The Activity of Different Transfer Factors Introduced into the Same Plasmid-containing Strain of *Escherichia coli* K12

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SUMMARY

Non-transferring plasmids containing the genetic determinants for resistance to streptomycin (Sm), sulphonamides (Su) and ampicillin (Ap), colicine production (Col) and α -haemolysin production (Hly) were incorporated in the same strain of *Escherichia coli* K12. A plasmid coding for tetracycline (Tc) resistance was then co-transferred to it by eleven transfer factors, including F and I.

Some of the transfer factors differed greatly in the rate at which they transferred the determinants from this Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺ donor strain to another strain of K12 and some of the determinants differed greatly in the rate at which they were transferred. Only F and one other transfer factor were found to transmit Hly. Some transfer factors were not detected to transfer Col or Ap. Linkage was demonstrated between Tc and three transfer factors, including F, and between Sm and Su. The other plasmids and transfer factors appeared to exist as separate units. In mating studies between the donor strain possessing both F and I, and recipient strains possessing different combinations of these transfer factors, the determinants most greatly affected by the choice of recipient were the F-linked Tc and Hly.

When a multiple antibiotic resistance plasmid, instead of the Tc one, was co-transferred to the donor strain by I or F, then I, but not F, established a linkage with this plasmid. Although this linkage was probably responsible for the higher rate at which the plasmid was transferred from the I⁺ than from the F⁺ strain, its actual transfer rate was lower than that of the unlinked Col plasmid also possessed by the I⁺ strain.

INTRODUCTION

By means of the transfer factor F, Smith & Gyles (1970) mobilized plasmids possessing genetic determinants for tetracycline (Tc), streptomycin (Sm) and sulphonamide (Su) resistance and colicine E₁ (Col) and α -haemolysin (Hly) production in wild strains of *Escherichia coli* and transferred them to the same strain of *E. coli* K12. In mating experiments between this strain and another K12 strain, linkage was demonstrated between F and Tc and between Sm and Su but not between them, Col, and Hly. They were transmitted as four independent units in no predetermined order and, consequently, strains were obtainable that were Col⁺Hly⁺Sm⁺ and Su⁺ but F⁻. By exploiting this technique, strains possessing additional plasmids have been prepared. These strains have allowed us to compare the behaviour of different transfer factors introduced into the same plasmid-containing environment. F and I (the transfer factor of colicine I) were also studied when both were present at the same time in the same plasmid-containing environment. The results are reported in this paper.

METHODS

Only those methods not described previously by Smith & Gyles (1970) and Smith & Linggood (1970), and the mating procedure employed, are given below.

Escherichia coli K12 strains. These were HPR1ap^r (ampicillin-resistant by mutation) prototrophic lac⁺, HPR2spe^r (spectinomycin-resistant by mutation) prototrophic lac⁺ and HPR3 colicine E1-resistant (col^r) nalidixic acid-resistant (nal^r) auxotrophic lac⁻.

Escherichia coli wild strains. Nine carried transfer factors A2-A10 in the absence of characters known to be transferable. Another three did not carry transfer factors (Tra⁻) and, of these, one (w1Ap) carried an Ap plasmid, one (w2Tc) a Tc plasmid and the third (w3 multiple resistant) a plasmid conferring resistance to TcSmSuCmNeoSpe (Tc = tetracycline, Sm = streptomycin, Su = sulphonamides, Cm = chloramphenicol, Neo = neomycin).

Transfer (Tra) factors. These were F (Lederberg, Cavalli & Lederberg, 1952; Hayes, 1953), Idrd16(I) (Meynell, 1968) and A2-A10.

Non-transferring plasmids. These conferred resistance to Tc, Sm and Su, Ap or (TcSmSuCmNeoSpe) or determined colicine E1 (Col) or α -haemolysin (Hly) production. All were derived initially from wild strains.

fi character of transfer factors. An F-linked Tc determinant was transferred to K12 strains containing transfer factors A2-A10 (strains that became available during the course of the work) and Tc⁺ ex-conjugants of these strains were tested for susceptibility to visible lysis by the F-specific phage MS2 (Davis, Strauss & Sinsheimer, 1961). The transfer factors in those that were lysed, A3-A10, were designated *fi*⁻ (fertility inhibition⁻) and in the one that was not, A2, *fi*⁺.

Construction of donor strains. Smith & Gyles (1970) had introduced plasmids determining resistance to Sm, Su and Tc and also Hly and Col, together with F, into *Escherichia coli* K12 HPR1. This culture was used as donor with HPR2 as recipient, and an ex-conjugant selected that had acquired Sm, Su, Hly and Col but not F. Ap, without F, was transferred into this HPR2 ex-conjugant from strain w1ApF⁺. The HPR2 line, the prospective donor, now carried five plasmid-determined characters and no transfer factor. It was converted into a donor by introducing various transfer factors, Tc from strain w2Tc being introduced at the same time (for technique see Smith & Gyles, 1970). Acquisition of Tc by HPR2 indicated that conjugation had occurred, and introduced a sixth plasmid-controlled character. That the transfer factor was also acquired is shown in Results. The F⁺ HPR2 strain prepared in this manner was subsequently mated with a K12I⁺ strain and an I⁺F⁺ form of HPR2 selected, the presence of I and F⁺ being judged on phage susceptibility (see later).

Two donor strains with F and I as transfer factors were made using w3 multiple resistant as the indicator instead of w2Tc. The complete resistance pattern (TcSmSuCmNeoSpe) was transferred en bloc.

Mating procedure. Nutrient broth (10 ml) was inoculated with 0.02 ml each of 24 h broth cultures of a donor strain and the nal^r recipient strain HPR3 and incubated at 37 °C for 24 h. The number of recipient organisms that had acquired the different genetic determinants from the donor was then estimated from viable counts (Miles & Misra, 1938) on MacConkey agar containing 20 µg sodium nalidixate/ml and 15 to 20 µg of one or other of the antibiotics to which the donor was resistant/ml. The proportion that had acquired Hly and Col was estimated in relation to the total viable count on MacConkey agar containing sodium nalidixate only. A total of 100 colonies from this medium was also picked, purified by streaking on plain MacConkey agar and the characters they had acquired assessed. In addition,

Table 1. The rate of transfer of characters to *Escherichia coli* K12 *nal^r* HPR3 during mating with strains of *E. coli* K12 HPR2 *spe^r* *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing different transfer factors

Transfer factor (Tra) in donor strain	No. of organisms of prospective recipient strain log ₁₀ /ml								
	That acquired, during mating								
	Total	Tra	Tc	Col	Hly	Sm	Su	Ap	<i>spe</i>
A2	8.3	8.3	8.3	8.2	N	8.2	8.2	7.3	N
I	8.2	8.1	8.0	7.7	N	7.5	7.5	2.7	1.3
F	8.2	7.8	7.8	7.7	7.4	6.5	6.5	4.6	1.7
A3	8.0	7.6	7.3	7.5	N	7.0	7.0	5.9	N
A4	8.7	7.7	7.7	6.3	N	6.4	6.4	6.0	N
A5	8.0	7.0	6.7	6.0	N	5.7	5.7	5.6	N
A6	8.0	N	5.7	N	N	4.0	4.0	3.7	N
A7	8.4	N	5.4	5.8	N	4.0	4.0	4.0	N
A8	8.0	N	5.7	N	5.7	4.8	4.8	N	N
A9	8.3	N	5.3	N	N	5.1	5.1	N	N
A10	8.1	N	5.0	N	N	4.2	4.2	3.8	N

Tra = Transfer factor. Tc, Sm, Su, Ap, *spe*, Col, Hly = genetic determinants for resistance to tetracyclines, streptomycin, sulphonamides, ampicillin, spectinomycin (chromosomal), and for production of colicine E1 and α -haemolysin respectively.

N = None found. This was approximately < three organisms/ml in the case of antibiotic resistance determinants, < 1/100th of the total number of prospective recipient organisms in the case of transfer factors and < 1/1000th in the case of Col and Hly.

groups of 20 recipient colonies, selected as having acquired one or other of the characters, were tested for possession of the remainder. The presence of F and I was recognized by susceptibility to visible lysis by the F-specific phage, MS2, and the I-specific phage, If1 (Meynell & Lawn, 1968). The presence of the other transfer factors in recipient organisms was recognized by ability to transmit the acquired determinants. Their presence in recipient organisms that had not acquired determinants was recognised by ability to mobilize an SmSu plasmid in a Tra⁻ K12 strain.

RESULTS

Mating of strains of Escherichia coli K12 HPR2 *spe^r* *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing different transfer factors with *E. coli* K12 *nal^r* HPR3

The results of mating strains of *Escherichia coli* K12 HPR2 *spe^r* *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing different transfer factors with the *nal^r* mutant of *E. coli* K12 HPR3 are summarized in Tables 1 to 3. Those shown in Table 3 are of matings for which no, or very few, positive results were quotable in Table 2; some transfer rates were too low to permit even this. Each of the eleven matings was repeated at least once with similar results on each occasion. There was a great difference between some of the transfer factors in the rate they transferred determinants and between some determinants in the rate at which they could be transferred. In general, A2 was the most efficient transfer factor, followed by I and F; these three were greatly superior to A6, A7, A8, A9 and A10. In nine of the eleven matings Tc was transferred at a higher rate than the other determinants, Col being transferred at a higher rate than this determinant in the matings mediated by A3 and A7. Sm and Su and, particularly, Ap were usually transferred at much lower rates; no Ap transfer by A8 and A9 was detected. From many of the matings, colonies selected as Sm⁺ or Su⁺ were mostly Ap⁻, whereas those selected as Ap⁺ were mostly Sm⁻ and Su⁻. Only two transfer factors,

Table 2. *The transfer of characters to Escherichia coli* K12 *nal^r* HPR3 during mating with strains of *E. coli* K12 HPR2 *spe^r* *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing different transfer factors

		No. of 100 colonies of the prospective recipient strain that acquired the character or character pattern during mating with the donor strain possessing transfer factor						
Character		A2	I	F	A3	A4	A5	A6-A10
Tra		100	100	48	39	9	9	0
Tc		99	96	48	19	9	3	0
Col		86	51	43	30	0	1	0
Hly		0	0	25	0	0	0	0
Sm		69	37	3	11	0	1	0
Su		69	37	3	11	0	1	0
Ap		4	0	0	0	1	0	0
None		0	0	49	61	91	91	100
Character pattern								
Tra	Tc	Col	Hly	Sm	Su	Ap		
+	+	+	+	+	+	—	0	0
+	+	+	—	+	+	+	4	0
+	+	+	—	+	+	—	58	24
+	+	+	+	—	—	—	0	0
+	+	—	—	+	+	—	6	13
+	+	+	—	—	—	+	0	0
+	—	+	—	+	+	—	1	0
+	+	+	—	—	—	—	23	25
+	+	—	+	—	—	—	0	0
+	+	—	—	—	—	+	0	0
+	+	—	—	—	—	—	8	34
+	—	+	—	—	—	—	0	2
—	—	+	+	—	—	—	0	0
—	—	+	—	—	—	—	0	0
—	—	—	+	—	—	—	0	0
+	—	—	—	—	—	—	0	2
—	—	—	—	—	—	—	0	0
							49	61
							91	91
							91	100

+ Present; — absent. For other abbreviations see Table 1.

F and A8, transferred Hly and only seven transferred Col; this was even the case when organisms selected as having acquired antibiotic resistance determinants were examined (Table 3). The relatively low rate of Sm and Su transfer by F and of Ap transfer by I compared with other factors was a constant finding.

A variety of character patterns was acquired by recipient organisms in the eleven matings. Sixteen different ones were found during the examination of the 600 colonies from the matings mediated by F, I, A2, A3, A4 and A5 (Table 2), nine being found amongst the 100 colonies from the F mating. During the examination of the colonies from all the matings, Sm was always found with Su. A fairly close association was usually found between Tc and the transfer factors but only in the case of F, A4 and A9 was it complete. No other linkages between determinants or between determinants and transfer factors were detected. Recipient colonies that had acquired a determinant were usually Tra⁺. Exceptions were noted in the matings mediated by F, A4, A8, A9 and, particularly, by A7; of the 20 colonies selected as Sm⁺ in the A7 mating, only six were Tra⁺ (Table 3). Recipient colonies that had acquired Tra but no determinants were found in the matings mediated by I, A3 and A5.

During the matings mediated by F and I, but not by the other transfer factors, the

Table 3. *The characters acquired by recipient colonies of Escherichia coli* K12 *nal^r* HPR3 mated with strains of *E. coli* K12 HPR2 *spe^r* *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing different transfer factors and selected for different characters

The results for colonies selected because they were *Su⁺* are not included because they were similar to those of the *Sm⁺*-selected colonies.

Transfer factor of donor strains	Character selected	Characters possessed by 20 selected recipient colonies						
		Tra	Tc	Col	Hly	Sm	Su	Ap
A2	Ap	20	20	19	0	16	16	20
I	Ap	20	20	8	0	4	4	20
	<i>spe</i>	20	20	14	0	8	8	0
F	Sm	20	20	19	7	20	20	0
	Ap	20	20	10	11	1	1	20
	<i>spe</i>	17	17	20	8	3	3	0
A3	Ap	20	18	19	0	8	8	20
A4	Sm	19	19	10	0	20	20	1
	Ap	20	20	3	0	2	2	20
A5	Sm	20	17	4	0	20	20	1
	Ap	20	18	7	0	1	1	20
A6	Tc	20	20	0	0	0	0	0
	Sm	20	4	0	0	20	20	1
	Ap	20	14	0	0	0	0	20
A7	Tc	20	20	2	0	0	0	0
	Sm	6	7	14	0	20	20	2
	Ap	15	15	2	0	0	0	20
A8	Tc	20	20	0	0	0	0	0
	Hly	20	0	0	20	0	0	0
	Sm	14	14	0	4	20	20	0
A9	Tc	20	20	0	0	4	4	0
	Sm	20	11	0	0	20	20	1
	Ap	20	11	0	0	0	0	20
A10	Tc	20	20	0	0	6	6	0
	Sm	16	16	0	0	20	20	0

For abbreviations see Table 1.

chromosomal spectinomycin resistance of the donor transferred at a very low rate. The distribution of the plasmid-located determinants in the *spe^r* recipient organisms (Table 3) broadly resembled that in the *spe^s* recipient organisms.

Mating of Escherichia coli K12 *spe^r* HPR2 *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing both *F* and *I* with *E. coli* K12 *nal^r* HPR3 possessing different combinations of these two transfer factors

The results of mating *Escherichia coli* K12 HPR2 *spe^r* *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* *F⁺I⁺* with strains of K12 *nal^r* HPR3 that were *F⁻I⁻*, *F⁺I⁻*, *F⁻I⁺* and *F⁺I⁺* are summarized in Tables 4 to 6; those in Table 6 are of matings for which no or very few results were quotable in Table 5. *I* and *F* transferred themselves to the *F⁻I⁻* strain at approximately the same rates as they had transferred from the donor strains in which they existed separately (Table 1). *Sm* and *Su* were transferred to this strain at the high rate characteristic of *I*-mediated transfer; *Col* was transferred at a rate even higher than that characteristic of *I*-mediated transfer. *Tc* and *Ap*, by contrast, were transferred at the rate characteristic of *F*-mediated transfer; *Hly* transfer was rather lower than that expected on this basis. All the determinants and

Table 4. *The rate of transfer of characters to strains of Escherichia coli* K12 *nal^r* HPR3 *possessing different combinations of F and I from E. coli* K12 HPR2 *spe^r Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing both of these transfer factors

Transfer factor status of prospective recipient strain before mating	No. of organisms of prospective recipient strain, log ₁₀ /ml								
	That acquired during mating								
	Total	F	I	T	Col	Hly	Sm	Su	Ap
F-I ⁻	8.4	8.0	8.4	8.0	8.3	7.3	7.9	7.9	4.9
F-I ⁺	7.6	6.4	...	6.4	7.1	6.4	6.0	6.0	4.0
F+I ⁻	7.7	...	7.7	4.6	7.7	N	7.2	7.2	3.6
F+I ⁺	8.1	...	...	3.2	N	N	4.7	4.7	N

... = Character already possessed by recipient organisms. For abbreviations see Table 1.

Table 5. *The transfer of characters to strains of Escherichia coli* K12 *nal^r* HPR3 *possessing different combinations of F and I during mating with E. coli* K12 HPR2 *spe^r Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* possessing both of these transfer factors

		No. of 100 colonies of prospective recipient strain that acquired the character or character pattern during mating, its transfer factor status previously being			
Character		F-I ⁻	F-I ⁺	F+I ⁻	F+I ⁺
F		43	7	...	...
I		100	...	100	...
Tc		43	7	0	0
Col		95	28	100	0
Hly		9	5	0	0
Sm		32	2	32	0
Su		32	2	32	0
Ap		0	0	0	0
None		0	70	0	100

Character pattern										
Tra*	Tc	Col	Hly	Sm	Su	Ap				
+	+	+	-	+	+	-	12	0	0	0
+	-	+	+	+	+	-	4	0	0	0
+	+	+	+	-	-	-	3	2	0	0
+	-	+	-	+	+	-	15	0	32	0
+	+	+	-	-	-	-	28	4	0	0
+	-	+	+	-	-	-	2	0	0	0
+	-	+	-	-	-	-	31	0	68	0
+	+	-	-	-	-	-	0	1	0	0
+	-	-	-	+	+	-	1	0	0	0
-	-	+	-	+	+	-	0	2	0	0
-	-	+	+	-	-	-	0	2	0	0
-	-	+	-	-	-	-	0	18	0	0
-	-	-	+	-	-	-	0	1	0	0

* = F, I or both.

... = Character already possessed by the recipient organisms. For other abbreviations see Tables 1 and 2.

Table 6. The characters acquired by prospective recipient colonies of strains of *Escherichia coli* K12 *nal^r* HPR3 mated with *E. coli* K12 HPR2 *spe^r* *Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* and selected for different characters; before mating, the recipient strains possessed different combinations of F and I and the donor strain both of these transfer factors

The results for colonies selected because they were *Su⁺* are not included because they were similar to those of the *Sm⁺* selected colonies.

Transfer factor status of recipient strain before mating	Character selected	Characters acquired by 20 selected recipient colonies								
		F	I	Tc	Col	Hly	Sm	Su	Ap	<i>spe</i>
F-I ⁻	Hly	11	20	11	20	20	8	8	0	0
	Sm	10	20	10	20	1	20	20	0	0
	Ap	20	20	20	19	1	11	11	20	0
F-I ⁺	Hly	5	...	5	19	20	0	0	0	0
	Sm	7	...	7	19	5	20	20	0	0
	Ap	20	...	20	9	2	1	1	20	0
F ⁺ I ⁻	Tc	...	20	20	20	0	10	10	0	0
	Sm	...	20	0	20	0	20	20	0	0
	Ap	...	20	0	20	0	12	12	20	0
F ⁺ I ⁺	Tc	...	...	20	2	0	0	0	0	0
	Sm	...	...	0	6	0	20	20	0	0

... = Character already possessed by the recipient organisms. For abbreviations see Table 1.

F itself were transferred to the F-I⁺ strain at a lower rate than that at which they were transferred to the F-I⁻ strain from the F⁺ donor (Tables 1 and 2), a feature of the mating being the high proportion of recipient organisms that had acquired determinants but not F; for example, of the 100 colonies examined from this mating, only six of the 28 that had acquired Col had also acquired F. The main feature of the mating involving the F⁺I⁻ recipient strain was the low rate of Tc (F-linked) transfer. This was several thousand times less than that observed in the case of the F-I⁻ recipient. As expected, the transfer rate of all determinants to the F⁺I⁺ recipient was low. A similar low rate was noted in an additional mating in which both donor and recipient were F⁺I⁻, the number of organisms that had acquired Tc, Sm and Su being, log₁₀/ml, 3.2, 2.7 and 2.7. The corresponding figures for a mating in which both donor and recipient were F-I⁺ and in which, unlike the previous mating, the transfer factor was not Tc-linked was 6.3, 4.8 and 4.8 respectively.

Mating of strains of Escherichia coli K12 *spe^r* HPR2 *Col⁺Hly⁺SmSu⁺* (*Tc Sm Su Cm Neo Spe*)⁺ possessing I or F with *E. coli* K12 *nal^r* HPR3

The results of mating with *Escherichia coli* K12 *nal^r* HPR3 strains of K12 HPR2 *spe^r* *Col⁺Hly⁺SmSu⁺Tra⁻* into which determinants for resistance to tetracycline, streptomycin, sulphonamides, chloramphenicol, neomycin and spectinomycin had been introduced *en bloc* from a wild strain of *E. coli* by means of I or F are illustrated in Tables 7 to 9. Both transfer factors transmitted this group of resistance determinants intact to 275 of 286 recipient colonies indicating that they were probably integrated in the same plasmid. Of the remaining eleven, six were Tc⁻, two Tc-Cm⁻, one Tc-Neo⁻, one Neo⁻ and one Sm-Su-Neo-Spe⁻. In the mating mediated by I, only the recipient colonies that had acquired the multiple resistance plasmid had acquired I. There was no evidence of such a plasmid/transfer factor linkage in the F⁺ donor strain, the rate of transfer of the plasmid from that strain being

Table 7. *The rate of transfer of characters to Escherichia coli K12 nal^r HPR3 during mating with strains of E. coli K12 HPR2 spe^r Col⁺Hly⁺SmSu⁺ (TcSmSuCmNeoSpe)⁺ possessing transfer factor I or F*

Transfer factor (Tra) in donor strain	No. of organisms of prospective recipient strain, log ₁₀ /ml											
	Total	That acquired during mating										
		Tra	Col	Hly	SmSu*	Ap†	Tc	Sm	Su	Cm	Neo	Spe
I	8.6	8.3	8.4	N	7.3	3.7	8.3	8.3	8.3	8.3	8.3	8.3
F	9.0	8.8	8.5	8.7	7.9	N.O.	6.6	6.6	6.6	6.6	6.6	6.6

* Determinants of the SmSu plasmid, distinguishable from those of the Tc Sm Su Cm Neo Spe plasmid by coding for a higher degree of streptomycin resistance (Cm, Neo, Spe = chloramphenicol, neomycin and spectinomycin).

† The I⁺ donor strain also possessed the Ap determinant possessed by the donor strains referred to in Tables 1 to 6.

N.O. = No observations. For other abbreviations see Table 1.

lower than that of F itself and, incidentally, much lower than that at which it was transferred from the I⁺ strain. Neither was there evidence of linkage between it and the Col, Hly, SmSu, or, in the case of the I⁺ strain, the Ap plasmid, also present in this donor strain. Col was transferred from the I⁺ strain at a higher rate than the I-linked plasmid but Hly transfer was not detected. By contrast, Hly was transferred from the F⁺ strain at a very high rate; the SmSu plasmid was transferred at a rate higher than that at which it was transferred from the I⁺ strain, the reverse of that found in the case of the Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺ strains (Tables 1 and 2).

DISCUSSION

Because of their method of preparation, some of the donor strains might have contained more than one transfer factor. The transfer factors, too, might have co-transferred unidentifiable genetic determinants when they were incorporated in these strains. Although no evidence was found to suggest that either of these possibilities actually occurred, they must be borne in mind when considering our results. Examination of the colonies of the *nal^r K12 HPR3* strain that had been mated with the eleven *spe^r K12 HPR2 Tc⁺Col⁺Hly⁺Sm⁺Su⁺Ap⁺* donor strains that differed only in respect of the particular transfer factor they contained, revealed a fairly close association between Tc and the transfer factor in several of the donor strains. Only in three, those harbouring transfer factors F, A4 or A9, however, did it appear to be complete. No such linkage was established between the transfer factors and the other determinants in any of the strains, a transfer factor/determinant relationship resembling that originally described by Anderson (1965, 1968) and Anderson & Lewis (1965). Apart from confirming the linkage previously reported between Sm and Su (Smith & Gyles, 1970), the determinant patterns of the recipient colonies revealed that Tc, Col, Hly, Sm, Su and Ap, after their incorporation in the donor strains, had remained as separate units, i.e. as plasmid 'aggregates' (Clowes, 1972). By contrast, the mobilization by I of the plasmid possessing determinants coding for resistance to tetracyclines, streptomycin, sulphonamides, chloramphenicol, neomycin and spectinomycin from the wild Tra⁻ strain w3 and the linkage established between I and this plasmid in the prospective donor strain into which they were transferred provided an example of the transfer factor/determinant relationship described by Watanabe (1963) in which the determinants and the transfer factor are all integrated in the same plasmid, i.e. as plasmid 'co-integrates' (Clowes, 1972). Because

Table 8. The transfer of characters to *Escherichia coli* K12 *nal^r* HPR3 during mating with strain *E. coli* K12 HPR2 *spe^r* *Col⁺* *Hly⁺* *SmSu⁺* (*TcSmSuCmNeoSpe⁺*)⁺ possessing transfer factor I or F

										No. of 100 colonies of the prospective recipient strain that acquired the character or character pattern during mating with the donor strain possessing transfer factor	
Character										I	F
Tra										52	74
Col										57	38
Hly										0	52
SmSu*										6	11
Tc										50	0
Sm										52	0
Su										52	0
Cm										52	0
Neo										51	0
Spe										52	0
Ap†										0	N.O.
Character pattern											
Tra	Col	Hly	SmSu*	Tc	Sm	Su	Cm	Neo	Spe		
+	+	—	+	+	+	+	+	+	+	6	0
+	+	—	—	+	+	+	+	+	+	44	0
+	+	—	—	—	+	+	+	+	+	1	0
+	+	—	—	—	+	+	+	—	+	1	0
+	+	+	+	—	—	—	—	—	—	0	7
+	—	+	+	—	—	—	—	—	—	0	3
+	+	+	—	—	—	—	—	—	—	0	17
+	—	—	+	—	—	—	—	—	—	0	1
+	+	—	—	—	—	—	—	—	—	0	10
+	—	+	—	—	—	—	—	—	—	0	21
—	+	+	—	—	—	—	—	—	—	0	1
—	—	+	—	—	—	—	—	—	—	0	3
—	+	—	—	—	—	—	—	—	—	5	1
+	—	—	—	—	—	—	—	—	—	0	15
—	—	—	—	—	—	—	—	—	—	43	21

For details and abbreviations see Tables 1 and 7.

this donor strain also possessed Hly, Col, Ap and SmSu plasmids as separate units, it presents an example of plasmid co-integrates and aggregates, one of which (Hly) was non-transmissible, existing in the same bacterial cell. The position of the corresponding donor strain whose transfer factor was F, not I, was different in that F had remained as a separate unit, a fact that might have accounted for the relatively low transfer rate of the co-integrated determinants from this donor strain compared with the I⁺ one.

Apart from the general relationships noted, more specific ones appeared to exist between some transfer factors and individual determinants. For example, A8 and, especially, F transferred Hly at a high rate, whereas all the other nine transfer factors, including A2 and I, in general the most efficient transfer factors, were not detected to do so. Some transfer factors, too, transferred Col at a high rate, whereas others appeared to be unable to transfer

Table 9. *The characters acquired by recipient colonies of Escherichia coli* K12 *nal^r HPR3* mated with strains of *E. coli* K12 *HPR2 spe^r Col⁺Hly⁺SmSu⁺ (TcSmSuCmNeoSpe)⁺* possessing *I* or *F* and selected for different characters

Transfer factor of donor strain	Character selected	Characters possessed by 20 selected recipient colonies										
		Tra	Col	Hly	SmSu*	Tc	Sm	Su	Cm	Neo	Spe	Ap†
I	Col	14	20	0	1	14	14	14	14	13	14	0
	Sm	20	20	0	2	20	20	20	20	20	20	0
	Su	20	20	0	4	20	20	20	20	20	20	0
	Tc	20	20	0	1	20	20	20	20	19	20	0
	Cm	20	20	0	1	20	20	20	20	20	20	0
	Neo	20	20	0	1	19	20	20	20	20	20	0
	Spe	20	20	0	3	20	20	20	20	20	20	0
	Ap†	20	20	0	2	16	20	20	20	20	20	20
F	Col	20	20	20	5	0	0	0	0	0	0	N.O.
	Hly	20	12	20	2	0	0	0	0	0	0	N.O.
	Sm	17	14	15	20	0	0	0	0	0	0	N.O.
	Su	20	12	16	19	1	1	1	1	1	1	N.O.
	Tc	20	14	14	1	20	20	20	20	20	20	N.O.
	Cm	19	11	17	3	20	19	19	20	19	19	N.O.
	Neo	20	15	14	4	18	20	20	18	20	20	N.O.
	Spe	20	8	12	6	20	20	20	20	20	20	N.O.

For details and abbreviations see Tables 1 and 7.

it. The method of selecting *Hly*⁺ and *Col*⁺ organisms, however, was by no means as sensitive as that employed for detecting organisms that had acquired antibiotic resistance determinants. Consequently, it is possible that the transfer factors recorded as failing to transfer *Col* and *Hly* might really have been transferring them at rates too low to be detected. The relationship between transfer factors and *Ap* appeared more complex. Two failed to transfer it, whereas *A2* transferred it at a high rate and *I* transferred it at a much lower one, lower than that attained by transfer factors generally much less efficient than *A2* and *I*. Such differences cannot be explained in the present state of knowledge. Nor can the smaller but constantly observed ones, like the lower rate of transfer of the *SmSu* plasmid by *F* than by *I* despite the fact that they transfer themselves, *Tc* and *Col* at more closely similar rates. Transfer factor linkage provided an explanation of the rate of transfer of *Tc* from the donor strains possessing transfer factors *F*, *A4* and *A9*. Being that of the transfer factor itself, this ensured a fairly high rate of transfer. Its linkage to *F*, though, accounted for the rate of *Tc* transfer to the *F*⁻*I*⁻ recipient from the *F*⁺*I*⁺ donor (Tables 4 and 5) being less than half that from the *F*⁻*I*⁺ donor (Tables 1 and 2) and for its greatly reduced rate of transfer from the *F*⁺*I*⁺ donor to the *F*⁺*I*⁻ recipient, the transfer of the unlinked determinants in this mating being unaffected by the presence of *F* in the recipient organisms. The presence of *I* in the *F*⁻*I*⁺ recipient strain, too, slightly interfered with *F* transfer from the *F*⁺*I*⁺ donor in that the rate of transfer of *F* itself, and hence of *Tc*, was decreased and many recipient organisms had acquired determinants, particularly *Col*, in the absence of *F*. It is noteworthy that recipient organisms possessing plasmid determinants but not transfer factors were isolated from the matings mediated by four of the nine wild transfer factors (Tables 2 and 3) as well as by *F* and that the unlinked *Col* determinant was actually transmitted at a higher rate from the donor strain containing the *I*⁺ co-integrate than was the co-integrate itself.

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