Specific Chromosomal Affinity of a Resistance Factor

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SUMMARY

R factors, responsible for transmissible drug resistance in Enterobacteriaceae, convert their hosts into donors of genetic material, but fewer recombinants for chromosomal genes are produced than with F. For one R factor, at least, this was merely a consequence of less frequent conjugation. With a mutant where conjugation was not repressed in contrast to the wild type, this R factor, R₁, was shown to behave like an F' factor and to transfer the bacterial chromosome at high frequency from a fixed origin near the *try* genes.

INTRODUCTION

The factors responsible for transmissible drug resistance in Enterobacteriaceae resemble the F factor of *Escherichia coli* K 12 in conferring on their hosts the ability to donate the factor by conjugation to other bacteria (Watanabe, 1963). The existence of F was originally detected by the ability of an F⁺ bacterium to produce genetic recombinants with an F⁻ recipient (Lederberg, Cavalli & Lederberg, 1952; Hayes, 1953), but the presence of an R factor may be detected directly by transfer of the drug-resistance determinants as a part of the factor itself. The recipient is thus converted to a drug-resistant bacterium at the same time as it in turn becomes able to conjugate and to pass on its resistance. R factors occasionally bring about transfer of chromosomal genes and the formation of genetic recombinants between bacteria both of which are F⁻ (Sugino & Hirota, 1962). In contrast to the situation with F, however, chromosomal recombinants appear at very low frequency, corresponding ordinarily only to about 10⁻⁷ to 10⁻⁸ of the R⁺ population instead of 10⁻⁴ to 10⁻⁵ of an F⁺ population.

Several different R⁺ strains of *E. coli* K 12 58-161/*sp met*⁻ were tested for their ability to give genetic recombinants with an F⁻ recipient, J6-2 *pro*⁻ *his*⁻ *try*⁻, and one of the factors, R₁, was remarkable for the relatively large number of *try*⁺ recombinants produced. While *pro*⁺ and *his*⁺ recombinants occurred at a frequency of 10⁻⁷ to 10⁻⁸ per R⁺ donor cell, there were more than 10⁻⁵ *try*⁺ colonies. Tryptophan independence was not conferred, like drug resistance, by the R factor itself; crosses between R₁⁺ F⁻ bacteria and a recipient strain mutated in the genes *pyrF*, *try* and *purB* (Signer, Beckwith & Brenner, 1965; see Fig. 1) showed that it was indeed this region of the bacterial chromosome that was transferred.

Almost every F⁺ bacterium can conjugate, but with other factors conjugation is

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repressed and happens with only a minority of the cells (Ozeki, Stocker & Smith, 1962; Meynell & Datta, 1966*a*; Meynell & Lawn, 1967). Since, when conjugation occurs it only occasionally leads to chromosome transfer, when it is infrequent the numbers of genetic recombinants must be correspondingly small. Recombination with R I was first studied with the wild-type R factor, but the nature of the preferential transfer of the *try* region could be investigated only after the isolation of a derepressed mutant, R I *drd* (Meynell & Datta, 1967), with which conjugation occurred about as often as with F, and which therefore allowed the rates of production of different classes of chromosomal recombinants with the two sex factors to be directly compared.

METHODS

Culture media. Defined medium was the mineral salts medium described by Tatum & Lederberg (1947), with the omission of asparagine and solidified with 1.5 % (w/v) Davis N.Z. agar. In a few experiments, M9 medium (Adams, 1959) was used. These were appropriately supplemented with amino acids, purines and pyrimidines at 20 µg./ml., thiamine at 1 µg./ml. and sugars used as carbon source at 0.2 % (w/v).

Fig. 1. The genetic map of *Escherichia coli* showing the loci which are referred to.

Broth was either Oxoid broth No. 2, or L-broth (Lennox, 1955) adjusted to pH 7.4; for nutrient agar, these were solidified with 1.35 % (w/v) Davis N.Z. agar.

Bacteria. These were all derivatives of *Escherichia coli* K12 and are described in Table 1.

R factor. R I (Meynell & Datta, 1966*a*) confers resistance to chloramphenicol, kanamycin, ampicillin, streptomycin and sulphonamide. Mutants of R I whose conjugation function is no longer repressed have been isolated by Meynell & Datta (1967), and one of these, R I *drd-19*, was used in addition to the wild-type R factor. The R factor was introduced into a strain by conjugation, selecting for resistance to chloramphenicol (25 µg./ml.) added to defined medium supplemented to allow growth of the recipient, but not the donor, strain.

Bacterial crosses and measurements of frequency of R factor transfer. Donor and recipient strains were grown overnight in broth without shaking. Next day, the culture of the donor strain was diluted 1/10 in fresh broth and incubated for 2 hr without aeration until the bacterial concentration reached $2-5 \times 10^8$ /ml. The temperature of incubation greatly affected the donor ability of the culture; at 35° or less very little chromosome or R transfer occurred. Mating mixtures contained 0.5 ml. of the donor

Table 1. *Bacterial strains: all derivatives of Escherichia coli K12*

Strains used as donors	Characters*
58-161 F ⁺	<i>met-1</i> (Lederberg, 1947)
58-161/sp	Spontaneous F-defective mutant of 58-161 F ⁺ (Hayes, 1953; Meynell & Datta, 1966b)
58-161 F ⁻	58-161 F ⁺ cured of F by acridine orange
J5-3 F ⁻	<i>pro-1 met-2</i> (Clowes & Rowley, 1954), cured of F by acridine orange
J5-3 F ⁺	J5-3 carrying F transferred from 58-161 F ⁺
K12 F	Wild-type strain K12 cured of F by acridine orange
w1655 F ⁻	<i>met-1</i> (λ) ⁻ λ- <i>res</i> (Lederberg & Lederberg, 1953), cured of F by acridine orange
C600	<i>thr-leu⁻ thi-lac⁻</i> (λ) ⁻ F ⁻ (Appleyard, 1954)
w1177	<i>thr-leu⁻ thi-lac⁻ str-r</i> F ⁻ (Lederberg, 1951)
w677	<i>thr-leu⁻ thi-lac⁻</i> F ⁻ (Lederberg, 1950)
HfrC	Hfr Cavalli derived from 58-161 F ⁺ by integration of F giving <i>pro</i> as a leading marker (Cavalli-Sforza, 1950)
HfrB10	Derived from w1655 F ⁺ by integration of F giving <i>try</i> as a leading marker (Broda, 1967)
Strains used as recipients	
J6-2	<i>pro-2 his-1 try-1 lac⁻</i> (Clowes & Rowley, 1954), cured of F by acridine orange
J6-2 <i>str-r</i>	streptomycin-resistant mutant of J6-2
PA 309	<i>thr-leu⁻ thi-try⁻ his-arg⁻ lac⁻ gal⁻ mal⁻ xyl⁻ str-r</i> (λ) ⁻ λ- <i>res</i> F ⁻ (kindly provided by Dr W. Hayes)
X195	<i>pro⁻ his⁻ try⁻ met⁻ purB⁻ pyrF⁻ tyr⁻ lac⁻ str-r</i> F ⁻ (from the collection of the M.R.C. Laboratory of Molecular Biology, Cambridge, by courtesy of Dr J. Shapiro)
MSO	<i>pyrD⁻ his⁻ str-r</i> F ⁻ (Shapiro, 1967)

* Location of genetic markers shown in Fig. 1.

culture and 4.5 ml. of the overnight recipient culture, and were rotated at 37° in a $\frac{1}{2}$ oz. screw-cap bottle on an inclined turntable revolving at 33 rev./min. With wild-type R1 and with F⁺ donors, the mixture was incubated for 60 min. and dilutions in buffer were then plated on appropriate media to isolate recombinants. In all except the preliminary experiments with R1 *drd-19*, the mating mixture was incubated for only 5 min. to allow cell-to-cell contacts to form, and was then gently diluted 1/100 into fresh warm broth (de Haan & Gross, 1962). This was held at 37° either for 60 min. or for the time stated in the experiments where mating was interrupted. Before plating, the mating pairs were separated by agitating the sample with a 'Whirlimixer' (Fisons Scientific Apparatus Ltd., Loughborough, Leicestershire). Samples were plated in a layer of soft agar, using 2.5 ml. volumes of 0.6% (w/v) agar in water; control experiments showed that as many recombinant colonies were produced by this method as by spreading the sample on the surface of the medium. Ability to synthesize a given nutrient was tested by omitting it from an otherwise complete defined medium; ability to utilize a particular sugar was tested by providing it as sole energy source.

Contraselection against the R⁺ donor strain with streptomycin. The bacteria used as recipients were one-step mutants to high level streptomycin resistance and, although

streptomycin is one of the antibiotics to which the R factor R1 confers resistance, the maximum concentration which allows normal growth of R⁺ bacteria on nutrient agar is about 20 µg./ml. The concentration of streptomycin initially used to select against the donor strain was 250 µg./ml., but when this was shown to be insufficient to abolish growth of J5-3(R1) on defined medium (Pearce & Meynell, 1968), the concentration was increased to 5000 µg./ml., which was the least concentration needed to prevent completely the growth of the R⁺ donor, but which still allowed normal growth of the highly streptomycin-resistant recipient strains.

RESULTS

Selected cases of recombinants

In preliminary tests of R⁺ donors with a recipient strain J6-2 *pro⁻ try⁻ his⁻ str^{-r}* the factor R1 gave 10⁻⁷ to 10⁻⁸ *pro⁺* and *his⁺* recombinants per donor cell, while *try⁺* colonies appeared much more frequently, at a rate of 10⁻⁴ to 10⁻⁵. The original donor strain, 58-161/*sp met⁻* carries a defective F factor (Meynell & Datta, 1966*b*) which might have been responsible for the observed recombination, so R1 was re-tested in strain J5-3 *pro⁻ met⁻*, an F⁻ strain cured of F by acridine orange. Since donor and recipient were both *pro⁻* here as a result of mutations in closely linked genes, only the numbers of *his⁺* and *try⁺* recombinants could be compared; however, in crosses between J5-3 (R1) and J6-2 *str^{-r}* large numbers of *try⁺* colonies were again obtained (Table 2). Their *try⁺* gene was not an integral part of the R factor itself because about 200 times more recipients became drug-resistant than become tryptophan-independent, while any *try⁺* strain was converted into a high-frequency donor of the character when R⁺ derivatives were selected for drug-resistance alone. While most *try⁺* recombinants were drug-resistant, 17 out of 423 were R⁻; in addition, R⁻ segregants of R⁺*try⁺* recombinants remained *try⁺*.

In the first crosses with strain J6-2 *str^{-r} pro⁻ his⁻ try⁻* as recipient, only 250 µg./ml. streptomycin was added to the medium to inhibit the donor (Pearce & Meynell, 1967) and it was observed that the plates without tryptophan used for scoring *try⁺* recombinants were covered with non-recombinant bacterial growth in contrast to those in which either proline or histidine had been omitted. This indicated considerable cross-feeding for tryptophan and suggested that the high incidence of *try⁺* recombinants might have resulted from mating on the plates. However, when strain J6-2 *str^{-r}* was replaced as recipient by strain X195 carrying *pyrF⁻* and *purB⁻*, loci closely linked to *try⁻*, and the concentration of streptomycin was increased to 5000 µg./ml., the numbers of *pyrF⁺* and *purB⁺* recombinants were as high as those of *try⁺* recombinants, although when adenine or uracil was omitted there was no background growth, and presumably no cross-feeding and continued mating on the plates. Moreover, when the derepressed mutant of R1, which gives a much higher rate of conjugation, was substituted for wild-type R1, *try⁺* recombinant colonies could be counted after plating dilutions of the mating mixture which did not give any background growth.

Table 2 shows the frequencies of the different recombinant classes obtained with the various conjugation factors. With F, the frequency of recombinants for all markers was 1 × 10⁻⁵ to 2 × 10⁻⁴ and, although it was not unusual to find more recombinants for *purF*, *try* or *purB* than for other loci, the difference was seldom more than twofold. The small numbers of *pro⁺* recombinants using strain J5-3 *met⁻ pro⁻* as donor was

Table 2. *Escherichia coli* K12: frequencies of different classes of selected recombinants expressed as number of recombinants per donor

Factor	Donor	Cross	Recipient	<i>thr</i> <i>leu</i>	<i>pro</i>	<i>lac</i>	<i>gal</i>	<i>pyrD</i>	<i>purB</i>	<i>try</i>	<i>pyrF</i>	<i>his</i>	<i>tyr</i>	<i>arg</i>
R1	J5-3		J6-2 <i>str-r</i>	—	$<1 \times 10^{-8}$	—	—	—	—	$10^{-4} \times 10^{-5}$	—	1×10^{-8}	—	—
R1	J5-3		PA309	1×10^{-7}	—	—	—	—	—	$10^{-4} \times 10^{-5}$	—	$10^{-7} \times 10^{-8}$	—	—
R1	J5-3		X195	—	—	$<1 \times 10^{-7}$	—	—	1×10^{-5}	1.5×10^{-5}	2.5×10^{-5}	1×10^{-7}	1×10^{-7}	5×10^{-8}
R1	K12F-		PA309	2.5×10^{-7}	—	—	—	—	—	1.5×10^{-5}	—	—	—	—
R1	J5-3		X195	—	—	2×10^{-5}	—	—	2.8×10^{-3}	3.8×10^{-3}	6×10^{-3}	—	1×10^{-5}	—
R1	K12F-		PA309	5×10^{-4}	—	—	—	—	—	8×10^{-3}	—	2.5×10^{-5}	—	—
R1	58-161/ <i>sp</i>		J6-2 <i>str-r</i>	—	7×10^{-4}	—	—	—	—	2×10^{-2}	—	3×10^{-5}	—	—
R1	58-161 F-		J6-2 <i>str-r</i>	—	2×10^{-4}	—	—	—	—	6×10^{-3}	—	2×10^{-5}	—	—
R1	w1655 F-		J6-2 <i>str-r</i>	—	2×10^{-4}	—	—	—	—	7×10^{-3}	—	5×10^{-6}	—	—
R1	C600		J6-2 <i>str-r</i>	—	8×10^{-6}	—	—	—	—	4×10^{-3}	—	3×10^{-6}	—	—
R1	w1177		X195	—	—	—	3×10^{-5}	—	9×10^{-4}	1×10^{-3}	1.2×10^{-3}	—	3×10^{-5}	—
R1	w677		X195	—	—	—	—	2×10^{-4}	4×10^{-4}	—	—	1×10^{-5}	1×10^{-5}	—
R1	J5-3		MSO	—	—	—	—	—	—	—	—	1×10^{-5}	—	—
F	J5-3		PA309	1×10^{-5}	—	—	—	—	—	2.5×10^{-5}	—	6×10^{-6}	—	—
F	J5-3		X195	—	6×10^{-6}	5×10^{-5}	—	—	7×10^{-5}	1×10^{-4}	1.5×10^{-4}	1×10^{-4}	2×10^{-5}	—
F	58-161 F ⁺		J6-2 <i>str-r</i>	—	2×10^{-4}	—	—	—	—	2×10^{-4}	—	—	—	—
F	w1177		X195	3.5×10^{-5}	—	—	—	—	—	1×10^{-5}	—	—	—	—
F	w677		X195	1×10^{-5}	—	—	—	—	2×10^{-5}	2×10^{-5}	—	—	7×10^{-6}	—
F	HfrC		J6-2 <i>str-r</i>	—	1.5×10^{-1}	—	—	—	—	4×10^{-5}	—	5×10^{-5}	—	—
R1	HfrC		J6-2 <i>str-r</i>	—	1.3×10^{-1}	—	—	—	—	1×10^{-2}	—	5×10^{-5}	—	—

and F

due to close linkage of *pro*⁻ in donor and recipient. With R1, recombinants for *pyrF*⁺, *try*⁺ and *purB*⁺ occurred at a frequency of 10⁻⁴ to 10⁻⁵, about 100 times higher than for other markers.

R1 *drd-19* is a mutant of the wild type R1 in which conjugation is no longer repressed (Meynell & Datta, 1967). The frequency of recombinants for chromosomal genes with R1 *drd-19* was increased to the same extent as the frequency of conjugation

Table 3. *Escherichia coli* K12: comparison of recombination between strain J5-3 as donor and strain X195 as recipient mediated by R1 *drd-19*, wild-type R1 and F

Factor	Ratio of frequencies of R transfer	Ratios of frequencies of selected recombinants				
		<i>lac</i>	<i>purB</i>	<i>try</i>	<i>pyrF</i>	<i>tyr</i>
R1 <i>drd-19</i> compared with F	—	1	42	29	46	1
R1 <i>drd-19</i> compared with R1	250	—	343	280	325	—
F compared with R1	—	—	8	9	7	—

Table 4. *Escherichia coli* K12: patterns of segregation of unselected markers in crosses between J5-3 and X195

Pattern of segregation of markers					Recombinants with					
					J5-3 F ⁺		J5-3 (R1 <i>drd-19</i>)		J5-3 (R1)	
<i>lac</i>	<i>purB</i>	<i>try</i>	<i>pyrF</i>	<i>tyr</i>	No.	%	No.	%	No.	%
S	o	o	o	o	39	67.2	48	55.75	—	—
S	1	1	1	o	14	24.0	29	33.7	—	—
S	1	o	o	o	3	5.0	3	3.5	—	—
S	1	1	o	o	2	3.4	1	1.15	—	—
S	o	o	1	o	o	o	2	2.3	—	—
S	o	1	1	o	o	o	2	2.3	—	—
S	1	o	1	o	o	o	1	1.15	—	—
o	S	o	o	o	21	34.4	11	12.6	6	9.5
o	S	1	o	o	2	3.3	3	3.45	2	3.2
o	S	1	1	o	18	29.5	69	79.3	53	84.0
o	S	o	1	o	1	1.6	1	1.15	1	1.6
1	S	1	1	o	9	14.7	2	2.3	1	1.6
1	S	1	o	o	4	6.5	1	1.15	o	o
1	S	o	o	o	4	6.5	o	o	o	o
o	S	o	o	1	2	3.3	o	o	o	o
o	1	S	1	o	30	49.2	29	33.7	22	40.7
o	o	S	1	o	23	37.7	49	57.0	28	51.8
o	1	S	o	o	1	1.6	3	3.5	o	o
1	1	S	1	o	6	9.8	o	o	o	o
o	o	S	o	o	1	1.6	5	5.8	4	7.4
o	1	1	S	o	25	43.0	23	26.1	21	30.0
o	o	1	S	o	14	24.0	50	56.8	28	40.0
o	o	o	S	o	7	12.0	13	14.7	20	28.6
o	1	o	S	o	o	o	1	1.15	o	o
1	1	1	S	o	12	20.6	1	1.15	1	1.4
o	o	o	o	S	52	88.0	24	43.7	5	100.0
o	1	1	1	S	5	8.4	25	45.45	o	o
o	o	1	1	S	2	3.4	3	5.4	o	o
o	1	1	o	S	o	o	2	3.6	o	o
o	1	o	o	S	o	o	1	1.3	o	o

1 = allele inherited from donor; o = allele inherited from recipient (Lederberg, 1951); S = donor allele selected.

measured by transfer of the R factor (Table 3), indicating that the numbers of genetic recombinants obtained with wild-type R 1 was limited principally because conjugation was infrequent. The mutant R 1 *drd-19* behaved exactly like the wild-type R 1 in giving about 100 times more recombinants for the chromosomal region *pyrF...try...purB* than for other genes, and the relative frequencies of three classes of recombinants for genes *pyrF*, *try* and *purB* were the same with R 1 *drd-19* as with R 1 (Table 2). Chromosomal recombinations brought about by the R factor could therefore be compared directly with that due to the sex-factor F, provided that the mutant R 1 *drd-19* was used, for then the frequencies of recombinant production were then no longer complicated by repression of mating in the wild type. In Table 3, R 1, R 1 *drd-19* and F are compared in crosses between strain J5-3 as donor and strain X195 as recipient. R 1 *drd-19* gave nearly as many recombinants as F for genes outside the *try* region, but about 30 times more for *pyrF*, *try* and *purB*. The same relationship between the numbers of recombinants for the markers *pyrF*, *try* and *purB*, and for other markers was observed with all lines of *E. coli* K12 tested as donors (Table 2).

Unselected markers

The patterns of segregation of unselected markers are shown in Tables 4 and 5. Most of the alleles were those of the R⁻ parent indicating that, as with the F factor,

Table 5. Pattern of segregation of unselected markers in crosses between F⁺ and R1⁺ donor strains and strain PA309

Pattern of segregation of markers										Recombinants with						
										58-161 (F)		J 5-3 (F)		J 5-3 (R 1)		
<i>str</i>	<i>mal</i>	<i>xyl</i>	<i>man</i>	<i>met</i>	<i>arg</i>	<i>thr</i> <i>leu</i>	<i>lac</i>	<i>try</i>	<i>his</i>	No.	%	No.	%	No.	%	
C	o	o	o	C	S	o	o	o	o	—	—	—	—	8	57.15	
C	o	o	o	C	S	1	o	o	o	—	—	—	—	3	21.45	
C	o	o	o	C	S	1	1	1	1	—	—	—	—	2	14.3	
C	o	o	o	C	S	o	o	o	1	—	—	—	—	1	7.15	
C	o	o	o	C	o	S	o	o	o	27	96.4	14	100.0	5	50.0	
C	o	o	o	C	o	S	o	1	o	1	3.6	o	o	3	30.0	
C	o	o	o	C	1	S	o	1	o	o	o	o	o	1	10.0	
C	o	o	o	C	1	S	o	o	o	o	o	o	o	1	10.0	
C	o	o	o	C	o	o	o	o	S	o	75	77.4	37	90.0	233	92.0
C	1	o	o	C	o	o	o	o	S	o	o	o	o	o	7	2.7
C	o	o	1	C	o	o	o	o	S	o	o	o	o	o	4	1.58
C	o	o	o	C	o	o	o	o	S	1	4	4.1	4	10.0	o	o
C	o	o	o	C	o	1	o	o	S	1	o	o	o	o	1	0.39
C	o	o	o	C	o	1	o	o	S	o	18	18.55	o	o	8	3.15
C	o	o	o	C	o	o	o	o	S	6	75.0	—	—	4	57.0	
C	o	o	o	C	o	o	o	1	S	2	25.0	—	—	1	14.3	
C	o	o	o	C	1	o	o	1	S	o	—	—	—	1	14.3	
C	o	o	o	C	o	1	o	o	S	o	o	—	—	1	14.3	

1 = allele inherited from donor; o = allele inherited from recipient (Lederberg, 1951); S = donor allele selected; C = donor allele contraselected.

transfer occurred from the R⁺ to the R⁻ cell. In the initial experiments, not shown in Tables 4 and 5, with wild-type R 1, using strain J6-2 *str-r pro⁻ his⁻ try⁻ lac⁻* as recipient, only 6 out of 196 recombinants selected for *try*⁺ also received *lac*⁺, and when *pyrF*,

try or *purB* were selected, the chromosome fragment transferred did not often extend beyond this region. The relative numbers of recombinants for *pyrF*, *try* and *purB* (Table 2) suggest that these three genes were transferred in ordered fashion $0 \dots \textit{pyrF} \dots \textit{try} \dots \textit{purB}$, starting from a fixed origin as in an Hfr strain; further support for a fixed gradient of transfer, in which *pyrF* regularly preceded *purB*, was provided by the observation that amongst recombinants selected for *purB*⁺ (the distal marker), 80–90% were also *pyrF*⁺, whereas only 30% of the recombinants selected for *pyrF*⁺ received *purB*⁺. A strain, MSO, mutated in *pyrD*, situated about the same distance as *pyrF* to the other side of *purB* (Signer *et al.* 1965), was kindly provided by Dr J. Shapiro, and when crossed with strain J5-3 (R1 *drd-19*), *pyrD*⁺ recombinants were produced at intermediate frequency, as expected (Table 2).

Fig. 2

Fig. 3

Fig. 2. Comparison of the relative frequencies of recombinants for *pyrF*, *try*, *purB*, *pyrD* and *gal* with J5-3 (R1 *drd-19*) and HfrB10 as donors. The crosses, J5-3 (R1 *drd-19*) × x195 and HfrB10 × x195, were performed as described in Methods, the mating mixtures being diluted 1/100 after 5 min., and then allowed to stand for 1 hr before blending and plating. Map distances on the abscissa are as proposed by Signer *et al.* (1965), and the percentage of recombinants obtained with J5-3 (R1 *drd-19*) (●) and HfrB10 (×), relative to the frequency of *pyrF* recombinants in each case, are plotted on a logarithmic scale.

Fig. 3. Comparison of the frequencies of inheritance of the donor *try* and *purB* genes as unselected markers in recombinants selected for *pyrF*, and of the frequency of *purB* in recombinants selected for *try*, with J5-3 (R1 *drd-19*) and HfrB10 as donors. The percentage of recombinants with the marker in the cross J5-3 (R1 *drd-19*) × x195 (●) and the cross HfrB10 × x195 (×) are plotted on a logarithmic scale, the map distances on the abscissa being those proposed by Signer *et al.* (1965). Curves A and B selected marker *try*; curves C and D selected marker *pyrF*.

Comparison of strain J5-3 (R1 *drd-19*) with an Hfr strain

The donor behaviour of strain J5-3 (R1 *drd-19*) was compared with that of an Hfr strain by using HfrB10, which transfers in the order $0 \dots \textit{try} \dots \textit{lac} \dots \textit{pro} \dots$ (Broda, 1967) and which is therefore likely to initiate chromosome transfer near the presumptive origin of strains carrying R1 *drd-19*.

Crosses of the donors HfrB10 and J5-3 (R1 *drd-19*) with the recipient x195 are compared in Figs. 2 and 3. In Fig. 2 the relative frequencies of recombinant classes selected for *pyrF*, *try*, *purB*, *pyrD* and *gal* are plotted logarithmically against the corresponding map distances as determined by Signer *et al.* (1965), and it can be seen that although the gradient of transfer was steeper than the simple exponential function

of distance from the origin (de Haan & Gross, 1962), precisely the same gradient was observed with J5-3 (R1 *drd-19*) as with HfrB10. Figure 3 shows the proportion of recombinants selected for the proximal gene, *pyrF*, that also received *try* and *purB*, and the proportions selected for *try* that received *purB*. Once again, essentially the same result is given by the two donor strains.

Times of entry of pyrF and purB genes

The production of *pyrF*⁺ and *purB*⁺ recombinants was measured in a cross between J5-3 (R1 *drd-19*) and x195 with interrupted mating. Figure 4 shows the curves obtained when successive samples were plated for recombinants inheriting *pyrF*⁺ and *purB*⁺. Extrapolating the ascending parts of the curves back to the abscissa to measure the time taken for transfer of a particular segment of chromosome (Wollman & Jacob,

Fig. 4. Times of entry of *pyrF* and *purB* genes. The mating mixture consisted of J5-3 (R1 *drd-19*) 5×10^7 /ml. and x195 5×10^8 /ml. and was diluted 1/100 into fresh broth after 5 min. Samples taken at the times given on the abscissa were blended and then plated for recombinants inheriting *pyrF*⁺ (x) and *purB*⁺ (●), which are plotted as number of recombinants per 10^5 donor cells. The number of *purB*⁺ recombinants, 350 per 10^5 donors, produced by a sample of the mating mixture which had been incubated without dilution for 120 min., showed that at least half of the total possible number of cell pairs had formed within 5 min. The curve for R factor transfer (O) plotted as number of R⁺ recipients per 10^8 donors was obtained by plating samples on defined medium fully supplemented for growth of strain x195 and containing chloramphenicol 20 μ g./ml.

1958; Wollman, Jacob & Hayes, 1956), the times of entry for *pyrF*⁺ and *purB*⁺ recombinants were shown to be separated by 6 min. The rare recombinant colonies appearing in the earliest samples presumably arose from mating pairs which had failed to separate. The maximum number of recombinants for *pyrF* was reached by about 40 min., and for *purB* by about 50 min., and by that time, there were about 1.6 times as many for *pyrF* as for *purB*, which agreed more or less with the relative numbers obtained when mating was uninterrupted for 60 min.

Figure 3 also shows the curve for R transfer. The actual numbers of drug-resistant colonies differed somewhat between experiments, depending largely on the time allowed for the R⁺ recipient cells to express their resistance before plating on medium containing the drug. However, the shapes did not differ significantly.

Behaviour of an Hfr strain also carrying R1 drd-19

The orientation of chromosomal transfer by Hfr donors is determined by the position of the sex-factor F. In strain HfrC, where F is between *pro* and *gal*, *pro* is one of the first genes to be transferred and *try* one of the last. Thus, many recombinants inherit the *pro* gene of the donor, whereas very few acquire *try*. Crosses were made between HfrC, both R⁻ and carrying R1 *drd-19*, and strain J62 *str-r* allowing 5 min. for cell contacts to form and then incubating a 1/100 dilution of the mixture for 60 min. before blending and plating to select *pro*⁺, *his*⁺ and *try*⁺ recombinants. The presence of R1 *drd-19* in HfrC did not appreciably alter the frequency of *pro*⁺ recombinants (Table 2); however, the number of *try*⁺ recombinants increased about 250-fold, and thus the R factor evidently directed the transfer of this particular chromosomal segment in HfrC just as it did in an F⁻ strain. When wild-type R1 was present in HfrC, the results were altogether comparable to those with R1 *drd-19*, although the effect was masked to a large extent by the repression which R1 exerts on conjugation, which greatly lowered the numbers of all classes of recombinants.

DISCUSSION

Although genetic recombinants for chromosomal markers are known to be produced during conjugation brought about by R factors (Sugino & Hirota, 1962), a distinction is drawn between conjugation factors like R factors and *col* I (Ozeki, Howarth & Clowes, 1961) on the one hand, and F on the other, in that recombination is more frequent with F. The number of recombinants, however, necessarily depends on the number of bacteria that conjugate, and both *col* I and R factors also differ from F in producing a repressor which prevents synthesis of the specific pilus, and thus conjugation, in all but a small minority of bacteria (Stocker, Smith & Ozeki, 1963; Monk & Clowes, 1964; Meynell & Datta, 1966a; Meynell & Lawn, 1967). In the case of R1 at least, the lower rate of recombination observed with the wild-type R factor was largely due to less frequent mating, as was shown by using a mutant, R1 *drd-19*, which does not produce repressor (Meynell & Datta, 1967).

The striking feature of recombination with R1 is the relatively high frequency with which the *try* region of the donor chromosome appears among recombinants. This peculiarity of R1 was difficult to analyse with the wild-type R factor, since it gave few recombinants of any kind, but using the de-repressed mutant R1 *drd-19* it became clear that a genetic donor carrying R1 transferred the bacterial chromosome as though

it were an Hfr strain with the F factor integrated between the *try* region and *his*, not far from *pyrF*. This phenomenon is distinct from the high frequency of recombinants for *met*, reported by Sugino & Hirota (1962), when R factors bring about conjugation in the Richter φ_3 strains. The φ_3 strain, though losing ability to conjugate, nevertheless retained the chromosomal origin near *met* characteristic of its parent Hfr strain (Richter, 1961); thus, when conjugation factors are introduced which restore its donor ability, recombinants for *met* are produced at high frequency. The same thing is observed when 'F-refractory' Hfr strains (Lederberg & Lederberg, 1956) are infected with R factors (Hirota, Fujii & Nishimura, 1966). With R 1, on the other hand, the oriented transfer of the *try* region is a characteristic feature of the R factor and is independent of the particular bacterial strain used as donor.

Using as recipients with R 1⁺ donors, strain x195 *pyrF*⁻ *try*⁻ *purB*⁻ which was also *gal*⁻ and the uracil-requiring strain MSO *pyrD*⁻, it was possible to show that the relative numbers of recombinants inheriting the various donor markers between *pyrF* and *gal* coincided with their order on the genetic map as determined by Signer *et al.* (1965). That the order of transfer was *pyrF*...*try*...*purB*...*gal*, starting from a fixed origin, was supported by the patterns of unselected markers. Most recombinants selected for the more distal *purB*, also acquired the proximal *pyrF*; but selected for *pyrF*, fewer recombinants had *purB*. Finally, by using the de-repressed mutant, R 1 *drd-19*, an R 1⁺ donor could be directly compared with a known Hfr strain, and also examined in an interrupted mating experiment for the relative times of entry of *pyrF* and *purB*. These tests showed that an R 1⁺ donor transferred its chromosome in the way associated with the Hfr state. There were, however, two differences. First, recombinants were rather fewer; this may be misleading, as pointed out above, since, in comparing recombination rates with different conjugation factors, one may in reality be comparing the frequencies at which conjugation takes place, rather than chromosome transfer itself. Second, and more important, the recombinants had nearly always received the R factor also. These are precisely the two features which distinguish recombination brought about by F' factors such as F 2 (Adelberg & Burns, 1960) which alternate rapidly between the autonomous state and integration at a particular chromosomal site, from recombination with a typical Hfr donor where the only F factor present is integrated and seldom transferred. The relative frequencies of sex-factor transfer and production of the most frequent class of recombinant with R 1 are altogether comparable to those reported for F 2 by Adelberg & Burns (1960). R 1 thus appears as another genetic element with a specific affinity for a particular chromosomal site, which it probably acquired in the same way as an F' factor; that is, by recombination with the chromosome of a previous host. The ultimate source of the resistance determinants carried by R factors remains unknown, but the region of R 1 homologous with the chromosome did not lie in its resistance genes: one segregant without kanamycin-resistance, as well as another which lost all except resistance to kanamycin, continued to give large numbers of recombinants for the *try* region as before. If the homologous region of R 1 is indeed associated with the conjugation factor proper, it is worth noting that its site of integration is one of those where F integrates (to give the Hfr strain HfrB 10 described by Broda, 1967).

Integrated and autonomous F' factors can co-exist in the same cell because of the instability of chromosomal attachment, which does not last long enough to cause the autonomous F to disappear (Adelberg & Burns, 1960). Presumably the same explana-

tion applies to R1, which must therefore alternate frequently between the integrated and the autonomous state. Transient integration, if it lasted for a few generations, might lead to detectable fluctuation in the numbers of recombinants produced by different donor cultures, provided that young enough cultures were examined. In fact, a significant degree of variation appeared in an experiment where replicate 1 ml. broth cultures of J5-3 (R1 *drd-19*) grown from an inoculum of 200 bacteria/ml. to a concentration of 2×10^5 /ml. were tested for the production of *pyrF*⁺ recombinants after mixing with strain X195 at 5×10^8 /ml. for 1 hr. When 19 individual 0.02 ml. samples from each of two cultures were plated, the average numbers of recombinants were 15.3 and 16.5 with variances of 14.1 and 19.5 equal to 0.92 and 1.2 times the mean respectively. However, with single samples from each of 130 further cultures, the average number of recombinants was 12.47 and the variance 40.7; that is, considerably greater than the mean, as expected for a non-random distribution.

The frequency with which different Hfr types arise, as well as their stability, depends directly on the length of the homologous region (Broda, 1967) and, on this argument, the region in R1 homologous with the chromosome of *Escherichia coli* K12 should be of considerable length. The members of that class of R factors comprising R1 so resemble each other and the F factor in the conjugation pilus and the genetic control of its synthesis that all are likely to have evolved from a common ancestor (Meynell & Datta, 1966*a*). Nevertheless, the conjugation factor of R1 is not F itself, for, apart from several perhaps only insignificant differences such as in susceptibility to acridines and in restriction of different phages (see Watanabe, 1966) they manifest no super-infection immunity towards each other. In contrast to the mutual exclusion between two F factors (Scaife & Gross, 1962), or between F and *col* V (Macfarren, 1966), bacteria carrying either F or R1 readily accept the other, and both factors are able to co-exist stably in the same cell.

The characteristic behaviour of R1 in an F⁻ strain could also be observed with Hfr bacteria, although here again it was necessary to use the de-repressed mutant to avoid repression of conjugation mediated by F as well as by R. When R1 *drd-19* was introduced into HfrC, two kinds of recombinant were produced at high frequency, the usual *pro* recombinants resulting from the oriented transfer of the Hfr chromosome and, superimposed on these, the *try* recombinants due to a second point of origin determined by the R factor. Strain HfrC (R1 *drd-19*) thus resembled the 'double male' strain of Clark (1963), which carries two F factors integrated at different chromosomal sites. Watanabe & Ogata (1966) also refer to the similar behaviour of an Hfr strain carrying, as well as the integrated F factor, a particular hybrid of an R factor with F.

It has been questioned whether the term 'episome' (Jacob & Wollman, 1958) should be applied to conjugation factors like *col* factors and R factors which have never been shown with certainty to integrate with the bacterial chromosome in the same way as the F factor (Clowes & Moody, 1966). However, from the way in which R1 brings about the transfer of the chromosome from the donor cell, it now seems that this is one R factor which may be described as an episome within the original definition.

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