2 (value marked <sup>*</sup>), when ED4430 was used to test zygotic induction.

The last column contains the percentage Tra<sup>+</sup> recombinant progeny occurring during the complementation test.

130.
The insertion of \(\lambda_{\text{ptrp}}^+\) BG2 has clearly caused a polar mutation. Since \(\text{traK}^4\) is a polar mutation, defective for genes \(\text{traK}\) through \(\text{traG}\), the position of the \(\lambda_{\text{ptrp}}^+\) BG2 prophage has been defined between the promoter proximal side of \(\text{traK}\) and \(\text{traB}\). The use of \(P_1\) transductional complementation would provide more precise information on the location of the prophage, but this was not considered necessary.

Expression of the tryptophan operon in ED4481

Tryptophan independent lysogens of \(\lambda_{\text{ptrp}}^+\) BG2 were explained by assuming illegitimate insertion of the bacteriophage, so as to allow expression of the tryptophan operon from a bacterial promoter (section 6.1). If such an event occurred in the formation of EDPL262 then expression of the tryptophan operon would be controlled by the transfer operon, and hence tryptophan operon expression should parallel that of the transfer operon. The predicted control by the transfer operon was tested by measuring the effect of R100 (\(\text{finO}^+\)) and R100-1(\(\text{finO}^-\)) on tryptophan operon expression by ED4481.

Unexpectedly, neither R factor affected the ability of ED4481 to grow on minimal medium lacking tryptophan. Furthermore, the extent of growth inhibition by \(3\) methyl-tryptophan and the specific activity of anthranilate synthetase were not changed by the presence of R100 (table 8.4). The effectiveness of \(\text{FinO}\) transfer inhibition on EDPL262 was confirmed by use of R136 mutants. Derivatives of ED4481 were made carrying a \(\text{finO}^-\text{finP}^+\) R136 element (R136\(_1^-\)) or a \(\text{finO}^+\text{finP}^-\) R136 element (R136\(_2^+\)). Both R136 mutants
Table 8.4  Tryptophan expression by ED4481

<table>
<thead>
<tr>
<th>B factor in ED4481</th>
<th>Growth on minimal medium without tryptophan</th>
<th>Radius of zone of 6-methyl tryptophan inhibition</th>
<th>Specific activity of anthranilate synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>+</td>
<td>1.6</td>
<td>0.24</td>
</tr>
<tr>
<td>R100</td>
<td>+</td>
<td>1.7</td>
<td>0.18</td>
</tr>
<tr>
<td>R100-1</td>
<td>+</td>
<td>1.8</td>
<td>0.19</td>
</tr>
</tbody>
</table>

a. Ability to grow on minimal medium without tryptophan was tested. No difference in colony size between the strains tested was detected.
b. The radius of the zone of inhibition caused by 6-methyl tryptophan was measured (see chapter 2).
c. Specific activity of anthranilate synthetase was measured and expressed as described in chapter 2.

produce an uninhibited transfer system, but whilst the finO\textsuperscript{+} mutant R136p\textsuperscript{−} inhibits Flac\textsuperscript{+} transfer, the finO\textsuperscript{−} mutant R136q\textsuperscript{−} does not. Since the traA products of R136 and F can be distinguished by the plating efficiencies of male specific phage (see chapter 3), the presence of the EDFL262 traA product in the two derivatives could be tested. The R136r\textsuperscript{−} derivative (finO\textsuperscript{−}) had a high plating efficiency for the male specific phage MS2 (60%, relative to ED4431) showing that the EDFL262 traA product was produced. In contrast the R136p\textsuperscript{−} derivative (finO\textsuperscript{+}) had a low plating efficiency for the male specific phage MS2 (1%, relative to ED4431), showing that FinOp fertility inhibition prevented expression of the traA gene of EDFL262.
Further study prompted by these results, revealed that a
culture of B4401 contained a proportion of cells that were unable
to form single colonies on minimal medium lacking tryptophan.
These Trp⁻ derivatives of B4401 maintained the other proportion
of λ(p15Α₂) E2 lysogenic; they were Trp⁺, and had 434 immunity.
They reverted to Trp⁺ at a relatively high frequency when plated
on minimal medium without tryptophan; one Trp⁺ colony per 10⁶
Trp⁻ colonies being observed. This indicates that Trp⁻
cells retain a potentially active tryptophan operon.

The unusual tryptophan operon expression of B4401 has been
incorporated in a hypothesis which also explains the proportion
of B4401 that appear to conflict (section 8.4).

In vivo transformation and from donor activation by B4401

The E417293 is mobilized by λ(p15Α₂), as described in
section 8.3 or as in complementation tests, transfer to a lysogenic
recipient occurs. From the recipient one or one-lysogens, transfer
occurred at the same frequency, suggesting that replicative infection
did not occur (see table 8.3).

A culture of B4401 contains free phage particles, that
plate on E417293, but not on the λ(p15Α₂) lysogen E4408. This
shows that the λ(p15Α₂) E2 present on E417293 is capable of vegeta-
tive growth when liberated, and in a mating might be expected
to kill recipient cells if these are not 434 immune.

None, the absence of vegetative infection by E417293 conflicts
with its ability to liberate plaque forming bacteriophages.
9.4 Model for the structure of EDV1282

To account for the properties of ED4481, it is suggested that tryptophan operon expression has remained under control of the $\lambda$ promoter $P_\lambda$ and that it is repressed by the lambda repressor, product of gene $oI$.

When a wild type $oI$ gene is present, tryptophan operon expression could occur if the lysogenic $\lambda^{pTRP^+}$ BG2 enters the anti-immune phase. Certain $\lambda$ lysogens are able to exist in two alternative phenotypic states, the immune ($im^+$) and the anti-immune ($im^-$), (Eisen et al., 1968, Calef et al., 1971).

For this to be possible three conditions must be fulfilled:

1. Certain genes to the left of $N$ must not be transcribed.

2. $\lambda$ DNA must not replicate upon derepression.

3. The $cro$ gene must be active.

The usual lysogen used to meet these requirements has a thermo-inducible prophage with two additional mutations. One is in gene $N$, to prevent complete leftward transcription, and the other, in either gene $O$ or $P$, prevents bacteriophage DNA synthesis.

To become anti-immune, a $\lambda^{pTRP^+}$ BG2 lysogen does not need an $N$ mutation, since the genes between the site of $N$ action ($t_\lambda$) and $att$ are deleted and replaced by the tryptophan operon and $trpB$ gene. Davison et al. (1974) have recently shown that deletion of genes $O$ and/or $P$ of a $\lambda^{pTRP^+}$ BG2 prophage is sufficient for establishment of the anti-immune phase.
A diagram of the structure of Flac$^+$ (λptp$^+$ BG2) resulting from insertion of λptp$^+$ BG2 into the transfer region of F by illegitimate recombination at site 2 (figure 8.1).
Figure 8.3. A possible structure for the Flac\(^{+} (\Lambda pt\text{trp}\^{+}BG2)\) co-integrate EDFL262.
The properties of E441 would be as found, if \( \lambda_{\text{pT}} \) D23 had inserted within the transfer gene of E441D23, by illegitimate recombination, at a point between genes \( g \) and \( f \) on the phage genome. A diagram of the structure postulated for E441D23 is given in figure 1.3. In a lysogen of this type, genes \( g \) and \( f \) would be intact, maintaining the potential for liberation of plaque-forming bacteriophages. However, expression of \( g \) and \( f \) by transcription from \( \lambda_{\text{pT}} \) would be impossible, accounting for the absence of cryptic induction, and the ability to establish the anti-immune phase. Transcription of the tryptophan operon would be initiated from \( P_\lambda \), and the \( \text{Trp}^+ \) form in which E441 was isolated represents the anti-immune phase of the lysogen. \( \text{Trp}^+ \) revertants of E441 occur when the \( \lambda_{\text{pT}} \) prophage spontaneously shifts into the immune phase.

The possibility that the prophage had inserted as described, but with the reverse polarity is unlikely. Such an orientation would allow expression of \( \lambda \) genes \( g \) and \( f \) from the transfer operon promoter. No expression was detected, since E441 failed to plate heteroimmune \( \lambda \) phage mutant in genes \( g \) or \( f \).

Furthermore, with the prophage orientation reversed, genes of the transfer operon (on the transfer promoter distal side of the prophage) would be expressed under the control of the \( \lambda \) \( P_\text{Pm} \) promoter. Such expression would only occur in the anti-immune (\( \text{Trp}^+ \) ) phase, but not when the \( \lambda \) repressor was effective, as in the immune (\( \text{Trp}^- \) ) phase. As the complementation tests showed (table 1.3) the state of tryptophan operon expression (which indicates the phase of the lysogen) had no effect on transfer gene expression.
9.1 Introduction

An attempt has been made to generate fusions of the transfer region of F to a well understood bacterial operon, by isolating deletions of suitable F-prime elements. The process is shown diagramatically in figure 7.1, and the essential properties of suitable F-prime elements and well studied operons are presented as a series of conditions:

1. The bacterial operon to which the transfer genes are to be fused must be transcribed in the same direction as the transfer operon. Since the bacterial DNA carried by an F-prime is located between co-ordinates 0 and 17.6 on the physical map of F (see figure 1.1), the deletion, selected to generate fusions to the transfer genes, will not remove essential F genes.

2. Between the well studied bacterial operon and the transfer operon, an independent marker is required. Selection for the loss of this marker should be possible to increase the selection pressure for deletions of the F-prime element.

3. A further marker is required, that will not be deleted during the fusion process, and which consequently can be used independently to select cells carrying the F-prime element.
4. A mutation which inactivates the structural genes of the chosen well studied operon, but not their potential for activity (i.e. the structural genes of the operon must be non mutant) is required. The exception to this is where only some of the promoter distal structural genes of the operon are required for selection of fusions. In this case the promoter proximal genes, which will be deleted, can be mutant. This type of mutation is essential for selection of expression of the structural genes of the operon (or part of the operon) upon deletion of the mutation and fusion of the operon to a new promoter.

5. At least one of the structural genes of the chosen operon must have an easily assayable gene product. Also a transducing phage carrying structural genes of the operon must be available, to allow mRNA hybridisation studies. These requirements allow control of transfer gene expression to be investigated after isolation of fusions, by assay of the gene products and mRNA produced by the well studied operon.

The diagram in figure 9.1 illustrates the deletion event and fusion process and illustrates the first four conditions. Table 9.1 tabulates the features of plasmids fulfilling the conditions. These F-prime elements are considered individually below.
Figure 9.1 Formation of gene fusions by the isolation of deletions.

- Selection of deletions

- Condition 1

- Condition 2

- Condition 3

- F DNA

- Bacterial DNA

- XYZ well studied

- Bacterial operon
### Table 9.1 Characteristics of the F-prime elements used

<table>
<thead>
<tr>
<th>F-prime element</th>
<th>Condition 1 (operon in correct orientation)</th>
<th>Condition 2 (marker for selection of deletions)</th>
<th>Condition 3 (marker to select the F-prime element)</th>
<th>Condition 4 (mutation to inactivate the operon)</th>
<th>Condition 5 (assay of gene product)</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELF44</td>
<td>lacZ,Y,A</td>
<td>tax selecting T&lt;sub&gt;6&lt;/sub&gt; resistance</td>
<td>leu</td>
<td>promoter deletion lac&lt;sup&gt;11&lt;/sup&gt; strong polar lac&lt;sup&gt;+&lt;/sup&gt;</td>
<td>lac&lt;sup&gt;Z&lt;/sup&gt; product β-galactosidase</td>
<td>λ&lt;sup&gt;plac&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELF47</td>
<td>galE,T,K</td>
<td>att selecting survivors of temperature induced lysogen (coli57)</td>
<td>none . use gal&lt;sup&gt;+&lt;/sup&gt;</td>
<td>promoter mutation gal&lt;sup&gt;-&lt;/sup&gt;</td>
<td>galK product β-galactoside transacetylase</td>
<td>λ&lt;sup&gt;gal&lt;/sup&gt;</td>
</tr>
<tr>
<td>KLF5</td>
<td>argE</td>
<td>bfe selecting EF23 resistance</td>
<td>met9</td>
<td>promoter deletion arg&lt;sup&gt;sup102&lt;/sup&gt;</td>
<td>arg&lt;sup&gt;E&lt;/sup&gt; product acetylornithase</td>
<td>g&lt;sup&gt;50&lt;/sup&gt; arg&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>KLF13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
0.2 Fusion to the lactose operon

The F-prime element KLF44 (Low, 1972) was the only available F-prime element carrying the lactose operon, and fulfilling the conditions given in section 9.1. The leucine marker could be used to select the plasmid, and the \( T_6 \) resistance gene, \( \text{tax} \), provides an independent selection for deletions. A \( \text{tax}^{-} \) host carrying KLF44 (\( \text{tax}^{-}/\text{tax}^{+} \)) is \( \text{Tax}^{+} \) since the wild type allele is dominant. Deletion of the wild type allele of the plasmid leads to the \( \text{Tax}^{-} \) phenotype, which can be selected by resistance to \( T_6 \).

Two types of mutation to inactivate the lactose operon, whilst maintaining the potential of its structural genes for expression, were available. Promoter deletions such as LI (Miller et al., 1968) would allow subsequent selection for Lac\(^+\) strains, in which the lactose genes \( \text{lacZ} \), \( \text{lacY} \) and \( \text{lacA} \) were expressed by transcription initiated at the transfer operon promoter. The \( \text{lacZ} \) gene product, \( \beta \)-galactosidase, could ultimately be assayed to study transfer gene control. In addition, strong polar mutations of gene \( \text{lacZ} \) are suitable for selection of fusions. Mutations such as \( \text{lacZ}_{1116} \) and \( \text{lacZ}_{281} \) inactivate the promoter distal genes \( \text{lacY} \) and \( \text{lacA} \) by polarity. Deletions extending into the \( \text{lacZ} \) gene to remove the polar mutation could be selected. Expression of \( \text{lacY} \), which encodes the lactose permease, can be selected by the ability of derivatives to utilize the carbon source melibiose at 42\(^\circ\) (Beckwith, 1970). Fusions to the transfer operon, selected in this way, could be further studied by assay of the \( \text{lacA} \) gene product, \( \beta \)-galactoside transacetylase. The former of these types of mutation, promoter deletions, has the advantage of providing a clean selection for fusions (the melibiose selection is relatively
poor), and a very simple assay for the lacZ gene product, once fusions have been isolated (the assay for β-galactoside trans-
acetylase is relatively complex). For these reasons the I1 pro-
mitter deletion was chosen for attempts to generate transfer-
lactose operon fusions.

Isolation of KLF44 lac₄₄₄

The parental strain required for this experiment is a derivative of KLF44 carrying the deletion lac₄₄₄₄₄.

Before attempting to construct this, the KLF44 plasmid was tested to ensure that it was a sufficiently good donor to be used in studies of transfer gene control. The donor ability of KLF44 was 27% when using the RecA⁻ strain K1251 as donor, and the RecA⁺ recipient strain ED3543, and selecting Lac⁺ Leu⁺[Pro⁺,
Met⁺, Spo⁺] progeny. The plasmid rendered host cells sensitive to male specific phages f1, f2 and QB. Hence KLF44 is suitable for use in the fusion experiments.

Construction of a derivative of KLF44 which carries the lac₄₄₄₄₄ mutation was attempted by homozygotisation. This involves the isolation of a homozygote (both plasmid and chromosome encode the same allele of a given gene) from a heterozygote (plasmid and chromosome encode different alleles of a given gene). For the plasmid KLF44 homozygotisation involved, the isolation of the F-prime element in a recA⁺ lac₄₄₄₄₄ strain by selecting a Lac⁺ derivative. Subse-
quently a Lac⁻ derivative would be isolated in which both KLF44 and the bacterial chromosome carry the lac₄₄₄₄₄ mutation. Numerous attempts to isolate a derivative of XA6001 (recA⁺ lac₄₄₄₄₄) carrying KLF44 were unsuccessful. The experiment involved a 60 minute
mating between K1251 (KLF44) and XA8001, selecting Lac⁺[Met⁺, Pro⁺, Trp⁺] transconjugants. These were patched and tested for ability to transfer KLF44, in a plate mating with ED3842, selecting Lac⁺ [SpcR] progeny, and for sensitivity to male specific phages using the Giemsa-μ2 replica plating technique. Most transconjugants were non-transferring, male specific phage resistant, recombinants, whilst the few male specific phage sensitive clones isolated did not transfer lac⁺ to ED3842. (These could be instances of F-prime insertion into the chromosome by general recombination. KLF44 carries a large segment of chromosomal DNA, providing a considerable area of homology between the F-prime and the bacterial chromosome). The plasmid probably acts as an Hfr during transfer, generating large numbers of recombinants between the genes it carries and their equivalents on the bacterial chromosome. When the whole KLF44 molecule is transferred and recircularized it is probably extremely unstable, because of the large area of homology with the bacterial chromosome. This difficulty prevented further study of KLF44 as a possible source of transfer-lactose operon fusions.

As an alternative approach, an HfrH strain carrying the lac⁻ mutation was used in an attempt to isolate an F-prime element equivalent to KLF44, but already carrying the lac⁺ mutation. Exponential phase cultures of CA8001 (HfrH, lac⁻, lac⁺, T₆, Str⁰) and ED3887 (F⁻, recA, lacZ, leu, proC, T₆, Str⁰) were mated for sufficient time to allow entry of the lac and proC genes, but not the recA⁺ gene of the Hfr donor strain. Mating was interrupted by the addition of concentrated UV (to 10⁻⁴ phu/ml), U.V. irradiated T₆ and/or by agitation in a mechanical interrupter,
before plating to select Leu$^+$ Pro$^+$ [Str$^R$] trans-conjugants. These were screened for ability to transfer leu$^+$ to ED3542, selecting Leu$^+$ [Spe$^R$] progeny in a plate mating, and for sensitivity to a specific phage using the Gieman-m$^2$ replica plating technique. No fertile clones were isolated amongst 300 trans-conjugants tested in this way and insufficient time was available to continue this experiment.

9.3 Fusion to the galactose operon

One plasmid, KLF47, is suitable for the isolation of transfer-galactose operon fusions. Three isolates of strains carrying KLF47 were obtained from Dr. B. Bachmann. In each case the plasmid was unstable, segregating Gal$^-$ derivatives, even when carried by a RecA$^+$ host. This instability prevents use of KLF47 for complex experiments of this type. In the event of a similar F-prime element being isolated the following fusion experiment, planned for KLF47, might profitably be undertaken.

The attachment site for phage $\lambda$ is between the correctly oriented galactose operon and the transfer operon of KLF47. This enables a well established technique for the isolation of deletions to be used. Lysogenisation by $\lambda$ a1887 of a strain deleted for the att site on the bacterial chromosome, but carrying KLF47, would lead to prophage insertion at the attachment site carried by the plasmid. The lysogens would be temperature sensitive, because of the a1887 mutation, and growth at 42$^\circ$ would cause induction of $\lambda$, and killing of host cell. Deletions of the $\lambda$ a1887 prophage would be selected amongst survivors of such a temperature induction. It is known that deletions extending into the bacterial (or plasmid)
DNA on either side of the prophage can be obtained in this way (Adhya et al., 1968). This selection of deletions could be coupled with a selection for those deletions generating operon fusions. For this the mutation gal-9 (probably a promoter mutation, J. Davison, personal communication) could be used to prevent expression of the galactose operon. Reactivation of the promoter distal genes galt and gali by fusion to the transfer genes of F could then be selected by either of two methods.

By performing the selection in a host strain in which the gale gene is present, but the galk and galt genes are deleted, galactose fermenting derivatives would be generated by expression of the plasmid carried galt and galk genes.

The properties of bacteriophage C21 provide an alternative selection for expression of the promoter distal genes of the galactose operon. The receptor site for C21 is blocked by a build up of UDP galactose, which, as the following diagram shows, is a metabolite involved in reactions controlled by galactose operon gene products:

```
<table>
<thead>
<tr>
<th>galK</th>
<th>galt</th>
<th>gale</th>
<th>promoter</th>
</tr>
</thead>
<tbody>
<tr>
<td>galactose ←→ galactose phosphate ←→ UDP galactose ←→ UDP glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

C21 receptor
The central position of UDP galactose in the reactions of galactose operon encoded enzymes causes \( \text{gal}^E \) mutants grown on galactose to be resistant to phage C21. A strain with a total galactose operon deletion, but carrying a plasmid equivalent to KLF47 \( \text{gal}^O \), would be sensitive to C21 whether grown on galactose or any other carbon source. Deletions of KLF47 \( \text{gal}^O \), removing part of \( \text{gal}E \) and the \( \text{gal}O \) mutation, might fuse \( \text{gal}T \) and \( \text{gal}K \) to another operon, such as the transfer operon. A derivative of this type would express genes \( \text{gal}T \) and \( \text{gal}K \), causing a build up of UDP galactose within the cells when grown on galactose. Consequently it would be resistant to phage C21, and could therefore be selected as such.

### 3.4 Fusion to the arginine operon

The F-prime elements KL5 and KL12 are suitable for fusion experiments involving the argE gene of the argECBH cluster. These plasmids can be selected by using the metB marker, and the gene \( \text{bfe} \), which is between the transfer genes and the argECBH cluster, provides an independent selection for deletions. Mutants of \( \text{bfe} \) are resistant to phage EF23, and are recessive to the wild type allele. Hence EF23 resistant derivatives of a \( \text{bfe}^- \) host, carrying KL5 or KL12 (EF23 sensitive strains), will include deletions of the plasmid encoded wild type \( \text{bfe} \) gene.

The organisation of the argECBH cluster is complex. Genes \( \text{arg}E, \text{arg}D \) and \( \text{arg}H \) form one operon, transcribed in the opposite direction to a second operon, comprising solely gene \( \text{arg}G \). The \( \text{arg}E \) operon is transcribed with the same polarity as the transfer
operon of F. A deletion which inactivates argE by deleting the promoter is available. This deletion, sup102, also removes genes argC and argB of the other operon, but argH remains and is expressed (Elseviers et al., 1973). The structure of the two operons of the argECEH cluster, and the position of deletion sup102 is as follows:

![Diagram showing the structure of the arg operon with sup102 deletion]

Selection for reactivation of the argE gene, in the absence of argC and argE, is possible because the argE gene product, acetylornithinase, can metabolise artificial substrates. In addition to the in vivo role of the enzyme (conversion of acetylornithine to ornithine) the substrates, acetylhistidine, acetylmethionine and acetylarginine can be converted to histidine, methionine and arginine, respectively. These activities of argE enable His<sup>−</sup>, Lys<sup>−</sup> and Arg<sup>−</sup> auxotrophs to grow in the absence of the relevant amino acid supplements, and forms the basis of a selection for reactivation of argE expression, by fusion to another operon.
F-prime elements KLF8 and KLF12

The plasmids KLF8 and KLF12 were derived from the very unstable Ra-2 Hfr in which F has inserted into the chromosome at a sex factor affinity locus (Low, 1972). The F factor in both the Ra-2 Hfr and the plasmids KLF8 and KLF12 is separated from the chromosomal DNA by a repeated DNA sequence, YS (Chibsbo et al., 1974). This sequence causes high frequency, RecA independent, dissociation of F from the bacterial DNA. As a result the F prime elements KLF8 and KLF12 segregate F+ derivatives at a high frequency and are not stabilized in a RecA- host. Despite this instability, these plasmids were suitable for fusion studies provided that selection for a chromosomal gene carried by them was maintained.

Isolation of KLF12 arg sup102 by homozygotisation

KLF5 and KLF12 were transferred to strain AS2, which carries the arg sup102 mutation, selecting Arg⁺ Met⁺ [Leu⁺ Pro⁺ Thr⁺] progeny. The derivatives were grown overnight and plated on minimal medium to select Met⁺ clones (arginine was present), which carried the plasmid. Plates with well separated colonies were replica plated onto minimal medium without arginine. If homozygotisation had occurred during overnight growth, the derivatives homozygous for arg sup102 would be arginine dependent. No colonies unable to grow without arginine supplement were found in this way.

Consequently penicillin enrichment was used to select homozygotes (see chapter 2). Colonies growing after enrichment were screened for arginine dependent clones carrying KLF5 or KLF12.
Accept 10 colonies carrying 1576, none were arginine dependent. However, from 64 colonies carrying 15712, 4 were arginine dependent and able to transfer the plasmid to the Rec - strain 11369 when selecting nat + (41') progeny. The derived plasmids expressed pro A+ (characteristic of 17 A+) enabling host cells with a total deletion of argE65X to utilize thymine for growth in the absence of arginine. Such derivatives did not revert to Arg', providing additional evidence that the argE65X deletion had been transferred to the 15712 plasmid.

One derivative, E21410, was further studied. The plasmid was transferred to ED107, selecting Nat + Can + Phe + [A p ] progeny. Tumor ability of E21410 from ED107 to ED1368 selecting Nat + Can + Phe + [A p ] progeny was 6%. E21410 rendered host cells sensitive to only specific shapes 21, 12 and 42.

Deletion of E21410: losing the transfer operon to the 157/3

Deactivation of 15714X: in the ED107 derivative carrying E21410, can be selected by growth of this Rif' auxotroph on minimal medium lacking histidine, but containing acetylhistidine and arginine. Similarly growth of this strain on acetylarginine requires expression of 1576 but not the other 157 genes. In a preliminary experiment ED407 carrying E21410 was plated on minimal medium supplemented with acetylhistidine and arginine, but not histidine, and on medium containing acetylarginine and histidine, but not arginine. Colonies growing on the plates appeared at a frequency of 10^-3 to 10^-6 and 10^-7 when selecting for utilization of acetylhistidine and acetylarginine, respectively. In both cases colonies were slow growing, arising after incubation for
4 days and 5 to 6 days, respectively. Colonies resulting from these selections were patched and screened, by replica plating techniques, for ability to transfer met\(^+\) to ED4500, and for resistance to EF23. None of the 203 clones tested (69 from the acetylhistidine selection and 134 from the acetylarginine selection) were resistant to EF23, and all were transfer proficient.

The experiments were repeated with the additional selection for EF23 resistant deletions. Cells were treated as above but 10\(^{10}\) EF23 phage per plate were also added. The few colonies growing on the plates were tested for loss of fertility (by plate mating to ED4500 selecting Met\(^+\) Pco\(^+\) [Spc\(^B\)] progeny, and by the Giemsa \(\mu 2\) replica plating technique for male specific phage sensitivity). All were fertile showing that no deletions causing transfer-\(\text{arg}\) fusion operons had been selected.

In an attempt to increase the number of deletion events cells were exposed to ultra violet irradiation or to nitrous acid mutagenesis prior to plating (chapter 2). However, the few colonies growing after mutagenesis were fertile, again showing that the required fusion event had not occurred.

Time was not available to continue this experiment but two refinements of the selection procedure for \(\text{arg}\) expression have been considered. In a report by Elseviers et al., (1972) deletions of a strain carrying the \(\text{arg}\)\(^{\text{meta}102}\) mutation were isolated by growth of a Met\(^-\) auxotroph on acetyl methionine and arginine. These deletions were presumed to be fusions of \(\text{arg}\) to another bacterial operon. This selection may be better because the \(\text{arg}\) enzyme utilizes acetylmetionine more efficiently than either
acetylhistidine or acetylarginine (S. Baumberg, personal communication). Also a deletion of argE which leaves the argCBU operon active is available (S. Baumberg, personal communication). If this was incorporated in the host strain carrying EDFA265, selection for expression of argE by growth on arginine would be possible.
10.1 Introduction

Although the attempts to generate fusions described in Chapters 8 and 9 were unsuccessful, a direct study of transfer gene expression is also possible. The mRNA produced by transfer gene transcription can be assayed if a suitable source of homologous DNA is available. DNA for use in mRNA-DNA hybridisation studies could be provided by lambda transducing phages which carry transfer genes.

10.2 Transducing phages generated in vivo

Introduction

Flac+ (λ) co-integrates have been isolated in this laboratory by Dr S. McIntyre, using the technique developed by Shimada and Weisberg (1972), which involves selection of lysogens in a host deleted for the attachment site. In some cases the λ prophage is inserted within the transfer region of Flac+. Such transfer defective Flac+ (λ) co-integrates provide a source from which transducing phages carrying transfer genes can be isolated.

Flac+ (λ) co-integrates used

The two Flac+ (λ) co-integrates EDFL224 and EDFL223 have the prophage located within genes tetA and trpX or trpB respectively. For reasons not important here the λ bacteriophages used in construction of the three co-integrates were different. EDFL224 has a λ s1687 cro− sus0 prophage whereas EDFL223 has a
Isolation of \( \lambda \) transducing phage from 177 lysogen has
relied upon the 7-prime element 7109. This 7109 phage carries
the attachment site and hence can receive the \( \lambda \) prophage upon
lymphodation. Then the 7109 phage is carried by BB149, which
has a chromosomal deletion of \( \lambda g^+ \) and \( \lambda 122 \), lymphodation leads to
preferential insertion of the prophage at the plasmid-form attachment
site.

Point mutations in host transfer gene have been transferred to
BB100 by recombinant with \( \lambda g^+ \) elements by Dr. H. Wittenbo
using the technique described for \( \lambda g^+ \) in Chapter 7. If BB149
carrying BB100 in lymphodation by a \( \lambda g^+ \) transducing phage,
it is possible for the transfer defective BB100 to be transferred
together with the \( \lambda g^+ \) transducing phage, thus both selecting
and isolating the latter. However, this procedure depends upon
expression of the \( \lambda g^+ \) gene carried by the \( \lambda \) prophage. Since the
\( \lambda \) precursors \( P_8 \) and \( P_9 \) are expressed in lymphodation, it is not possible
to select transfer proficient lymphodation in which the transfer gene
are expressed by transcription from \( \lambda \) promoter. Hence, a transfer
gene promoter must also be carried by the \( \lambda g^+ \) transducing phage,
to allow transfer gene expression. This restriction leaves the
method applicable only to transfer gene alone enough to the transfer
opera promoter to be incorporated, together with the promoter, in
a \( \lambda \) transducing phage (\( \lambda \) transducing phage carrying 1717 or 1714
were therefore exact).

BB104 and BB105, which are BB149 derivatives carrying
EI222 and EI224 respectively, were subjected to temperature
infection (Chapter 2) to produce L77 lysates. Transfer of
YAC (EMD) and 7200 (EMD) was collected after infection with the
L77 lysates from E5762 and E5764 in the following way.

Overnight cultures of E5762 carrying YAC (EMD) or
7200 (EMD) (E5210 and E5211B respectively) were centrifuged and
resuspended in a half volume of 0.01 M KCl. Three suspension
were started by shaking for 10 minutes at 39°. Equal volumes
(0.1 ml
of this culture, the relevant L77 lysates
(m.o.i. 1-10) and an E574 lysate to act as helper phase, were
incubated for 60 minutes at 39°, added to L broth (1.0 ml), and
grown to exponential phase. This culture was used in a 10 minute
infection at 39° with exponential phase E5777 (1.0 ml + 1.0 ml), and
the other culture plated to select 01* (8ML) progeny. 39° was
used for all inoculations to prevent induction of lysogeny, so the λ
prophages carry all23 mutations. The recipient E5777 was λ 1200,
preventing lysogenic induction.

Progeny were pelleted and plated with the recipient, the
L52 strain E57640, selecting O6* (Lys t Lys) progeny, and the
L52 strain E57640, selecting O6* (Lys t Lys) progeny. Transfer proficient 7200 Lys* (λ Lys) originations would transfer
to E5764 but not to E57640, because of lysogenic induction.

And transfer proficient lysogeny were obtained using E52110
infected with L77 lysate from E5764, but not using E52110 after
infection with L77 lysate from E5764. The frequency of transfer
proliferation originations of E5210 was 3 x 10^{-9} per plaque (see table
10.1).
Table 10.1 Selection for λ\textsuperscript{tra}\textsuperscript{+} transducing phages generated in vivo

<table>
<thead>
<tr>
<th>Phage lysate</th>
<th>F100 element</th>
<th>Tra\textsuperscript{+}F100 derivatives</th>
<th>Multiplicity of infection</th>
<th>Tra\textsuperscript{+}F100 derivatives per input phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>tested</td>
<td>tested</td>
<td>per 2x10\textsuperscript{8} tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ex HP3764</td>
<td>HP163(traAl)</td>
<td>10</td>
<td>10\textsuperscript{5}</td>
<td>3 x 10\textsuperscript{-3}</td>
</tr>
<tr>
<td>HD\textsuperscript{-}4 control</td>
<td>HP163(traAl)</td>
<td>&lt; 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ex HP3764</td>
<td>HP160(traJ90)</td>
<td>&lt; 1</td>
<td>0.7\textsuperscript{a}</td>
<td>&lt; 3 x 10\textsuperscript{-3}</td>
</tr>
<tr>
<td>HD\textsuperscript{-}4 control</td>
<td>HP160(traJ90)</td>
<td>&lt; 1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Multiplicity of infection varied because the lysogens HP3764 and HP3765 produced lysates with different titres upon temperature induction. HP3764 was a double lysogen, giving a larger burst size upon induction (S. McIntyre, personal communication).

Four transfer proficient derivatives of F100 traAl were transduced to ED2149 using the temporary immunity technique (chapter 2), selecting Cal\textsuperscript{+} [His\textsuperscript{+}Trp\textsuperscript{+}Lys\textsuperscript{+}] progeny. A further 29 transfer proficient derivatives were transferred on 

| to ED2250, and purified by streaking on galactose tetrazolium plates. Temperature induction (chapter 2) of these derivatives produced putative HFT lysates. These were used in a repeat of the selection procedure described above. The large input of λ\textsuperscript{traA}\textsuperscript{+} transducing phages was expected to cause a high frequency of F100 traAl transfer. Surprisingly, none of the 33 lysates tested produced more transfer than an HD\textsuperscript{-}4 control lysate.

The isolation of transfer proficient F100 derivatives which were not found in a control experiment using only an HD\textsuperscript{-}4 lysate, implies that λ\textsuperscript{traA}\textsuperscript{+} transducing phages are present in the HFT lysates produced.
from ED3764. The only explanation for the inability to form HFT lysates is that the \( \lambda \text{tra}^+ \) transducing phages generate F100 \( \text{tra}^+ \) derivatives by general recombination, in preference to F100 \( \text{tra}^- (\lambda \text{tra}^+) \) derivatives by lysogenisation.

To circumvent this problem the selection procedure is being performed in this laboratory using \( \text{reoA}^- \) strains to eliminate general recombination. Indeed, similar selection methods used for \( \lambda \text{trp}^+ \) (W. Brummar, personal communication) and \( \lambda \text{rif}^+ \) (Ikazuki et al., 1975) transducing phages, have revealed problems due to general recombination. In the latter case, \( \lambda \text{rif}^+ \) transducing phages were isolated with ease when \( \text{reoA}^- \) strains were substituted.

In addition, the technique to be described in section 10.3 is currently being applied to the selection of \( \lambda \text{tra}^+ \) transducing phages generated \textit{in vivo}. This technique selects lysogens by the ability of \( \lambda \text{tra}^+ \) transducing phage to recombine with F100 by virtue of transfer gene DNA homology. It has the important advantage of not demanding that the transfer genes be expressed.

10.3 Transducing phages generated \textit{in vitro}

\textbf{Introduction}

The use of restriction enzymes to produce \( \lambda \) transducing phages \textit{in vitro} has been developed in this department. The method involves the digestion of DNA with restriction enzymes, such as \( \text{EcoRI} \) or \( \text{HindIII} \), which generate specific staggered breaks to produce fragments with 'sticky ends'. The fragments can be rescued by insertion into a receptor molecule, which has also been treated by restriction enzyme. The 'sticky ends' produced by the enzyme are
identical, regardless of the DNA source which acts as substrate, allowing the joining with ligase of fragments from different sources. 

\( \lambda \) phage having only a single site at which a particular restriction enzyme produces a nick are used in this department as receptors for DNA fragments from various sources to generate \( \lambda \) transducing phages. The donor DNA and receptor phage are treated with restriction enzyme, mixed, ligased, and used to transform a bacterial strain. Reconstituted \( \lambda \) phages (some being transducing phages) form plaques on this bacterial strain, and these can be screened for whatever transducing phages are required. (K. Murray, N. Murray, W. Brammar, personal communication).

DNA of the plasmid KLFI has been treated in this way by Dr J. Beggs, with the intention of isolating \( \lambda \) transducing phages carrying bacterial chromosome genes. The transducing phages formed in this experiment may also include the \( \lambda \text{tra}^+ \) phages needed for study of transfer gene control.

\( \lambda \) transducing phages carrying Hind III generated KLFI fragments

Mixed lysates of phage NM \( \lambda \) 5-40 (imm\(^{21}\)), reconstituted after treatment with Hind III restriction enzymes, and mixing with Hind III generated fragments of KLFI, were supplied by Dr J. Beggs. They were used in a selection experiment for \( \lambda \text{tra}^+ \) and \( \lambda \text{traA} \) transducing phages, as described in section 10.2. Since NM\(^{0}:6:40 \) has the immunity region of phase 21, the recipient strain ED4471, a lysogen of \( \lambda \text{imm} \), was used in this experiment. Twenty four mixed lysates were tested in pairs for the presence of \( \lambda \text{tra}^+ \) and \( \lambda \text{traA} \) transducing phages, by infection of ED2213 and ED2219 and performing a mating with ED4471, selecting Gal\(^+\) [Str\(^R\)] progeny (section 10.2). Ex-conjugant colonies were screened for transfer proficient derivatives.
of 7601 from and 7601 from plate resistant to E4470, colonies
and (E4470) progeny. Except a total of 163 colonies from the
λ E4470 resistant experiment 1 transfer proficient clone was
isolated. In the λ E4470 resistant experiment, three lysates
produced 0 transfer proficient clones (0 were free in experiment).

Lysates from both clones were prepared by E.T. induction
after purification and confirmation that the clones were both
transfer proficient and mature to radioactive phase A1,
A4 and A6. Two plates of 17 lysates were used to infect
bacteria carrying F69 (strain 204) or F69 (strain 319) respectively.

The bacteria were prepared as described for E.coli derivatives
carrying R100 (Section 19.3) to give optimal conditions for
lymphocytosis. The infected cells were cultured out on
bacteriophage indicator plates and les° colonies patched.
They were replica plated to test lyogamy (similarly to λ
was tested), and fertility by plate mating to E4470 resistant
les° (E4470) progeny. 163 lysates of from each 17 lysates were
tested, but one was transfer proficient.

It is concluded that if the transfer proficient lysogen
was not revertant, λ les° transforming phage was present in
the lysates tested, but that the les° derivatives isolated were
produced by general recombination. This problem, discussed in
section 19.3, might be overcome by the use of less° strains. A
second type of selection technique was developed because of the
problem encountered with R100, and because the R100 technique
required that a transfer non-predictor rescued any transfer
gene selected on transforming phage. This technique is being
186.
used by Dr. N.S. Willette to select \( \lambda^{\text{tra}+} \) transducing phages generated both \textit{in vivo} and \textit{in vitro}.

\( \lambda \) transducing phages carrying Eco RI generated KLP1 fragments

Lysates of NM\( \lambda \) 461 carrying EcoRI generated fragments of KLP1 DNA at the EcoRI target within the red genes of \( \lambda \) were supplied by Dr. J. Beggs. Lysogens of these phages were isolated by insertion of the prophage at the region of homology with the host DNA, using general recombination. By using a host strain with a deletion of the \( \lambda \) attachment site and carrying \( \text{Flac}^+ \), transducing phages carrying F DNA were inserted within this \( \text{Flac}^+ \) element. Such lysogens were identified by screening for transfer properties of the \( \text{Flac}^+ \) element. \( \text{Flac}^+ \) elements carrying \( \lambda \) prophages exhibit zygotic induction upon transfer to a non-lysogenic recipient. Hence lysogens were screened, by plate mating, for transfer ability to a lysogenic recipient (ED2143) and to a non-lysogenic recipient (ED2144). Lysogens of transducing phages carrying bacterial chromosome DNA transfer to both recipients, whereas lysogens of transducing phages carrying F DNA transfer to the lysogenic recipient but less efficiently to the non-lysogenic recipient because zygotic induction occurs. The only exception to this is that lysogens of transducing phages carrying transfer genes, without including the transfer operon promoter or the last essential gene of the transfer operon, will be transfer defective, transferring neither to a lysogenic nor a non-lysogenic recipient. Figure 10.1 illustrates the lysogens and their transfer properties.
Lambda lysogens formed by general recombination and their transfer properties are shown. The transducing phages shown carry DNA of:

1. the bacterial chromosome.
2. F without transfer operon genes.
3. F with the promoter proximal end of the transfer operon.
4. the middle of the transfer operon.
5. F with the promoter distal end of the transfer operon.

The formation of these lysogens and their properties are further discussed in the text.
Figure 10.1 Formation of lysogens by general recombination.

\[ \lambda \text{ transducing phage} \]

\[ \text{host DNA} \]

\[ \text{lysogen} \]

<table>
<thead>
<tr>
<th>transfer to</th>
<th>tra(^+)</th>
<th>tra(^+)</th>
<th>tra(^-)</th>
<th>tra(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lysogenic recipient</td>
<td>Tra(^+)</td>
<td>Tra(^+)</td>
<td>Tra(^-)</td>
<td>Tra(^-)</td>
</tr>
<tr>
<td>non-lysogenic recipient</td>
<td>Tra(^+)</td>
<td>Tra(^-)</td>
<td>Tra(^-)</td>
<td>Tra(^-)</td>
</tr>
</tbody>
</table>
The lysates of transducing phages supplied by Dr. J. Beggs were screened, by isolation of lysogens and testing the transfer properties of the lysogens, to ascertain which transducing phages carried fragments of PDNA. Isolation of lysogens by general recombination was complicated by the fact that NM 461 is recA+, causing a reduction in the efficiency of RecBC recombination (Unger and Clark, 1972; Unger et al., 1972). However, lysogens could be selected by plating infected host cells with a mixture of λ b26+ and b s0 c a a 9.

Two λ transducing phages, amongst those supplied by Dr. J. Beggs, carried PDNA (revealed by the properties of their lysogens, see below) and were subsequently shown to allow complementation of transfer defective mutants, under some circumstances. The properties of these phages, JBλ6 and JBλ7, are discussed below.

**Transductional complementation tests**

Lysates to be tested were added to exponential phase cultures of ED149 (StrR) carrying transfer defective F lac+ elements (O.1ml + 0.1ml) at a multiplicity of infection of 1. After incubating the infected cells for 60 minutes, 0.8ml of an exponential phase culture of recipient strain JC3272 (Str R, iml λ) was added. After a 60 minute mating period, 0.05ml samples were spread onto minimal plates to select Lac+ (StrR) progeny. A control experiment, in which EDλ4 replaced the test lysate, was included for each complementation test.

**JBλ7**

JBλ7 is a transducing phage derived in vitro from EMλ461.
and Eco RI generated fragments of HindI. The phage carries a fragment of HindI at the Eco RI target within the sal gene of K1241. After an in vivo recombination experiment (J. Rogen, personal communication), a lysate in which a J197 prophage was inserted within J510 was isolated as described above. The lysate was transfer proficient, but transferred at a greatly reduced frequency, to a non-lysogenic recipient, because syngentic induction occurred. As figure 10.1 indicates, such properties are expected for lysates of non transducing phage carrying transfer genes. Accordingly, this phage was tested for its ability to complement transfer mutations, using transductional complementation tests, also described above.

Temperature induction of this J197 lysate yielded a lysate which complemented point mutations in J510, and in the proximal gene of the transfer operon, J510 through J515 (table 10.3). However, the level of complementation was low.

The original J197 lysate, used to construct the lysagen, did not complement any transfer gene mutations (table 10.1). Also, when the temperature induced lysate was recycled on the RecA strain C600, or the recA strain C600, complementation no longer occurred (table 10.3).

An explanation for the properties of J197 is that the receptor phage K1241 carries an Eco RI generated fragment of 7 Eco just to the left of the SalI gene. Such a phage would not complement transfer gene mutations. A lysate formed by general recombination with P197 would be transfer proficient, carrying a J197 prophage to the left of sal1.
(Footnote to Table 10.2 and 10.3)

Numbers are the progeny of a complementation test after subtraction of the background level determined in control experiments using ED\(\lambda\) 4. The values represent progeny per \(10^7\) phage particles. Donor strains were ED2149 derivatives carrying the plasmids given and the recipient strain was JC3272. Trans-conjugants in all cases were tested for Tra\(^+\) recombinants. No significant levels of recombination were detected.

a. The \textit{traJ50} mutation is leaky causing a high background transfer of \textit{Flac} \(^+\) in the absence of complementation. Hence these results, which are not considered to represent complementation, probably reflect variation in the level of background transfer.

b. Similar results were obtained for two other single plaque lysates.
### Figure 10.2 Complementation by λ 7

<table>
<thead>
<tr>
<th>Plasmid tested</th>
<th>Mutation carried</th>
<th>Phage lysate tested</th>
<th>Cycle on C600</th>
<th>Cycle on GR48</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Original lysate</td>
<td>Induced lysogen</td>
<td></td>
</tr>
<tr>
<td>JCF190</td>
<td>traJ50</td>
<td>0 (^a)</td>
<td>222</td>
<td>6 (^a)</td>
</tr>
<tr>
<td>JCF121</td>
<td>traA1</td>
<td>1</td>
<td>173</td>
<td>1</td>
</tr>
<tr>
<td>EDF12</td>
<td>traL311</td>
<td>1</td>
<td>103</td>
<td>1</td>
</tr>
<tr>
<td>JCF118</td>
<td>traE16</td>
<td>1</td>
<td>56</td>
<td>1</td>
</tr>
<tr>
<td>JCF1105</td>
<td>traK105</td>
<td>NT</td>
<td>143</td>
<td>NT</td>
</tr>
<tr>
<td>JCF12</td>
<td>traB2</td>
<td>1</td>
<td>57</td>
<td>1</td>
</tr>
<tr>
<td>JCF13</td>
<td>traC5</td>
<td>1</td>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>JCF113</td>
<td>traF13</td>
<td>1</td>
<td>1.6</td>
<td>1</td>
</tr>
<tr>
<td>Plasmid tested</td>
<td>Mutation carried</td>
<td>Plasmid tested</td>
<td>Mutation carried</td>
<td>Phage lysate used</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>----------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>JCFL50</td>
<td>traB0</td>
<td>JCFL71</td>
<td>traG71</td>
<td>JCFL63</td>
</tr>
<tr>
<td>Number</td>
<td>Induced lysate</td>
<td>Cycle on</td>
<td>Cycle on</td>
<td>Two cycles on</td>
</tr>
<tr>
<td></td>
<td>lysogen</td>
<td>C500</td>
<td>QR45</td>
<td>QR48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>2</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>8</td>
<td>11,500</td>
<td>274</td>
<td>522</td>
<td>347</td>
</tr>
<tr>
<td>1</td>
<td>1,670</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>1</td>
<td>128</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>
Upon induction of such a lysogen, an LFT lysate would be formed. A small proportion of transducing phages carrying transfer genes would account for the observed complementation (table 10.2). If defective transducing phages were lost or their proportion reduced upon vegetative growth of the induced lysates, this low level of complementation would no longer occur (table 10.2).

**JB λ 8**

**JB λ 8** is a transducing phage derived *in vitro* as described above for **JB λ 7**. A lysogen, in which a **JB λ 8** prophage was inserted within JC710 by general recombination, was isolated. This lysogen was transfer defective, transferring neither to a lysogenic recipient nor to a non-lysogenic recipient. It was sensitive to male specific phages fl and Qβ, but was resistant to f2. This phenotype is characteristic of a **traD**− mutant. The lysogen probably has a prophage inserted within or to the left of **traD**, which would cause a **traD**−, **traI**− polar defect.

After temperature induction of this lysogen, a lysate that complemented point mutations in **traD** and **traI** was obtained (table 10.3). Complementation was greater for **traD** than **traI**, possibly because the complementabilities of mutations in different genes vary (this may reflect different quantity requirements for the various transfer gene products). After vegetative growth of the induced lysate on the RecA+ strain C600, or the RecA− strain QM48, the level of complementation was reduced (table 10.3). A second cycle of vegetative growth on
QD did little effect on this reduced level of complementation for JλD. Three single plasmids from the temperature induced lysate were grown on QD. These lysates showed no complementation of JλD or JλL (table 10.3), suggesting that the majority of phage particles present in the temperature induced lysate were not transducing phage.

An explanation for these properties of Jλλ D is that the phage carries a small Eco RI generated fragment of 720A that is between F6 and F7 (see Eco RI fragment map in figure 10.8; Jλλ D may carry F6 or F7). Lycomplementation by general recombination into FJpG would insert the prophage at this point, causing a fin" mutant, as found. Upon induction, an IF7 lysate may be produced, to account for the observed complementation of F6 and F7. The level of complementation of Jλλ D by the temperature induced lysate was fairly high. This may reflect the fact that only small quantities of Jλλ D product are needed for complementation, (see Chapter 7), or a more complex explanation for the properties of Jλλ D may be required.

Conclusions

Further study of Jλλ D and Jλλ E has been postponed, because their complementation properties suggest that the original phage do not carry entire transfer gene.

Recent research has revealed the approximate position of Eco RI targets within the transfer region of 7 (Obinata, P., personal communication; Murray and Clark, 1979; see figure 10.8 for Eco RI fragment map of 7). It shows that useful fragments of 7 Eco, carrying transfer gene, should be generated.
This fragmentation map was adapted from Skurray and Clark (1975). The pRE numbers refer to work by these authors. The properties of JβA5 and JβA7 are consistent with those of transducing phages carrying EcoR1 fragments f17 and/or f19, and f3 respectively.
Figure 10.2. EcoR1 fragmentation map of F.
by **Eco RI** digestion. Such a digestion of F DNA, and the
construction of λ transducing phages carrying F DNA is being
conducted in this laboratory by Dr. N.S. Willetts.

( The experiments using JBλ 5 and JBλ 7 were carried out in
collaboration with Dr. N.S. Willetts.)
The mechanisms of action of the five systems for inhibition of F transfer (Fin\textsuperscript{OP}, Fin\textsuperscript{Q}, Fin\textsuperscript{U}, Fin\textsuperscript{V} and Fin\textsuperscript{W}), as far as is known, are illustrated in figure 11.1.

Most Fin\textsuperscript{+} F-like plasmids inhibit F transfer simply because their transfer control systems are similar to those of F itself. It is curious that three non-F-like plasmids (R62, JR66a and R456) falling into the I\textalpha and X incompatibility groups and probably having transfer systems quite unrelated to that of F, should carry Fin\textsuperscript{+} systems. Similar situations have also been found for pairs of non-F-like plasmids. The X group R factor R6K causes a 400-fold reduction in the transfer level of the N group R factor R46 (Pinney and Smith, 1974) and further examples amongst F, N, X and W group plasmids have recently been described by Olsen and Shipley (1978). It is possible that such phenomena result from recombination between different types of plasmid during evolution (Olsen and Shipley, 1978). There may be some selective advantage in the Fin\textsuperscript{+} phenotype, as the convergent evolution of five Fin\textsuperscript{+} systems suggests. A co-existing Fin\textsuperscript{+} plasmid may be protected from the prevalent F specific bacteriophages that would otherwise attack a cell carrying a genetically or physiologically derepressed F-like plasmid (Hedges et al., 1973). Alternatively the Fin\textsuperscript{+} property may allow a plasmid to monopolize a bacterial population by reducing the transfer frequency of a competing plasmid (Casson and Willetts, 1975).
Figure 11.1. Possible mechanisms for the systems of F fertility inhibition.
The attempts to provide a technology for the biochemical study of transfer gene expression are being continued in this laboratory. In addition to further characterization of fertility inhibition systems, such a technology would allow direct demonstration of the positive control of the transfer operon by \textit{traJ}.

Also gene expression within the transfer operon could be studied. It is likely that some genes within the operon code for structural proteins (e.g. \textit{traA}, which encodes the pilin protein) whilst others code for catalytic enzymes (e.g. \textit{traI}, which probably encodes an endonuclease). Hence some control within the transfer operon may exist to regulate the amounts of different gene products that are produced.
REFERENCES


Achtman, M. and Holcorth, H. 1974. The F factor carries an operon of more than 15 x 10^6 daltons coding for deoxyribonucleic acid transfer and surface exclusion. Microbiology 1: 05-103.


Bonhoeffer, F. 1968. DNA transfer and DNA synthesis during bacterial conjugation. Z. Vererbungsl. 98: 141-149.


Brinton, C.C., Gorski, P. and Carnahan, J. 1964. A new type of
bacterial pilus genetically controlled by the fertility factor
of E. coli K12 and its role in chromosome transfer.

Daichari, A.I. and Zipsor, D. 1972. Random insertion of Mu-1 DNA

Barton, A. and Sinsheimer, R.L. 1969. The process of infection
with bacteriophage \( \phi X 174 \). VII. Ultracentrifugal analysis

Cairns, J. 1963. The bacterial chromosome and its manner of
replicating as seen by autoradiography. J. Mol. Biol. 6:
205-213.

Calef, E., Avitabile, A., del Giudico, L., Marchelli, C., Monna, T.,
Neubauer, Z. and Seller, A. 1971. The genetics of the anti-
immune phenotype of defective lambda lysogens. "The bacterio-


Caro, L.G. and Schmüs, M. 1966. The attachment of the male-
specific bacteriophage f1 to sensitive strains of Escherichia

infectious factor controlling sex compatibility in Bacterium

Chakrabarty, A.K. 1972. Genetic basis of the biodegradation of

Clark, A.J. 1963. Genetic analysis of a "double male" strain


Cooke, M. and Haynell, E. 1969. Chromosomai transfer by
14: 79-87.

Crawford, E.M. and Costeland, R.F. 1974. The adsorption of


100: 1091-1104.

A transferable kanamycin resistance plasmid isolated from
126A: 397-399.

Datta, N. and Hodges, R.W. 1972. Trimethoprim resistance conferred
by V plasmids in Enterobacteriaceae. J. Gen. Microbiol. 72:
345-355.

with anomalous compatibility properties, mobilizing a

Datta, N., Lawn, A.M. and Haynell, E. 1966. The relationship of
F type piliation and F phage sensitivity to drug resistance
48: 365-76.

Datta, N. and Olarte, J. 1974. R factors in strains of Salmonella
 typhi and Shigella dysenteriae I isolated during epidemics in
The DNA sequence organisation of F and of F-primes; and the 
sequences involved inHdr formation. in "Microbiology I".  
Davis, B.D. 1950. Non-filterability of the agents of genetic 
Davison, J., Brammar, W.J. and Brunel, F. 1974. Quantitative 
aspects of gene expression in a λ-trp fusion operon. 
De Haan, P.C. and Stouthamer, A.H. 1963. F-prime transfer and 
and adsorption of the N-specific filamentous phage 1Ke. 
Dunn, N.W. and Gunsalus, I.C. 1973. Plasmid transfer 
and adsorption of the N specific filamentous phage 1Ke. 
Echols, H. 1963. Properties of F' strains of Escherichia coli 
superinfected with F-lactose and F-galactose episomes. 
Egawa, R. and Hirota, Y. 1962. Inhibition of fertility by 
multiple drug resistance factor in Escherichia coli K12. 
Eisen, H.L., Pereira de Silva, L. and Jacob, F. 1968. The 
regulation and mechanism of DNA synthesis in bacteriophage lambda. 

172.


Hsu, S., Ohtsubo, E. and Davidson, N. 1975a. Electron microscope heteroduplex studies of sequence relations among plasmids of Escherichia coli: structures of F13 and related F-primes.


Escherichia coli B into Hfr and F- strains of Escherichia

MacFarren, A.C. and Clowes, R.C. 1967. A comparative study of
two F-like colicin factors, ColV2 and ColV3 in Escherichia


Heynoll, E. and Cooke, N. 1969. Repressor-minus and operator-
constitutive de-repressed mutants of F-like R factors: their
14: 309-313.

Heynoll, E. and Datta, N. 1966. Functional homology of the sex-
207: 684-685.

Heynoll, E. and Datta, N. 1966. The nature and incidence of
Camb. 7: 141-148.

Heynoll, E. and Datta, N. 1967. Mutant drug resistance factors


Heynoll, E., Heynoll, G.G. and Datta, N. 1968. Phylogenetic
relationships of drug resistance factors and other trans-


Cold Spr. Harb. Laboratory.


163.


185.


186.


Five Control Systems Preventing Transfer of *Escherichia coli* K-12 Sex Factor F

M. J. GASSON and N. S. WILLETTS

MRC Molecular Genetic Unit, Department of Molecular Biology, University of Edinburgh

Edinburgh EH9 3JR, Scotland

Received for publication 30 December 1974

The transfer inhibition systems of 28 Fin plasmids have been characterized, using Flac mutants insensitive to inhibition by R100 or R62. All F-like plasmids (except R455) and one N group plasmid determined systems analogous to that of R100; this is designated the FinOP system. None of these plasmids could supply a FinP component of the transfer inhibitor able to replace that of F itself. In addition to the FinOP and R62 transfer inhibition systems described previously, new systems were encoded by the F-like plasmid R455, the I-like plasmid JR66a, and the group X plasmid R485. Besides inhibiting F transfer, JR66a also inhibited F pilus formation and surface exclusion, whereas R485 inhibited only pilus formation and R455 inhibited neither. All three R factors inhibited transfer of J-independent Flac elements, indicating that they act directly on one or more genes (or products) of the transfer operon, rather than indirectly via *traJ*. The *traJ* products and transfer origin sequences of two Fin* F-like plasmids, CoB2 and R124, appear to have similar specificities to those of F itself.

Transfer of the *Escherichia coli* K-12 sex factor F can be substantially reduced by the presence of a second plasmid in the same cell. This phenotype, which we designate Fin* for F fertility inhibition, has been used extensively in the classification of new plasmid isolates (24, 32). It is a frequent characteristic of F-like plasmids that encode transfer systems related to that of F in that they determine pili sensitive to the same male-specific bacteriophages (19, 24). Such plasmids may belong to any one of at least four incompatibility groups *F*1, *F*11, *F*111, or *F*IV (16). More recently several plasmids which are not F-like and belong to other incompatibility groups have been shown to be Fin*. These include the I-like plasmids R62, JR66a, and TP101 to TP109 (6, 12, 21) and plasmids belonging to the N, and X incompatibility groups (13, 18). Furthermore, many other Fin* plasmids have been reported that are not *R* or Col factors, and that have not yet been assigned to incompatibility groups; these include plasmids determining synthesis of enterotoxin, hemolysin, and the K88 antigen (20, 42, 43, 44), as well as an otherwise cryptic plasmid present in many *Salmonella* strains (30).

We have screened a total of 28 Fin* plasmids of all the types mentioned above in a preliminary analysis of their mechanisms of F transfer inhibition. It has already been shown that the inhibition mechanisms of the F-like plasmids R100 and R136 are different from that of the I-like plasmid R62 (21, 40), and for our analysis we have exploited Flac mutants insensitive to inhibition by R100 or by R62. Mutations in *traO* and *finP* (previously *traP*) prevent inhibition by R100 (8), whereas mutations in *traQ* prevent inhibition by R62 (40). *traO* and *traQ* mutations are dominant and are presumed to affect the sites of action of the R100 and R62 transfer inhibition systems, respectively, whereas *finP* mutations are recessive and probably inactivate an F-specified component (FinP*) required for F inhibition by R100 (8, 40). Fin* plasmids having the same transfer inhibition mechanisms as R100 or R62 should therefore not inhibit transfer of Flac *traO* or of Flac *traQ* mutants, respectively, whereas plasmids determining other mechanisms should inhibit transfer of both.

By these means, three plasmids with new transfer inhibition system(s) were identified. These were characterized further by determining whether F pilus formation and/or surface exclusion were also inhibited. The genes encoding these two functions, together with those for conjugal deoxyribonucleic acid metabolism, form a single operon (36, 37; R. Helmuth and M. Achtman, personal communication) and simultaneous control of all three functions is therefore possible. Furthermore, expression of this operon requires the product of the gene *traJ*
MATERIALS AND METHODS

Terminology. We have tried to rationalize the symbols concerned with fertility inhibition systems. The gene previously denoted \( i \) (7), \( f i \) (32), or \( f i n \) (9) we now write as \( finO \). The gene specifying the second component of the transfer inhibitor, previously written as \( traP \), we have renamed \( finP \). Together, the products of these two genes constitute the FinOP system for transfer inhibition, which acts at \( traO \) (8) to prevent synthesis of the \( traJ \) product. Analogous symbols can be used for other fertility inhibition systems: for example, \( R62 \) carries a gene \( finQ \) which acts at \( traQ \) (40) to prevent \( F \) transfer.

Bacterial strains. These are described in Table 1.

Bacterial plasmids. The \( Fin^+ \) plasmids which were tested are listed in Tables 2 and 3. References describing the \( Fin^+ \) nature of the plasmid, the antibiotic resistance pattern or other phenotype, and, where known, the incompatibility group are given.

We are grateful to E. S. Anderson, N. Datta, E. Meynell, E. E. M. Moody, F. Ørskov, and H. Williams-Smith for gifts of strains carrying these plasmids.

The wild-type \( Flac \) element JCFL0 was used (2). EDFL67 (\( Flac \) traO3O4) and EDFL51 (\( Flac \) finP301) are mutants of JCFL0 insensitive to inhibition by \( R100 \) (8), and WPFLL39 (\( Flac \) traQ312) is a mutant insensitive to inhibition by \( R62 \) (40). The \( J \)-incompatible \( Flac \) elements JCFL119 and JCFL130 were isolated and described by Achtman (1).

Media. These have been described (8).

Mating techniques. The donor abilities of plasmid-carrying strains were measured in 30-min matings as described by Finnegam and Willetts (8). Surface exclusion was measured in matings between the Hfr strain KL98 and the JC3272 derivative classified into incompatibility groups, strains were always tested to ensure that >95% of the cells carried the relevant plasmid(s).

The exponential cultures of plasmid-carrying strains were measured in 30-mm matings as described by Finnegam and Willetts (8). Surface exclusion was measured in matings between the Hfr strain KL98 and the JC3272 derivative classified into incompatibility groups, strains were always tested to ensure that >95% of the cells carried the relevant plasmid(s).

The relationships of the plasmid transfer inhibition systems to those of \( R100 \) and \( R62 \) are apparent from the results in the last three columns of Table 2. All \( F_1 \), \( F_{111} \), \( F_{111} \), and \( N \) group plasmids tested inhibited transfer of \( Flac \) traQ\(-\), but not of \( Flac \) traO\(-\) or of \( Flac \) finP\(-\). The transfer inhibition systems of all these plasmids are therefore related to that of \( R100 \); for convenience, we shall refer to this common system as FinOP. However, none of the plasmids synthesized a \( finP \) product able to replace that of \( F \) itself, and thus inhibit \( Flac \) finP\(-\) transfer. They may either synthesize one of different specificity (as is known to be the case for \( R100 \), \( R1 \), \( R136 \), and \( R6 \) [9, 30]), or, especially in the case of the \( N \) group plasmids, not synthesize one at all.

Three other \( Fin^+ \) plasmids (\( JR66a \), \( R485 \), and \( R455 \)) each inhibited the transfer of \( Flac \) traO\(-\), \( Flac \) finP\(-\), and \( Flac \) traQ\(-\) mutants to the same extent as that of wild-type \( Flac \) (Table 2). Their \( F \) transfer inhibition systems must therefore be different from those of \( R100 \) and \( R62 \). It is particularly interesting that the two \( I \)-like plasmids \( R62 \) and \( JR66a \) specify different transfer inhibition systems (although they also be-
### Table 2. Inhibition of Flac transfer by Fin+ plasmids

<table>
<thead>
<tr>
<th>Fin+ plasmid</th>
<th>Reference</th>
<th>Incompatibility group</th>
<th>Flac mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>tra*</td>
</tr>
<tr>
<td>R455</td>
<td>18</td>
<td>F¹</td>
<td>0.2</td>
</tr>
<tr>
<td>R1</td>
<td>16, 23</td>
<td>F₁₁</td>
<td>0.8</td>
</tr>
<tr>
<td>R6</td>
<td>33</td>
<td>F₁₁</td>
<td>0.2</td>
</tr>
<tr>
<td>R100</td>
<td>16, 31</td>
<td>F₁₁</td>
<td>0.06</td>
</tr>
<tr>
<td>R136</td>
<td>22</td>
<td>F₁₁</td>
<td>0.06</td>
</tr>
<tr>
<td>R192</td>
<td>22</td>
<td>F₁₁</td>
<td>0.2</td>
</tr>
<tr>
<td>R444</td>
<td>18</td>
<td>F₁₁</td>
<td>0.2</td>
</tr>
<tr>
<td>R538-1</td>
<td>28</td>
<td>F₁₁</td>
<td>0.2</td>
</tr>
<tr>
<td>ColB2</td>
<td>11, 15</td>
<td>F₁₁</td>
<td>2.3</td>
</tr>
<tr>
<td>ColB4</td>
<td>4, 16</td>
<td>F₁₁⁺</td>
<td>0.3</td>
</tr>
<tr>
<td>ColB1</td>
<td>4</td>
<td>F₁₁⁺</td>
<td>0.3</td>
</tr>
<tr>
<td>R124</td>
<td>16</td>
<td>F₁Ⅳ</td>
<td>9.4</td>
</tr>
<tr>
<td>R62</td>
<td>14, 21</td>
<td>Fα</td>
<td>1.1</td>
</tr>
<tr>
<td>R455</td>
<td>13, 22</td>
<td>N</td>
<td>0.02</td>
</tr>
<tr>
<td>R389</td>
<td>3</td>
<td>(W)⁺</td>
<td>0.2</td>
</tr>
<tr>
<td>R404</td>
<td>5</td>
<td>(W)⁺</td>
<td>0.2</td>
</tr>
<tr>
<td>R485</td>
<td>18</td>
<td>X</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Except for R100 and R62 (these results are taken from reference 40), the donor strains were derivatives of JC5455 and the recipient strain was JC3272, ED57 (ColVB¹) or ED26 (Col¹) as appropriate. The number of Lac⁺ progeny per 100 donor cells is given. R6, R62, R124, R128, R389, R404, R444, R455, R485, and JR66a were supplied by N. Datta; R1, R192, ColB1 (ColB-CA18), ColB2 (ColB-K77), and ColB4 (ColB-K98) by E. E. M. Moody; R100 and R538-1 by E. Meynell; and R136 by E. S. Anderson.

The incompatibility groups of these plasmids is questionable. In our hands, cells carrying Flac together with either R455 or R124 segregated at similar low rates, but our criterion that >95% of the cells in the donor population should carry both plasmids was satisfied when cultures were inoculated from fresh single colonies. All the antibiotic resistance markers of R455 were transferred or lost simultaneously with the Fin character. CoIB1 has not been grouped, and Col134 is sometimes incompatible with Flac group R factors (9). R389 and R404 are probably composite R factors, the Flac and Tet characters being carried by plasmids not of the W incompatibility group (5).

long to different incompatibility groups) and that the F transfer inhibition system of the Flac plasmid R455 is different from that of the other Flac plasmids (see below).

The results for R455, JR66a, and R485 were confirmed by showing that second examples of each type of inhibitor-insensitive mutant (Flac tra0305, Flac finP303, and Flac traQ316) were also subject to transfer inhibition by these R factors. Furthermore, the Flac elements transferred (alone) from cells carrying an inhibitor-insensitive Flac mutant together with one of these R factors were shown to retain the mutations leading to inhibited sensitivity.

**Inhibition of F pilus formation.** Strains carrying both an Flac element and one of the Fin+ plasmids described in the preceding section were tested for their sensitivities to f1, f2, and Qf. Resistance was taken as an indication of the absence of the F pilus.

As expected, those plasmids with transfer inhibition systems of the FinOP type all prevented pilus formation by wild-type Flac and Flac traQ⁻, but not by Flac traO⁻ or Flac finP⁻ (Table 3). Of the three plasmids with new transfer inhibition systems, JR66a and R485 both inhibited pilus formation by wild-type Flac, Flac traO⁻, Flac traP⁻, and Flac traQ⁻: these R factors must therefore inhibit expression of at least one of the genes required for both pilus formation and transfer.

R455, on the other hand, did not inhibit pilus formation by either the wild-type or mutant Flac elements, despite its approximately 650-fold reduction in the transfer levels of these elements. This result was surprising, since cells carrying R455 alone transferred the R factor at a low level and were resistant to F-specific phages; superficially, then, R455 resembles other wild-type F-like R factors. Although in our hands R455 showed only limited incompatibility with Flac, it has previously been assigned to the Flac incompatibility group (18). We therefore tested its effects on R1-19 (carrying a finO mutation) and R1-16 (carrying a finP or tra0 mutation), mutants of the Flac group plasmid R1 which with R455 is fully compatible. R455 did not inhibit transfer or pilus formation by either
Table 3. Inhibition of Flac pilus formation of Fin+ plasmids

<table>
<thead>
<tr>
<th>Fin+ plasmid</th>
<th>Reference</th>
<th>Flac mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tra⁺</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>R455</td>
<td>S⁺</td>
<td>S⁺</td>
</tr>
<tr>
<td>R100</td>
<td>R⁺</td>
<td>R⁺</td>
</tr>
<tr>
<td>ColB4</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>R124</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R62</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>JR66à</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R128</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R404</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>R485</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>MP10-36</td>
<td>30</td>
<td>R</td>
</tr>
<tr>
<td>Ent-H19⁺</td>
<td>43, 44</td>
<td>R</td>
</tr>
<tr>
<td>Ent-P3⁺</td>
<td>43, 44</td>
<td>R</td>
</tr>
<tr>
<td>Ent-P307⁺</td>
<td>43, 44</td>
<td>R</td>
</tr>
<tr>
<td>Hly-P233⁺</td>
<td>42, 44</td>
<td>R</td>
</tr>
<tr>
<td>Vir-S5</td>
<td>41</td>
<td>R</td>
</tr>
<tr>
<td>K88</td>
<td>20, 44</td>
<td>R</td>
</tr>
<tr>
<td>Tra-A14</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Tc-D1122</td>
<td>25</td>
<td>R</td>
</tr>
<tr>
<td>H,S-D1148</td>
<td>25</td>
<td>R</td>
</tr>
</tbody>
</table>

* Sensitivity or resistance to the male-specific bacteriophages f1, f2, and Q5 was determined by spot tests and is indicated by the letters S and R, respectively. The host strains were: JC5455 for the plasmids described in Table 2; S. typhimurium 36 for MP10-36; Nal⁺ or Str⁺ derivatives of RC711 for the Ent, Hly, Vir, and Tra plasmids; and W1067 for Tc-D1122, H,S-D1148 and K88.

* Sources and references for the first group of plasmids are given in footnote (c) to Table 2. MP10-36 was supplied by E. S. Anderson; Ent-P3, Ent-H19, Ent-P307, Hly-P233, Vir-S5, and Tra-A14 by H. Williams-Smith; and Tc-D1122, H,S-D1148 and K88 by F. Ørskov. Tra-A14 is a temperature-sensitive transfer factor (H. Williams-Smith, personal communication).

* It is possible that the Fin⁺ character of the strains carrying Ent and Hly plasmids is determined by a second, coexisting plasmid (44).

* Cells taken from the lawns on which these results were obtained were >95% Lac⁺ Tet⁻, proving that male-specific phage sensitivity was not due to the segregation of R⁺(Flac⁺) cells.

* R1, R6, R136, R192, R444, R538-1, ColB2, ColB1, and R389 also gave this result.

R1-19 or R1-16, although R455 elements reisolated from these strains retained the ability to inhibit their own transfer and pilus formation, and to inhibit transfer (but not pilus formation) by Flac. We conclude that, unlike other F-like plasmids, the control system responsible for inhibition of transfer and pilus formation by R455 does not involve a nonspecific finO product. Possibly R455 does determine a FinOP system acting at its traJ gene, but with an R455-specific finO product. Alternatively, it may determine a completely different transfer control system. Transfer inhibition of F might be due to some component of the latter, or to a second R455 transfer inhibition system. In either case, the transfer inhibition system for F (which does not inhibit R1-19) must prevent expression of a gene required for F transfer but not for F pilus formation. These results indicate that the best initial test for the Fin⁺ or Fin⁻ character of a newly isolated plasmid is inhibition of F transfer, rather than of F-specific phage sensitivity.

Since (except for R455) the tests for F-specific phage sensitivity gave the results expected from the donor abilities of the four Flac elements in the presence of the Fin⁺ plasmids, this simple procedure was used to screen a variety of other Fin⁺ plasmids for the nature of their transfer inhibition systems. All of these, although of widely diverse origins and phenotypes, possessed systems of the FinOP type (Table 3). Again, none of them synthesized a FinP component able to replace that of F itself.

Effects of R455, JR66a, and R485 on surface exclusion. Transfer inhibition requires that expression of at least one of the F transfer genes be prevented. However, since these genes form a single operon, several or all of them might be controlled simultaneously. An indication that this is the case can be obtained by showing that surface exclusion is also inhibited. The gene(s) responsible for surface exclusion (traS), although a component of the transfer operon, is not required for transfer per se (35; M. Achtman and N. S. Willetts, manuscript in preparation). It has been shown previously that both R100 and R62 inhibit surface exclusion by F, and that this is because they prevent expression of all or part of the transfer operon (10, 38; Gasson and Willetts, unpublished data).

Of the three plasmids with new transfer inhibition systems, JR66a prevented surface exclusion by Flac, whereas R455 and R485 did not (Table 4). In fact, cells carrying both R455 and Flac had a particularly high level of surface exclusion, perhaps due to a contribution from R455 itself. As found for donor ability and for pilus formation, the presence of a traO, finP, or traQ mutation on the Flac element had no effect.

We conclude that JR66a might affect expression of the entire F transfer operon, whereas R455 and R485 do not. Since it was shown in the preceding section that F transfer inhibition by R455 differs from that by R485 in that pilus formation is not prevented, the transfer inhibition systems of the three plasmids must be different from each other, as well as from those

TABLE 3. Inhibition of Flac pilus formation of Fin+ plasmids
of R100 and R62.

Transfer inhibition of J-independent Flac elements. Expression of the transfer operon can be prevented either directly or indirectly via inhibition of the synthesis or function of the tral product. One way to determine which alternative is correct is to study the effect of the Fin* plasmid on transfer of J-independent Flac elements. These carry an amber mutation together with a further mutation allowing partial expression of the transfer operon in the absence of the tral product; the resulting low levels of transfer are therefore called J-independent (1). Transfer of such elements was not inhibited by R100 (1, 40) consistent with the finding that tral is the primary site of action of the FinOP system (10), but was reduced by R62, which acts directly on the transfer operon (40; Gasson and Willetts, manuscript in preparation).

R455, JR66a, and R485 each inhibited transfer of two J-independent Flac elements (Table 5). All three R factors must therefore act directly, and not via tral, to prevent synthesis or function of one or more genes of the transfer operon. This is consistent with the absence of any effect of R455 on surface exclusion and pilus formation, or of R485 on surface exclusion, since neither of these F phenotypes would be expressed in the absence of the tral product.

Specificity of the plasmid transfer systems. When R or Col factors with transfer inhibition systems of the FinOP type were present in cells together with Flac tralO− or Flac finP− elements, a fully active F transfer system was synthesized (Table 2). From these cells, transfer of the R or Col factor would also take place at high frequency, despite inhibition of the synthesis of its own transfer system, if it carried a site on its deoxyribonucleic acid recognized as the origin of transfer by the F transfer system. The plasmid specificities of the tral products of various F-like plasmids have been described previously (3, 37), and these have been correlated with the specificity of the F origin of transfer (27).

In the mating experiments described in Table 2, therefore, transfer of the R or Col factor was also measured; results for FinOP+ plasmids are given in Table 6. The transfer levels of most plasmids were increased slightly (to frequencies of less than 1%) in the presence of Flac tralO− or Flac finP−, as compared to transfer from F− cells or cells carrying wild-type Flac. This could be due to recombination between the Flac elements and the R or Col factors, or to some overlap in specificity of the F tral and/or tral products (37), or to partial expression of the plasmids tral gene even during transfer inhibition.

ColB2 and R124, however, transferred at frequencies similar to those of the Flac elements in all cases: in particular, their transfer from cells carrying Flac tralO− or Flac finP− was around 100% (Table 6). This result suggests that the origin sequences of F, ColB2, and R124 are closely related and predicts that their tral products have similar specificities. Alternatively (or in addition), the tral products of these three plasmids could be interchangeable; that of the inhibitor-insensitive Flac element would then allow expression of the R or Col factor tral gene.

The prediction that the tral products of F and of ColB2 are interchangeable was confirmed by showing that a finO+ finP− mutant of ColB2

### Table 4. Inhibition of Flac surface exclusion

<table>
<thead>
<tr>
<th>Fin+ plasmid</th>
<th>Flac element</th>
<th>Flac tralO</th>
<th>Flac finP</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>R100</td>
<td>2.6</td>
<td>6</td>
<td>550</td>
</tr>
<tr>
<td>R62</td>
<td>1.2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>R455</td>
<td>13</td>
<td>1900</td>
<td>2200</td>
</tr>
<tr>
<td>JR66a</td>
<td>0.9</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>R485</td>
<td>0.8</td>
<td>620</td>
<td>640</td>
</tr>
</tbody>
</table>

*The figures are surface exclusion indices, defined as the ratios of the number of His+ recombinants obtained with the F− strain JC3272 to the numbers obtained with the JC3272 derivative carrying the plasmid(s) indicated, in crosses with Hfr KL98. The results for strains carrying Flac elements together with no R factor, R100, or R62, are taken from Willetts and Paranchych (40).

### Table 5. Transfer inhibition of J-independent Flac elements

<table>
<thead>
<tr>
<th>Fin+ plasmid</th>
<th>Flac element</th>
<th>JCFL119</th>
<th>JCFL130</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.3</td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>R100</td>
<td>1.6</td>
<td>0.988</td>
<td></td>
</tr>
<tr>
<td>R62</td>
<td>0.038</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>R455</td>
<td>0.0004</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>JR66a</td>
<td>0.003</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>R485</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Derivatives of JC6256 carrying either of the two J-independent Flac elements JCFL119 or JCFL130 together with the Fin+ plasmid were used as donors in crosses with ED2197. The figures represent the number of Lac+ [Nal+] progeny per 100 donor cells. The results for no plasmid, R100, and R62 are taken from Willetts and Paranchych (40).
Our results suggest that the majority of F-like plasmids specify analogous mechanisms for inhibition both of their own transfer and of the F factor. We shall refer to this mechanism in which two separate proteins (the finO and finP products) act together to inhibit the synthesis or function of the trad control protein (8, 10) as the FinOP system. Furthermore, one Fin\(^+\) plasmid of the N incompatibility group, and twelve of as yet unidentified incompatibility groups and pilus types, also carried a finO gene capable of inhibiting F transfer. The similarities of the transfer inhibition systems of the Salmonella plasmid MP10 and of the N group plasmid R128 to those of F-like R factors has already been noted (13). Confirming previous observations (9, 24), the finO components appear to be relatively nonspecific, serving to inhibit transfer both of the plasmid itself and of the F factor (which probably carries a finO\(^-\) deletion). However, the extents by which the various finO\(^+\) plasmids reduced F transfer ranged from 14- to 5,000-fold, and this might reflect some degree of specificity of the finO product. This could also explain why, although most finO\(^+\) plasmids inhibited transfer of themselves and of F to similar extents, R124 was much more effective at inhibiting its own transfer, whereas R538-1 and ColB4 were much less effective.

The other component of the FinOP transfer inhibitor, the finP product, is much more specific (9). In fact none of the 24 finO\(^+\) plasmids tested determined a finP product able to replace that of F itself. It is possible, of course, that some of these plasmids, particularly those of the N and W incompatibility groups, do not carry a finP gene. By taking into consideration the plasmid specificities of the finP components of F-like plasmids, as well as those of their trad products, we can explain the apparently unusual properties of the “derepressed” mutants ColB2 fdr (15) and R124 drd (E. Meynell and A. M. Lawn, Proc. Soc. Gen. Microbiol. 1:2, 1973). The basis for this is that both are finP\(^-\) mutants, and that, whereas the trad products (and transfer origins) of F, ColB2, and R124 are interchangeable (see Results), the finP product of F differs from that of ColB2 and R124. As a result, (i) F is transferred at a high frequency in the presence of ColB2 fdr (15) or R124 drd (Proc. Gen. Soc. Microbiol. 1:2, 1973), because although expression of the F transfer system is inhibited, the ColB2 fdr and R124 drd transfer systems can themselves be efficiently used for F transfer; (ii) transfer of finO\(^-\) R factor mutants is inhibited by R124 drd (Proc. Gen. Soc. Microbiol. 1:2, 1973), and this is seen because these finO\(^-\) R factors require trad products of different specificities and cannot be transferred by the R124 transfer system; (iii) ColB2 fdr transfer is not inhibited by R100 (8) or by the finO\(^+\) F group plasmid R386 (our unpublished data), but is inhibited by R124 (our unpublished data) since FinP\(_{R124} \neq FinP\(_{ColB2}\) = FinP\(_{R124} \neq FinP\(_{R386}\); (iv) R124 drd transfer is not inhibited by ColB4 or R100 but is inhibited by ColB2 (Proc. Gen. Soc. Microbiol. 1:2, 1973) since FinP\(_{ColB2}\) \neq FinP\(_{ColB2}\) = FinP\(_{R124} \neq FinP\(_{R100}\).

In addition to the FinOP transfer inhibition system and that of R62 (21, 40), we have characterized three new Fin systems, those of R455, J666a, and R485. It had been suggested previously that the Fin\(^+\) systems of J666a and R485 might be different from those of most F-like plasmids (6, 18). The properties of all five systems are summarized in Table 7, and our present understanding of their mechanisms can be summarized as follows: (i) the FinOP system acts at \(traO\) to prevent synthesis of the \(traJ\) product which is in turn required for expression of the transfer operon (10, 35); (ii) the R62

---

**DISCUSSION**

The donor strains were derivatives of JC5455, and the recipient strain was JC3272. The markers selected are listed for each plasmid in Materials and Methods, and \([Str]\) was contraselected. The numbers of plasmid-carrying \([Str]\) progeny per 100 donor cells are given.

(15; see Discussion) transferred a coexisting Flac \(tra65\) element with a high frequency (77%), similar to that of its own transfer (160%).

---

**TABLE 6. Transfer of FinOP plasmids in the presence of Flac elements\(^a\)**

<table>
<thead>
<tr>
<th>Fin(^+) plasmid</th>
<th>Flac element</th>
<th>Flac</th>
<th>Flac trad(_{3034})</th>
<th>Flac finP(_{301})</th>
</tr>
</thead>
<tbody>
<tr>
<td>R455</td>
<td>None</td>
<td>0.001</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>R1</td>
<td>0.07</td>
<td>0.08</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>R6</td>
<td>0.2</td>
<td>0.3</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>R100</td>
<td>0.3</td>
<td>0.3</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>R136</td>
<td>0.005</td>
<td>0.006</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>R192</td>
<td>0.4</td>
<td>0.2</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>R444</td>
<td>0.1</td>
<td>0.1</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>R538-1</td>
<td>4.9</td>
<td>2.2</td>
<td>3.8</td>
<td>2.9</td>
</tr>
<tr>
<td>ColB2</td>
<td>1.3</td>
<td>1.5</td>
<td>86</td>
<td>79</td>
</tr>
<tr>
<td>ColB4</td>
<td>10</td>
<td>31</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>ColB1</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>R124</td>
<td>0.6</td>
<td>13.3</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>R128</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>R389</td>
<td>0.07</td>
<td>0.01</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>R404</td>
<td>0.04</td>
<td>0.09</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\(^a\) The donor strains were derivatives of JC5455, and the recipient strain was JC3272. The markers selected are listed for each plasmid in Materials and Methods, and \([Str]\) was contraselected. The numbers of plasmid-carrying \([Str]\) progeny per 100 donor cells are given.
system acts at \( \text{tra}Q \) to give premature termination of transcription of the transfer operon; \( \text{tra}Q \) is located near to \( \text{tra}C \) (40; Gasson and Willetts, unpublished data); (iii) the \( R455 \) system inhibits \( F \) transfer without affecting pilus formation or surface exclusion, and may therefore prevent synthesis or function of a transfer product (such as that of \( \text{tra}R \)) which is not required for these properties; (iv) the \( J R66a \) system directly prevents expression of all or part of the transfer operon, but by a mechanism different from that of \( R62 \); (v) the \( R485 \) system inhibits pilus formation but not surface exclusion, and since \( \text{tra}S \) is transcribed after the pilus-forming genes this system cannot prevent transcription of the transfer operon, but may prevent translation or product function of one or more pilus-forming genes.

Most \( F^+ \) \( F \)-like plasmids inhibit \( F \) transfer simply because their transfer control systems, like their transfer systems, are similar to those of \( F \) itself. It is therefore curious that three \( F \)-like R plasmids (\( R62, J R66a, \) and \( R485 \)) falling into the \( I_\alpha, I_\omega \) and \( X \) incompatibility groups and probably having quite unrelated transfer systems should carry genes able to accomplish the same purpose but by different mechanisms. It seems unlikely that their \( F \) transfer inhibition systems also prevent their own transfer, especially in the case of \( R62 \) (21). Rather, the convergent evolution of at least five different systems for inhibiting the fertility of \( F \)-like plasmids suggests that this confers some selective advantage on a coexisting plasmid. This might be because of the prevalence of \( F \)-specific plages which would otherwise attack a cell carrying a genetically or physiologically derepressed \( F \)-like plasmid, as suggested by Hedges et al. (18). Alternatively, further reduction in the transfer frequency of a competing \( F \)-like plasmid by a different transfer inhibition system might enable the \( F^+ \) plasmid to monopolize a bacterial population. For example, \( J R66a \) reduced the transfer frequency of \( R100 \) by 1,000-fold (our unpublished data), of \( R124 \) by 100-fold, and of \( R136 \) by 10-fold (6). A similar situation, in which transfer of an \( N \) group plasmid was reduced 400-fold by an \( X \) group plasmid, has been described by Finney and Smith (26).

### ACKNOWLEDGMENTS

M.J.G. is the holder of an M.R.C. Scholarship for Training in Research Methods. A preliminary account of this work was presented at the First International Congress of the International Association of Microbiological Societies, held in Tokyo, Japan, during September 1974.

### LITERATURE CITED


### Table 7. Summary of the characteristics of the transfer inhibition systems

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Incompatibility group</th>
<th>Pilus formation</th>
<th>Surface exclusion</th>
<th>J-independent Flac transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>R100*a</td>
<td>( F_{I1} )</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>R128</td>
<td>( N )</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>R389, R404</td>
<td>( (W) )</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>R62</td>
<td>( I_\alpha )</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>R455</td>
<td>( F_1 )</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>JR66a</td>
<td>( I_\omega )</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>R485</td>
<td>( X )</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*a All \( F_{I1}, F_{I1H}, \) and \( F_{X} \) group plasmids tested, as well as those as yet unclassified, also determined transfer inhibition systems with these characteristics. We call this the FinOP system.