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The relationship between the transfer systems of some bacterial plasmids

By GUILLERMO ALFARO* and NEIL WILLETTS
M.R.C. Molecular Genetics Unit, Department of Molecular Biology, University of Edinburgh, Scotland

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SUMMARY

The relationship between the transfer systems of several plasmids was investigated, using as criteria complementation of a series of transfer-deficient F*lac* mutants, the efficiency of plating of F-specific phages, and identification of surface exclusion systems. The transfer systems of ColV2 and ColVB*trp* were similar to that of F except for surface exclusion: ColVB*trp* specified a surface exclusion system different from that of F, and ColV2 did not specify any detectable system. The transfer system of R1–19 was also similar to that of F*lac*, but the products of *traA*, *traI* and *traJ* were plasmid-specific. R1–19 determined the same surface exclusion system as ColVB*trp*, and this was under the control of the transfer inhibitor. The transfer systems of ColIb*drd* and F*lac* were unrelated. ColE1 was transferred by F*lac* *traI*$^-$ mutants, but not by mutants in other cistrons.

1. INTRODUCTION

Amongst the transmissible plasmids which can use *Escherichia coli* K12 as host, many specify a pilus related to that of the F factor in serving as the site of adsorption of F-specific bacteriophages; these plasmids have been called F-like (Meynell, Meynell & Datta, 1968). However, differences have been found in the detailed serological and F-specific phage adsorption properties of pili produced by different F-like plasmids (Lawn & Meynell, 1970; Nishimura *et al.* 1967; Willetts, 1971). We have compared the transfer systems of the F-like plasmids ColV2, ColVB*trp* and R1–19 with that of F*lac* at a genetic level by determining the ability of these plasmids to complement a series of transfer-deficient F*lac* mutants. Complementation was measured both for transfer and for formation of the F-pilus (using the efficiency of plating of F-specific phages as criterion). The extent of surface exclusion in matings between strains carrying these plasmids was also measured; surface exclusion is closely related to the transfer properties of F*lac* (Willetts & Finnegan, 1970; Finnegan & Willetts, 1971; Willetts & Achtman, 1972).

Members of a second group of plasmids, called I-like, specify a pilus which adsorbs a different class of bacteriophages (Meynell *et al.* 1968). The prototype of

* Present address: Departamento de Genética, Centro de Investigación y de Estudios Avanzados del I.P.N., México, 14, D.F., México.

this group is ColIb, and complementation experiments between a 'derepressed' mutant of this and the Tra⁻F*lac* mutants were therefore performed, to determine whether the difference in pilus type reflected a complete dissimilarity of their transfer systems.

Finally, the transfer abilities of cells carrying the non-transmissible plasmid ColE1 and a Tra⁻ F*lac* mutant were investigated. ColE1 differs radically from most transmissible plasmids both in its small size and in the large number of copies per chromosome found in *E. coli* (Helinski & Clewell, 1971). It is of particular interest since it is transferred at a very high frequency in the presence of F (Clowes, 1963; Nagel de Zwaig & Puig, 1964).

2. MATERIALS AND METHODS

Bacterial and phage strains

The bacterial strains are described in Table 1. Plasmids were obtained from the following sources: F*lac* and F*lac tra*⁻ mutants from the laboratory collection (Achtman, Willetts & Clark, 1971, 1972; Willetts & Achtman, 1972); ColV–K94 (here called ColV2; Kahn & Helinski, 1964; MacFarren & Clowes, 1967) from Dr R. C. Clowes; ColVB*trp* (Fredericq, 1969) from Dr C. Yanofsky; R1, R1–19 (Meynell & Datta, 1967), R1–19K⁻ (a kanamycin-sensitive segregant of R1–19), ColIb*drd* and ColE1 (Meynell *et al.* 1968; Nagel de Zwaig & Puig, 1964) from Dr E. E. M. Moody; R100 and R100–1 (Egawa & Hirota, 1962) from Dr E. Meynell; R100–99, a tetracycline- and chloramphenicol-sensitive mutant of R100–1 (Hashimoto & Hirota, 1966), from Dr T. G. B. Howe; and R100Tet^{s}, a tetracyline-sensitive segregant of R100, from N. D. F. Grindley.

Bacteriophages f1, f2 and Qβ were obtained from Dr R. C. Valentine, and M12 from Dr P. Hofschneider. Methods for preparing lysates of these phages and of T6, and of assaying them, have been described previously (Achtman *et al.* 1971, 1972).

Table 1. *Bacterial strains*

Strain no.	Lac	His	Trp	Str	Spc	T6	Other markers
ED28	−	−	−	R	S	R	Lys⁻ ColVB^{R}
ED55	+	+	+	S	S	S	Hfr, ColVB^{R}
ED56	−	+	−	S	S	S	ColVB^{R}
ED664	−	−	−	R	S	R	ColE1^{R}
JC3051	−	−	−	R	S	R	.
JC3272	−	−	−	R	S	R	Lys⁻
JC5455	−	−	−	S	R	R	.
JC6256	−	+	−	S	S	S	.
RC580	+	+	+	S	S	S	Met⁻ (ColV2)⁺

The nomenclature is that recommended by Taylor (1970). ED28, ED55 and ED56 are ColVB^{R} derivatives of JC3272, KL98 and JC6256 respectively. ED664 is a ColE1^{R} derivative of JC3051. JC3051, JC3272, JC5455 and JC6256 are related, and have been described previously (Achtman *et al.* 1971). RC580 is a derivative of 58–161 (MacFarren & Clowes, 1967).

Media

These have been described previously (Willetts & Finnegan, 1970). Antibiotics were used at the following concentrations to select for cells carrying R factors: tetracycline, 40 μg/ml; ampicillin, 25 μg/ml; chloramphenicol, 25 μg/ml; kanamycin, 50 μg/ml; sulphadimidine, 100 μg/ml.

Bacterial matings

To determine the frequency of plasmid transfer, 0·5 ml of an exponential culture of the donor strain at 2×10^8 cells/ml was mixed with 4·5 ml of a similar culture of the recipient strain in a 100 ml conical flask, and the mixture incubated at 37 °C for 30 min. Mating was then interrupted either by violent agitation (Low & Wood, 1965) or by mixing 1 ml of the mating mixture with 1 ml of u.v.-irradiated T6 phage at 5×10^{10} particles/ml and incubating for a further 15 min. Dilutions were plated by the soft agar overlay technique, using 2·5 ml molten 0·7 % agar to which 0·1 ml L broth had been added to avoid step-down conditions (Gross, 1963). The parental cultures were always checked to confirm that $\geqslant$ 98 % of the cells carried the relevant plasmid(s).

Progeny carrying the various plasmids were selected as follows: F*lac*, Lac$^+$ derivatives of a Lac$^-$ strain; ColVB*trp*, Trp$^+$ derivatives of a Trp$^-$ strain; R1–19, kanamycin resistance; R1 and R1–19K$^-$, ampicillin or chloramphenicol resistance; R100 and R100–1, tetracycline or chloramphenicol resistance. Clones carrying ColE1 or ColIb*drd* were detected by the stab or triple-layer overlay techniques (Monk & Clowes, 1964), and those carrying ColV2 by patching and replica-plating on to a non-selective minimal medium plate spread with 0·1 ml of an overnight culture of an indicator strain. Other methods for detection of clones carrying ColV2 gave poor results.

Matings to measure surface exclusion were performed as described by Achtman, Willetts & Clark (1971). Plasmid derivatives of strains resistant to the colicins produced by ColVB*trp* and ColV2 were used in these experiments, to prevent killing of donor or recipient cells in the 1:1 mating mixtures. Selection was applied only for acquisition of the plasmid present in the donor strain, not for retention of that originally present in the recipient strain.

3. RESULTS

Complementation of F lac tra$^-$ *mutants by ColV2*

Since ColV2 does not coexist with F*lac* (Kahn & Helinski, 1964; MacFarren & Clowes, 1967), a method similar to that of Achtman, Willetts & Clark (1972) was used. This measured complementation in transient populations of cells carrying both ColV2 and an F*lac tra*$^-$ mutant. In brief, ColV2 was transferred from RC580 (T6^s Spc^s) to F$^-$ phenocopied cells of JC3272 (T6^R Spc^s) carrying an F*lac tra*$^-$ mutant. After killing the donor strain with T6, diluting into fresh broth and incubating to allow expression of the *tra*$^+$ cistrons, the transfer ability of the resultant cells carrying both ColV2 and the F*lac tra*$^-$ mutant was determined in matings

Table 2. *Complementation of F*lac tra⁻ *mutants*

F*lac* mutation	F-specific phage group	Complementing plasmid			
		ColV2	ColVB*trp*	R1–19	ColIb*drd*
tra⁺	III	70	100	110	125
traA1	I	20	35	75	0·04
traB16	I	10	45	20	0·09
traC12	I	40	50	30	0·07
traD83	II	135	60	110	0·04
traE18	I	20	85	25	0·10
traF13	I	25	75	45	0·07
traG42	III	1·1	45	75	0·04
traG81	I	25	65	50	0·06
traH88	I	20	35	140	0·10
traI65	III	10	75	0·19	0·11
traJ90	I	20	80	0·14	0·20
traK105	I	30	55	20	0·003

The efficiency of complementation by ColV2 was measured in transient populations of heterozygous cells, and by the other (compatible) plasmids in established derivatives of JC6256. The figures indicate the number of Lac⁺ progeny per 100 donor cells carrying both plasmids.

The F-specific phage groups are: I, $f1^R$ $f2^R$ $Q\beta^R$; II, $f1^S$ $f2^R$ $Q\beta^S$; III, $f1^S$ $f2^S$ $Q\beta^S$.

with JC5455 ($T6^R$ Spc^R). The number of such cells in the donor culture (of JC3272) was measured by a sectored colony technique (Achtman *et al.* 1972) and the number of Lac⁺ [Spc^R] progeny expressed as a percentage of this.

The results are shown in Table 2, column 3. High levels of complementation of F*lac tra*⁻ mutants in all eleven cistrons were observed, suggesting that the transfer systems of ColV2 and F are functionally identical. F*lac traG42* was only poorly complemented by ColV2; however, it was also poorly complemented by F*his* (Achtman *et al.* 1972), and both results may be due to the partial dominance of the *traG42* mutation under these experimental conditions. In *stable* derivatives, F*lac traG42* was fully complemented by the compatible plasmids ColVB*trp* and R1–19 (Table 2; see below).

*Complementation of F*lac tra⁻ *mutants by ColVB*trp, *R1–19 and ColIb* drd

These three plasmids are compatible with F*lac*, and derivatives of JC6256 carrying one of them, together with an F*lac tra*⁻ mutant, were constructed. The abilities of these strains to transfer the F*lac* mutant in crosses with JC3051 are shown in Table 2, columns 4–6.

ColVB*trp* complemented F*lac tra*⁻ mutants in all eleven cistrons. 10–50 % of the Lac⁺ progeny had inherited the F*lac* mutant but not ColVB*trp*, indicating that complementation rather than mobilization or formation of F*lac tra*⁺ recombinants was involved. The transfer system of ColVB*trp* therefore appears to be functionally identical to that of F*lac*.

R1–19, on the other hand, complemented F*lac tra*⁻ mutants in nine cistrons, but not those mutant in *traI* or *traJ*. Assuming that R1–19 specifies products analo-

gous to those determined by the F*lac traI* and *traJ* cistrons, then these must be plasmid-specific. This behaviour of R1–19 is similar to that previously described for R100–1 (Willetts, 1971).

ColIb*drd* did not complement any of the F*lac tra*$^-$ mutants. The results are similar to those obtained previously (Willetts, 1970) for another I-like plasmid, R64–11, which specifies a pilus serologically distinguishable from that of ColIb*drd* (Lawn & Meynell, 1970).

F-specific phage sensitivity

Cells carrying the F-like plasmid R100–1, although sensitive to F-specific phages, show a low efficiency of plating of some RNA phages in comparison with cells carrying an F factor (Nishimura *et al.* 1967; Willetts, 1971). The efficiency of plating of several F-specific phages on strains carrying ColV2, ColVB*trp* or R1–19 was therefore determined (Table 3).

Strains carrying ColV2 or ColVB*trp* plated all the phages tested with efficiencies similar to, or slightly greater than, a strain carrying F*lac*. Similar results for the efficiency of plating of the F-specific phage $\mu 2$ on strains carrying ColV2 have been reported (MacFarren & Clowes, 1967). These results corroborate the genetic similarity between the transfer systems of ColV2, ColVB*trp* and F*lac* found in transfer complementation tests.

Table 3. *Efficiency of plating of F-specific phages*

Plasmid	f1	f2	M12	Qβ
F*lac*	100	100	100	100
ColV2	105	135	125	160
ColVB*trp*	100	125	100	90
R1–19	10	60	45	3

Derivatives of JC6256 were used as the indicator bacteria for plating the DNA-containing phage f1 and the RNA-containing phages f2, M12 and Qβ. The plaque count is expressed as a percentage of that found using the F*lac* derivative as indicator.

Table 4. *Efficiency of plating of Qβ*

F*lac* mutation	Efficiency of plating
tra$^+$	60
traA1	5
traB16	60
traC12	75
traE18	55
traF13	100
traG81	65
traH88	80
traJ90	4
traK105	50

Cultures of derivatives of JC6256 carrying R1–19 and an F*lac tra*$^-$ mutant were used as the indicator bacteria. About 200 Qβ phage particles were plated, and the plaque count is expressed as a percentage of that found using the F*lac* derivative of JC6256 as indicator strain.

A strain carrying R1–19 plated f2 and M12 with efficiencies only slightly lower than those on a strain carrying F*lac*; however, Qβ was plated with a markedly reduced efficiency. The R1–19 pilus therefore differs from the F-pilus. It also differs from the R100–1 pilus; strains carrying R100–1 plated f2 and M12, as well as Qβ, with markedly reduced efficiencies (Willetts, 1971).

The genetic basis for this reduced efficiency of plating of Qβ was then sought, by measuring the efficiency of plating on strains carrying R1–19 together with an F*lac tra*$^-$ mutant defective in F-pilus synthesis (Table 4). A strain carrying wild-type F*lac* and R1–19 gave a high efficiency of plating, although slightly less than a strain carrying F*lac* alone, perhaps due to the formation of 'mixed pili' (Lawn, Meynell & Cooke, 1971). All strains carrying R1–19 and an F*lac tra*$^-$ mutant gave high efficiencies except those carrying an F*lac* element mutant in *traA* or *traJ*. Similar results were found for strains carrying R100–1 and F*lac tra*$^-$ mutants (Willetts, 1971). Since the *traJ* product is probably necessary for synthesis or function of the *traA* product (Willetts, 1971; Willetts & Finnegan, 1972), the efficiency of plating of RNA phages is probably determined by the precise nature of the *traA* allele. Both R1–19 and R100–1 presumably specify *traA* products since they complemented an F*lac traA*$^-$ mutant for transfer.

Surface exclusion

Exponential phase cells carrying an F factor are poor recipients in conjugation experiments (Lederberg, Cavalli & Lederberg, 1953); this phenomenon is called surface exclusion, and may be due to a reduced level of mating pair formation (Achtman *et al.* 1971). Genetically, surface exclusion is closely related to transfer ability, since the gene responsible (*traS*) seems to form part of an operon with six transfer cistrons (Willetts & Achtman, 1972; N. S. Willetts, unpublished data). Also, F*lac traJ*$^-$ mutants have simultaneously lost surface exclusion and transfer ability (Achtman *et al.* 1971, 1972), and surface exclusion, as well as transfer and pilus formation, is inhibited by *fin*$^+$ R factors (Willetts & Finnegan, 1970). Since the complementation tests described above showed that the transfer systems of the F-like plasmids tested were closely related or identical to that of F, surface exclusion between cells carrying these plasmids was measured.

First, surface exclusion was measured in a series of crosses between an Hfr donor strain and plasmid-carrying recipient strains, selecting chromosomal His$^+$ [Str^R] recombinants (Table 5, column 2). Cells carrying F*lac* showed the expected high level of surface exclusion, but those carrying other F-like plasmids gave low, probably insignificant, levels. However, the absence of surface exclusion in crosses between a strain carrying an F factor, which is known to determine a surface exclusion system, and a strain carrying a Col or R factor, shows only that the recipient cells must determine the *corresponding* surface exclusion system for the phenomenon to be observed. Crosses between strains carrying phenotypically distinguishable derivatives of the same plasmid are necessary to determine unambiguously whether a surface exclusion system is made. Incompatibility between such derivatives should not affect the results if both resident and incoming plasmids

Table 5. *Surface exclusion indices*

Plasmid in recipient strain	Hfr ED55	Plasmid in donor strain					
		F*lac*	ColV2	ColVB*trp*	R1–19	R1–19K⁻	R100–1
None	1	1	1	1	1	1	1
F*lac*	470	(360)*	(3)	4	4	6	2
ColV2	5	(2)	.	1	.	2	1
ColVB*trp*	5	1	.	.	60	70	1
R1	3	1	1	17	.	.	(1)
R1–19K⁻	4	1	2	175	(120)	.	(2)
R100	2	1	1	1	.	(1)	(15)†
R100–1	3	1	1	1	.	(2)	(25)‡

Except for ED55 (a ColVB^R derivative of the Hfr strain KL98 (Achtman *et al.* 1971)) donor strains were derivatives of ED56. Recipient strains were derivatives of ED28. The surface exclusion index indicates the ratio of the number of progeny obtained using ED28 as recipient, to the number obtained using a strain carrying the plasmid indicated. Figures in parentheses indicate that the plasmids in the donor and recipient strains are incompatible.

* The recipient strain carried F*his*, not F*lac*.

† The recipent strain carried R100Tet^s, not R100.

‡ The recipient strain carried R100–99, not R100–1.

are inherited with equal frequency by daughter cells; the lack of apparent surface exclusion in crosses between pairs of strains carrying the incompatible plasmids F and ColV2, or R100–1 and R1–19 (Table 5; see below) indicates that this is the case.

Phenotypically distinguishable derivatives were available for three plasmids: F*lac* and F*his*, R1–19 and R1–19K⁻, and R100–1 and R100–99. Crosses between strains carrying these plasmids were performed (Table 5). The level of surface exclusion in crosses between the strains carrying the two F prime factors was similar to that determined using an Hfr donor strain. Further, both R1–19 and R100–1 seemed to determine surface exclusion systems, since transfer of R1–19 to cells carrying R1–19K⁻ was reduced 120-fold, and transfer of R100–1 to cells carrying R100–99 was reduced 25-fold.

Surface exclusion was then measured in crosses between strains carrying F*lac*, R1–19K⁻, or R100–1, to confirm that the surface exclusion system specified by F is different to those specified by R1–19K⁻ and R100–1, and to determine whether the latter two systems are the same (Table 5). No surface exclusion was found in any case, indicating that all three surface exclusion systems are different.

Phenotypically distinguishable derivatives of ColV2 and ColVB*trp* were not available for testing as described above, and strains carrying one of these plasmids were crossed with strains carrying the other, or F*lac*, R1–19K⁻, or R100–1, to discover if these two Col factors specified detectable surface exclusion systems (Table 5). Strains carrying ColV2 showed no surface exclusion in any crosses, and it remains to be determined whether this plasmid specifies a surface exclusion system. However, high levels of surface exclusion were found in crosses between strains carrying ColVB*trp* and R1–19K⁻. These two plasmids, although compatible and distinct in their other properties, therefore determine similar surface exclusion

systems. This result also confirms that surface exclusion, rather than incompatibility, is responsible for the reduced frequency of transfer of R1–19 to cells carrying R1–19K⁻.

Surface exclusion by F has been shown to be under the control of the transfer inhibitor (Willetts & Finnegan, 1970; Finnegan & Willetts, 1971; Negrotti & Nagel de Zwaig, 1972). The levels of surface exclusion in crosses between recipients carrying the transfer-inhibited plasmids R1 or R100Tet^s, and donors carrying ColVB*trp* or R100–1, respectively, were therefore measured (Table 5). Cells carrying R1 showed only 10% of the surface exclusion of cells carrying R1–19K⁻, indicating that this surface exclusion system is also controlled by the transfer inhibitor. Although the level of surface exclusion produced by R100Tet^s was similar in absolute terms to the inhibited levels produced by F and R1, that produced by R100–99 was only twofold greater. It is difficult to interpret this result, but one explanation, consistent with the findings for F and R1, is that the comparatively low level of surface exclusion produced by R100–99 is the result of an adventitious mutation introduced during mutagenesis of R100–1, and that the level produced by R100–1 itself may be similar to the levels produced by F and R1–19.

*Strains carrying ColE1 and an F*lac tra⁻ *mutant*

These strains were constructed by transferring the F*lac tra*⁻ mutants to a strain carrying the compatible non-transmissible plasmid ColE1, using the techniques described by Achtman *et al.* (1972). Their transfer abilities are shown in Table 6. There was no increase in transfer of any of the F*lac tra*⁻ mutants, showing that although ColE1 is efficiently transferred by the F transfer system, it does not itself carry any transfer cistrons functionally related to those of F. Similarly, with the exception of *traI*, there was no transfer of ColE1 by any of the F*lac tra*⁻ mutants:

Table 6. *ColE1 transfer by F*lac tra⁻ *mutants*

F*lac* mutation	Lac⁺	ColE1⁺
tra⁺	53	80
traA1	$< 5 \times 10^{-5}$	< 0·2
traB16	$< 5 \times 10^{-5}$	< 0·2
traC12	2×10^{-2}	< 0·2
traD83	2×10^{-4}	< 0·2
traE18	$< 5 \times 10^{-5}$	< 0·2
traF13	$< 5 \times 10^{-5}$	< 0·2
traG42	$< 5 \times 10^{-5}$	< 0·2
traG81	2×10^{-4}	< 0·2
traH88	$< 5 \times 10^{-5}$	< 0·2
traI40	5×10^{-3}	105
traI65	$< 5 \times 10^{-5}$	13
traJ90	1×10^{-4}	< 0·2
traK105	$< 5 \times 10^{-5}$	< 0·2

Donor strains were derivatives of JC6256 carrying ColE1 and an F*lac tra*⁻ mutant, and the recipient strain was ED664. The figures indicate the number of Lac⁺ and ColE1⁺ progeny, respectively, per 100 donor cells.

all these *tra*$^+$ products are therefore required for ColE1 transfer, including those of *traG* and *traD* which are not necessary for pilus formation (Achtman *et al.* 1971, 1972; Willetts & Achtman, 1972).

However, ColE1 *was* transferred from cells carrying F*lac traI*$^-$ mutants, with approximately the same frequency as from cells carrying wild-type F*lac*, despite the absence of concomitant transfer of the F*lac* mutant. Either ColE1 or the host chromosome may determine a product which replaces the F*lac traI* product for ColE1 transfer. One possible mechanism for ColE1 transfer, mobilization by covalent linkage of ColE1 and F*lac*, is excluded by this result: the appreciable level of ColE1 transfer alone, from cells carrying ColE1 and a wild-type F*lac* factor (Clowes, 1972; Alfaro, unpublished data) leads to a similar conclusion.

4. DISCUSSION

The relationships between the transfer systems of several plasmids were investigated using several criteria: complementation of a series of transfer-deficient mutants of F*lac*, identification of the pilus type by measuring the efficiency of plating of several F-specific phages, and characterization of the surface exclusion system. The transfer systems of the F-like plasmids tested, but not that of the I-like plasmid ColIb*drd*, were closely related to the transfer system of F.

In particular, the transfer systems of ColV2 and ColVB*trp* were indistinguishable from that of F in transfer complementation tests, and the pili specified by these three plasmids allowed similar efficiencies of plating of all F-specific phages tested. Lawn & Meynell (1970) have shown that the pili specified by F and by ColV2 are indistinguishable serologically. However, ColVB*trp* determined a surface exclusion system different to that of F, and ColV2 did not determine a system either of the F type or of the ColVB*trp* type.

R1–19 behaved similarly to R100–1 (Willetts, 1971) in giving transfer complementation of F*lac* mutants in all cistrons except *traI* and *traJ*. Our interpretation of this is that the *traJ* product is a plasmid-specific protein required for synthesis or function of *traI*, and that *traI* determines a plasmid-specific product required for DNA transfer (Willetts, 1971; Willetts & Finegan, 1972). The efficiency of plating pattern for F-specific RNA phages was different on strains carrying R1–19, F*lac*, or R100–1, showing that the pili specified by these three plasmids are different. Serological differences have also been detected (Lawn & Meynell, 1970). As found for R100–1 (Willetts, 1971), the cistron directly responsible for the changes in efficiency of plating is probably *traA*. R1–19 determined a surface exclusion system different to that of F, but similar to that of ColVB*trp*; as in the case of F, this system was under the control of the transfer inhibitor. Results with R100–1 were somewhat equivocal, but this plasmid may determine a third surface exclusion system.

In summary, the products of eight *tra* cistrons of the F-like plasmids tested were functionally identical, whereas those of *traA*, *traI*, *traJ* and *traS* showed some degree of plasmid-specificity. Particular alleles of *traA*, *traI* and *traJ*, but not of

traS, were associated in the plasmids tested, but this association may not be obligatory.

The relationships between the transfer systems of these F-like plasmids were not necessarily reflected by their incompatibility properties. Thus although F and ColV2 have similar transfer systems (except for *traS*) and fall into the same incompatibility group (Kahn & Helinski, 1964; McFarren & Clowes, 1967), ColVB*trp* which also has a transfer system differing from that of F only in the *traS* component, falls into a second incompatibility group (G. Alfaro & N. S. Willetts, unpublished data). Although R1–19 and R100–1 both belong to a third incompatibility group, they specify different *traA* and *traS* components, and possibly different *traI* and *traJ* components as well.

The transfer of ColE1 by F*lac traI*$^-$ mutants is of particular interest since the *traI* product is not required for pilus synthesis, but probably for F DNA metabolism during conjugation (Willetts & Achtman, 1972). There may be different pathways for plasmid DNA metabolism during transfer of F and ColE1, perhaps related to the different pathways for vegetative replication of these plasmids (Kingsbury & Helinski, 1970; Goebel, 1972).

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