HOST SPECIFICITY OF DNA PRODUCED BY ESCHERICHIA COLI. VI. EFFECTS ON BACTERIAL CONJUGATION

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BACTERIAL host cells endow the DNA of bacteriophage λ and that of the transducing phage λ*dg* with host specificity (ARBER and DUSSOIX 1962; ARBER 1964). This label on the DNA plays an important role in phage infection. In the absence of the required host specificity, the bacteria degrade the DNA of the infecting phage, which is then said to be restricted (DUSSOIX and ARBER 1962).

Preliminary evidence has been given by ARBER and DUSSOIX (1961) and ARBER (1962) that bacterial DNA is also subject to this same control mechanism. The enzymatic system endowing λ DNA with host specificity would thus act on the host DNA as well, and the host specificity could play a role in the decision of whether DNA transferred in conjugation from male to female bacteria is accepted or rejected. This last action can be checked by measuring the frequency of formation of recombinants, provided other factors antagonistic to genetic integration, such as nonhomology of the male and female DNA molecules, are also considered. The present paper gives an account of experiments carried out to investigate restriction in bacterial conjugation involving Hfr, F^+, F-*gal*$^+$, F-*lac*$^+$ and $(RTF)^+$ donor strains. Since our first experiments the results have been confirmed and extended by various other investigators (BOICE and LURIA 1963; HOEKSTRA and DE HAAN 1963; GLOVER, SCHELL, SYMONDS and STACEY 1963; PITTARD 1964; BOYER 1964).

MATERIALS AND METHODS

Table 1 gives the bacterial strains used. Two strains carrying resistance transfer factors were kindly supplied by DR. T. WATANABE. One of them was of type 1, or *fi*$^+$ (inhibition of expression of fertility factor F), and we call this particular factor RTF-1. The other was of type 2, or *fi*$^-$, and we call it RTF-2 (WATANABE 1963; WATANABE, NISHIDA, OGATA, ARAI and SATO 1964).

The phages were the same as described by ARBER and DUSSOIX (1962).

Media: (a) Tryptone broth: 1 percent Difco Bacto tryptone, 0.5 percent NaCl, pH 7.0; for solid medium completed with 1.5 percent agar. (b) Top agar: 1.0 percent Difco Bacto tryptone, 0.5 percent NaCl, 0.7 percent Difco Bacto agar, pH 7.0. (c) LB medium: 1.0 percent Difco Bacto tryptone, 0.5 percent Difco yeast extract, 1.0 percent NaCl, 0.1 percent glucose, pH 7.0. (d) Davis minimal agar: 0.7 percent K_2HPO_4, 0.2 percent KH_2PO_4, 0.05 percent Na_3 citrate · 5 H_2O, 0.1 percent $(NH_4)_2SO_4$, 0.02 percent $MgSO_4$, 0.1 percent asparagine, 0.1 percent glucose or other sugar, 1.4 percent Difco Bacto agar. (e) EMB lactose or galactose agar: 1.0 percent Difco Bacto

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TABLE 1

Strains of Escherichia coli *employed*

Strain numbers	Characteristics and origin
(a) Derivatives of *E. coli* K-12	
106	Hfr H: order of genes transferred: O *thr*+*leu*+*lac*+*gal*+(λ)+ *str*^{s} (HAYES 1953)
101	C600: F$^-$*thi*$^-$ *thr*$^-$*leu*$^-$*lac*$^-$(λ)$^-$ (APPLEYARD 1954)
102	C600 *str*^{r}
342	C600(P1) *str*^{r}, giving strong restriction of λ·K
348	C600(P1) *str*^{r}, giving weak restriction of λ·K (DUSSIOX and ARBER 1962)
374	Hfr C: (λ)+*met*$^-$: order of genes transferred: O *lac*+*leu*+*thr*+ *met*$^-$*str*^{s} (CAVALLI, LEDERBERG, and LEDERBERG 1953), obtained from DR. L. CAVALLI-SFORZA
372	Hfr C(P1)
512	CR34: *thy*$^-$*thr*$^-$*leu*$^-$*lac*$^-$λ^s(λ)$^-$, obtained from DR. J. WEIGLE
639	Hfr derived from CR34 by DR. D. PRATT: *thy*$^-$. Order of genes transferred: O *thr*+*leu*$^-$*lac*+*gal*+(λ)$^-$*his*+*thy*$^-$
432	PA209 *str*^{r}: *thr*$^-$*leu*$^-$*thi*$^-$*his*$^-$*try*$^-$*lac*$^-$*gal*$^-$*mal*$^-$*xyl*$^-$*mtl*$^-$*ara*$^-$λ^r*str*^{r} (λ)$^-$, obtained from DR. F. JACOB
603	432(P1)
110	W3110 prototroph
147	W3110 F+
115	W3350: *gal*$_1^-$*gal*$_2^-$
377	W3350 F+
338	C600(P1)
411	W3101(F$_2$*gal*+), obtained from DR. E. WOLLMAN
596	W4520(F$_8$*gal*+)*met*$^-$(λ)+λ^r, obtained from DR. J. GROSS
598	596(P1)
602	W3350(P1)
430	200PS(F-*lac*+), obtained from DR. F. JACOB
634	C600(F-*lac*+), obtained from a cross of 430 and 101
561	C600 *str*^{r} R10 of DR. R. THOMAS, obtained from him
573	561(P1) giving strong restriction for λ·K
495	CSH-2(RTF–1): *sul*^{r}*str*^{r}*cm*^{r}*tc*^{r}, obtained from DR. T. WATANABE
505	C600(RTF–1), obtained from a cross of 495 with 101
520	C600(P1) (RTF–1)
496	58–161 F+ (RTF–2): *sul*^{r}*str*^{r}, obtained from DR. T. WATANABE
501	C600(RTF–2), obtained from a cross of 496 with 101
540	C600(P1) (RTF–2)
(b) Derivatives of *E. coli* B	
251	Bc *mal*±λ^s(λ)$^-$ prototroph (ARBER and LATASTE-DOROLLE 1961)
258	251 *gal*$^-$
(c) Derivatives of *E. coli* C	
518	C (BERTANI and WEIGLE 1953)

Symbols: +, ability to synthesize or utilize; presence of prophage. −, inability to synthesize or utilize, absence of prophage. *r*, resistance. *s*, sensitivity. *F*, fertility factor (numbers signify different isolates). RTF, resistance transfer factor. λ, lambda phage. P1, phage P1.

Auxotrophic markers: threonine (*thr*); leucine (*leu*); thiamine (*thi*); histidine (*his*); tryptophan (*try*); methionine (*met*); thymine (*thy*). *Carbohydrate utilizations:* Galactose (*gal*); lactose (*lac*); maltose (*mal*); xylose (*xyl*); mannitol (*mtl*); arabinose (*ara*). *Drug-resistance markers:* streptomycin (*str*); sulfonamide (*sul*); chloramphenicol (*cm*); tetracycline (*tc*).

tryptone, 0.1 percent Difco yeast extract, 0.5 percent NaCl, 1.5 percent Difco Bacto agar, 2.0 percent lactose or 1.0 percent galactose, and 0.25 percent of a mixture prepared with 20 g K_2HPO_4, 4 g Eosin Y and 0.65 g methylene blue.

For the crosses, bacteria were grown in slightly aerated LB medium or in tryptone broth to a concentration of 2×10^8 to 10^9 cells per ml. The donors and the recipients were then mixed, sometimes after resuspension in fresh LB medium, in the chosen proportions. Further procedure is indicated in each experiment.

The bacterial strains involved in all crosses were tested for their host specificity type with either phage λ or phage 82. These tests consisted in determination of the efficiency of plating (e.o.p.) of standard phage stocks grown on K-12, K-12(P1), B and C and plated on the strain involved on the one hand, and of the e.o.p. of phage grown on the strain involved and plated on standard indicators of K-12, K-12(P1), B and C on the other hand.

The presence of the F factor was scored according to the method applied before (ARBER 1960), which is based on the capacity of F+ strains to give rise to bacterial recombinants.

RESULTS

Hfr crosses: A λ-lysogenic Hfr strain of *E. coli* K-12 was crossed with λ-sensitive F⁻K-12 or F⁻K-12(P1), respectively, and the frequencies of zygotic induction (production of phage λ in the F⁻ cells) and of formation of bacterial recombinants were measured (Table 2). In the cross of Hfr K-12 × F⁻ K-12 one Hfr in 15 gave rise to zygotic induction, but when the same Hfr strain was crossed with F⁻K-12 (P1) only one infective center of λ phage was found per 2600 Hfr cells. A similarly large reduction was observed in the cross with P1-lysogenic F⁻ when selection was made for either *thr*⁺*leu*⁺*str*^{r} or *lac*⁺*str*^{r} recombinants. These findings suggest that the prophage P1 in the female exerts control over acceptability of the DNA transferred to the cell. The DNA of prophage λ and the DNA of the bacterial chromosomes as well would thus be required to possess the proper specificity in order to be accepted upon entry into another cell, exactly as it is known for infection with DNA of phage λ.

The *str*^{r} markers used in the crosses with restricting hosts were of the type that do not influence the frequency of acceptance of restricted λ phage (DUSSOIX and ARBER 1962). If Hfr K-12 was crossed to the other type of *str*^{r} mutants, e.g. strain 348, an F⁻ K-12(P1) *str*^{r}, about the same recombination frequencies were observed as with the nonrestricting F⁻ K-12 females.

TABLE 2

Restriction in the cross of Hfr K-12 with F⁻K-12(P1)

Cross	Zygotic induction Infective centers per ml	Bacterial recombinants per ml	
		thr⁺ *leu*⁺ *str*^{r}	*lac*⁺ *str*^{r}
Hfr K-12 × F⁻K-12	10^6	6.2×10^5	3.4×10^5
Hfr K-12 × F⁻K-12(P1)	5.7×10^3	3×10^3	5×10^2

1.5×10^7 Hfr cells (washed) were mixed with 2.5×10^8 F⁻ bacteria in 1 ml of LB medium and incubated at 37°C without aeration. After 85 min, infective centers of λ were measured by plating appropriate dilutions with additional F⁻ cells as indicator. After 2 hr total incubation other aliquots were diluted 50-fold into fresh LB medium containing streptomycin (150 μg/ml) and further incubated for 2 hr. Then the *thr*⁺*leu*⁺*str*^{r} recombinants were assayed by spreading on Davis minimal agar supplemented with thiamine (1 μg/ml) and streptomycin (150 μg/ml), and the *lac*⁺*str*^{r} recombinants on EMB lactose agar with thiamine and streptomycin.

The Hfr K-12 is strain 106, an Hfr H derivative with order of chromosomal transfer: O *thr*⁺*leu*⁺*lac*⁺(λ)⁺*str*^{s}. Both F⁻ strains are derivatives of C600, and are *thr*⁻*leu*⁻*lac*⁻(λ)⁻*str*^{r}*thi*⁻. F⁻K-12 is strain 102, F⁻K-12(P1) is strain 342.

TABLE 3

Restriction in the cross of Hfr K-12 with F⁻K-12(P1). Effect on number of recombinants

Cross	Bacterial recombinants per ml	
	lac⁺str^r	*leu⁺thr⁺met⁺str^r*
Hfr K-12 × F⁻K-12	7.5×10^5	3.1×10^5
Hfr K-12 × F⁻K-12(P1)	5×10^3	5×10^3
Hfr K-12(P1) × F⁻K-12	1.1×10^6	3.3×10^5
Hfr K-12(P1) × F⁻K-12(P1)	5.3×10^5	10^5

7.5×10^8 cells were mixed with 1.5×10^8 F⁻ bacteria in 1 ml of LB medium and incubated at 37°C without aeration. After 2 hr, a 50-fold dilution was made into LB containing streptomycin (150 μg/ml) and incubated for another 90 minutes. Then the recombinants were scored as in Table 1.

The Hfr K-12 is strain 374, and Hfr C with order of chromosomal transfer: O *lac⁺leu⁺thr⁺met⁻str^s*; its P1-lysogenic derivative is strain 372. The F⁻ strains are derivatives of C600 and are *lac⁻leu⁻thr⁻met⁺str^r*. F⁻ K-12 is strain 102, F⁻K-12(P1) is strain 342.

The same results were obtained in crosess involving other Hfr and F⁻ strains and other genetic markers. Two examples are given in Table 3 and Figure 1. It should be pointed out that the probability of DNA acceptance is usually higher in crosses with restricting females than that of acceptance of infecting restricted λ phage.

Example: For strain K-12(P1), the probability of DNA acceptance, calculated as the number of *lac⁺ str^r* recombinants with markers from Hfr K-12 divided by the number of recombinants with markers from Hfr K-12(P1), is 10^{-2} (Table 3); while the ratio of plaques obtained by infection with λ·K and λ·K(P1), respectively, is 2×10^{-5}.

Crosses involving P1-lysogenic Hfr strains yield about the same number of recombinants as a comparable Hfr K-12 × F⁻K-12 cross, independently of whether the females are P1-lysogenic or not (Table 3). This suggests that DNA donated by K-12(P1) is nonrestricted in both K-12 and K-12(P1) cells, in agreement with the results obtained with phage λ.

In the experiment presented in Figure 1 similar degrees of restriction were obtained for markers being transferred early and late, and for various times of contact. But this type of experiment has not been repeated with other strains and markers, and does not, therefore, permit any conclusions about whether the acceptance is a function of the marker involved, of its location with respect to the origin of chromosome transfer, or of the time of its transfer.

F⁺ crosses: F⁺ cells of a *gal⁺* strain were mixed with F⁻*gal⁻* bacteria at a proportion of about 10 to 1 and left in contact for 2 hours. Then *gal⁻* colonies grown from the exconjugant female population were tested for whether they had acquired the fertility factor. It is seen in Table 4 that in the cross F⁺K-12 × F⁻K-12 most cells of the female parent had become carriers of the F factor. But not so in the cross F⁺K-12 × F⁻K-12(P1), since none out of 16 exconjugant colonies tested was F⁺. This indicates that the transfer of the fertility factor from K-12 F⁺ to the P1-lysogenic F⁻ strain is greatly reduced. On the other hand, F⁺ K-12(P1) donates the factor F with high probability to both F⁻K-12 and F⁻K-12(P1).

In the F⁺K-12 × F⁻K-12(P1) cross it was observed that the recovery of exconjugant K-12(P1) *gal⁻* genotypes was very poor. We investigated this effect further

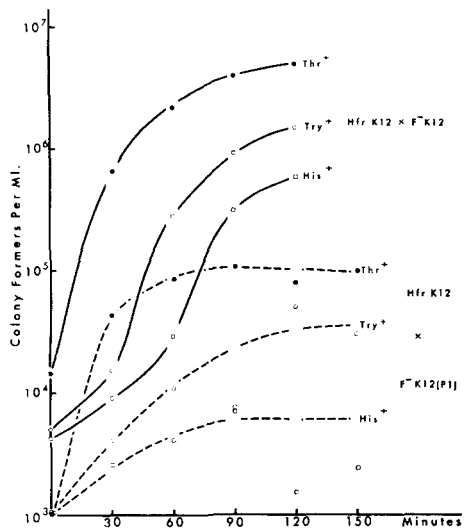

FIGURE 1.—Cross of Hfr K-12 with F⁻K-12 and F⁻K-12(P1). 4×10^7 Hfr cells were mixed with 6×10^7 F⁻ bacteria in 1 ml of LB medium, and 0.1 ml aliquots distributed into test tubes and incubated at 37°C. At various times *t*, the mating was interruped by adding 5 ml of phosphate buffer to one of the tubes and by stirring violently. Samples were then plated on Davis minimal agar with supplements appropriate for the selection of the three classes of recombinants desired. The Hfr K-12 is strain 639, with order of chromosome transfer: O *thr*+*leu*⁻*try*+ *his*+*thy*⁻. The F⁻ strains are 432 *thr*⁻*leu*⁻*try*⁻ *his*⁻*thy*+ and its P1-lysogenic derivative 603.

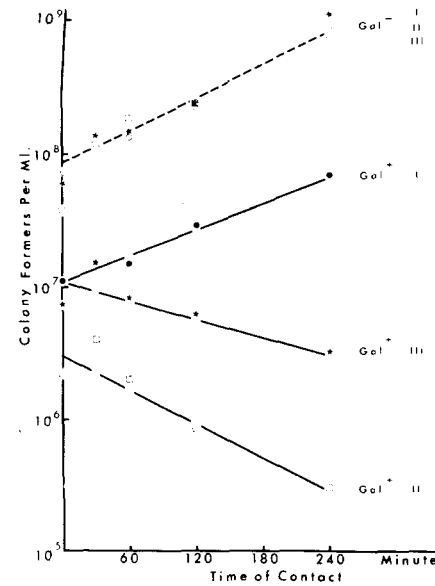

FIGURE 2.—Killing of restricting females in F+ crosses. 10^8 F+ cells were mixed with about 10^7 F⁻ bacteria in 1 ml of LB medium and incubated at 37°C without aeration. At various times aliquots were spread on EMB gal agar for viable colony count assay of both genotypes. After the first 2 hours of incubation the mixtures were diluted 4-fold into fresh LB medium. F+K-12 is the *gal*⁻ strain 377. The three F⁻ recipient strains are all *gal*+: I, K-12, strain 101; II, K-12(P1), strain 338; III, B, strain 251.

in the experiment shown in Figure 2. Viable colony formers of both parental genotypes were assayed at various times after the mixture of the F+ with the F⁻ cells. In cross I (F+K-12 *gal*⁻ × F⁻K-12 *gal*+), titers of both *gal*⁻ and *gal*+ increased in parallel as a function of incubation time. In the crosses II and III with restricting females (F+K-12 *gal*⁻ × F⁻K-12(P1) *gal*+, and F+K-12 *gal*⁻ × F⁻ B *gal*+, respectively), the number of *gal*⁻ colonies increased exponentially, but the viable count of *gal*+ cells decreased as a function of the incubation time. This suggests that in these crosses the transfer of the fertility factor to a restricting F⁻ is lethal for the females. It should be pointed out that this effect does not seem to be caused by the P1 prophage only, since it occurs also with restricting B female. No killing was observed in F+K-12(P1) × F⁻K-12, or F+K-12(P1) × F⁻K-12(P1) crosses.

Upon prolonged incubation of an F+K-12 × F⁻K-12(P1) mixture with periodic dilutions into fresh medium in order to keep the cell titer about constant and allow

TABLE 4

Test of the fertility type of exconjugant colonies in F+ crosses

Cross	Exconjugant *gal*⁻ colonies		
	total tested	F+	F⁻
F+K-12 gal+ × F⁻K-12 gal⁻	12	11	1
F+K-12 gal+ × F⁻K-12(P1) gal⁻	16	0	16
F+K-12(P1) gal+ × F⁻K-12 gal⁻	12	12	0
F+K-12(P1) gal+ × F⁻K-12(P1) gal⁻	12	10	2

10^8 F+ cells were mixed with 10^7 F⁻ bacteria in 1 ml of LB medium and incubated for 2 hr at 37°C without aeration. Then the appropriate dilutions were spread on EMB gal agar. *gal*⁻ exconjugant colonies were tested for their fertility type. The *gal*+ strain was 147 and the F⁻ strain was 115.

TABLE 5

Cross of F-gal+ donors with restricting and nonrestricting F⁻ recipients

Cross	Strains and markers involved		Bacterial recombinants		
	F⁻*gal*+	F⁻	Time of contact (minutes)	Selected markers	Titer per ml
(a) F-*gal*+K-12 × F⁻K-12	411 *gal*⁻/*gal*+*str*^{s}	432 *gal*⁻*str*^{r}	90	*gal*+*str*^{r}	3.7×10^7
(b) F-*gal*+K-12 × F⁻K-12(P1)	411	603	90		1.2×10^5
(c) F-*gal*+K-12 × F⁻K-12	596 *gal*⁻/*gal*+*met*⁻	115 *gal*⁻*met*+	60	*gal*+*met*+	1.1×10^8
(d) F-*gal*+K-12 × F⁻K-12(P1)	596	602	60		1.2×10^6
(e) F-*gal*+K-12(P1) × F⁻K-12	598	115	60		1.2×10^8
(f) F⁻*gal*+K-12(P1) × F⁻K-12(P1)	598	602	60		8.8×10^7
(g) F⁻*gal*+K-12 × F⁻B	596	258	60		3.7×10^6

The donors were mixed in LB medium with the recipients at a proportion of 3 to 1 to yield a total cell density of about 2×10^8 per ml. The mixtures were incubated at 37°C with slight aeration for the indicated time of contact. Then the recombinants were scored for the crosses (a) and (b) by spreading on EMB gal agar supplemented with streptomycin (150 μg/ml); and for the crosses (c) through (g) by plating on Davis minimal agar.

growth, the F^- genotypes finally resume growth. In one experiment the 1 percent survivors started to grow in *parallel* to the F^+ parent. All survivors were still P1-lysogenic and they still restricted λ·K, but most of them were now shown to carry the F factor. Hence the fertility factor seems to be accepted in the restricting strain with a low probability, similar to that allowing formation of recombinants in the Hfr crosses.

F-gal$^+$ *crosses:* The transfer of F-*gal*$^+$ from K-12 donors into K-12, K-12(P1) or B recipients was investigated with two different F-*gal*$^+$ factors (Table 5). After 60 to 90 minutes incubation of the mixture of three F-*gal*$^+$ K-12 cells per one F^-K-12, most of the recipient-type cells had become carriers of F-*gal*$^+$; but in crosses of F-*gal*$^+$ K-12 with F^-K-12(P1) or F^-B, only about 1 percent of the recipient cells had accepted the F-*gal*$^+$ factor, indicating a restriction against acceptance of the episome. No restriction was noted in the control cross F-*gal*$^+$ K-12 (P1) × F^-K-12(P1). A more detailed study with one of the donors is shown in Figure 3. It is seen that restriction persists upon prolonged incubation and that here again, as found in F^+ crosses, the conjugation seems to be lethal to the restricting female.

F-lac$^+$ *crosses:* F-*lac*$^+$K-12 was crossed with F^-K-12 and F^-K-12(P1), respectively. Transfer of the F-*lac*$^+$ factor occurred about ten times less frequent to the K-12(P1) recipient than to the nonrestricting F^-K-12 (Figure 4). Again, there was a slight decrease in the number of viable cells of the recipient genotype. When the donor was rendered P1-lysogenic no restriction was observed in the acceptance of the F-*lac*$^+$ factor by the F^-K-12 or F^-K-12(P1).

RTF crosses: The transfer of resistance transfer factors (RTF) was explored between K-12 or K-12(P1) donors and K-12, K-12(P1), B and C recipients (Table 6) and the following results were obtained: the laws of restriction established in phage infection and bacterial conjugation hold true also for the transfer of RTF-1 and RTF-2, i.e., lower acceptance rates are measured in a cross between K-12(RTF) donor with K-12(P1) or B recipients than in a cross of the same donor with K-12 or C recipients. Evidence for killing of the recipient cells was obtained only in the cross K-12(RTF-2) × K-12(P1). It was also noted that the transfer of RTF-1 occurred as a rule about ten times more frequently than that of RTF-2, independently of whether there was restriction or not because of difference in host specificity.

Host specificity produced and controlled by RTF-2: Strains carrying RTF-2 were shown to restrict phage lambda grown on strains that do not carry RTF-2 (Table 7). It may be noted that the restriction in B is particularly strong: only 4×10^{-6} λ·B formed plaques on B(RTF-2), while the efficiency of plating of λ·K on K-12(RTF-2), of λ·K(P1) on K-12(P1)(RTF-2), or of λ·C on C(RTF-2) was about 10^{-2}. The restriction procured by RTF-2 is additive to the other restrictions. We like to recall here the finding of Dussoix (1964), that the DNA of restricted λ·K is degraded upon infection of K-12(RTF-2) (60 percent acid-soluble breakdown products after 15 min adsorption followed by 15 min incubation in growth medium).

Phage λ undergoes host-controlled modification upon growth on strains carry-

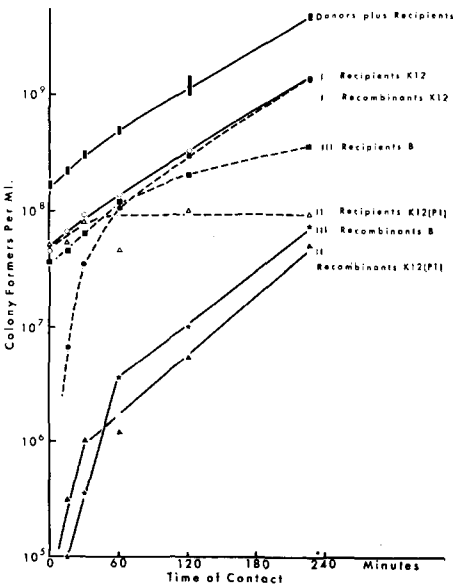

FIGURE 3.—Cross of F⁻ *gal*+ K-12 donor with restricting and nonrestricting F⁻ recipients. The donors were mixed in LB medium with the recipients at a proportion of 3 to 1 to yield a total cell density of about 2×10^8 per ml. The mixtures were incubated at 37°C with slight aeration and, in order to prevent exhaustion of the medium, diluted 3-fold into fresh LB medium after 30 min and again after 90 min of contact. At various times aliquots were plated on complete medium (= donors plus recipients, graph gives variation in experiments I through III), on Davis minimal agar with glucose (= *met*+ genotypes of recipient) and on Davis minimal agar with galactose (= *gal*+ *met*+ recombinants). F⁻*gal*+K-12 is strain 596 F⁻ strains: Curve I, K-12 (strain 115); curve II, K-12(P1) (Strain 602); curve III, B (strain 258).

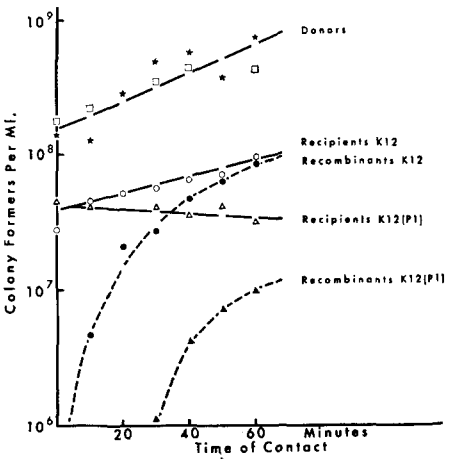

FIGURE 4.—Cross of F⁻*lac*+ donor with restricting and nonrestricting F⁻ recipients. The *str*^{s} donors were mixed in LB medium with the *lac*⁻*str*^{r} recipients at a proportion of about 4 to 1 to yield a total cell density of about 2×10^8 per ml. The mixtures were incubated at 37°C with slight aeration. At various times samples were plated on EMB lactose agar and on EMB lactose agar supplemented with streptomycin (150 μg/ml) and the titers of the two parental genotypes and of the *lac*+*str*^{r} recombinants determined. F⁻*lac*+K-12 is strain 634. The F⁻ strains: K-12, strain 561; K-12(P1), strain 573.

ing RTF-2: as seen in Table 7, phage λ·K-12(RTF-2) is not restricted anymore on K-12(RTF-2) or C(RTF-2).

No host-controlled modification was noted in strains carrying RTF-1, nor do such strains display any restriction specific to RTF-1.

In the course of our experiments, a mutant arose from the RTF-2 factor, such that strains carrying the mutant factor did not restrict λ·K any more; but phage grown in the presence of the mutant transfer factor was still provided with the host specificity typical for RTF-2. The other genetic markers in this mutant were unchanged: it was still *fi*⁻ (no inhibition of the expression of the fertility factor F) and had the resistance factors *str*^{r} and *sul*^{r}. This indicates that the genes in RTF-2 for control of host specificity are independent of the characters mentioned.

TABLE 6

Transfer of RTF by conjugation with nonrestricting and restricting recipients

Cross	Strains and markers involved		Exconjugant cells per tube (4 ml)			
	Donor *lac⁻thr⁻leu⁻* $(RTF)^+$	Recipient *lac⁺thr⁺leu⁺* $(RTF)^-$	Donor *lac⁻* (a)	Recipient *lac⁺* (b)	Recombinant *lac⁺* $(RTF)^+$ (c)	Frequency (c)/(b)
K-12(RTF-1) × K-12	505	115	7.4×10^8	1.9×10^8	2.7×10^7	1.4×10^{-1}
K-12(RTF-1) × K-12(P1)	505	602	1.1×10^9	3.2×10^8	10^6	3.1×10^{-3}
K-12(RTF-1) × B	505	251	5.1×10^8	1.6×10^8	3×10^6	1.9×10^{-2}
K-12(RTF-1) × C	505	518	9×10^8	10^8	6.4×10^6	6.4×10^{-2}
K-12(P1)(RTF-1) × K-12	520	115	6.7×10^8	3×10^8	2×10^7	6.7×10^{-2}
K-12(P1)(RTF-1) × K-12(P1)	520	602	7×10^8	1.4×10^8	1.8×10^7	1.3×10^{-1}
K-12(RTF-2) × K-12	507	115	6.4×10^8	2.2×10^8	2.6×10^6	1.2×10^{-2}
K-12(RTF-2) × K-12(P1)	507	602	7.2×10^8	2×10^7	5.6×10^4	2.8×10^{-3}
K-12(RTF-2) × B	507	251	6×10^8	2.3×10^8	9×10^5	3.9×10^{-3}
K-12(RTF-2) × C	507	518	6.9×10^8	1.5×10^8	1.8×10^6	1.2×10^{-2}
K-12(P1)(RTF-2) × K-12	540	115	5.3×10^8	3×10^8	1.2×10^6	4×10^{-3}
K-12(P1)(RTF-2) × K-12(P1)	540	602	6.4×10^8	1.8×10^8	1.1×10^6	6×10^{-3}

The donors were mixed in 1 ml of LB medium with the recipients at a proportion of about 3 to 1 to yield a total cell density of 4×10^8 per ml. The mixtures were incubated at 37°C without aeration. Fresh LB medium was supplied twice: 1 ml after 30 min and 2 ml after 60 min incubation. After a total of 2 hr incubation aliquots were plated on EMB lactose and EMB lactose supplemented with streptomycin (10 μg/ml) and, in the experiments with RTF-1, with chloramphenicol (25 μg/ml). Most of the results were confirmed by parallel plating on Davis minimal agar appropriately supplemented.

TABLE 7

Restricted acceptance of infecting DNA by strains carrying RTF-2

Phage type	Efficiency of plating on host strain							
	K-12	K-12(RTF-2)	K-12(P1)	K-12(P1)(RTF-2)	B	B(RTF-2)	C	C(RTF-2)
λ·K	1	2×10^{-2}	2×10^{-5}	10^{-6}	10^{-4}	6×10^{-6}	1	10^{-2}
λ·K(P1)	1	10^{-2}	1	10^{-2}	10^{-4}	3×10^{-6}	1	5×10^{-3}
λ·K(RTF-2)	1	1	3×10^{-5}	6×10^{-5}	10^{-4}	2×10^{-4}	1	1
λ·B	10^{-3}	2×10^{-6}	6×10^{-8}	10^{-8}	1	4×10^{-6}	1	3×10^{-4}
λ·C	10^{-3}	4×10^{-5}	10^{-6}	4×10^{-8}	10^{-4}	10^{-5}	1	3×10^{-2}

The host bacteria were grown in aerated tryptone broth to about 5×10^8 cells per ml and starved by 1 hr aeration in 0.01M $MgSO_4$. Appropriately diluted phage was preadsorbed to the host bacteria for 15 min at 37°C before plating on tryptone agar.

DISCUSSION

The control exerted by bacteria over acceptance of foreign DNA was first studied for phage infection (DUSSOIX and ARBER 1962) and for infection with purified phage DNA (DUSSOIX and ARBER 1965). The rules found in those systems are now found to hold true more generally, applying also to the transfer of DNA, chromosomal and episomal, from a donor cell to a recipient bacterium. The consequence of this control is manifest in the low probability of formation of genetic recombinants. It would be interesting to know whether restricted bacterial DNA is degraded upon its penetration into the recipient cell, similarly to the observed breakdown of DNA from infecting phage. Unfortunately this question cannot be answered yet by direct experimental approach. DNA breakdown to some extent has been suggested by PITTARD's observation (1964) of unlinking of genetic markers under restricting conditions. The efficiency of restriction in conjugation, as judged from the number of recombinants formed or from the number of cells having acquired an episome, does not appear to be as high as the restriction measured in phage infection by the probability of plaque formation. We do not attach great importance to these quantitative differences, the reasons for which may be complex and may involve, for example, physiological conditions, the mechanism and speed of transfer of the donated DNA, the particular distribution of host specificity over the DNA molecules, the size of the donated DNA molecule and the size of the scored genes, the location in the cell of the restricting and modifying enzymes. A more elaborate study involving a great number of genetic markers and their transfer with several independent Hfr donors would perhaps shed more light on some of these questions.

Restriction of DNA with inadequate host specificity may be one of the reasons why conjugation is frequently unsuccessful between bacterial strains which are not closely related. It is obvious that other factors such as inefficient copulation and nonhomology of donor and recipient DNA molecules may also cause important hindrance in exchange of genetic material. We were fortunate to have at hand a system—that of the strains K-12 and K-12(P1)—in which these factors do not exist. It is very likely that the formation of genetic recombinants in an Hfr K-12 × F⁻K-12(P1) cross is only inhibited by the control exerted by the female

on the host specificity of the transferred DNA. If K-12 is mated with B females, nonhomology may already play a role as shown for the region of galactose markers in the transduction experiments with λ*dg* (ARBER 1964).

Donation of episomes such as F, F-*gal*, F-*lac* or RTF which can express their functions without integration into the bacterial chromosome, should not be affected as much by nonhomology, and it is possible that here the control of the host specificity determines whether a cell acquires an episome from another strain. We do not understand yet the reasons why the infection with a restricted episome should be lethal to the recipient cell, as our observations suggest. For practical reasons we were obliged to do these measurements in mixtures with donor to recipient ratios higher than one. We do not know if the killing is a function of this ratio; lethality was never observed, however, in control crosses with non-restricting females. It should be remembered that λ·K phage does not kill K-12 (P1) bacteria upon infection (ARBER and DUSSOIX 1962). No killing was observed in conjugation with Hfr donors. Perhaps this might suggest that the lethal effect is obtained only with those cell pairs that persist after the end of donation of the transferred DNA molecule.

Since production and control of host specificity of the DNA are bacterial functions, the genetic information for these functions is also transferred in the course of conjugation from one cell to another, as has been observed by GLOVER and COULSON (personal communication), and PRELL (personal communication). Partial diploids which result by such a transfer may display changed phenotypic properties with respect to modification and restriction of DNA, and they may also give rise to hybrid recombinants with the host specificity type of the donor or perhaps of a new arrangement.

Genetic information for host specificity may also be carried by episomes, as shown by the example of phage P1 and now by that of a resistance transfer factor. It is interesting to note that various independently isolated *fi*$^-$ RTF have been shown by WATANABE, *et al.* (1964) to provide an additional host specificity, while none of several *fi*$^+$ transfer factors did. But host specificity control by the *fi* gene is made unlikely by WATANABE's observations as well as our own.

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SUMMARY

Zygotic induction of bacteriophage λ and the formation of recombinants for several genetic markers tested are "restricted" in crosses between Hfr K-12 donors of *Escherichia coli* and F$^-$K-12(P1) recipients: they occur about 100 times less frequent than in crosses Hfr K-12 × F$^-$K-12. No restriction is observed in crosses of P1-lysogenic Hfr donors with either K-12 or K-12(P1) recipients. Prophage P1 carried by strain K-12(P1) also restricts the acceptance of the fertility factor F, of the episomes F-*gal* and F-*lac* and of resistance transfer factors (RTF) if

they are donated by nonlysogenic K-12 strains, while no restriction occurs for P1-lysogenic donors. The same episomes also are restricted upon transfer from K-12 donors into *E. coli* B recipients. No restriction is noted with recipients *E. coli* C. The similarity of these restrictions with those found for the acceptance of phage λ suggests that the bacterial DNA carries, like that of phage λ, host-specificity determinants.

Conjugation appears to be lethal to the restricting females mating with F^+ donors or donors carrying some other episomes. One of the resistance transfer factors was shown to carry genetic information for production and control of host specificity itself; phage λ is restricted in strains carrying this RTF, and those phages which are accepted undergo host-controlled modification.

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