CHROMOSOMAL TRANSFER FROM "RECOMBINATION-DEFICIENT" STRAINS OF *ESCHERICHIA COLI* K-12

R. C. CLOWES[1] AND E. E. M. MOODY[1]

Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London, W. 12., England

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CERTAIN strains of Enterobacteria harbor infectious genetic elements known as sex factors (HAYES 1964) or transfer factors (GROSS 1965) which enable them to act as genetic donors. That is, cells of such donor strains are able to form stable unions with other cells and transfer to them extrachromosomal material (which may include the sex factor itself) and, more rarely, segments of bacterial chromosome.

The first discovered (HAYES 1953; CAVALLI, LEDERBERG and LEDERBERG 1953) and best documented (JACOB and WOLLMAN 1961) of these factors is the F fertility factor of *Escherichia coli* K-12. Since that time other genetic elements have been found which control other cellular properties in addition to sex factor activities. Among these elements are the *colicin factors* (OZEKI and HOWARTH 1961; CLOWES 1961) and the multiple *drug-resistance transfer factors* (SUGINO and HIROTA 1962).

The F sex factor, together with the temperate bacteriophage λ, formed the model on which the "episome" concept of JACOB and WOLLMAN (1958) was based; an episome being defined as a nonessential genetic element which could exist either autonomously or integrated with the chromosome. Although in the wild state, the F sex factor is an autonomous, extrachromosomal element (in F^+ donors), stable Hfr strains can be isolated from cultures of F^+ strains, which transfer their chromosome with high frequency and in an oriented manner. In each Hfr strain, the F sex factor is suggested to be integrated at one of a number of alternative sites on the K-12 chromosome by a mechanism involving pairing between the F factor and chromosome, followed by a reciprocal genetic exchange (ADELBERG and PITTARD 1965; GROSS 1965). It has moreover been suggested that the entire fertility of F^+ strains may reside in the small number of Hfr mutants present in every large F^+ population.

It was at one time concluded (ALFOLDI, JACOB, WOLLMAN and MAZÉ 1958) that certain colicin factors also occupy a chromosomal site and thus qualify as episomes, but the conclusions of these experiments have been questioned (CLOWES 1963b) and a model of colicin factors as stable, autonomous plasmids has been proposed (MONK and CLOWES 1964b). Nevertheless, it could still be suggested that occasional integration of a colicin sex factor with the chromosome might

[1] Present address: Division of Biology, Graduate Research Center of the Southwest, Dallas, Texas, 75230.

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occur which would account for its ability to mediate the transfer of chromosomal markers.

More recently, "recombination-deficient" (*rec*$^-$) mutants of K-12 have been isolated by CLARK and MARGULIES (1965) which are suggested to be unable to catalyze one or more steps involved in the recombination process. The extent to which these *rec*$^-$ mutants can act as genetic donors when infected with one of

TABLE 1

Bacterial strains (all strains are E. coli *K-12 sub lines except where noted)*

Strain	Genotype: Chromosome	Genotype: Sex factor	Origin or derivation
(a) *Sources of sex factors*			
501	(58–161) *met*	F	Original strain (HAYES 1952)
770	(W1655) *met lac*+	F-*lac*+	SCAIFE and GROSS (1962)
519	(58–161) *met*	colI	MONK and CLOWES (1964a)
414	(*E. coli* K94) prot	colV2	Original strain K94 (FRÉDÉRICQ, 1963) see MACFARREN and CLOWES (1966)
810	(J62) *his try thy str*^{r}	colV3	MACFARREN and CLOWES (1966)
(b) *Strains derived from AB1157 (thr leu thi pro his arg lac*$^-$*xyl*$^-$*T6*^{r} *str*^{r}*)*			
(i) "Recombination-active" (rec+) *strains*			
97	*rec*+	0(F$^-$)	Original strain (ADELBERG 1962)
112	*rec*+	F	97 × 501
101	*rec*+	F-*lac*+	97 × 770
100	*rec*+ *colI*^{r}	0(F$^-$)	97 × colicin I
108	*rec*+ *colI*^{r}	colI	100 × 519
110	*rec*+	colV2	97 × 414
131	*rec*+	colV3	97 × 810
(ii) "Recombination-deficient" (rec$^-$) *strains*			
96	*rec*$^-$	0(F$^-$)	HOWARD-FLANDERS and THERIOT (AB 2463)
111	*rec*$^-$	F	96 × 501
107	*rec*$^-$	F-*lac*+	96 × 770
99	*rec*$^-$ *colI*^{r}	0(F$^-$)	96 × colicin I
106	*rec*$^-$ *colI*^{r}	colI	99 × 519
109	*rec*$^-$	colV2	96 × 414
130	*rec*$^-$	colV3	96 × 810
(c) *Other strains*			
902	*(Salmonella typhimurium)* LT-2 *cys36*	colI	OZEKI and HOWARTH (1961)
750	Hfr C (*met*)	F	Original strain (CAVALLI 1950)
C600	*thr leu thi lac*$^-$	0(F$^-$)	Original strain (APPLEYARD 1954)
502	(58–161) *met str*^{r}	0(F$^-$)	Original strain (HAYES 1952)
510	(58–161) *met str*^{r} *colI*^{r}	0(F$^-$)	502 × colicin I

prot indicates growth on minimal medium without amino acid or vitamin supplements. *met, thr, leu, thi, pro, his, arg, cys, thy:* requirement for methionine, threonine, leucine, thiamine, proline, histidine, arginine, cysteine, or thymine. *str*^{s} (*str*^{r}): sensitivity (resistance) to streptomycin (200 μg/ml). *lac*+ *xyl*+ ability to ferment the sugars lactose or xylose; *lac*$^-$, *xyl*$^-$: inability to ferment these sugars. Strain 96 is a mutant isolated from strain 97 by P. HOWARD-FLANDERS which is hypersensitive to ultraviolet and also to X-irradiation. F is the F fertility factor of K-12; colI, colV2, colV3 are colicin factors which determine the ability of a strain to produce colicin I or colicin V. colI^r indicates strains selected resistant to colicin I and which are also cross-resistant to colicin V.

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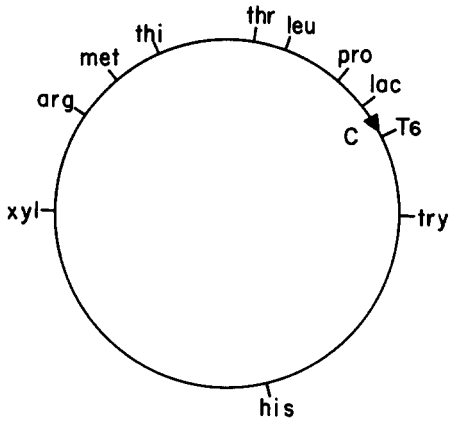

FIGURE 1.—Disposition of markers on the circular chromosome of K-12. "C" represents orientation of transfer of Hfr Cavalli.

several alternative sex factors might be expected to clarify whether recombination between a sex factor and the chromosome is a necessary prerequisite for chromosomal transfer. This would, of course, require that infection by sex factors does not promote the formation of recombinants in the *rec*⁻ strain. This paper reports an investigation of these properties using a mutant of the *rec*⁻ type (HOWARD-FLANDERS and THERIOT 1966) infected with F, or with one of several distinct colicin factors.

MATERIALS AND METHODS

Bacterial strains: The strains used in this study are listed in Table 1.

Media: All media used have been described in a previous publication (MONK and CLOWES 1964a).

Techniques of infection with F, and with colicin factors, of F testing and of colicinogeny testing are described elsewhere (MONK and CLOWES 1964a). Standard crossing techniques were used in bacterial matings (see CLOWES 1961; MONK and CLOWES 1964a; MACFARREN and CLOWES 1966).

RESULTS

Efficiency of rec⁻ *strains as recipients:* The first experiments tested the ability of the parental *rec*⁻ strain to act as a recipient when mated with the Hfr donor, Hfr CAVALLI. When one of a number of sex factors had been introduced into this *rec*⁻ strain, its capacity as a recipient was similarly investigated with the results shown in Table 2. It can be seen that in contrast to the control crosses using *rec*⁺ recipients, where transfer of a proximal marker, *pro*⁺, led to about 5% recombinants, the frequency of *pro*⁺ recombinants issuing from *rec*⁻ recipients was reduced by a factor of at least 1 in 5000. Moreover, when the factors F or F-*lac*⁺ were present in the recipient, the recombinants were reduced even further, as would be expected from crosses between two donor strains (see HAYES 1964).

Nonchromosomal transfer from rec⁻ *donors:* The extent to which *rec*⁻ strains, when infected with one of the several sex factors, can conjugate and transfer

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TABLE 2

Efficiency of rec⁻ *strains as recipients*

Donor × Recipient

Hfr Cavalli × AB 1157

Recipient	Mean number *pro*⁺ colonies per plate	Total count Hfr ($\times 10^8$)	Overall dilution	Frequency *pro*⁺ recombinants per input Hfr
rec⁺ F⁻	304	3	10^{-3}	5×10^{-2}
colI⁺	298	2.4	10^{-3}	6.2×10^{-2}
colV3⁺	358	3	10^{-3}	6×10^{-2}
rec⁻ F⁻	623	3	$\times 10$	1×10^{-5}
F⁻	592	2	$\times 10$	1.5×10^{-5}
F⁺	17/5	2	$\times 10$	8.5×10^{-8}
F-*lac*⁺	13/5	2	$\times 10$	6.5×10^{-8}
colI⁺	538	2.4	$\times 10$	1.1×10^{-5}
colI⁺	135	2	$\times 10$	3.4×10^{-6}
colV3⁺	601	3	$\times 10$	1×10^{-5}

1 ml young (ca. 2×10^8) Hfr C culture mixed with 9 ml (0/N dil. 1/10=ca. 2×10^8) AB1157 recipient culture was incubated for 2 hr at 37° unshaken, washed twice in 10 ml buffer, resuspended in 1 ml ($\times 10$) and 0.2 ml plated in quintuplicate either direct (*rec*⁻ crosses) or after 10^{-4} dilution (*rec*⁺ controls), on minimal medium supplemented with threonine, leucine, thiamine, histidine, arginine and streptomycin (to select *pro*⁺ recombinants).

their sex factor to the same normal (*rec*⁺) recipient strain (C600 F⁻) was next investigated and compared with the transfer from the corresponding *rec*⁺ donor strains. The results shown in Table 3 show that although transfer of either F or F-*lac*⁺ from *rec*⁻ donors is reduced, it still remains at a value greater than a half that from *rec*⁺ donors. Transfer of the three colicin factors however was almost equally efficient from either *rec*⁻ or *rec*⁺ strains. In the case of the colI factor, transfer was similar whether measured at the low level found from stably colicinogenic cells (low frequency colicinogeny transfer-LFC) or from a strain newly infected with colI from which transfer is enhanced (high frequency colicinogeny transfer-HFC; see STOCKER, SMITH and OZEKI 1963; MONK and CLOWES 1964a).

Chromosomal transfer from rec⁻ *donors:* The transfer of chromosomal markers from these *rec*⁻ strains, each infected with one of the several sex factors, to the same common recipient strain, was next investigated. These experiments, together with the control crosses using the corresponding *rec*⁺ donors, are shown in Table 4. The multiply-deficient auxotrophic genotype (*thr leu thi pro his arg*) of the *rec*⁺ and *rec*⁻ parental strains, limited the number of strains with suitable markers that could be used as recipients, and the strain chosen carried a *met* mutation. Selection for *met*⁺ recombinants was therefore made on minimal agar supplemented with arginine and thiamine. This avoided counter selection of the recipient markers *arg*⁺ and *thi*⁺, which are closely linked to either side of *met*⁻ and would otherwise have reduced the overall yield of recombinants.

Several features can be seen in the data of Table 4. First, with the notable exception of colI-mediated transfer, the numbers of *met*⁺ recombinants produced by *rec*⁻ donors were much inferior to those derived from the corresponding *rec*⁺ donors, ratios ranging from only 0.13% of the *rec*⁺ level in F-*lac*⁺ crosses to

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TABLE 3

Nonchromosomal transfer from rec⁻ *donors*

Donor × Recipient

AB 1157 (*xyl*⁻*lac*⁻) C600 (*xyl*⁺*lac*⁻) F⁻

Donor			Sex factor transfer		
			μ^s		
F+		*rec*+	37/40 (93%)		
		rec⁻	22/40 (56%)		
			μ^s *lac*⁺	μ^s *lac*⁻	μ^r *lac*⁺
F-*lac*+		*rec*+	39/40 (98%)	0/40	0/40
		rec⁻	21/40 (54%)	0/40	0/40
			colI+		
colI+	LFC	*rec*+	4/48 (8%)		
	LFC	*rec*⁻	4/48 (8%)		
	HFC	*rec*+	46/50 (92%)		
	HFC	*rec*⁻	45/50 (90%)		
			μ^scolV2+	μ^rcolV2+	μ^scolV2⁻
colV2+		*rec*+	30/48 (63%)	0/48	0/48
		rec⁻	26/48 (54%)	0/48	0/48
			μ^scolV3+	μ^rcolV3+	μ^scolV3⁻
colV3+		*rec*+	48/48 (100%)	0/48	0/48
		rec⁻	47/48 (98%)	0/48	0/0

Equal volumes young cultures (ca. 2×10^8) of both donor and recipient strains were mixed for 2 hr at 37°* and then diluted and plated on EMB xylose for single colonies. A number of well isolated *xyl*⁺ colonies were then purified by streaking and tested for transfer of the sex factor concerned: F in F+ and F-*lac*+ strains by sensitivity (μ^s) to the male specific phage, μ_2, colI by overlay with C600F⁻ as a colicin I-sensitive strain; and colV2 and colV3 by both overlay with the colicin V sensitive strain (C600F⁻) and also by sensitivity of each colony to the male specific phage, μ_2. (All strains carrying colV2 and colV3 are also sensitive to male-specific phage, μ, – see MACFARREN and CLOWES 1966). A proportion of the μ^s and μ^r colonies were tested for fertility. 20/20 μ^s colonies from F-*lac*+ crosses were fertile; 0/20 μ^r colonies were fertile. 12/12 μ^s colonies from colV2 crosses were fertile; 0/5 μ^r colonies were fertile. 8/8 μ^s colonies from colV3 crosses were fertile, one μ^r colony was infertile.

* 200 μg/ml trypsin was added to cultures to prevent lethal effects of free colicins on the recipient.

10.1% in the colV2 cross. Secondly, irrespective of the frequency of *met*⁺ recombinants produced by these various *rec*⁺ donors (from 1.5×10^{-5} in the case of F-*lac* to 8×10^{-8} with colV3), the frequency from the corresponding *rec*⁻ donors was very similar (within the range 0.61 to 2.1×10^{-8}). Finally, this low level of transfer from *rec*⁻ donors was found in *all* crosses involving sex factors, and was at least 200-fold the maximum possible level in mixtures when *no* sex factor was present (whether *rec*⁺ or *rec*⁻ mixtures) and was in fact very similar to the level of transfer from colI donors in the HFC state, whether *rec*⁺ or *rec*⁻.

The relative frequencies with which the two unselected markers, *xyl*⁻ and *T6*^{r} lying on opposite sides of the circular K-12 chromosome (see Figure 1) appear in *met*⁺ recombinants in these various crosses is shown in Table 5. It has been previously demonstrated that there is no obvious preference for the transfer of markers from any one segment of the chromosome, in crosses from normal *rec*⁺ strains mediated either by *autonomous* F (CLOWES and ROWLEY 1954; CAVALLI-SFORZA and JINKS 1956) by ColI (CLOWES 1961) or by colV2 (MACFARREN and CLOWES 1966). In contrast, the transfer due to an F prime factor such as F-*lac* is biased owing to the preferred integration of the factor at the site of the chromosomal fragment which was previously incorporated as part of the F-prime struc-

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TABLE 4

Chromosomal transfer from rec⁻ *donors*

Donor AB 1157 (*thr leu thi pro his arg T6ʳ xyl⁻*) × Recipient* 58–161 (*met T6ˢ xyl⁺*) *F⁻*

Donor			Experiment number	Total cells plated ($\times 10^9$)	*met⁺arg±thi±* colonies	Rec. freq. ($\times 10^{-8}$)			Efficiency *rec⁻/rec⁺*
F+		*rec*+	1	4	1853	46	44		
			3	10	4078	41		2.7%	
		rec⁻	1	4	54	1.3	1.2		
			3	10	109	1.1			
F-*lac*+		*rec*+	5	10	157,400	1574		0.13%	
		rec⁻	1	4	127	3.2	2.1		
			5	10	103	1.0			
colI+	LFC	*rec*+	1	4	11	0.28	0.25	0.92%	
			2	10	24	0.24			
			3	10	23	0.23			
	LFC	*rec*⁻	1	4	9	0.22	0.23		
			2	10	22	0.22			
			3	10	28	0.28			
	HFC	*rec*+	6	10	182	1.82	1.80	0.69%	
			8	10	177	1.77			
	HFC	*rec*⁻	6	10	86	0.86	1.24		
			8	10	161	1.61			
colV2+		*rec*+	4	6	1567	26.2	16.8	10.1%	
			7	10	1121	11.2			
		rec⁻	4	6	168	2.8	1.7		
			7	10	101	1.0			
colV3+		*rec*+	7	20	1688	8.4		7.3%	
		rec⁻	7	20	122	0.61			
F⁻ (control)		*rec*+	4	10	0	< .003			
			7	20	0				
		rec⁻	4	10	0				
			7	10	0				

50 ml young cultures (ca. 2×10^8) of both donor and recipient strains are mixed at 37° for 2 hr (static), washed twice in 100 ml buffer and resuspended in 5 ml (×20). Volumes of 0.2 ml are then plated on minimal medium supplemented with arginine, thiamine, and streptomycin and incubated 48 hr at 37°.

* Resistant to colicin I (*colI*ʳ) and cross resistant to colicin V2 and V3.

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TABLE 5

Unselected markers among recombinants

Donor AB 1157 (*thr leu thi pro his arg xyl⁻ T6ʳ*) × Recipient 58–161 (*met xyl⁺ T6ˢ*) F⁻

met⁺ recombinants with donor marker*	Donor									
	F-lac⁺		F⁺		colI⁺		colV2⁺		colV3⁺	
	rec⁺	*rec*⁻	*rec*⁺	*rec*⁻	*rec*⁺	*rec*⁻	*rec*⁺	*rec*⁻	*rec*⁺	*rec*⁻
xyl⁻	10/48	9/48	17/63	9/63	6/63	9/63	24/80	33/128	16/90	22/90
	(21%)	(19%)	(27%)	(14%)	(10%)	(14%)	(30%)	(26%)	(18%)	(24%)
T6ʳ	1/366	6/103	5/63	3/63	8/63	6/63	6/80	7/128	10/90	13/90
	(0.25%)	(6%)	(8%)	(5%)	(12%)	(10%)	(8%)	(6%)	(11%)	(14%)

* Pooled recombinants from crosses shown in Table 4 were tested for these unseleced markers.

ture, in this case *lac*, which lies between *pro* and *T6*. This particular F-prime transfers *pro*⁺ as an early (proximal) marker and *T6* as a late (distal) marker (SCAIFE and GROSS 1962). The results from *rec*⁺ donors shown in Table 5 are in line with these observations, the donor *xyl* marker being found in between 10 and 30% of the *met*⁺ recombinants in all crosses, whereas although the *T6* marker is found in between 8 and 12% recombinants from F, colI, colV2 and colV3 mediated crosses, it is found in only 0.25% of the recombinants from F-*lac* donors.

In contrast, the incorporation of both markers is more nearly the same from all *rec*⁻ donors, including those from F-*lac rec*⁻ donors; *xyl* appearing in 14 to 26% of the recombinants and *T6* in 5 to 14%.

DISCUSSION

The results of Table 2 show that the *rec*⁻ strain 96 (AB2463 of HOWARD-FLANDERS and THERIOT) is defective *when used as a recipient*. It has been assumed that this defect is probably due to the inability to integrate the genetic material which is received from the donor, as has been concluded in the case of the similar strain isolated by CLARK and MARGULIES (1965), and it is *not* due to the lack of ability to accept this genetic material from the donor. This latter aspect is supported by the finding that no difficulty was experienced in infecting AB 2463 with the various sex factors used in this study. Moreover, this strain has been shown by HOWARD-FLANDERS and THERIOT to be hypersensitive to both ultraviolet and X-irradiation to the same extent as the strain of CLARK and MARGULIES. It may also be concluded from Table 2 that infection of this *rec*⁻ strain with any of the sex factors used in this study does not compensate this defect, and that if this is due to lack of recombination, then recombination is still not possible in the *rec*⁻ donor strains.

When used as donors, all the *rec*⁻ strains infected with various sex factors were able to form contacts and transfer their sex factor with near-normal efficiency to the standard recipient C600F⁻ strain (Table 3). This suggests that the donor

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properties including the formation of stable unions and the transfer of non-chromosomal elements are not greatly affected by the *rec*⁻ mutation. The production of recombinants, and by inference, chromosomal transfer is, on the other hand, markedly reduced from all donor strains carrying the *rec*⁻ mutation, with the exception of those carrying colI (Table 4). This is consistent with the idea that the major part of chromosome transfer mediated either by F (or F-*lac*), colV2 or colV3 depends on the recombination of the sex factor and the chromosome (as has also been concluded in the case of F or F-*lac* by ADELBERG and PITTARD (1965) and by SCAIFE and GROSS (1964)) and this recombination is precluded by the *rec*⁻ mutation.

The results from the study of unselected markers (Table 5) support this main conclusion. Here it is seen that transfer from an F-*lac rec*⁻ donor does not show the bias against a distal marker such as *T6*, as is shown by the F-*lac rec*⁺ donor. It can be concluded that transfer from the *rec*⁺ donor arising from recombination of the F-prime element with the preferred (*lac*) site, is no longer possible, and that the greatly reduced and nonpolarized transfer from the F-*lac rec*⁻ donor is due, as is that from all *rec*⁻ donors, to events which do not involve recombination of sex factor and chromosome. These results would also seem to eliminate a transfer mechanism involving a transient, unstable structural association of sex factor and chromosome, not necessarily involving recombination, since this would also be likely to show a bias for the preferred site in F-prime transfer. The finding of a constant level of recombinants from all *rec*⁻ donors, irrespective of the differences shown by the corresponding *rec*⁺ donors, supports the idea of a low level transfer from *all* donors, which is independent of interaction of sex factor and chromosome. "Leakiness" in the *rec*⁻ mutation, on the other hand, would be expected to lead to a constant *rec*⁺/*rec*⁻ ratio of recombination.

In the case of colI, it appears that the low level of chromosomal transfer normally seen (from colI⁺ *rec*⁺ donors) is not due in any extent to recombination of sex factor and chromosome. This is supported by the fact that this low level transfer, shown by colI donors in the HFC state, whether in *rec*⁺ or *rec*⁻ strains, occurs at a similar level to that which is shown by all other *rec*⁻ donors. (The level shown by colI in the LFC state is further reduced due to the lower frequency with which this factor exhibits all sex-factor properties, including formation of unions and transfer of extrachromosomal elements, when stabilized in a cell [see Table 3 and MONK and CLOWES 1964a]).

Chromosome transfer from colI donors therefore seems at all times to be independent of interaction of sex factor and chromosome. This is a situation parallel to that involving the transfer of other noninfective extrachromosomal elements such as colE1 and colE2 that can be brought about by sex factors like F or colI. In these cases, there is also apparently no structural association between the mediating and the passive extrachromosomal elements (e.g. transfer of colE1 or colE2 by colI in Salmonella (SMITH, OZEKI and STOCKER 1963) or of colE1 and colE2 by F (CLOWES 1964). Chromosome transfer by colI might depend only upon spontaneous fragmentation of the normally circular K-12 chromosome to form a linear structure, as has been suggested in an earlier publication (CLOWES

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1963a), and the colI factor seems to have no demonstrable ability for association or integration with the chromosome as have the other sex factors used in this study.

We are indebted to DR. P. HOWARD-FLANDERS for the *rec*+ and *rec*⁻ parental strains and for unpublished information.

SUMMARY

Chromosome transfer mediated by certain sex factors (F and F-primes, colV2 and colV3) is greatly reduced from donor strains which have a "recombination-deficient" (*rec*⁻) mutation, when compared to transfer from the corresponding normal (*rec*+) donors. It is concluded that the major part of chromosome transfer mediated by these factors is dependent upon recombination of sex factor and chromosome. Nevertheless, chromosomal transfer *is* in fact found in all cases at a decreased level which is fairly constant in crosses involving any of these sex factors. Unselected marker transfer supports the idea that this low level transfer does not depend upon recombination or on a transient association of sex factor and chromosome. Chromosome transfer by another factor such as colI is similar whether from *rec*+ or *rec*⁻ donors from which it is concluded that such factors may be incapable of structural association or integration with the bacterial chromosome.

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