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Specificities of IncF plasmid conjugation genes

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Summary

The conjugation regions of IncF plasmids are closely related in that they share extensive DNA homology, and that they specify related pili. Variations between individual conjugation gene products of different IncF plasmids have, however, been noted. We have extended these observations by carrying out a systematic survey of twelve such plasmids, to examine the numbers and the groupings of the plasmid-specific alleles of several genes required for conjugation and its control.

Using vector plasmids carrying cloned origins of transfer (*oriT*), four different specificities were recognized, and these were correlated with the specificities of the genes with products that may act at this site (*traM*, *traY* and *traZ*). The *traY* gene is the first gene of the major transfer operon, and is therefore located close to the site at which the *traJ* protein acts to induce expression of the operon: correspondingly, correlation was observed between the *oriT*/*traMYZ* and *traJ* specificities in most of the plasmids. In turn, *traJ* is negatively regulated by the *finO* and *finP* products acting in concert: the *finO* product was relatively non-specific, but six *finP* alleles were identified, again with specificities correlated with those of *traJ*. Our explanation for this unexpectedly large number of *finP* alleles derives from the concept that the *finP* product is an RNA molecule rather than a protein. Although the conjugative pili encoded by IncF plasmids are closely related, they confer different efficiencies of plating of the various F-specific bacteriophages. We distinguished four groups on this basis, presumably resulting from differences in the primary amino-acid sequences of the pilin proteins. These groups could be related to the surface exclusion system specificities, consistent with the hypothesis that surface exclusion acts at least in part by preventing interaction between the pilus and the recipient cell surface.

From these data, information about the evolutionary relationships between the twelve IncF plasmids can be deduced.

1. Introduction

A common property determined by bacterial plasmids falling into a variety of incompatibility groups (Datta, 1979), is the ability to transfer their DNA by conjugation to a recipient cell. For naturally occurring plasmids, it has generally been observed that the conjugation systems of plasmids belonging to the same incompatibility group are closely related, while those of plasmids in different incompatibility groups are dissimilar. The criteria for relationships between conjugation systems include the morphology and serology of the pilus and the particular pilus-specific bacteriophages that they adsorb (Bradley, 1980*a*, *b*), genetic complementation between transfer genes (Ohtsubo, Nishimura & Hirota, 1970; Foster & Willetts, 1976, 1977), ability to initiate transfer at a given origin of transfer (*oriT*) sequence (Willetts & Wilkins, 1984), the effectiveness of the surface exclusion system against another plasmid (Alfaro & Willetts, 1972), and the extent of homology between the transfer (*tra*) region DNAs (Sharp, Cohen & Davidson, 1973; Ingram, 1973; Falkow *et al.* 1974).

Using as criteria the formation of similar pili giving sensitivity to the same pilus-specific phages, *tra* DNA homology, and ability to inhibit transfer of the F plasmid, the conjugation systems of IncF plasmids are closely related. However, in the course of our studies of these systems we have observed important differences, indicating the existence of different alleles for genes with products required for control of conjugation (Willetts, 1977; Finnegan & Willetts, 1972; Alfaro & Willetts, 1972), for processing of

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plasmid DNA (Willetts, 1981; Willetts & Wilkins, 1984) and for surface exclusion (Alfaro & Willetts, 1972). Minor variations have also been found for the genes specifying the pilin sub-unit protein (Lawn & Meynell, 1970; Willetts, 1971; Alfaro & Willetts, 1972). This paper surveys the plasmid-specific properties of twelve commonly studied F-like plasmids.

2. Materials and Methods

(i) *Bacterial strains*

The *E. coli* K12 host strains were: ED57 (ColVB^R derivative of JC3272); ED397 (*recA56* derivative of W3110); ED2030 (His$^-$ Trp$^-$ Lac$^-$ Spc^R RecA$^-$; Foster & Willetts, 1976); ED2196 (His$^-$ Trp$^-$ Lac$^-$ Nal^R; Gasson & Willetts, 1977); ED3818 (Nal^R derivative of JC3272); ED3872 (Nal^R derivative of ED57); JC3272 (His$^-$ Trp$^-$ Lys$^-$ Lac$^-$ Str^R; Achtman, Willetts & Clark, 1971); JC6256 (Achtman *et al.* 1971) and W3110 (prototroph; Bachmann, 1972).

(ii) *Bacterial plasmids*

The naturally occurring plasmids used in this study are listed in Table 1, as are the transfer-depressed mutants derived from these. JCFL40 (F*lac traI40*) JCFL102 (F*lac traM102*) and JCFL90 (F*lac traJ90*) were described by Achtman *et al.* (1971, 1972), and Willetts & Achtman (1972). Transfer-deficient mutants isolated from other transfer derepressed plasmids are described in the text and in Table 6. The *oriT* clones were pED822 (pED825 *oriT*-F cloned on a 373 bp *Bgl*II–*Hae*II fragment; Everett & Willetts, 1982); pED221 (pED825 *oriT*–R1 on a *ca* 1 kb *Msp*I fragment) and pED222 (pED825 *oriT*–R100 on a 1·6 kb *Bam*I fragment).

(iii) *Media*

These have been described (Willetts & Finnegan, 1970).

(iv) *Mating techniques*

The donor abilities of plasmid-carrying strains were measured in 30-min matings as described by Finnegan & Willetts (1971). Surface exclusion indices were measured as described by Willetts and Maule (1974). Re-transfer experiments were carried out as described by Finnegan & Willetts (1973), except that the number of intermediate cells (ED2196) that had received the plasmid from the donor strain was measured by selecting the appropriate Nal^R transconjugants. All matings were at 37 °C.

(v) *Male-specific phage techniques*

The efficiencies of plating of f1, f2 and Qβ were measured as described by Achtman *et al.* 1971).

Table 1. *Bacterial plasmids*

FinOP$^+$ plasmid	IncF	Markers	Ref	Transfer-derepressed mutant	Type	Ref
—	I	*lac*$^+$		F*lac*[a]	*finO*	1
—	I	*lac*$^+$		EDFL51	*finO finP301*	2
—	I	*col*$^+$		ColV2-K94	*finO*	3
R386	I	Tc^R	4	pED202	*finP* or *traO*[b]	5
R1	II	Ap^{R}Cm^{R}Km^{R}Sm^{R}Su^R	6	R1-19[c]	*finO*	6
R6[d]	II	Cm^{R}Hg^{R}Km^{R}Sm^{R}Su^{R}Tc^R	7	pED204	*finP*[e]	8
R100[f]	II	Cm^{R}Hg^{R}Sm^{R}Su^{R}Tc^R	9	R100-1[g]	*finO*	9
R136	II	Tc^R	10	pED241	*finO*	11
R538-1	II	Cm^{R}Hg^{R}Sm^{R}Su^R	12	pED207	*finO*	13
ColB2-K77	II	*col*$^+$	14	pED236	*finP*	14
ColB4-K98	II[h]	*col*$^+$	15	pED203	*finO*[i]	8
R124	IV	Tc^R	10	pED200	*finP*[e]	5
—	IV[j]	*col*$^+$*trp*$^+$		ColVB*trp*	*finO*	16

References are: (1) Achtman *et al.* 1971; (2) Finnegan & Willetts, 1971 (3) McFarren & Clowes, 1967; (4) Dennison, 1972; (5) S. Sangsoda & N. S. Willetts, unpublished data; (6) Meynell & Datta, 1967; (7) Watanabe *et al.* 1964; (8) Maule, J. & Willetts, N. unpublished data; (9) Egawa & Hirota, 1962; (10) Meynell & Datta, 1966; (11) Grindley *et al.* 1971; R136*i*-1 is re-numbered pED241; (12) Romero & Meynell, 1969; (13) Willetts & Maule, 1974; (14) Hausmann & Clowes, 1971; ColB2 Fdr is re-numbered pED236; (15) Clowes *et al.* 1969; (16) Fredericq, 1969.

[a] F*lac* = JCFLO. The F*his* plasmid F57 (Achtman *et al.* 1971) was occasionally used in its place. [b] Although R386 has a relatively high level of transfer, it is *finO*$^+$ (see text), and its mutant derivative pED202 transfers 30-fold more efficiently. pED202 is still *finO*$^+$ since it inhibits R100-99 transfer; because of R386's unique *finP* specificity, we were not able to determine if the mutation carried by pED202 was recessive (*finP*) or dominant (*traO*). [c] pED219 is a derivative of R1-19 that determines only Km^R. [d] R6-5 is the *tet*::IS*10* derivative of R6 (Sharp *et al.* 1973). [e] Found *finO*$^+$ (by inhibition of F*lac* transfer) *finP*$^-$ (recessive mutation in re-transfer experiments). [f] R100 Tc^S was described by Finnegan & Willetts (1972). [g] R100-99 is a Cm^{S}Tc^S mutant of R100-1. [h] We invariably observed incompatibility between ColB4 and all IncFII R factors. [i] Failed to inhibit F*lac* transfer. [j] S. Sangsoda & N. Willetts, unpublished data.

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3. Results

(i) *Variation in the* finO *product*

Classically, the *finO* products of FinOP plasmids have been considered interchangeable, since they all serve to inhibit transfer of the *E. coli* K12 sex factor F. This is of course the definition of their 'fi$^+$' phenotype (Watanabe & Fukasawa, 1962; Hirota *et al.* 1964; Nishimura *et al.* 1967). F itself is *finO*$^-$, probably due to insertional inactivation by the IS*3*a sequence (K. C. Cheah & R. Skurray, pers. comm.).

We have tested the quantitative abilities of representative *finO*$^+$ plasmids to inhibit the transfer of those *finO*$^-$ plasmid mutants that were available. Some tests were not carried out because the two plasmids belonged to the same incompatibility group, or had no distinguishing marker; also the *finO*$^-$ plasmid ColV2 was not used because of the difficulty of measuring low levels of Col factor transfer. Nevertheless, amongst those tests that were possible, quite marked variations in the level of transfer inhibition were observed (Table 2). The inhibition ratios ranged from the extremes of 2200 (for the inhibition by the R1 *finO*$^+$ gene product of R1 transfer itself) to only 6 (for the inhibition by R386 or by R124 of pED207 transfer). In the latter cases, the low inhibition ratios were reflected in the partial sensitivity of these strains to the F-specific phages f1, f2 and Qβ. Interestingly, plasmid *finO* gene products were not necessarily most efficient in self-inhibition: that of R538-1, for instance, inhibited R538-1 transfer only 58-fold, but inhibited transfer of JCFL0 and ColVB*trp* 530- and 750-fold, respectively.

Comparison of the transfer inhibition ratios indicates that there may be two general types of *finO* products: those of R1, R6, R100, R136, R538-1 and ColB4 that inhibit transfer of F*lac* and ColVB*trp* by 100–1000-fold, and those of R386, ColB2 and R124 that inhibit transfer of F*lac* and ColVB*trp* by only 20–50-fold.

It is appropriate to mention here that the supposedly F-like plasmid F$_0$*lac* (Falkow & Baron, 1962) determines a system for inhibition of its own transfer totally different from those of true IncF plasmids. Thus, although itself transferring at a low level (0·2%), it did not reduce transfer of either F*his* or of R100-1 (data not shown). Furthermore, transfer of pED208, a mutant of F$_0$*lac* that transfers at 99%, was not inhibited by R100. Cells carrying pED208, although sensitive to phage f1, were totally resistant to the F-specific RNA phages f2 and Qβ, and this, together with the absence of a serological relationship between F pili and F$_0$*lac* pili (Bradley, 1980*b*; Armstrong *et al.* 1980) and the lack of homology between F and F$_0$ (H. Smith, pers. comm.), confirms that F$_0$*lac* is not an IncF or F-like plasmid.

(ii) *Alleles of the* finP *gene*

Transfer inhibition of F-like plasmids requires the products of two genes, *finO* and *finP* (Finnegan & Willetts, 1971, 1972). Both products are required to prevent expression of *traJ*, the positive inducer of the transfer operon, although the way in which they interact together is not yet understood (Finnegan & Willetts, 1973; Mullineaux & Willetts, 1984). Although the *finO* products of FinOP plasmids are largely interchangeable, the *finP* products are much more specific, and four alleles have already been reported (see Table 5 for references). The products of these different *finP* alleles, for example those of F and R100, are not interchangeable. We have here extended this classification to include the others of the twelve plasmids being surveyed.

Table 2. *Variation in* finO

FinO$^-$ plasmid	Transfer frequency (%)	Transfer inhibition ratio[a]							
		R386	R1	R6	R100	R538-1	ColB2	ColB4	R124
Self[d]	—	28	2200	520	180	58	68	26	906
F*lac*	105	i	120[b]	530	1300[b,c]	530[c]	46[c]	420[b,c]	43
R1-19	130	650	2200[b,d]	i	i	i	i	(1·1)[b,e,f]	960
R100-99	50	700	i	i	180[b,d]	i	i	(50)[b,e,g]	1300
pED207	260	6[f]	i	i	i	58[c]	i	i	(6)[e,f]
pED203	260	26	i	i	i	i	NT	26[c]	16
ColVB*trp*	60	29[e]	75[b]	(15)[e]	130[b]	750	19	600[b]	i

The donor strains were derivatives of ED2196 and the recipient strain was JC3272 or ED57 (ColVB^R), as appropriate, except where results are taken from Finnegan & Willetts (1972) or Gasson & Willetts (1975) where the donor host strain was JC5455.

'i' signifies incompatible plasmids, and NT not tested.

[a] Defined as the transfer frequency of the FinO$^-$ plasmid from ED2196 divided by its transfer frequency in the presence of the *finO*$^+$ plasmid. [b] Taken from Finnegan & Willetts (1972). [c] Taken from Gasson & Willetts (1975). [d] Ratio of the transfer frequencies of derepressed and repressed (wild-type) forms of the plasmid. [e] Strains carrying these pairs of plasmids were unstable, and the results are therefore shown in parentheses. [f] In cases where the inhibition ratio was < 10, sensitivity to the male-specific phages was tested by a spot test method. These strains were slightly sensitive, giving turbid spots. [g] R100-1 was used in place of R100-99.

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Table 3. finP *specificity by complementation*

	finP⁻ plasmid			
finP⁺ plasmid	EDFL51[a] (F*lac finP301*)	pED236 (ColB2 Fdr)	EDR200 (R124 *finP*)	EDR204 (R6-5 *finP*)
None	150	120	140	47
R386	i	85	NT	38[b]
R1	180	i	130	i
R100	160	i	160[c]	i
R538-1	110	i	220	i
ColB2	81	1·3[d]	4[d]	i
ColB4	115	i	125	i
R124	125	21[d]	0·6[d]	18
ColVB*trp*	NT	8[d]	i	NT

The numbers give the % frequencies of transfer of the *finP*⁻ plasmid from derivatives of ED2196 carrying this together with a *finP*⁺ plasmid, in 30 min matings with JC3272 or ED57 as appropriate. NT indicates not tested, and i that tests were not possible because of incompatibility.

[a] Taken from Gasson & Willetts (1975). [b] Unstable combination. [c] R100 Tc^S was used. [d] Fertility inhibition was confirmed by showing that these strains were resistant to phages f1, f2 and Qβ.

The most direct method for determination of *finP* specificity requires the isolation of *finP*⁻ mutants, followed by complementation tests using other, *finP*⁺, plasmids. Such mutants were available for JCFL0, R6–5, ColB2 and R124. Two further requirements are that the *finP*⁻ and *finP*⁺ plasmids should be compatible, and that they should be distinguishable from each other by suitable genetic markers. Results for pairs of plasmids where these requirements are satisfied, are presented in Table 3. In particular, they show that the *finP* products of ColB2, R124 and ColVB*trp* are interchangeable. It is not clear why the inhibition of pED236 transfer by R124 was only 6-fold, whereas that of pED200 by ColB2 was 35-fold; however, both strains were resistant to F-specific phages, confirming that complementation of *finP*⁻ did in fact occur. The results also showed that the *finP* alleles for many other pairs of plasmids are dissimilar, but further tests were necessary to distinguish between these.

In cases where *finP*⁻ mutants were not available or the plasmids were incompatible, re-transfer of a *fin*⁻ plasmid mutant from 'intermediate donor' cells carrying the second, *finP*⁺, plasmid was measured. Under these conditions, the *fin*⁻ plasmid in the primary donor strain can be either *finO*⁻ or *finP*⁻; after transfer of plasmid DNA from the primary donor cells (which are then killed with T6), inhibition of re-transfer is found only when the intermediate cells *already* carry appropriate *finO*⁺ and *finP*⁺ alleles. Otherwise, transfer inhibition is delayed for several hours (Finnegan & Willetts, 1971, 1972; Willetts, 1974). Since *finP* specificity was to be tested, it was essential that a functional *finO*⁺ product be provided in the intermediate cells, either by the *finP*⁺ plasmid itself, or, if the *finP*⁺ plasmid was *finO*⁻ or its *finO* product was not very successful in preventing transfer of the *fin*⁻ donor plasmid (see Table 2), by the presence of a second, suitable *finO*⁺ plasmid whose *finP* product would not interfere.

The results of such re-transfer experiments demonstrated that the *finP* products of R538-1 and ColB4, of ColVB*trp* and R124, and of R100 and R6–5, are interchangeable with each other, since re-transfer of the incoming *finO*⁻ plasmid was immediately inhibited (Table 4). High values for re-transfer were obtained for the other pair of plasmids, similar to those found for re-transfer through the plasmid-free strain. This indicated that the *finP* alleles of all these plasmid pairs are different from each other.

The above results, plus those available in the literature, are summarized in Table 5 and serve to define six *finP* alleles.

(iii) *Alleles of the* traJ *gene*

The *traJ* product is required for expression of the *traY→Z* operon, and perhaps also of *traM* (Finnegan & Willetts, 1973; Willetts, 1977; Gaffney *et al.* 1983; Mullineaux & Willetts, 1984). It was demonstrated previously that neither R100-1 (Willetts, 1971) nor R1-19 (Alfaro & Willetts, 1972) complement JCFL90 (F*lac traJ90*), and thus, making the reasonable assumption that these plasmids do have a *traJ* gene, that different *traJ* alleles exist.

Such complementation experiments were extended, to show that the *traJ* products of R6-5, R136, R538-1 and ColB4 also differ from that of F (Table 6(*a*)). The other five F-like plasmids all apparently complemented JCFL90 (data not shown); however, such results can be interpreted as showing complementation of *traJ* and/or of all the genes with products acting at the plasmid-specific *oriT* site (see below). These alternatives

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Table 4. finP *specificity by retransfer*

Donor plasmid	Plasmid in intermediate host					
	None	R124	R538-1	ColB4	R6-5	R386
F*lac*	35	NT	63	35	69	197[b]
ColVB*trp*	23	< 0·5	43	30[a]	66	77[b]
R1-19	50	43	79	69	170	121
R100-1	28	16	26	20[a]	0·1	150[c]
pED207	644	313	NT	< 0·03	NT	524
pED204	29	NT	26	81	NT	NT

The numbers give the % frequencies of retransfer of the donor plasmid from the intermediate host carrying the plasmid noted. NT denotes not tested.

[a] Taken from Finnegan & Willetts, 1972. [b] Similar results were obtained if R100 Tc^S (*finO*$^+$) was also present in the intermediate strain. [c] R100-99 was used in place of R100-1.

Table 5. *Summary of* finP *specificities*

Plasmid	I	II	III	IV	V	VI
Representative plasmids						
F*lac*	Prototype					
ColVB*trp*	≠[a]	Prototype				
R1	≠[a,b]	≠[a]	Prototype			
R100	≠[a,b]	≠[a]	≠[a,c]	Prototype		
R538-1	≠[b,d]	≠[d]	≠[d]	≠[d]	Prototype	
R386	≠[d]	≠[d]	≠[a,d]	≠[a,d]	≠[d]	Prototype
Other plasmids						
ColV2	=[a]	≠[a]	≠[a]	≠[a]	NT	NT
R124	≠[b]	=[d,e]	≠[d,e]	≠[d,e]	≠[d,e,f]	NT
ColB2	≠[b]	=[b,e,f]	NT	≠[g]	NT	≠[b,e]
R6–5	≠[b,d]	≠[d,e]	≠[d]	=[d]	≠[d]	≠[e]
R136	≠[a,b]	≠[a]	≠[a,c]	=[a,c]	≠[a]	NT
ColB4	≠[a,b]	≠[a,f,e]	≠[a,d]	≠[a]	=[d]	NT

'=' and '≠' signify that the *finP* products are interchangeable or not, respectively. NT signifies not tested.
References are: [a] Finnegan & Willetts, 1972. [b] Gasson & Willetts, 1975. [c] Grindley *et al.* 1973. [d] Table 4. [e] Table 3. [f] Meynell & Lawn, 1973. [g] Hausmann & Clowes, 1971.

were distinguished for ColVB*trp*, ColB2 and R124 by isolating and testing *tra*$^-$ pilus-specific phage-resistant mutants; complementation by these of F*lac traJ90* showed that the *traJ* products of these plasmids must serve to induce the F transfer system (Table 6(*b*)). A second ColVB*trp* *tra*$^-$ mutant (pED206) did not complement JCFL90, and presumably carries a *traJ* mutation; consistent with this, surface exclusion was lost by pED206, but not pED205. ColV2 and R386 are incompatible with F, preventing direct complementation tests between *tra*$^-$ derivatives, but pED237 (ColV2*tra*) was shown to complement pED206, confirming the similarity of their *traJ* genes. R386 could not be tested since its derepressed mutant pED202 is *finO*$^+$, and would have inhibited pED206 transfer.

Information about *traJ* specificities can be obtained from some re-transfer experiments. For example, re-transfer of R1-19 (*finP*-III) from cells carrying R538-1 or ColB4 (*finP*-V) took place as expected, at high frequency. Furthermore, R538-1 and ColB4 were *also* transferred at high frequency from these transient heterozygotes (data not shown): this requires that the *traJ* proteins of these three plasmids, and/or their *oriT*-specific proteins (which would be induced by the *traJ* protein) have similar specificities. The *oriT* specificities of R538-1 and ColB4 differ from that of R1-19 (see below), hence all three plasmids must have similar *traJ* specificities. In contrast, although R100-1, pED204 and pED241 *finP*-IV) re-transferred at high frequency from cells carrying R538-1 or ColB4 (*finP*-V), the latter plasmids transferred from the transient heterozygotes at low frequency: the specificities of both *traJ* and *oriT* of R538-1 and ColB4 must, therefore, differ from those of the other three plasmids.

(iv) *Specificity of* oriT *and of gene products acting at that site*

The origin of transfer site is expected to interact with several proteins involved in the initiation of

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Table 6. traJ *specificity*

Fin⁻ plasmid	Transfer frequency (%)	
	Fin⁻ plasmid	F*lac traJ90*
(*a*) Tra⁺		
R1-19	155[a]	0·14[a]
pED204	20[b]	0·024[b]
R100-1	130[c]	0·9[c]
pED241	61	0·05
pED207	190	0·4
pED203	180	0·28
(*b*) *Tra*⁻		
ColV2 *tra*	63[d]	20[d]
pED236 *tra*	43[b]	29[b]
pED200 *tra*	96[b]	105[b]
pED205[e]	50	95
pED206[e]	0·7	1·1

[a] Taken from Alfaro & Willetts, 1972. [b] EDFL50 (F*lac traO304 traJ90*) was used in place of F*lac traJ90*, to prevent transfer inhibition of the *finO*⁺*finP*⁻ plasmid. [c] Taken from Willetts, 1971. [d] pED206 (ColVB*trp traJ348*) was used in place of F*lac traJ90*, because of incompatibility. [e] pED205 and pED206 are independently isolated *tra*⁻ mutants of ColVB*trp*.

conjugation. In particular, these include the *traYZ* endonuclease, thought to catalyse interconversion of covalently closed and open circular forms of plasmid DNA by reversibly nicking and ligating at *oriT* (Everett & Willetts, 1980), and a protein triggering DNA transfer in response to mating pair formation (possibly the *traM* product; Willetts & Wilkins, 1984). It has been observed previously that the products of the *traMYZ* genes, and of *traI* (see below) are not always interchangeable amongst IncF plasmids, leading to the hypothesis that the *oriT* sequences of these plasmids differ (Willetts & Maule, 1979; McIntire & Willetts, 1978; Everett & Willetts, 1980; reviewed by Willetts & Skurray, 1980).

This hypothesis has been tested directly, by cloning the *oriT* sites of F, R1-19 and R100-1 on small DNA fragments into the *oriT*-free vector pED825, and measuring the frequencies of mobilisation of these chimeras by the transfer-derepressed IncF plasmids. The results showed that the *oriT* sites of F, R1-19 and R100-1 differed from each other, and the most of the other IncF plasmids mobilized one of the three (Table 7). The exception were pED207 and pED203, which mobilized none of the three *oriT* chimeric plasmids: R538-1 and ColB4 must therefore determine at least one further *oriT* specificity.

Point mutations in *traY* and *traZ* were not available to test whether, as expected, only those plasmids able to mobilize from the corresponding *oriT* were able to complement. However, application of a *λoriT* nicking assay has shown previously that the R1-19 and R100-1 *traY* products will not substitute for that of F, while the *traZ* product of R1-19, but not of R100-1, will do so (Everett & Willetts, 1980). The sequences of the *oriT* regions of F (Thompson *et al.* 1984) and of R1 (Ostermann, Kricek & Hogenaner, 1984) have recently been published, and that part covering the potential nick sites (from within gene X to 28 base-pairs past nick 1 of Thompson *et al.*) is identical. Presumably the plasmid-specific *traY* product must bind outside the region of sequence identity, and therefore it may be the *traZ* protein component of the proposed *traYZ* endonuclease that actually nicks at *oriT*.

Complementation of F*lac traM102* by the IncF plasmids was tested, and all those able to initiate transfer at the F *oriT* sequence gave positive results, while all the others gave negative results (Table 7). This is consistent with the idea that the *traM* protein binds at *oriT*, again outside the region of F-R1 sequence identity.

It has recently been found that the product of *traI* is DNA helicase I (Abdel-Monem, Taucher-Scholz & Klinkert, 1983). This enzyme has been extensively characterized *in vitro* using T7 DNA substrate (Abdel-Monem *et al.* 1977; Kuhn *et al.* 1979). It might at first sight, therefore, seem surprising that the *traI* product of R100-1 (and of pED241 and pED204) will not substitute for that of F (Table 7; note that JCFL65 used previously in such tests, also carries a *traM* mutation; Willetts & Maule, 1979). However, the specificity may be determined not by interaction with a DNA sequence, but with plasmid-specific transfer proteins, such as the *traYZ* endonuclease or possibly the *traJ* protein if this forms part of a membrane 'transfer complex'. If ATP consumption during unwinding provides the motive force for DNA transfer (Willetts & Wilkins, 1984), the helicase would of necessity have to be fixed to such a complex.

(v) *Variation in the pilin protein*

The pili of F-like plasmids are related in allowing infection by the same group of pilus-specific phages, and in their morphological and serological characteristics (Lawn *et al.* 1967). However, differences have been observed between some of them in their detailed serology (Lawn & Meynell, 1970) and in their quantitative efficiencies of plating of the various pilus-specific phages (Nishimura *et al.* 1967; Willetts, 1971; Alfaro & Willetts, 1972). The latter differences were ascribed to differences in the *traA* gene (Willetts, 1971; Alfaro & Willetts, 1972), and since the product of this gene is pre-pilin (Minkley *et al.* 1976; Frost, Paranchych & Willetts, 1984), it is likely that the amino-acid sequences of the pilin proteins of different F-like plasmids vary. In the case of ColB2, this has been directly demonstrated in sequencing studies (Finlay, Frost & Paranchych, 1984).

We have extended measurement of phage plating efficiencies to the twelve IncF plasmids of the present study. The representative F-specific phages chosen were f1 (filamentous, single-strand DNA), f2 (one of

Table 7. *Mobilization of* oriT *recombinant plasmids and complementation of F*lac traM *and* traI *mutants*

	Mobilization[a]			Complementation[b]	
	pED822 (*oriT*-F)	pED221 (*oriT*-R1)	pED222 (*oriT*-R100)	F*lac traM102*	F*lac traI40*
F*lac*	140	6×10^{-4}	2×10^{-3}	1	1
pED219	2×10^{-3}	112	4×10^{-3}	3×10^{-4}	0.38
R100-1	0.3	7×10^{-3}	205	9×10^{-4}	4×10^{-3}
ColV2	74	NT	NT	0.86[t]	0.20[t]
pED202	43	NT	NT	0.38[t]	0.21[t]
pED236	49	NT	NT	0.34	0.43
ColVB*trp*	78	NT	NT	0.06	1.4
pED200	61	NT	NT	0.69	0.50
pED207	$< 1 \times 10^{-2}$	0.12	$< 2 \times 10^{-2}$	1×10^{-3}	0.30
pED203	$< 1 \times 10^{-2}$	4.5×10^{-2}	$< 1 \times 10^{-2}$	2×10^{-3}	0.19
pED241	NT	NT	223	3×10^{-4}	1×10^{-3}
pED204	NT	NT	77	3×10^{-4}	1×10^{-3}

[a] The donor strains were derivatives of ED2030, and the recipient strain was ED57. Mobilization frequencies are expressed as a percentage of the transfer frequency of the conjugative plasmid. NT, not tested. [b] The donor abilities of derivatives of ED2196 carrying the two plasmids were measured in crosses with JC3272 (or ED57 where appropriate), except where the two plasmids were incompatible or the Fin$^-$ plasmid was not a *finO*$^-$ mutant; in these cases a transient heterozygote technique was used (Materials & Methods). The numbers give the ratio of F*lac* mutant transfer to IncF plasmid transfer. Results for plasmids complementing F*lac tra* mutants were re-checked in an equivalent *recA56* host strain; essentially similar data was obtained.

Table 8. *Efficiencies of plating of F-specific phages*

Plasmid	f1	f2	Qβ	Group	Serological group[a]
F*lac*	100	100	100	I	A
ColV2[b]	105	135	160	I	A
pED202	62	75	62	I	—
R1-19[c]	10	60	3	III	B
pED204	0[d]	4	0[d]	IV	—
R100-1	2	8	3	IV	D
pED241	1	3	0[d]	IV	D
pED207	90	70	95	I	C
pED236	0[d]	86	115	II	—
pED203	3	120	117	II	—
pED200	86	130	93	I	C
ColVB *trp*[c]	100	125	90	I	—

Efficiencies of plating were measured as described in Materials & Methods, using JC3272 as host; they are expressed relative to the plating efficiencies found using F*lac*.

[a] Taken from Lawn & Meynell, 1970. [b] Taken from Willetts, 1971. [c] Taken from Alfaro & Willetts, 1972. [d] Although the efficiencies of plating were zero, these strains gave positive results in spot tests.

a large group of closely related isometric RNA phages) and Qβ (a more distantly related isometric RNA phage). Using the data obtained, four groups of pilus types could be distinguished (Table 8). These groups correspond in part to those based upon minor variations in serological properties (Lawn & Meynell, 1970; last column, Table 8).

Despite these differences, the functional similarity of the pilin subunits of different F-like plasmids is emphasised by (*a*) the ability of any IncF plasmid pilus to transfer the F factor during complementation of an F*lac traA* mutant (Willetts, 1971; Alfaro & Willetts, 1972; and our unpublished data); (*b*) complementation of pililess F*tra* mutants by pililess R100-1 *tra* mutants (Ohtsubo *et al.* 1970; Foster & Willetts, 1976, 1977) and (*c*) the formation of mixed pili by cells carrying two F-like plasmids, with subunits of each type assembled together (Lawn, Meynell & Cooke, 1971). The underlying sequence differences may be relatively minor, as suggested by the isolation of F *traA* point mutants that are still transfer-proficient, but show reduced levels of adsorption and plating of F-specific

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Table 9. *Surface exclusion indices*

Plasmid in donor strain	Plasmid in recipient strain: pED202 (R386*drd*)	pED204 (R6-5 *finP*)	pED236 (ColB2 Fdr)	pED203 (ColB4 *finO*)	pED200 (R124 *finP*)
(*a*) Standard donors					
F*lac*	770	4	2	3	72
R538-1*drd*[a]	7	1[b]	1900	2500	69000
R1-19	7	2	1[c]	3	23
R100-1	4	64	3	2	11

Plasmid in donor strain	Plasmid in recipient strain: F*lac*	R538-1 *drd*[a]	R1-19	R100-1
(*b*) Standard recipients				
pED202	100	1	2	1[d]
pED204	4	1	4	21
pED203	1	21	1	1
pED200	4	120	1	1[d]

Surface exclusion was measured as described in Materials & Methods; the numbers given are the surface exclusion indices. Results considered to be positive are given in heavy type.

[a] R538-1*drd*(*finO*$^+$, *finP*$^-$ or *traO*$^-$; Meynell & Cooke, 1969) was used in place of pED207. [b] The donor plasmid was pED236. [c] The donor plasmid was ColVB*trp*. [d] The recipient plasmid was R100-99.

phages (Willetts, Moore & Paranchych, 1980), and by comparison of the amino-acid sequences of F and pED236 pili (Finlay *et.* 1984; see below).

(vii) *Surface exclusion systems*

All F-like plasmids so far examined determine surface exclusion systems that reduce conjugative transfer into the cell of either the same or a related plasmid. In the case of F, surface exclusion is determined by *traS* and *traT*, genes located within the major transfer operon (Achtman *et al.* 1980). Mating pair formation and DNA transfer are both blocked (Achtman, Kennedy & Skurray, 1977), and specificity with regard to the donor plasmid may be determined by the particular pilin sub-unit at the pilus tip (Willetts & Maule, 1974).

Different F-like plasmids determine surface exclusion systems of different specificities, and surface exclusion between two plasmids is only observed when their surface exclusion systems have the same specificities. Previously, four specificities have been distinguished, corresponding to the surface exclusion systems of F (Sfx-I), of ColV2 and R538-1 *drd* (Sfx-II), of ColVB*trp* and R1-19 (Sfx-III), and of R100-1 and pED241 (Sfx-IV) (Alfaro & Willetts, 1972; Willetts & Maule, 1973, 1974). Reciprocal crosses were carried out between donors carrying plasmids representative of each of these four groups, and recipients carrying the other plasmids under study and *vice versa* (Tables 9(*a*) and *b*)). The data show that pED202 encodes the Sfx-I system, pED236, pED203 and pED200 all encode the Sfx-II system, and pED204 determines the Sfx-IV system. Large differences in the efficiencies of the surface exclusion systems were observed, that of R124 being particularly high.

4. Discussion

A summary of the plasmid-specific alleles that were identified, is given in Table 10. There are several significant correlations that can be made.

Considering the regulation of conjugation first, it was found previously (Meynell, Meynell & Datta, 1968), and confirmed by our quantitative data that the *finO* product is relatively non-specific. In contrast, the *finP* product was highly specific. Since the *finO* and *finP* products act together to prevent transcription of *traJ* (Willetts, 1977; Gaffney, Skurray & Willetts, 1983), it was not surprising to find a correlation between *finP* and *traJ* alleles (*traJ*-I with *finP*-I or -II; *traJ*-III with *finP*-III or -V; *traJ*-IV with *finP*-IV). *finP* may be encoded by a segment of the DNA strand complementary to that specifying the *traJ* leader mRNA, giving close linkage of the two genes (Mullineaux & Willetts, 1984). It was, however, surprising to discover six *finP* alleles amongst the twelve plasmids tested, especially since *finP* is thought to be a small gene (Johnson, Everett & Willetts, 1981). This abundance of alleles can most easily be explained by the proposal of Mullineaux & Willetts (1984) that the product of *finP* is a short RNA molecule, as predicted by DNA sequencing experiments. Different alleles might then be due to single base-pair changes, if the specificity of fertility inhibition is determined by the interaction of a *finP* RNA (plus the *finO* product) with its complementary RNA or DNA sequence to

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Table 10. *Summary of plasmid-specific alleles*

Plasmid	Incompatibility group	*finP*	*traJ*	*oriT*	Sfx	Pilus
F	I	I	I	I	I	I
ColV2	I	I	I	I	II	I
R386	I	VI	—	I	I	I
R1	II	III	III	III	III	III
R6-5	II	IV	(IV)	IV	IV	IV
R100	II	IV	IV	IV	IV	IV
R136	II	IV	(IV)	IV	IV	IV
R538-1	II	V	III	II	II	I
ColB2	II	II	I	I	II	II
ColB4	II	V	III	(II)	II	II
R124	IV	II	I	I	II	I
ColVB*trp*	IV	II	I	I	III	I

Parentheses denote that the assignation is likely, but has not been tested definitively.

prevent transcription and/or translation of *traJ*. Such a system would be similar to the control of plasmid replication by small RNA molecules, where a single base-pair change can produce a change in incompatibility group (Tomizawa & Itoh, 1981; Lacatena & Cesareni, 1981).

Secondly, the *traJ*-I and -IV alleles were always paired with the same *oriT*/*traM*,*Y* specificities, while *traJ*-III was found in conjunction with *oriT*-II or -III. This correlation was again expected, since the *traJ* protein represses transcription of the *traY*→*Z* operon, the first gene of which is *traY*. The *traY* gene is hence tightly linked to the site of action of the *traJ* protein, which must of course have the same specificity as the protein itself. In contrast to the three alleles of *traM* and *traY*, which presumably act at the analogous *oriT* sequence, there were only two alleles of *traZ* which together with *traY* encodes the *oriT*-specific endonuclease: a possible explanation for this was given in the Results section, together with an explanation of why there might also be two equivalent alleles of the *traI* gene encoding helicase I.

Thirdly, the pilus variants II, III and IV were always found in conjunction with the corresponding surface exclusion system, although pilus variant I was encoded by plasmids determining surface exclusion systems of types, I, II or III. Willetts & Maule (1974) proposed that the surface exclusion system prevents interaction between the tip of the pilus and the recipient cell surface, hence some correlation might be expected. More precise understanding of the amino-acid and sequence differences between the different pili, and of the interaction between the pilus and the recipient cell that is prevented by surface exclusion, will be required before a more precise correlation can be made. For example, the altered N-terminus of ColB2 pilin (N-Ac-ala-gln-; Finlay *et al.* 1984) compared to F pilin (N-Ac-ala-gly-ser-ser-; Frost *et al.* 1984) is presumably responsible for the decreased sensitivity of the former to filamentous DNA phages, and might also be correlated with sequence changes in the Sfx-I and -II systems.

The sequence differences associated with plasmid-specificity might prevent heteroduplex formation between these regions of the plasmid DNA molecules. Such heteroduplexes of several pairs of IncF plasmids have been extensively studied (Sharp *et al.* 1973; Achtman, Kusecek & Timmis, 1978; Hansen, Manning & Achtman, 1982; Manning & Morelli, 1982). Overall, there is good agreement between the heteroduplex data and our genetic data. In particular, substitution loops are seen corresponding to the *oriT traM finP traJ traY* or *traST* (surface exclusion) regions with different specificities, and there are insertion–deletion loops, and restriction map differences, in *traI*. Three or four other regions of non-homology within the *traY*→*Z* operon have not so far been correlated with plasmid-specific genes. R100 and R6–5 are homologous over the entire transfer region, and this corresponds to their genetic similarity.

Finally, there is no *obligatory* association of conjugation system and incompatibility group, even though such an association is usually seen amongst naturally occurring plasmids (Bradley, 1980*a*, *b*). In the case of the IncF plasmids studied, belonging to three incompatibility groups, those in IncFI and IncFIV had the most closely related conjugation systems. The IncFII plasmids were the most varied, including two groups of three relatively similar plasmids (R1, R538-1, ColB4; R6-5, R100, R136) plus ColB2 which was more similar to IncFI and IV plasmids.

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