Genet. Res., Camb. (1969), **14**, *pp.* 79–87
With 1 *text-figure*
Printed in Great Britain

Chromosomal transfer mediated by de-repressed R factors in F⁻ *Escherichia coli* K12

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(*Received* 11 *April* 1969)

1. INTRODUCTION

R factors, the bacterial plasmids responsible for transmissible drug resistance (see Watanabe, 1963; Meynell, Meynell & Datta, 1968), promote bacterial conjugation and their own transfer to other bacteria in the same way as the sex factor, F, of *Escherichia coli* K12. The characteristic activity of F is the production of genetic recombinants, where segments of bacterial chromosome are transferred from the donor to a recipient. One way of testing whether chromosome transfer requires some special property of F itself, or is merely a consequence of the conjugation which F determines, is to examine the donor ability of bacteria carrying other transmissible plasmids. Chromosomal recombinants have been found with R factors (Sugino & Hirota, 1962; Hirota, Fujii & Nishimura, 1966) as well as with the colicin factor, ColI (Ozeki, Howarth & Clowes, 1961), although their numbers are extremely small compared to the numbers produced with F. However, the frequency of genetic recombination must necessarily be limited by the frequency of conjugation. With wild-type R and ColI factors, conjugation is limited by a repressor to 1% or less of the frequency with F (Meynell & Datta, 1966*a*). Thus, chromosomal recombinants, which appear at a rate of about 10^{-5} per cell of an F⁺ donor culture, may be expected to be considerably rarer with other sex factors, solely because of the lower frequency of conjugation. Conjugating efficiency may be increased to that with F by the use of de-repressed mutants of R factors (Meynell & Datta, 1967) and ColI (Edwards & Meynell, 1968). One such mutant R factor is R100-1, originally selected for loss of the normal inhibition of fertility due to F (Egawa & Hirota, 1962; Nishimura, Ishibashi, Meynell & Hirota, 1967); others, including R1*drd*, were directly selected for expression of their own conjugation function, leading to more frequent transfer of drug resistance (Meynell & Datta, 1967). With such mutants, it is possible directly to compare their respective abilities to bring about chromosome transfer. Both R100–1 (Sugino & Hirota, 1962; Hirota *et al.* 1966) and R1*drd* gave broadly the same numbers of recombinants as F for most chromosomal genes but R1*drd* was also observed to behave like an F′ factor with a preferential integration site which caused the donor to

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transfer the sequence of genes *pyr*F...*trp*...*pur*B nearly as frequently as an Hfr strain with its origin in that region (Pearce & Meynell, 1968*a*). The present paper describes the results of testing seven further R factors, three with F-like and four with I-like sex factors (see Meynell *et al.* 1968), for their ability to give recombinants for a variety of genes located throughout the chromosome of *E. coli* K12.

2. MATERIALS AND METHODS

R factors. R1*drd* (KmCmApSmSu), R136*drd* (TcSu), R192*drd* (TcCmSmSu) and R538–1*drd* (CmSmSu) were factors determining an F-like sex pilus; R64*drd* (TcSmSu), R144*drd* (Km*col*I), R163*drd* (TcKm*col*I) and R538–2*drd* (TcSm) an I-like pilus. All these R factors were de-repressed mutants selected for increased drug-resistance transfer, which occurred at a frequency of 0·3–1 per R^+ bacterium in 20 min under the conditions used to obtain chromosomal recombinants.

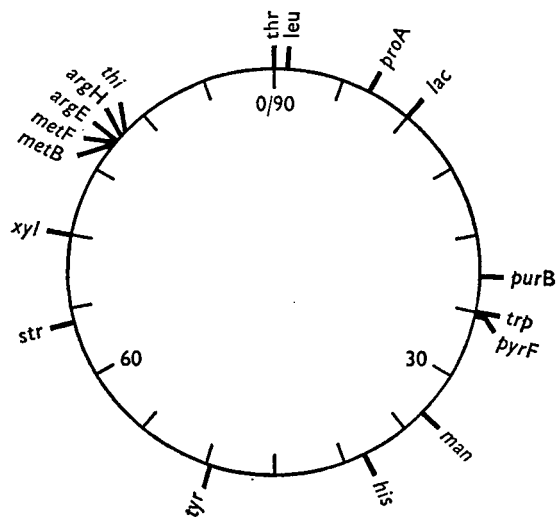

Fig. 1. Genetic map of *Escherichia coli* K12 with the positions of the genes referred to.

Bacteria. Two strains were used as donors: 58–161 (*met*B$^-$) and J53 (*met*F$^-$*pro*A$^-$). These were originally F^+ strains and were used after elimination of F by treatment with acridine orange (Hirota, 1960). Before introducing the R factors into them, the absence of F was confirmed by their failure to either act as genetic donors, propagate the F-specific phage MS2 (Meynell & Datta, 1966*b*) or restrict multiplication of phage T3 (Schell *et al.* 1963). The F$^-$R$^-$ recipient strains were J62 (*pro*A$^-$*trp*$^-$*his*$^-$*str*^{r}), PA309 (*thr*$^-$*leu*$^-$*trp*$^-$*his*$^-$*str*^{r}*xyl*$^-$*arg*H$^-$*thi*$^-$), X195 (*pro*A$^-$*lac*$^-$*pur*B$^-$*trp*$^-$*pyr*F$^-$*tyr*$^-$*str*^{r}) and AB1157 (*thr*$^-$*leu*$^-$*pro*A$^-$*his*$^-$*str*^{r}*arg*E$^-$*thi*$^-$). The information on the Arg$^-$ locus of PA309 was kindly provided by Dr Pearl Cooper. The positions of these markers are shown in Fig. 1, taken from Taylor & Trotter (1967).

https://doi.org/10.1017/S0016672300001877 Published online by Cambridge University Press

Mating mixtures consisted of 0·5 ml of the donor strain, freshly grown in nutrient broth (Oxoid No. 2) to a concentration of $1–4 \times 10^8$ bacteria/ml as determined by viable counts, mixed with 4·5 ml of an overnight broth culture of the recipient and incubated at 37 °C for 60 min in a screw-cap bottle slowly rotating on an inclined turntable. Generally, the bacteria were then centrifuged and resuspended in buffer to twice their original concentration. After agitating the suspension with a 'Whirlimixer' to separate mating pairs, dilutions in buffer were plated for recombinants on appropriately supplemented defined media containing streptomycin to kill the donor strain. The concentration of streptomycin was either 200 μg/ml, or 5000 μg/ml when the R factor carried an Sm determinant (Pearce & Meynell, 1968*b*); control experiments showed that this concentration did not impede the development of recombinant colonies. The frequency of recombinants was expressed with reference to the viable count of donor bacteria in the mating mixture at the time of mixing. Control cultures of donor and recipient alone, or of mixtures of recipient and either strain 58–161 or strain J53 without a sex factor produced no colonies on the selective media, with the exception of the histidine and tryptophan markers where an occasional colony appeared.

3. RESULTS

The different R factors were compared with one another and with F both for absolute frequencies of recombinants with strain 58–161 as donor (Table 1) and for the relative numbers for different genes, the latter being expressed with reference to *thr leu* combined in the case of the recipient PA 309 and to *pro*A with strains J62 and X195 (Table 2). If, following transfer, the probability of integration of any one marker is the same (Jacob & Wollman, 1961), then, with the exception of genes close to contraselected donor genes, observed differences should represent differences in transfer. Examples of the frequencies obtained for the various selected recombinant classes are given in Table 1. Crosses with any of the R factors produced as many recombinants as with F and the over-all variation between individual experiments was no greater with the R factors than with F. A limited number of recombinants were examined for unselected markers and showed no difference between any of the R factors and F. Table 2, compiled from all but a few experiments mentioned individually in the text, gives the ratios of the different classes of recombinants with 58–161 as donor bacterium, where both contraselected loci, *met*B and *str*, were distant from all the selected markers save *arg*H and *xyl*, accounting for the relatively small numbers of these recombinants. Amongst the other types of recombinant, the relatively high frequency for the *trp* region, previously noted with R1*drd*, was confirmed and observed to a lesser degree in some of the other factors, particularly R538–1*drd* and R144*drd* in crosses to PA309 and J62.

Table 3 shows the ratios of different classes of recombinants with J53 as donor; here, a contraselected donor gene, *pro*A, lies at 6 min distance from *leu* (Taylor & Trotter, 1967). The numbers of recombinants are expressed with reference to *his*

in J62 and *tyr* in X195, for, although these recipients have different Pro$^-$ mutations from the donor J53, phage P1 transductions and $F^+ \times F^-$ crosses of one strain with another, as well as crosses between each and the Hfr strain P4X-6 whose origin lies between *pro*A and *pro*B (Adelberg & Burns, 1960; kindly provided by Dr E. A. Adelberg), showed all the mutations to be closely linked within

Table 1. *Recombinant frequencies (for 10^6 donor cells) for 58–161 with different sex factors*

	Recombinants								
Sex factor	*thr*$^+$ *leu*$^+$	*pro*$^+$	*ade*$^+$	*trp*$^+$	*ura*$^+$	*his*$^+$	*tyr*$^+$	*xyl*$^+$	*arg*$^+$
				Recipient PA309					
F	14·0	—	—	18·0	—	10·0	—	2·5	0·4
R1*drd*	13·0	—	—	3000·0	—	5·0	—	3·0	5·0
R136*drd*	8·0	—	—	30·0	—	16·0	—	—	2·3
R192*drd*	140·0	—	—	550·0	—	79·0	—	3·6	2·5
R538–1*drd*	2·0	—	—	20·0	—	10·0	—	—	0·3
R64*drd*	190·0	—	—	250·0	—	46·0	—	2·9	5·0
R144*drd*	44·0	—	—	300·0	—	91·0	—	—	8·2
R163*drd*	22·0	—	—	65·0	—	8·0	—	6·0	0·5
R538–2*drd*	100·0	—	—	160·0	—	130·0	—	—	0·7
				Recipient X195					
F	—	28·0	8·3	6·0	14·0	—	20·0	—	—
R1*drd*	—	20·0	100·0	230·0	320·0	—	6·3	—	—
R136*drd*	—	7·0	3·0	14·0	13·0	—	9·0	—	—
R192*drd*	—	12·0	2·4	2·4	1·0	—	2·4	—	—
R538–1*drd*	—	1·0	2·0	3·0	5·0	—	0·4	—	—
R64*drd*	—	28·0	10·0	12·0	17·0	—	24·0	—	—
R144*drd*	—	37·0	15·0	45·0	56·0	—	26·0	—	—
R163*drd*	—	17·0	15·0	11·0	11·0	—	17·0	—	—
R538–2*drd*	—	53·0	46·0	42·0	54·0	—	53·0	—	—
				Recipient J62					
F	—	240·0	—	180·0	—	100·0	—	—	—
R1*drd*	—	60·0	—	3000·0	—	5·5	—	—	—
R136*drd*	—	14·0	—	24·0	—	43·0	—	—	—
R192*drd*	—	14·0	—	26·0	—	12·0	—	—	—
R538–1*drd*	—	44·0	—	17·0	—	10·0	—	—	—
R64*drd*	—	100·0	—	350·0	—	82·0	—	—	—
R144*drd*	—	140·0	—	360·0	—	85·0	—	—	—
R163*drd*	—	13·0	—	21·0	—	25·0	—	—	—
R538–2*drd*	—	43·0	—	84·0	—	44·0	—	—	—

*pro*A. There was more variation between individual experiments with J53 than with 58–161. The tendency towards a high proportion of *trp* recombinants may perhaps have been slightly greater with this strain, but with both donors, 58–161 and J53, it was difficult to distinguish from a second sort of inequality based on relatively reduced numbers of recombinants for *thr leu*, which produced an increased ratio for all the other markers. This was most noticeable with the sex

factors R538–1*drd* and R136*drd* but was also observed on four occasions with R1*drd*: in one experiment (Expt 156) with 58–161 (R538–1*drd*), the ratios of *trp* and *his* to *thr leu* recombinants were as high as 170 and 100, respectively, and in another (Expt 246) with J53 (R538–1*drd*) the corresponding ratios were 79 and 71. The result was not absolutely constant, however, for, in a further experiment (Expt 277), 58–161 (538–1*drd*) gave only 3 times as many recombinants for *trp* and for *his* as for *thr leu*.

Table 2. *Relative frequencies of recombinants for different markers*

Number of experiments shown in brackets.

Recombinants for:	Donor: 58-161 carrying sex factors								
	F	R1*drd*	R136*drd*	R192*drd*	R538–1 *drd*	R64*drd*	R144*drd*	R163*drd*	R538–2 *drd*
				Recipient PA309					
thr leu	1	1	1	1	1	1	1	1	1
trp	1·4–2·0	20–200	3·0–6·5	3·0–7·0	14–20	1·5–3·0	3·0–8·0	1·6–3·0	1·5–3·0
his	0·9–1·4	0·05–0·1	2·0–3·5	0·4–1·9	4·0–11	0·3–0·7	1·0–2·5	0·2–0·7	1·2–2·9
xyl	0·2–0·4	0·1–0·25	—	—	—	0·1–0·4	0·2–0·5	0·2–0·4	—
*arg*H	0·05–0·1	0·03–0·3	0·2–0·4	0·1–0·2	0·1–0·4	0·02	0·02	0·05	0·01–0·1
	(7)	(4)	(4)	(4)	(7)	(3)	(3)	(5)	(3)
				Recipient J62					
*pro*A	1	1	1	1	1	1	1	1	1
trp	0·7–1·7	23–33	2·0–3·5	0·8–2·0	0·5–2·5	1·5–7·0	2·0–3·0	2·0–2·5	1·8–2·0
his	0·5–1·0	0·1–0·3	1·0–3·0	0·8–1·5	0·2–2·5	0·6–2·0	0·5–1·0	0·4–0·8	1·0–1·1
	(6)	(4)	(4)	(4)	(5)	(5)	(3)	(3)	(3)
				Recipient X195					
*pro*A	1	1	1	1	1	1	1	1	1
lac	0·3–0·5	0·5	—	—	—	0·7–1·6	0·4–1·0	—	—
*pur*B	0·3–0·6	20–30	0·3–0·6	0·2–0·3	0·3–2·0	1·1–1·4	0·4–1·0	0·4–1·7	0·7–1·4
trp	0·5–1·0	20–40	1·1–1·8	0·2–0·8	0·3–3·0	1·0–2·0	1·2–1·5	0·5–1·2	0·7–1·6
*pyr*F	0·5–1·5	20–40	1·4–1·8	0·5–0·8	1·4–5·0	1·4–2·0	1·3–3·0	1·0–2·2	1·0–1·5
tyr	0·7–1·0	0·3	0·9–1·3	0·7–2·7	0·4–0·9	1·0	0·5–0·7	0·5–1·2	0·8–1·0
	(6)	(3)	(3)	(4)	(2)	(3)	(4)	(4)	(4)

In comparing the two bacterial strains used as donors, it was apparent that with many of the R factors, the relative numbers of *thr leu* recombinants were often even lower with J53 than with 58–161. The *leu* gene is situated at 6 min from *pro*A, contraselected in J53: crossing 58–161F$^+$ and AB1157 in tests for linkage between these two genes, 55% of recombinants selected for *leu*$^+$ had also inherited *pro*A$^+$ and 34% selected for *pro*A$^+$ were also *leu*$^+$. Linkage alone should thus have reduced the relative numbers of *thr leu* recombinants with J53 no more than twofold. In any event, no simple explanation based on linkage can account for the facts that the difference between J53 and 58–161 was not observed to any extent with F and was present to a variable extent with each of the other sex factors. A relatively low frequency of *thr leu* recombinants could theoretically be due either to the double selection for both markers or to the position of the markers in the chromo-

Table 3. *Relative frequencies of recombinants for different markers*

Number of experiments shown in brackets.

Recombinants for	Donor: J53 carrying sex factors								
	F	R1*drd*	R136*drd*	R192*drd*	R538–1*drd*	R64*drd*	R144*drd*	R163*drd*	R538–2*drd*
	Recipient PA309								
thr leu	1	1	1	1	1	1	1	1	1
trp	2·5–3·0	90–180	3·0–9·0	2·5–10	10–30	4·0–10	14–25	10–30	4·0–5·0
his	0·9–1·7	0·25–0·9	3·0–11	2·0–3·0	8–18	0·8–1·7	1·3–3·0	1·0–6·0	1·9–2·9
xyl	—	⩽ 0·7	—	—	—	0·4–0·5	0·07–1·0	< 0·6	—
*arg*H	0·05–0·1	0·1–1·0	0·06–0·6	0·1–0·3	0·1–0·5	0·05–0·07	0·2–0·3	< 0·1–0·3	< 0·05–0·1
	(5)	(4)	(4)	(3)	(4)	(3)	(3)	(3)	(3)
	Recipient J62								
*pro*A	—	—	—	—	—	—	—	—	—
trp	2·0–6·0	60–600	1·0–1·6	1·5–2·5	1·5–3·0	5·0–10	4·0–7·0	3·0	2·3–4·0
his	1	1	1	1	1	1	1	1	1
	(6)	(4)	(3)	(4)	(4)	(3)	(2)	(2)	(3)
	Recipient X195								
*pro*A	*	*	*	*	*	*	*	*	*
lac	0·3–2·5	1·0–4·0	—	—	—	0·3–2·0	0·2–0·5	—	—
*pur*B	0·8–3·0	55–300	0·6–1·5	1·0–2·0	2·0–5·5	1·0–2·5	0·5–1·5	0·5–1·5	0·8–1·5
trp	0·7–5·0	90–600	1·0–6·0	0·6–1·7	0·5–3·5	0·3–2·0	2·0–3·5	0·6–2·5	0·8–2·0
*pyr*F	0·6–3·0	90–700	3·5–6·0	1·0–2·0	1·0–5·0	0·5–0·8	1·5–4·5	0·3–2·5	1·5–2·2
tyr	1	1	1	1	1	1	1	1	1
	(7)	(6)	(4)	(3)	(2)	(3)	(3)	(4)	(4)

* See text.

some. To test the former possibility, the donors J53 (538–1*drd*), 58–161 (538–1*drd*), J53 (136*drd*), 58–161(136*drd*) and 58–161F$^+$ were compared with one another, selecting either *thr*$^+$ or *leu*$^+$, or both together. Recombinants for each marker alone were about twice as frequent as those for both *thr*$^+$ and *leu*$^+$, but there were no differences between the five donors. If the decreased frequency of *thr leu* recombinants were related to the position of these genes in the chromosome, recombinants for the neighbouring *pro*A might also show some reduction. Strain AB1157 is *pro*A$^-$ as well as *thr*$^-$ *leu*$^-$ and, with this recipient, fewer recombinants for *pro*A, as well as for *thr leu*, relative to its other markers were produced with 58–161(136*drd*) and 58–161 (538–1*drd*) than with 58–161F$^+$, although the differences were not large enough to be significant.

4. DISCUSSION

With the exception of R1, which transfers the chromosome predominantly from an origin near *pyr*F, conjugation brought about by the R factors we investigated led to broadly the same numbers and kinds of genetic recombinants as F. The same appears to be true of R100–1 (Sugino & Hirota, 1962).

Only limited information is provided on the mechanism of chromosome transfer by this type of experiment. In the first place, plating a mating mixture after relatively prolonged incubation does not distinguish single from repeated conjugations; in F$^+ \times$ F$^-$ crosses, the progressively increasing proportion of recombinants carrying autonomous F (Curtiss & Renshaw, 1969) suggests that new mating pairs are initiated throughout the period. The formation of Hfr variants is responsible for some, at least, of the recombinants produced by an F$^+$ donor strain (Jacob & Wollman, 1961). The number of recombinants is drastically reduced when an F$^+$ donor strain is a Rec$^-$ mutant (Clowes & Moody, 1966), just as would be expected if chromosome transfer depended entirely on the production of Hfr variants in which F had become integrated through the action of the bacterial Rec enzyme. Nevertheless, even in situations where stable Hfr variants are known to occur, it has been calculated that they are responsible for only 15% of the recombinants actually produced (Curtiss, Stallions, Mays & Renshaw, 1967); the sort of donor cell which gives rise to the remaining 85% remains unknown: either the Hfr state can be established transiently, or integration of chromosome and sex factor is not required. If the latter were the case, then the simple provision of a conjugation bridge might be expected to be sufficient, and every different sex factor should be equally efficient in leading to chromosome transfer. However, the frequency of recombinants with F and with R factors contrasts markedly with the behaviour of ColIb-P9 which gives very few recombinants in the wild-type state (Ozeki *et al.* 1961; Clowes & Moody, 1966) whose numbers remain below 10^{-7} per Col$^+$ donor cell even when the frequency of conjugation is increased by using de-repressed mutants (Edwards & Meynell, 1969; Cooke, unpublished experiments). Colicinogeny was unlikely to be responsible, for, on the one hand, R144 also determined production of colicin Ib and, on the other, the I-like sex factor

found with ColEla, which itself carries no *col* determinant, gave the same low numbers of chromosomal recombinants as ColIb-P9. There are, in addition, the relatively minor differences between F and R factors, and between some R factors and others, apparent from the data in Tables 1–3.

The types of recombinant produced with R1 strongly suggest a past history of chromosomal integration for this factor, for it now behaves, like an F′ factor, as if it had once acquired a segment of chromsome (Pearce & Meynell, 1968*a*). The presumed homologous region of chromosome lying between 26 and about 30 min contains few genes that have been identified (Taylor & Trotter, 1967), whose presence might be detected as part of R1. A gene for mannose fermentation lies at 33 min but although R1 has been maintained in mannose-positive bacteria, the presence of the positive allele of this gene could not be demonstrated upon transfer of the factor to the mannose-negative strain AT703 (Taylor & Trotter, 1967; kindly provided by Dr A. L. Taylor). The isolation of two donor clones, one with R538–1*drd* and the other with R163*drd*, giving predominantly *tyr*$^+$ recombinants, as well as a third, with R64*drd*, giving a relatively high frequency of recombinants for *thr leu* provided further evidence for possible integration of R factors and chromosome which is at present being examined.

SUMMARY

Conjugation brought about by each of eight different R factors was as efficient as with F in the production of genetic recombinants for a variety of genes located throughout the chromosome of *Escherichia coli* K12.

One of us (M.C.) is grateful to the University Grants Commission, New Zealand, for a post-doctoral fellowship held at the M.R.C. Microbial Genetics Research Unit.

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