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Plasmid Specificity of The Origin of Transfer of Sex Factor F

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The ability of F-like plasmids to promote transfer from the F origin of transfer was determined. Chromosome transfer was measured from plasmid derivatives of RecA$^-$ Hfr deletion strains which had lost all the F transfer genes but which in some cases retained, and in others had also lost, the origin sequence. ColV2 and ColVB*trp* could initiate transfer from the F origin, but R100-1, R1-19, and R538-1 *drd* could not. These results can be correlated with the plasmid specificity of the *traI* components of the different plasmid transfer systems, supporting the hypothesis that the origin of transfer is the site of action of the *traI* product. Most F-like plasmids, including R1-19 and R538-1 *drd*, could transfer ColE1, consistent with previous findings that the (plasmid-specific) *traI* product is not necessary for ColE1 transfer by F*lac;* ColE1 transfer may be initiated by a ColE1- or host-determined product. R100-1 and R136*fin*$^-$ could not transfer ColE1 efficiently, apparently because of differences residing in their pilus-forming genes.

During conjugation, deoxyribonucleic acid (DNA) is transferred from Hfr or F prime donor strains as a linear sequence of bases. The origin (*ori*), at which transfer begins, was first located outside the region containing the transfer (*tra*) genes (5, 22) and was then shown to lie within the F factor, between *traJ* and a locus (ϕ_{11}^R) specifying "resistance" to female-specific phages (22; Fig. 1). For the latter experiments, a series of RecA$^-$Hfr deletion mutants were constructed, and the presence or absence of the origin of transfer ascertained by measuring transfer of a chromosomal marker, the necessary transfer system being provided by an F*lac* element. Some ϕ_{11}^R deletion strains which lacked all the known *tra* genes retained the origin, and others had lost it.

In this study, we used those Hfr deletion mutants differing, as far as is known, only in the presence or absence of the origin of transfer to determine whether F-like plasmids with related transfer systems could initiate transfer from this origin. Also, we investigated the ability of these plasmids to transfer ColE1, which does not require the *traI* product for its transfer by F (3).

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1. ED2086 and ED2087 are *ori*$^+$ RecA$^-$Hfr deletion strains, and ED2089 and ED2091 are *ori*$^-$RecA$^-$Hfr deletion strains (22). ED3701, ED3702, ED3703, and ED3704 are their respective ColVB^R derivatives.

The plasmid host strains of their progenitors have been described previously (3, 22). ED57 is a ColVB^R derivative of JC3272, ED3707 is a Trp$^+$ transductant of JC6256, and ED3710 is a *bfe*$^-$ (colicin E-resistant) derivative of ED57. ED3724 is a ColVB^R derivative of ED267, and ED3815 is a ColVB^R derivative of ED369 (the RecA$^-$ derivative of the F$^-$ parent of the Hfr deletion strains). ColVB^R mutants were selected as resistant to the colicins produced by strains carrying ColVB*trp* and were not further characterized.

Plasmids and their detection. The sources of the colicinogenic factors ColV2 and ColVB*trp* and methods for detection of cells and progeny carrying them have been described (3). When ColE1 transfer by ColV2 or ColVB*trp* was measured, the appropriate ColE1^s ColVB^R (ED3724) and ColE1^R ColVB^s (ED664) indicator strains were used. The sources of the R factors have been given by Willetts (20), Alfaro and Willetts (3), and Finnegan and Willetts (8). R538-1 *drd* (16) was obtained from P. Beard. The presence of R100-1, R64-11, R136*fin*$^-$, and R386 was detected by resistance to tetracycline (20 μg/ml), and the presence of R1-19 and R538-1 *drd* was detected by resistance to spectinomycin (20 μg/ml).

The F*lac* element and the F*lac tra*$^-$ mutants were those described by Achtman et al. (1, 2) and Willetts and Achtman (24).

Media. The media used have been described (7, 9).

Bacterial matings. Plasmid and *pro*$^+$ transfer from established derivatives of the RecA$^-$Hfr deletion strains were measured by mixing 0.2 ml of an exponential-phase culture of the donor strain containing 2

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TABLE 1. *Bacterial strains*

Strain no.	His	Trp	Pro	Lac	T6[a]	Str	ColVB	RecA	Other
ED57	−	−	+	−	R	R	R	+	Lys⁻
ED267[b]	−	−	−	−	R	R	S	+	
ED369	+	+	+	−	R	S	S	−	
ED664[c]	−	−	+	−	R	R	S	+	Bfe^R
ED2086	+	+	+	−	R	S	S	−	
ED2087	+	+	+	−	R	S	S	−	
ED2089	+	+	+	−	R	S	S	−	
ED2091	+	+	+	−	R	S	S	−	
ED3707	+	+	+	−	S	S	S	+	
ED3710[c]	−	−	+	−	R	R	R	+	Lys⁻Bfe^R
ED3724	−	−	−	−	R	R	R	+	
ED3815	+	+	+	−	R	S	R	−	
JC6256	+	−	+	−	S	S	S	+	

[a] R, Resistant; S, susceptible.

[b] ED267 is a T6^S Str^{R}F⁻ derivative of J6-2 (4). It does not carry a *proC* mutation, but either a *proA* or a *proB* mutation (B. Bachmann, personal communication).

[c] The *bfe* mutation confers resistance to colicins of type E and bacteriophage BF23 (11).

× 10^8 cells per ml with 1.8 ml of a similar culture of either ED267 (for donors carrying R factors) or ED3724 (for donors carrying Col factors). After incubation at 37 C for 60 min, dilutions were plated on media selective for either plasmid-carrying or Pro⁺ progeny resistant to streptomycin. It was essential to use RecA⁻ derivatives of the Hfr deletion strains even for plasmids other than F, since large regions of homology between F and many F-like plasmids have been demonstrated (19).

Chromosome transfer by an incoming plasmid was measured as described by Willetts (22) for F*lac*. In addition, plasmid retransfer from the RecA⁻Hfr deletion strains was measured by selecting appropriate derivatives of the recipient strain.

Plasmid-promoted ColE1 transfer was measured by mixing 0.2 ml of an exponential-phase culture of a derivative of ED3707 carrying both ColE1 and the plasmid with 1.8 ml of a culture of ED3710. After incubation for 30 min at 37 C, dilutions were plated on the appropriate selective media. To obtain reproducible results for ColE1 transfer by cells carrying both R100-1 and F*lac* (or an F*lac tra*⁻ mutant), it was found to be necessary to grow the cells for at least eight generations in exponential phase before mating.

All results given are the averages of two or more experiments. The frequencies of *pro*⁺ transfer have been corrected for the (very low) spontaneous reversion frequency of the *pro*⁻ mutation.

RESULTS

Chromosome transfer by incoming plasmids. The *ori*⁺ and *ori*⁻ Hfr deletion strains used in this study still retain the site (*inc*) determining incompatibility towards a superinfecting F prime element (23; Fig. 1). In the case of F and possibly ColV2, which has a related incompatibility system (12, 15), it is therefore impossible to construct stable plasmid derivatives. For these plasmids then, and also for the other plasmids for purposes of comparison, the ability of the plasmid to mobilize the chromosomal *pro*⁺ marker was measured immediately after transfer of the plasmid to the RecA⁻Hfr deletion strain.

The results show that F*lac*, ColV2, and ColVB*trp* all promoted chromosomal transfer from the *ori*⁺ strains at 70 to 300 times the frequency of transfer from the *ori*⁻ strains (Table 2). The transfer systems of ColV2 and ColVB*trp* can, therefore, utilize the F origin of transfer with efficiencies similar to that of F itself. However, the plasmids R100-1, R1-19, and R538-1*drd* did not promote chromosomal transfer from either the *ori*⁺ or the *ori*⁻ strains, and their transfer systems must therefore recognize different origin sequence(s).

The low but significant levels of *pro*⁺ transfer from the *ori*⁻Hfr deletion strains by F*lac* and ColV2 were apparently due to a sequence carried by the remaining F DNA, since there was virtually no *pro*⁺ transfer from the isogenic RecA⁻F⁻ strain ED3815 (Table 2).

Chromosome transfer by established plasmids. Since the plasmids ColVB*trp*, R100-1, R1-19, and R538-1 *drd* are compatible with the F factor, stable derivatives of the RecA⁻Hfr deletion strains carrying these plasmids could be constructed. The ability of these derivatives to transfer the chromosomal *pro*⁺ marker were measured to confirm the results presented above.

Although transfer of ColVB*trp* itself by exponential cultures of these strains was low, it was again apparent that ColVB*trp* promoted chromosomal transfer from the *ori*⁺ strains (Table

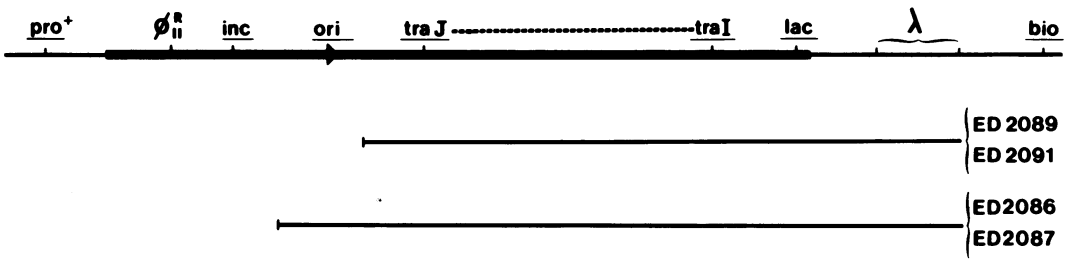

FIG. 1. *Hfr deletion strains. All four are* $\phi_{11}^{R}inc^{+}traJ^{-}$ *(22, 23).*

TABLE 2. *Chromosome transfer by incoming plasmids*[a]

Incoming plasmid	Intermediate strain	*ori*	Plasmid retransfer (%)	*pro*+ transfer (%)	*pro*+/plasmid transfer (%)
F*lac*	ED3701	−	5.9	2×10^{-3}	0.03
	ED3702	−	3.2	3×10^{-3}	0.09
	ED3703	+	2.7	0.32	12
	ED3704	+	3.4	0.62	18
	ED3815	−	32	3×10^{-4}	9×10^{-4}
ColV2	ED3701	−	21	1×10^{-2}	0.05
	ED3702	−	18	2×10^{-2}	0.11
	ED3703	+	15	0.7	4.7
	ED3704	+	17	1.1	6.5
	ED3815	−	105	3×10^{-4}	3×10^{-4}
ColVB*trp*	ED3701	−	2.2	3×10^{-4}	0.014
	ED3702	−	4.4	3×20^{-3}	0.016
	ED3703	+	0.19	5×10^{-3}	2.6
	ED3704	+	0.13	6×10^{-3}	4.6
R100-1	ED3701	−	0.8	$<10^{-4}$	
	ED3702	−	1.4	$<10^{-4}$	
	ED3703	+	1.3	$<10^{-4}$	
	ED3704	+	3.2	$<10^{-4}$	
R1-19	ED3701	−	4.6	$<10^{-4}$	
	ED3702	−	31	$<10^{-4}$	
	ED3703	+	20	$<10^{-4}$	
	ED3704	+	34	$<10^{-4}$	
R538-1*drd*	ED3701	−	21	$<10^{-4}$	
	ED3702	−	21	$<10^{-4}$	
	ED3703	+	6.5	$<10^{-4}$	
	ED3704	+	7.6	$<10^{-4}$	

[a] The donor strain was a derivative of JC6256, the intermediate strain was a ColVB^{R}RecA^{-}Hfr deletion strain (*ori*+ or *ori*−), and the recipient strain was ED267 (for crosses involving R factors) or ED3724 (for crosses involving Col factors).

TABLE 3. *Chromosome transfer by established plasmids*

Plasmid	Host strain	*ori*	Plasmid transfer (%)	*pro*+ transfer (%)	*pro*+/plasmid transfer (%)
ColVB*trp*	ED2086	−	0.64	$<10^{-4}$	
	ED2087	−	0.62	$<10^{-4}$	
	ED2089	+	0.26	0.0020	0.8
	ED2091	+	0.22	0.0019	0.9
ColVB*trp*[a]	ED2086	−	13	0.007	0.05
	ED2087	−	14	0.010	0.07
	ED2089	+	23	0.34	1.5
	ED2091	+	23	0.47	2.0
	ED369	−	80	$<10^{-4}$	
R100-1	ED2086	−	2.5	$<10^{-4}$	
	ED2087	−	2.3	$<10^{-4}$	
	ED2089	+	0.5	$<10^{-4}$	
	ED2091	+	1.9	$<10^{-4}$	
R1-19	ED2086	−	4.0	$<10^{-4}$	
	ED2087	−	4.5	$<10^{-4}$	
	ED2089	+	2.3	$<10^{-4}$	
	ED2091	+	3.0	$<10^{-4}$	
R538-1*drd*	ED2086	−	3.8	$<10^{-4}$	
	ED2087	−	4.4	$<10^{-4}$	
	ED2089	+	2.3	$<10^{-4}$	
	ED2091	+	3.0	$<10^{-4}$	

[a] These experiments used standing overnight cultures of the donor strains diluted 1:5 before use.

3). Unexpectedly, it was found that the frequencies of transfer of ColVB*trp* and the chromosomal *pro*+ marker were both increased by using standing overnight donor cultures, and these experiments confirmed that ColVB*trp* transferred *pro*+ from *ori*+, but not *ori*−, strains (Table 3). As with F*lac* and ColV2 previously, there was a low but significant level of transfer of *pro*+ by ColVB*trp* from the *ori*− RecA^{-}Hfr deletion strains. Again, there was no equivalent transfer from the isogenic RecA^{-}F^{-} strain ED369 (Table 3), and sequence carried by the remaining F DNA is presumed to be involved.

Transfer of the plasmids R100-1, R1-19, and R538-1 *drd* from the *ori*+ and *ori*− strains also took place at a fairly low frequency, but again there was no measurable transfer of the chromosomal *pro*+ marker from either type (Table 2).

In fact, some *pro*+ transfer by R1-19 was expected, as this has been reported to take place

at a frequency of about 10^{-2}% from both Rec$^+$ and RecA$^-$ donor strains (17, 18). Possibly the poor overall donor abilities of the RecA$^-$Hfr deletion strains reduced this below the detectable level. Plasmid derivatives of the RecA$^-$Hfr deletion strains gave 5- to 10-fold less plasmid transfer than equivalent derivatives of a RecA$^-$F$^-$ strain, themselves 5- to 10-fold less efficient than derivatives of a Rec$^+$F$^-$ strain.

Transfer of ColE1. The above data show that ColV2 and ColVB*trp*, both of which allow transfer of F*lac tral*$^-$ mutants (3), can initiate transfer at the F origin. On the other hand, R100-1, R1-19, and R538-1*drd*, which cannot supply a *traI* product able to allow F*lac tral*$^-$ transfer (3, 21; Willetts, unpublished data), cannot. This is therefore consistent with the proposal by Willetts (22) that the origin of transfer is recognized by the corresponding *traI* product.

ColE1 does not require the *traI* product for its transfer by F (3) and all other components of the F transfer system (excluding the *traJ* product, which is not directly involved in DNA transfer [9]) can be substituted for by those of all the other F-like Col and R factors so far tested. These F-like plasmids should therefore be able to transfer ColE1. In fact, transfer by ColV2 has been demonstrated previously (12, 25).

The abilities of several other F-like plasmids, and also of the I-like plasmid R64-11, to transfer ColE1 were therefore measured (Table 4). As predicted above, not only F*lac*, ColV2, and ColVB*trp*, all of which recognize the F origin of transfer, but also R1-19 and R538-1*drd*, which recognize different origin(s), allowed ColE1 transfer at frequencies similar to those for their own transfer. R386 transferred too poorly for measurement of the origin specificity of its transfer system, but it also gave ColE1 transfer at a frequency similar to that of its own. Even R64-11 allowed efficient ColE1 transfer, despite the fact that this I-like plasmid specifies a transfer system that is genetically dissimilar to that of F and does not recognize the F origin of transfer (20). ColE1 transfer by the prototype I-like plasmid ColI has also been demonstrated (6).

We conclude that ColE1 DNA must include either several origin sequences recognized by the various plasmid transfer systems, or an origin sequence recognized by a product specified by ColE1 itself or by the host chromosome. The ability of F*lac tral*$^-$ mutants to transfer ColE1 suggests that the second alternative is correct if, as we propose, it is the *traI* product which recognizes the origin of transfer.

The low frequencies of transfer of ColE1 by both R100-1 (24) or R136 *fin*$^-$ were unexpected (Table 3). An attempt was made to determine which F *tra* gene(s) was necessary for a high frequency of ColE1 transfer by measuring transfer from strains carrying R100-1, ColE1, and an F*lac* element mutant in one of the *tra* genes (Table 5). Comparison of the rates of transfer of ColE1 and of the F*lac tra*$^-$ mutants showed that all 10 *tra* gene products required for formation of the F-pilus were necessary to allow a high level of ColE1 transfer. The products of *traD* and *traI*, not necessary for pilus synthesis (2, 24) or, in the case of *traI*, for ColE1 transfer (3),

TABLE 4. *Transfer of ColE1 by various plasmids*

Plasmid	Plasmid transfer (%)	ColE1 transfer (%)	ColE1 transfer as % of plasmid transfer
F*lac*	65	65	100
ColV2	150	220	145
ColVB*trp*	90	105	115
R100-1	65	0.2	0.1
R136*fin*[a]	125	0.07	0.05
R1-19	140	55	40
R538-1*drd*	110	55	50
R386	1.5	1.1	75
R64-11	40	29	70

[a] This is the R136 derivative called 240*i*$^-$1 by Grindley et al. (10).

TABLE 5. *Transfer of ColE1 by R100-1/Flac tra*$^-$ *strains*

Plasmids in donor strain	Transfer (%)			ColE1 transfer as % of F*lac* transfer
	R100-1	F*lac*	ColE1	
— F*lac*		48	47	100
R100-1 —	33		0.17	
R100-1 F*lac*	32	42	36	86
R100-1 F*lac traA1*	39	15	0.85	6
R100-1 F*lac traB2*	38	13	2.0	15
R100-1 F*lac traC5*	41	38	3.8	10
R100-1 F*lac traD83*	42	12	12	100
R100-1 F*lac traE18*	72	42	4.1	10
R100-1 F*lac traF13*	20	24	2.5	10
R100-1 F*lac traG42*	18	9	0.5	6
R100-1 F*lac traG81*	56	36	3.6	10
R100-1 F*lac traH88*	47	21	2.1	10
R100-1 F*lac traI40*	47	0.13	21	
R100-1 F*lac traJ90*	38	0.08	0.27	
R100-1 F*lac traK105*	37	13	1.8	13
R100-1 F*lac traL311*	73	26	3.4	13

were not. The biochemical interpretation of these findings is at present unclear.

DISCUSSION

Both ColV2 and ColVB*trp* could initiate transfer from the F origin, whereas R100-1, R1-19, and R538-1*drd* could not. This can be correlated with the plasmid specificities of the *traI* products of these plasmids. Those of ColV2 and of ColVB*trp* could replace that of F, whereas those of R100-1, R1-19 and R538-1*drd* could not (3, 21; Willetts, unpublished data). The *traI* product is presumed to be the one which is relevant since *traI* and *traJ* are the only genes giving plasmid-specific products so far identified, and *traJ* is known to be a control gene required for expression of all the other *tra* genes, including *traI* (9). Also, the *traI* product is not required for pilus formation and is, therefore, likely to be involved in DNA metabolism during conjugation (24). This correlation between the specificities of the *traI* product and of the origin sequence supports the suggestion of Willetts (22) that the *traI* product is an endonuclease which recognizes the origin sequence. This possibility is now being tested directly.

The reason for the low level of chromosomal transfer by F, ColV2, and ColVB*trp* from the *ori*$^-$Hfr deletion strains, about 1% of that from the *ori*$^+$ strains, is not clear. There was almost no chromosome transfer from an equivalent F$^-$ strain, so that a sequence in the remaining F DNA is presumably involved, perhaps in a site-specific recombination event, since homologous sequences of F or chromosomal DNA cannot recombine in the absence of the *recA*$^+$ product (Willetts, unpublished data).

When an F-like plasmid and an F*lac tra*$^-$ mutant are present in the same cell, transfer of the F*lac* mutant might occur either by complementation (i.e., via the interchange of a single gene product) or by recognition of the F origin sequence by the (possibly quite different) transfer system of the F-like plasmid. Both mechanisms may contribute to transfer of F*lac tra*$^-$ mutants from cells also carrying ColV2 or ColVB*trp*, but only complementation can function for the R factors tested above since these did not recognize the F origin sequence.

Finally, the ability of all F-like and I-like plasmids tested (except R100-1 and R136*fin*$^-$) to transfer ColE1 at high frequency suggests that a product of either ColE1 itself or of the chromosome serves to initiate transfer of ColE1 DNA. This is consistent with the finding of Alfaro and Willetts (3) that the F *traI* product is not required and of Kingsbury and Helinski (13) that DNA polymerase I is required for transfer of ColE1, but not of ColV2. The inabilities of R100-1 and R136*fin*$^-$ to transfer ColE1 efficiently was apparently due to differences in the type of pilus. The pili of these two R factors are in fact closely related serologically (14) and show similar efficiencies of plating of F-specific phages (Willetts, unpublished data).

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