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Further Characterization of the F Fertility Inhibition Systems of "Unusual" Fin+ Plasmids

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Flac mutants insensitive to transfer inhibition by the R factors JR66a and R485 were isolated and characterized. Representative mutations were *cis* dominant and are therefore presumed to be at the sites of action, *fisU* and *fisV*, respectively, of the FinU and FinV transfer inhibition systems encoded by JR66a and R485. The mutants were used to confirm that the FinU and FinV fertility inhibition systems are different from each other and from the FinOP, FinQ, and FinW systems of R100, R62, and R455, respectively. Together with *traO* and *fisQ* mutants of *Flac*, the new mutants were also used to investigate the nature of the F fertility inhibition systems encoded by a further group of "unusual" Fin+ plasmids. Of these, two incompatibility group X plasmids were found to carry *finO*+ genes, and of five incompatibility group I plasmids, three encoded FinQ systems, one the FinU system, and one a new system (FinR). Transfer of a variety of derepressed F-like plasmids was inhibited by the FinQ, FinU, and FinV systems, but at quantitatively very different levels; this emphasizes the differences as well as the similarities between the conjugation systems of F-like plasmids.

Although most of the Fin+ plasmids that inhibit transfer of the *Escherichia coli* K-12 sex factor F are themselves IncF F-like plasmids, a few are unusual in belonging to other incompatibility groups and having non-F-like transfer systems. In a previous publication, we described the characteristics of the fertility inhibition systems encoded by the IncI plasmid JR66a and by the IncX plasmid R485 (10). These systems of F fertility inhibition are now denoted FinU and FinV, respectively. Their characteristics allowed the FinU and FinV systems to be distinguished from each other, from the FinOP system of most F-like plasmids, and from the FinQ system of the IncI plasmid R62. The characteristics also allowed possible mechanisms of action of the FinU and FinV systems to be deduced: the former may directly prevent expression of all or part of the transfer operon, whereas the latter may prevent translation or product function of one or more pilus-forming genes.

This paper describes the isolation and characterization of *Flac* mutants insensitive to the FinU and FinV fertility inhibition systems and the use of these, together with *traO* and *fisQ* mutants, to identify the Fin systems of a further group of "unusual" Fin+ plasmids. In addition, the response of derepressed F-like plasmids other than F to the FinQ, FinU, and FinV systems was tested.

MATERIALS AND METHODS

Nomenclature. We propose to introduce a new symbol, *fis*, to denote *f*ertility *i*nhibition *s*ite of action. In general, the fertility inhibition system FinX, encoded by the gene *finX*, acts at the site *fisX*; X is the letter identifying the particular system. For the purposes of the present paper, then, the FinU and FinV systems of JR66a and R485, respectively, act at the sites *fisU* and *fisV*, mutations of which are described. In conformity with this, *traQ* (10, 20), the site of action of the FinQ system of R62, is now written as *fisQ*. However, *traO*, the previously established symbol denoting the site of action of the FinOP fertility inhibition system, is presently retained.

Bacterial strains, plasmids, and phages. The properties of the bacterial host strains used are listed in Table 1.

JCFL0 is a wild-type *Flac* element; this and the F*his* element (F57) were described by Achtman et al. (1). EDFL67 (*Flac traO304*) was described by Finnegan and Willetts (7), and WPFL39 (*Flac fisQ312*) was described by Willetts and Paranchych (20). The Fin+ R factors used are described in Table 2, and the derepressed F-like plasmids are described in Table 9.

The F-specific bacteriophages f1, f2, and Qβ were from the laboratory collection.

Media. These have been described (7).

Mutagenesis. Mutagenesis using ethyl methane

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sulfonate has been described (18). Treatment with nitrosoguanidine was similar, except that the cells were resuspended in 5 ml of 0.1 M citrate buffer, pH 5.5, and nitrosoguanidine was added to a final concentration of 100 μg/ml.

Selection of inhibitor-insensitive F*lac* mutants. The technique for selecting F*lac* mutants insensitive to transfer inhibition by JR66a or R485 was analogous to that used previously to isolate R62 inhibitor-insensitive mutants (20).

Mating conditions. The efficiency of transfer from established strains was measured as described previously (10). Replica-plate matings were carried out as described by Clark and Margulies (3). Retransfer experiments were performed as described by Finnegan and Willetts (9), except that the number of ED2196 intermediate cells that had received the F*lac* element in the first mating was measured by plating for Lac$^+$ [Nal^r] transconjugants on lactose-minimal medium rather than on lactose-tetrazolium medium. This is simpler, gives similar results, and can be used even when the donor ability of the F*lac* element is low.

TABLE 1. *Bacterial strains*

Strain	Genotype	Origin/reference
ED24	*lacΔX74 tsx spc*	7
ED26	*lacΔX74 his trp lysA tsx str cib*^{r}	20
ED2196	*lacΔX74 his trp tsx spc nal*	Nal^r from JC5455
JC3272	*lacΔX74 his trp lysA tsx str*	1
JC5455	*lacΔX74 his trp tsx spc*	1
JC6256	*lacΔX74*	1

RESULTS

JR66a-insensitive F*lac* mutants. Eight independent JR66a-insensitive mutants of F*lac* were isolated after ethyl methane sulfonate or nitrosoguanidine mutagenesis, using the selection procedure described in Materials and Methods. These F*lac* elements are all presumed to carry mutations in *fisU*, the site of action of the FinU fertility inhibition system of JR66a.

The donor abilities and F-specific phage sensitivities of the mutants, either alone or in the presence of JR66a, are presented in Table 3. All eight mutants were still partially sensitive to fertility inhibition by JR66a, and some also had reduced donor abilities and F-specific phage sensitivities, even in the absence of JR66a. This difficulty in isolating a transfer-proficient F*lac* mutant fully resistant to JR66a makes it seem likely that the mutation affecting susceptibility to JR66a-encoded fertility inhibition also affects the synthesis or function of a component(s) of the F transfer system.

On the basis of their quantitative transfer frequencies and F-specific phage sensitivities,

TABLE 2. *Fin$^+$ bacterial plasmids*

Plasmid[a]	Incompatibility group	Drug resistance markers[b]	Reference
R62	Iα	Ap Cib Sm Su Tc*	16
R100	FII	Cm Hg Sm Su Tc*	6
R455	FI	Ap Cm Sm Su Tc*	14
R485	X	Su	14
R696[c]	?	Ap Km*	12
R699[c]	?	Ap Km*	12
R711b[c]	X	Km	12
R773	FI	Asa Sm Tc*	13
R778b[c]	X	Su	12
R805a[d]	Iα	Km	5
R820a	Iα	Ap* Sm	5
JR66a	Iα	Km	4
TP102	I	Km	11
TP108	I	Km	11
TP109	I	Km	11
pUM1[e]	F?	Su	2

[a] R62 was obtained from E. Meynell; TP102, TP108, and TP109 from H. Smith and E. S. Anderson; R100, R455, R485, R805a, R820a, and JR66a from N. Datta; R696, R699b, R711b, and R778b from W. Hedges; R773 from S. Baumberg; and pUM1 from J. Pittard. We are grateful for the generous provision of these strains.

[b] Abbreviations: Ap, ampicillin; Asa, arsenate; Cib, colicin Ib synthesis; Cm, chloramphenicol; Hg, mercuric ions; Km, kanamycin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline. The marker used to select for cells carrying the plasmid is marked with an asterisk.

[c] Strains carrying these plasmids were very unstable, and cultures were grown in L broth containing kanamycin (for R696, R699b, and R711b) or sulfadimidine (for R778b).

[d] R805a could not be transferred from the J53 derivative in which it was obtained.

[e] pUM1 carries a colicin determinant indistinguishable from that of ColB-K98 (ColB4). Incompatibility tests with F*lac*, R136, and ColVB*trp* showed that it did not belong to group FI, FII, or FIV.

the eight mutants could be divided into two groups: EDFL250 through EDFL253 retained relatively high levels of transfer and phage sensitivity, whereas those of EDFL254 through EDFL257 were more severely reduced. However, it is not known whether such grouping is significant.

R485-insensitive F*lac* mutants. Three independent F*lac* mutants insensitive to inhibition by R485 were isolated in a way analogous to that used for JR66a-insensitive mutants. The site of action of the FinV fertility inhibition system of R485 is designated *fisV*, and these three mutations are assumed to have occurred at this locus.

The properties of the mutants are presented in Table 4. As with the *fisU* mutants, these three R485-insensitive mutants showed reduced donor abilities and F-specific phage sensitivities when alone in the cell. However, unlike the *fisU* mutants, they were totally insensitive to the homologous FinV fertility inhibition system. Indeed, in the presence of R485 the donor abilities and F-specific phage sensitivities of the F*lac fisV* elements increased slightly.

Dominance of the *fisU* and *fisV* mutations. Retransfer experiments were carried out to determine (i) whether an F-specified component is required for F fertility inhibition by JR66a or R485 and (ii) whether representative *fisU* and *fisV* mutations are dominant.

If an F-specified component is required, fertility inhibition should not be seen when the cells in which it is measured have only just received the F*lac* element, and the F component has therefore not had time to reach inhibitory concentrations inside the cell. This is analogous to zygotic induction of prophage λ on transfer to a nonlysogen, or of β-galactosidase on transfer of *lacI* and *lacZ* to a *lac* deletion strain (15, 17). Such a situation was observed previously for R100, where the F *finP* gene product is required in conjunction with the R100 *finO* gene product for F fertility inhibition (7, 19).

Retransfer of JCFL0 was therefore measured from intermediate cells carrying no plasmid or from cells carrying JR66a or R485 alone or together with F*his* (Table 5, first row). The presence of JR66a or R485 substantially reduced the frequency of F*lac* retransfer, showing that an F-specified component is not required in either case. Although the absolute levels of retransfer were slightly higher than those found for transfer from established strains, this conclusion

TABLE 3. *Properties of Flac mutants insensitive to fertility inhibition by JR66a*

F*lac* element	Mutation	Donor ability (%)[a]		Phage sensitivity[b]	
		Alone	+JR66a	Alone	+JR66a
JCFL0	*fis*$^+$	110	0.02	S	R
EDFL250	*fisU321*	29	2.2	S	SR
EDFL251	*fisU322*	108	4.1	S	SR
EDFL252	*fisU323*	68	5.7	S	SR
EDFL253	*fisU324*	86	6.7	S	SR
EDFL254	*fisU325*	8.4	2.7	SR	SRR
EDFL255	*fisU326*	6.9	0.6	SR	SRR
EDFL256	*fisU327*	3.4	0.6	SR	SRR
EDFL257	*fisU328*	12.9	3.0	SR	SRR

[a] The host strain was JC5455, and the number of Lac$^+$ [Str^r] progeny per 100 donor cells was measured in crosses with JC3272.

[b] Sensitivities to the F-specific bacteriophages f1, f2, and Qβ were determined by spot tests. The letters indicate the degree of lysis observed: S, clear lysis; SR, turbid lysis; SRR, extremely turbid lysis; R, no lysis.

TABLE 4. *Properties of Flac mutants insensitive to fertility inhibition by R485*[a]

F*lac* element	Mutation	Donor ability (%)[a]		Phage sensitivity[b]	
		Alone	+R485	Alone	+R485
JCFL0	*fis*$^+$	110	0.5	S	R
EDFL259	*fisV329*	7.4	15.9	SRR	SR
EDFL260	*fisV330*	7.3	12.4	SRR	SR
EDFL261	*fisV331*	5.3	8.9	SRR	SR

[a, b] See Table 3.

TABLE 5. *Retransfer of JR66a-insensitive and R485-insensitive mutants of Flac*

F*lac* element in JC6256	Mutation	Retransfer frequency[a]				
		None	JR66a	JR66a, F*his*	R485	R485, F*his*
JCFL0	*fis*+	125	0.13	0.14	3.1	6.0
EDFL250	*fisU321*	73	4.6	2.1		
EDFL259	*fisV329*	8.2			7.5	5.3

[a] Derivatives of JC6256 carrying the F*lac* elements were mated with derivatives of ED2196 carrying the plasmids indicated. Retransfer frequencies from these intermediate strains to the recipient strain JC3272 were measured. The numbers represent the frequencies of Lac$^+$ [Str^r] derivatives of JC3272 per 100 intermediate ED2196 cells that had received the F*lac* element in the first mating.

was confirmed by the absence of any further reduction of F*lac* retransfer when F*his* was also present in the intermediate cells.

A representative *traU* mutant, EDFL250, was then tested in similar retransfer experiments to determine whether the *traU* mutation is dominant to the wild-type allele. For this, the frequencies of EDFL250 retransfer from cells carrying no plasmid, JR66a alone, or both JR66a and F*his* (which is *fisU*$^+$) were compared both with each other and with the equivalent frequencies of JCFL0 retransfer (Table 5). As expected from the residual partial FinU sensitivity of EDFL250 (and all other *fisU* mutants isolated), its retransfer from cells carrying JR66a was lower than its retransfer from plasmid-free cells. However, its retransfer from cells carrying both JR66a and F*his* took place at a frequency comparable to that from cells carrying JR66a alone and 15 times greater than the retransfer of JCFL0 from such cells. The JR66a insensitivity of EDFL250 transfer is therefore observed even in the presence of F*his fisU*$^+$ showing the *fisU321* mutation to be *cis* dominant.

Retransfer of a representative *fisV* mutant, EDFL259, took place at similar frequencies from cells carrying no plasmid, R485 alone, or both R485 and F*his*. The *fisV329* mutation of EDFL259 is therefore dominant over the *fisV*$^+$ allele carried by F*his*. In this instance, the similar absolute retransfer levels of both JCFL0 and EDFL259 from cells carrying R485 with or without F*his* are misleading because of the partial transfer deficiency of EDFL259 and other *fisV* mutants. Comparison with the frequencies of retransfer from cells carrying no plasmid shows that retransfer of JCFL0 is inhibited, whereas that of EDFL259 is not.

Inhibition of F*lac fisU* and F*lac fisV* mutants by heterologous Fin systems. To confirm that the fertility inhibition systems encoded by JR66a and R485 are distinct from each other and from the FinOP, FinQ, and FinW systems described previously (7, 10, 20), the susceptibility of representative *fisU* and *fisV* mutants to the four other Fin systems was tested. For this, the donor abilities and F-specific phage sensitivities of strains carrying an F*lac fisU* or F*lac fisV* mutant together with Fin$^+$ plasmids representing all five Fin systems were determined (Table 6).

Transfer of the *fisU* mutants EDFL250 and EDFL256 was inhibited by R100, R62, R485, and R455. Furthermore, their F-specific phage sensitivities were reduced by R100, R62, and R485, but not by R455. This is consistent with the properties of the FinW fertility inhibition system of R455, which prevents transfer but not pilus formation or surface exclusion by F*lac* (10). These results therefore confirm that the *fisU* mutants are specifically insensitive to inhibition by JR66a, and hence that the FinU fertility inhibition system is distinct from the FinOP, FinQ, FinV, and FinW systems.

Similarly, transfer and F-specific phage sensitivity of the *fisV* mutant EDFL259 was inhibited by R100, R62, and JR66a, whereas R455 again prevented transfer but not phage sensitivity. This confirms that the FinV fertility inhibition system of R485 is distinct from the four others.

More "unusual" Fin$^+$ plasmids. Originally, we screened 28 Fin$^+$ plasmids to determine the nature of their transfer inhibition systems and partially characterized the FinU and FinV systems that are further described above (10). Since that time, other "unusual" Fin$^+$ plasmids have been described or have become available to us. These include plasmids that are not F-like and/or do not fall into known F incompatibility groups; they also include R773, an F-like plasmid that belongs to the relatively rare FI group and determines arsenate resistance. The sources and properties of these plasmids, together with the references that first described

TABLE 6. *Fertility inhibition of JR66a-insensitive and R485-insensitive mutants of Flac by plasmids encoding heterologous Fin systems*

Fin$^+$ plasmid	Fin system	Donor ability of:[a]				Phage sensitivity of:[b]			
		JCFL0 *fis*$^+$	EDFL250 *fisU*	EDFL256 *fisU*	EDFL259 *fisV*	JCFL0 *fis*$^+$	EDFL250 *fisU*	EDFL256 *fisU*	EDFL259 *fisV*
None	None	110	29	3.4	7.4	S	S	SR	SRR
JR66a	FinU	0.02	2.2	0.6	0.002	R	SR	SRR	R
R485	FinV	0.5	0.06	0.006	16	R	R	R	SR
R62	FinQ	1.1	0.5	0.05	0.004	R	SRR	R	R
R100	FinOP	0.06	0.06	0.007	0.008	R	R	R	R
R455	FinW	0.2	0.1	0.01	0.03	S	S	SR	SR

[a, b] Footnotes as for Table 3, except that ED26 (ColI^r) was substituted for JC3272 when the donor strain carried the Cib$^+$ R factor R62.

their Fin$^+$ phenotype, are listed in Table 2.

The nature of the transfer inhibition systems of these plasmids has been investigated, taking advantage of the various F*lac* mutants specifically insensitive to four of the known fertility inhibition systems, including the F*lac fisU* and *fisV* mutants described above. Originally, it was our intention to carry out this screening simply by testing the F-specific phage sensitivities of strains carrying a Fin$^+$ plasmid and an inhibitor-insensitive F*lac* mutant. However, the partial JR66a sensitivity of *fisU* mutants and the reduced donor ability and F-specific phage sensitivity of *fisV* mutants precluded this, and quantitative donor ability measurements were also made.

For convenience, the Fin$^+$ plasmids are divided into two groups. First, several, including the F-like plasmids R773 and pUM1, the X-group plasmids R711b and R778b, and the plasmids of unknown incompatibility group R696 and R699b, carried *finO*$^+$ genes able (in association with the F-specified *finP* product) to inhibit F*lac* transfer. The Fin systems encoded by these plasmids were identified by their inability to inhibit the transfer or F-specific phage sensitivity of F*lac traO304*, which is insensitive to inhibition by the FinOP system (Table 7). Tests with the other inhibitor-insensitive F*lac* mutants were consequently not carried out, or the results are not shown.

When transfer of the Fin$^+$ R factors themselves was measured both when alone and when in the presence of F*lac traO304*, the transfer frequency of R773 increased dramatically from a very low unaided level to a frequency comparable to that of F*lac traO304* (Table 7). It is not known, incidentally, why this level was around 10% rather than 100%. This phenomenon shows that the plasmid-specific *traI* and/or *traJ* gene products of R773 and F are interchangeable such that those made by F*lac traO304* can be used to give efficient R773 transfer. The transfer systems of these two FI incompatibility group plasmids are therefore closely related. Transfer of the other plasmids in this group, including the IncF plasmid pUM1, was increased only slightly by the presence of F*lac traO304*. The mechanism of this relatively low-level and therefore nonspecific effect is not known, although some of the possibilities have been listed previously (10).

Plasmids of the second group all belong to the I incompatibility group; their abilities to inhibit the transfer and F-specific phage sensitivity of the various inhibitor-insensitive F*lac* mutants are shown in Table 8. Transfer and pilus formation by F*lac fisQ312* were not substantially affected by R805a, TP102, and TP108; all three plasmids must therefore determine FinQ transfer inhibition systems. As expected for these plasmids, which are unrelated to F, their transfer was not increased in the presence of F*lac fisO312*. In contrast, TP109 encoded a highly efficient transfer inhibition system that reduced the transfer of F*lac tra*$^+$ *fis*$^+$, *traO*, *fisQ*, and *fisV* elements to similar low levels. However, it was much less efficient at reducing the transfer and F-specific phage sensitivity of F*lac fisU321*, and since all *fisU* mutants are still partially sensitive to transfer inhibition by JR66a itself (Table 3), we conclude that TP109 determines the FinU type of transfer inhibition system.

R820a clearly inhibited the transfer and F-specific phage sensitivity of F*lac traO*, *fisQ*, and *fisU* mutants. However, although it also inhibited the F-specific phage sensitivity of the F*lac fisV* mutant, it apparently did not reduce its transfer level. We incline to interpret these data on the basis of the F-specific phage sensitivities by hypothesizing that the fertility inhibition system of R820a does in fact prevent expression of the F*lac fisV* transfer system, and

TABLE 7. *Transfer inhibition by plasmids with finO*$^+$ *genes*[a]

Fin$^+$ R$^+$ factor	Transfer (%)		R factor transfer (%) in presence of:	
	F*lac tra*$^+$	F*lac traO304*	No other plasmid	F*lac traO304*
None	110[b]	140[b]		
R773	0.020[c]	9.3[d]	0.0021	9.6
pUM1	0.038	150[b]	0.84	2.9
R711b	0.83	82[b]	0.0011	0.030
R778b	13[d]	150[b]	0.0027	0.037
R696	0.40	110[b]	<0.0001	0.006
R699b	0.28	62[b]	0.0016	0.023

[a] The plasmid host strain was ED2196, and matings with JC3272, selecting Str^r transconjugants, were carried out as described in Materials and Methods. Twenty-five clones from each of the donor cultures were tested for the presence of the appropriate plasmid(s), and ≥90% stability was found even in the case of strains carrying both R773 and an F*lac* element, or of strains carrying the unstable plasmids R711b, R778b, and R699b, grown in the presence of the appropriate antibiotic. Unless otherwise noted, strains were resistant to F-specific phages.

[b] These strains were fully sensitive to the F-specific phages f1, f2, and Qβ.

[c] Transfer of F*lac* elements carrying *traQ*, *traU*, and *traV* mutations was inhibited to a similar extent.

[d] These strains were partially sensitive (turbid spot test) to the F-specific phages.

TABLE 8. *Transfer inhibition by Fin⁺ I-like plasmids*[a]

Fin⁺ R factor	F*lac* transfer (%)					R factor transfer (%) in presence of:	
	tra⁺	*traO304*	*fisQ312*	*fisU321*[b]	*fisV329*	No other plasmid	F*lac fisQ312*
None	110[c]	140[c]	110[c]	29[c]	7.4[c]		
R805a[d]	5.8[e]	8.8[e]	31[c]			<0.0001	0.003
TP102	7.0[e]	4.1[f]	40[c]	1.7[e]	0.027	2.5	1.9
TP108	0.78[e]	1.2[f]	38[c]	0.14	0.010	0.21	0.11
TP109	0.0060	0.0025	0.0021	0.32[e]	0.0028	6.2	2.0[g]
R820a	8.0[e]	10[e]	5.8	4.4	7.5	0.9	4.6[g]

[a] The plasmid host strain was ED2196, and matings with JC3272, selecting Str[r] transconjugants, were carried out as described in Materials and Methods. Unless otherwise noted, strains were resistant to F-specific phages.

[b] Transfer of F*lac fisU327*, which has a lower donor ability than F*lac fisU321* (Table 3), was inhibited to a similar extent in all cases.

[c] These strains were fully F-specific phage sensitive (S).

[d] Since this R factor could not be transferred out of the J53 host in which it was obtained, J53 rather than ED2196 was used as host in these experiments.

[e] These strains were very slightly F-specific phage sensitive (RRS).

[f] These strains were partially F-specific phage sensitive (RS).

[g] Transfer of TP109 in the presence of F*lac fisU321*, or of R820a in the presence of F*lac fisV329*.

that F*lac fisV* transfer under these conditions is due to mobilization by the fairly efficient R820a transfer system. On this basis, R820a would determine a new transfer inhibition system, which we designate FinR. In agreement with this, R820a, unlike R485, inhibited surface exclusion by F*lac* by about 100-fold.

Transfer inhibition of other derepressed F-like plasmids. Although the FinQ, FinU, and FinV systems prevent the transfer of F, it does not necessarily follow that they will also prevent the transfer of other F-like plasmids. This depends upon the specificity of the interactions involved. To gain some knowledge of this, transfer of a variety of derepressed F-like plasmids in the presence of R62, JR66a, or R485 was measured (Table 9). It can be seen that the quantitative levels of transfer inhibition vary enormously for each of these three plasmids, presumably because the amino acid or nucleotide sequences at which the fertility inhibition systems act vary from F-like plasmid to F-like plasmid, despite the close relationship between their conjugation systems. We are presently investigating the nature of the sites of action of these three fertility inhibition systems, knowledge of which may allow more meaningful interpretation of the data in Table 9.

DISCUSSION

In most cases, the JR66a-independent *fisU* mutations and the R485-independent *fisV* mutations had simultaneously caused a partial transfer defect, and in addition all the *fisU* mutants retained partial sensitivity to JR66a transfer inhibition. We therefore suspect that the *fisU* and *fisV* mutations take place within a gene or site that is required for transfer and that high levels of transfer and JR66a or R485 independence are incompatible.

Despite these undesirable properties, it was possible to use the F*lac fisU* and *fisV* mutants to demonstrate the *cis* dominance of representative mutations: these have therefore probably occurred in the sites of action of the FinU and FinV fertility inhibition systems, respectively. They are equivalent to *traO* and *fisQ* mutations that prevent transfer inhibition by the FinOP and FinQ systems (7, 20). It was found that like R62 (20), but unlike FinOP plasmids such as R100 (7, 8), no F-specified component is required for FinU or FinV transfer inhibition. The *fisU* and *fisV* mutants were also used to confirm the dissimilarity of the five transfer inhibition systems decribed previously (10), by showing that heterologous Fin systems still inhibited their transfer and F-specific phage sensitivities.

In addition, the *fisU* and *fisV* mutants were used, together with F*lac traO* and *fisQ* mutants, to identify the fertility inhibition systems of a further group of unusual Fin⁺ plasmids. Two further IncX plasmids, R711b and R778b, carried *finO*⁺ genes and therefore differed from the IncX FinV plasmid R485. Three of the IncI plasmids, R805a, TP102, and TP108, resembled the IncI plasmid R62 in determining FinQ systems; the other Km[r] Fin⁺ I-like R factors described by Grindley and Anderson (11)

TABLE 9. *Transfer inhibition ratios*[a]

Fin⁻ plasmid[b]	Transfer inhibition ratio		
	R62	JR66a	R485
F*lac*	120	5,500	220
ColV2	7	>50	>60
EDR202	NT[c]	880	3,800
R1-19	18	2,300	37,000
R100-99	53	120	15[d]
EDR207	NT[e]	1.4[f]	43,000
EDR200	NT[c]	490	230,000
ColVB*trp*	21	51	17

[a] Numbers given are the transfer inhibition ratios, obtained by dividing the frequency of transfer from ED2196 derivatives carrying no other plasmid by the frequency from cells also carrying the Fin⁺ plasmid indicated.

[b] All these plasmids carry *finO* mutations except EDR200, which carries a *finP* mutation, and EDR202, which carries a *finP* or *traO* mutation. EDR202, EDR207, and EDR200 are derived from R386, R538-1, and R124, respectively (N. S. Willetts and J. Maule, unpublished data), and R100-99 is a Tet[s] Cml[s] derivative of R100-1. Plasmid sources are given in reference 8.

[c] Not tested since no distinguishing markers were available.

[d] EDR44, a deletion mutant of R100 that has lost *finO cml str sul*, was used in place of R100-99.

[e] Not tested since this pair of plasmids was unexpectedly incompatible.

[f] This strain was sensitive to F-specific bacteriophages. It was also rather unstable.

(except for TP109) may be similar, but were not tested. Another IncI plasmid, TP109, resembled the IncI plasmid JR66a in determining the FinU system, whereas the last IncI plasmid, R820a, determined a sixth transfer inhibition system differing from those previously described, and which we designate FinR.

Gasson and Willetts (10) made the point that the extents to which the various *finO*⁺ plasmids reduce F transfer vary over a wide range and that this might reflect some degree of specificity of the *finO* product. The effectiveness of the *finO*⁺ genes carried by the plasmids investigated here also varied, over a 600-fold range (Table 7). Similarly, the FinQ systems of R62 and TP108 were 10-fold more effective than those of R805a and TP102. In a reciprocal manner, some degree of specificity might be expected in the sites at which the various Fin systems act, so that a given Fin⁺ plasmid would inhibit the transfer of different derepressed F-like plasmids to different levels. N. S. Willetts and J. Maule (manuscript in preparation) have shown that this is the case for the FinO system, and the same wide variation was found for the FinQ, FinU, and FinV systems in the present paper (Table 9).

In conclusion, a pattern for unusual Fin⁺ systems is emerging, especially for those determined by IncI plasmids. We are continuing our investigations of the underlying biochemical mechanisms, both because of their intrinsic interest and because knowledge of these may also help our understanding of transfer gene expression.

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