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Novel Genotypes Among Transductants Made with Bacteriophage P1 Lysates from an F14 Merogenote Strain of *Escherichia coli* K-12[1]

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Among P1 transductants in *Escherichia coli* K-12 that were selected for the proximal and distal markers from the large F14 merogenote, a variety of unusual genotypes were found. As earlier workers had found, one class of these could transfer the proximal genes *(argH, metB)* and distal genes *(ilvEDAC)* of the F14 during conjugation. These F14 genes could be transferred into RecA recipients, indicating that they were carried on an F-merogenote rather than on an Hfr chromosome. The transduced F-merogenotes could transfer other F14 genes *(metE, rha)* as well. Transfer kinetic analysis showed that all of the latter transduced F-merogenotes that were examined were indistinguishable from the parental F14 in the order of transfer and the genetic distance between proximal and distal markers. This suggests that the whole F14 had been received somehow by the primary transductional recipients, a remarkable possibility since the F14 is much larger than the largest deoxyribonucleic acid segment normally transduced by P1. The mechanism of this phenomenon is not yet known. Many of the transductants did not transfer any of the F14 markers tested. Some of these transductants segregated certain F14 genes, indicating they were carried on self-replicating genetic elements, but others were not cured of F14 markers, even by acridine orange. Cotransductional analysis of this group was consistent with the hypothesis that the F14 markers in some of these strains had integrated into the chromosome in the expected manner, since in these latter the F14 alleles were linked to the expected chromosomal genes. Other strains among the stable transductants had acquired new linkages in that genes previously separated by several minutes could now be cotransduced. These latter included the novel cotransductional linkages of *rbs-ilv-argH*, *rbs-ilv-argH-metB*, and *ilvD-argH-purD*. Such strains might have been formed as a result of insertion into the chromosome of small circles derived from F14.

P1*kc*, a generalized transducing phage, has been widely used for fine-structure mapping of genetic markers on the *Escherichia coli* K-12 chromosome. There is a direct correlation between cotransduction frequency and the distance separating genetic markers. The physical and genetic limit of cotransductional linkage of chromosomal markers has been shown to be 100 kilobase (kb) pairs or slightly less than 2 min of the *E. coli* K-12 chromosome (14, 32). The transduced deoxyribonucleic acid (DNA) fragment is of the same molecular size as the P1 genome (6×10^7 daltons or 100 kb [12, 14, 25, 27]). The limiting size of these DNA fragments might be explained by the "headful" hypothesis of Streisinger et al. (22, 30).

Pittard used P1*kc* prepared on AB1206, an F' strain of *E. coli* K-12 haploid for the region of the chromosome carried on the F14 merogenote (Fig. 1 and 2) (21, 23), to transduce the genes controlling isoleucine-valine *(ilv+)* biosynthesis that are carried on F14 in this strain (J. Pittard and E. A. Adelberg, Bacteriol. Proc., p. 138, 1963). He found that 1 to 2% of the Ilv+ transductants were also MetB+. About 50% of these Ilv+,MetB+ transductants transferred *ilvD*, *metB*, and *argH* genes, all of which are carried on the original F14 in the P1*kc* donor strain. The genetic distance between *ilvD* and *metB* (161 kb pairs) plus the F (94.5 kb pairs) is greater than the established physical limits of joint transduction of genetic markers by P1*kc* (12, 14, 32), larger than the usual "headful" (22, 30).

The objective in the present study was to analyze genetically the nature of the transductants receiving these several F14 characteristics by P1 transduction as part of a continuing study on the mechanism for this seemingly impossible transduction.

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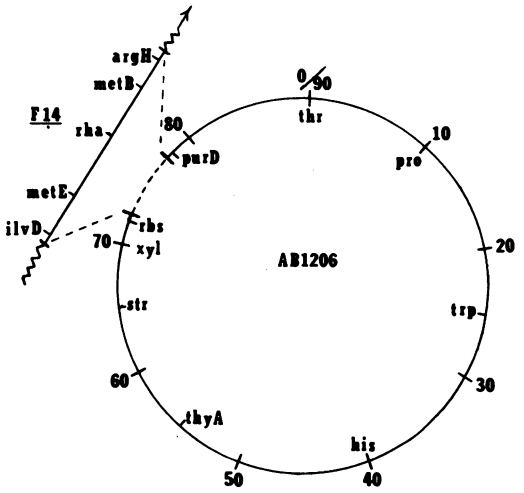

FIG. 1. *E. coli K-12 chromosome showing the segment of the chromosome carried by the merogenote in the haploid F14 strain AB1206.*

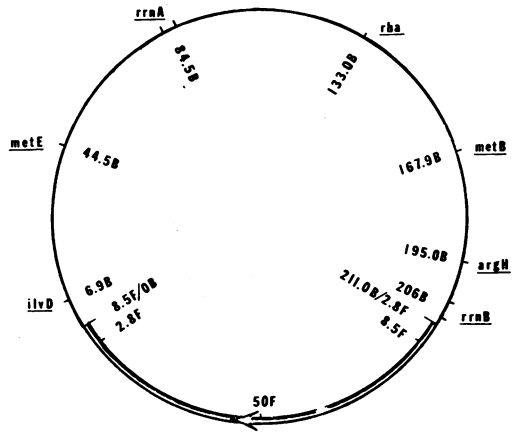

FIG. 2. *Fine-structure map of F14. Coordinates given in kilobase pairs. Kilobase coordinates followed by F are F factor coordinates; those followed by B are bacterial sequence coordinates. Modified from reference 16.*

MATERIALS AND METHODS

Media. All strains were routinely grown in Luria (L) broth (18) when a complex broth was required and with the addition of 2.0% agar for complex plating medium. Z broth (L broth with 2.5×10^{-3} M $CaCl_2$) (18) was used to grow recipient cultures for transduction with phage P1*kc*. Z agar (1% agar) was used for making P1*kc* lysates and for titering phage. SA-1 agar (0.7% agar and 1% NaCl) was used for overlaying Z agar and synthetic agar. Half-strength medium 56 (1) was the minimal medium routinely used for the selection of recombinants. All amino acids, purines, and pyrimidines were supplemented at a final concentration of 50 μg/ml; sugars were 20 mg/ml, and vitamin supplements were 0.2 μg/ml. In addition, the selective agar contained, as appropriate, streptomycin (170 μg/ml), rifampin (Calbiochem, 30 μg/ml), nalidixic acid (Calbiochem, 80 μg/ml), or trimethoprim (Burroughs, Wellcome and Co., 20 μg/ml). MacConkey agar base (Difco) containing 1% carbohydrate was used to test the fermentation capabilities of bacterial strains.

Bacterial strains. The bacterial strains used are described in Tables 1 and 2. The haploid F14 strains AB1206 and χ1254 were used as the donor strains in conjugation and transduction experiments. Each merogenote derived from F14 transduction was originally obtained in an Rec$^+$ recipient, either strain AB1450, KF100, or KF110. These merogenotes were transferred by conjugation to *recA1* recipient strains, KF104 or KF99. The F' *recA* strains were then used as donors in time-of-entry matings to be described. F14 *recA1* diploid strains, used in time-of-entry matings, were constructed by mating either AB1206 or χ1254 with KF104 and KF99.

Bacteriophage. Two stocks of the generalized transducing phage P1*kc* (15) were used. One, labeled P38, was obtained from Roy Curtiss III (University of Alabama at Birmingham). The second, P1*vir* (12), was obtained from J. Tomizawa (12) through Herbert Boyer (University of California at San Francisco).

The phage T6 was used to kill males in some time-of-entry matings (10).

Strain construction. Strain KF109 was constructed by cotransduction of *purD* and *rif*^{r} into AB1472, using a P1*kc* lysate grown on strain KF108. All other antibiotic-resistant strains were made by the usual procedures (20).

recA strains were made by using the following procedure. The recipient strain was first made *thyA* by trimethoprim selection (20), and then *thy*$^+$ and *recA* were conjugated into the recipient using a *recA* Hfr. *thy*$^+$ recombinants were tested for *recA* character. An alternate procedure (Anthony Pfister, personal communication) eliminated the *thyA* induction step. A *recA1,thyA* Hfr was mated with a *thy*$^+$ recipient, selecting for trimethoprim resistance on a medium containing thymine. Recombinants were patched onto homologous medium and replicated onto a medium containing ethyl methane sulfonate (500 ppm). *recA* recombinants failed to grow in the presence of ethyl methane sulfonate.

Production of phage lysates. Two methods for the production of phage lysates were used. (i) All P1*kc* lysates made on F14 haploid strains were made by the soft-layer method (31), using a simplified harvesting procedure (19). Lysates of haploid F14 strains were of a titer from 10^{10} to 6×10^{10} plaque-forming units (PFU)/ml. (ii) T6 lysates and P1*vir* lysates were made by lysing growing cultures in L and Z broth, respectively (19).

Verification of Rec characters. Six methods were used to check the Rec$^-$ characters: (i) sensitivity to ultraviolet light (150 to 200 ergs), (ii) sensitivity to methyl methane sulfonate or ethyl methane sulfonate (0.5 μl/ml), (iii) inability to form transductants wi th P1*kc*, (iv) inability to form recombinants in

TABLE 1. *E. coli K-12 strains*

Strain	Sex	Genotype	Source or derivation
AB1206	F14	(F-*ilv*$^+$, *metE*$^+$, *rha*$^+$, *metB*$^+$, *argH*$^+$)/ Δ (min 74 to 79) *thi-1*, *his-1*, *proA2*, *lacY1*, *tfr-3*, *str*	Brook Low, Yale Univ.
χ1254	F14	AB1206[a]	Norman Davidson, California Institute of Technology
KL-16-99	Hfr	*recA1*[b]	Anthony Pfister, New York Univ.
KF116	Hfr	As KL-16-99, also *thyA*	Anthony Pfister, trimethoprim selection *thyA*$^-$
AB2930	F$^-$	*ilvE12*, *argG12*, *his-42*, *thyA26*	This laboratory
KF99	F$^-$	*ilvE12*, *argG12*, *his-42*, *recA1*, *rha*, *metB1*	This laboratory
AB2990	F216	(F-*ilv EDAC*$^+$)/*ilvE12*, *argG12*, *his-42*, *recA1*	This laboratory
AB264	F$^+$	*ara*$^{mu-1(?)}$, λ$^+$	This laboratory
AB1450	F$^-$	*ilvD16*, *argH1*, *metB1*, *his-1*, *str*, *tsx*[c]	Barbara Bachmann, Yale Univ.
AB1472	F$^-$	*ilvD16*, *argH1*, *metB1*, *str*[d]	Barbara Bachmann
C600	F$^-$	*leu*, *thr*, *str*, λ$^-$	Phil Harriman, Duke Univ.
KF2201	F$^-$	*thi-1*, *metE46*, *trp-3*, *his-4*, *str*, *rha*	This laboratory
RK4101	F$^-$	*metE*, *argH1*, *str*, *rif*[e]	Robert Kadner, Univ. of Virginia
AB468	F$^-$	*purD13*, *proA*, *his*, *thi-1*[f]	Barbara Bachmann
KF100	F$^-$	As AB1472, also *thyA*	Trimethoprim selection of *thyA*$^-$
KF101	F$^-$	As AB1472, also *recA1*	KL-16-99 × KF100 selection for *recA*$^-$
KF102	F$^-$	As KF100, also *rha*, *rbs*	2-AP sequential selection for *rha*$^-$ and *rbs*$^-$
KF104	F$^-$	As AB1472, also *thyA*, *recA1*	KF116 × AB1472 selection for *thyA*$^-$ and *recA*$^-$
KF105	F$^-$	As AB1472, also *nalA*	Nalidixic acid selection
KF108	F$^-$	As AB468, also *rif*	Rifampin selection
KF109	F$^-$	As AB1472, also *purD13*, *rif*	Note text
KF110	F$^-$	As AB1450, also *nalA*	Nalidixic acid selection
KF275	F14	F14/KF104	χ1254 × KF104
KF400	F14 (?)	F14-like/AB1450	P1·χ1254 × AB1450
KF603	F14 (?)	F14-like/AB1450	P1·χ1254 × AB1450
KF636	F14 (?)	F14-like/AB1450	P1·χ1254 × AB1450
KF403	F14 (?)	F14-like/AB1450	KF603 × KF104
KF436	F14 (?)	F14-like/AB1450	KF636 × KF104

[a] χ1254 is a single colony isolate of AB1206, isolated by K. J. Roozen of Oak Ridge National Laboratory.
[b] O: *thyA-recA-his-ilv-serA*.
[c] This strain also carries *thi-1*, *xyl-7*, *malA1*, *lacY1*, *gal-6*, *tonA1*, λ^r, λ$^+$.
[d] This strain also carries *thi-1*, *malA1*, *gal-6*, λ^r, λ$^-$.
[e] This strain also carries *leu*, *pro*, *lysA*, *cyc*.
[f] This strain also carries *mtl1*, *xyl-5*, *galK2*, *lacY1*, λ$^-$.

Hfr matings, (v) ability to form colonies after matings with F′ strains, and (vi) lower growth rates than known Rec$^+$ strains.

Cross-streak test. The cross-streak screening test was used to test isolates of haploid F14 strains and to screen for transduced "F14-like" plasmids for preliminary determination of the genotype of their episomes. It was necessary that these strains transfer the *ilv* cluster, *metB*, and *argH* into an *recA* strain to verify that these genes were located on a merogenote. The donors were grown on minimal agar, picked, and incubated for a few hours at 37 C in 0.1 ml of minimal medium in wells of sterile microtiter dishes. Several glass pin heads (alcohol flamed and mounted on a holder) were then dipped into the wells containing each of the cultures and streaked across a recipient on selective medium (19). The mating plates were incubated for 2 days and observed for the presence of individual colonies along the streak.

Time-of-entry matings. Two methods were employed for time-of-entry matings. (i) The procedure of Adelberg and Burns (1) was used with the following modifications. The F′ males (grown to exponential phase in minimal media) and the F$^-$ females (grown to exponential phase in L broth) were mixed at a ratio of 1 male to 10 females. Matings were interrupted by subjecting 1-ml samples to a vibratory shaker (16) for 30 s. Further formation of mating pairs was prevented either by killing F′ strains by T6 infection (10) or by preventing their growth with either streptomycin or nalidixic acid in the

TABLE 2. *E. coli K-12 strains constructed by P1 (χ1254) transduction into AB1450 but which could not transfer F14 markers by conjugation*

Strain	Sex	Genotype
KF201	NT[a]	*ilvD*$^+$, *metB*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*[b]
KF205	NT	*ilvD*$^+$, *metB*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF209	NT	*ilvD*$^+$, *metB*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF213	NT	*ilvD*$^+$, *metB*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF217	NT	*ilvD*$^+$, *metB*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF220	NT	*ilvD*$^+$, *metB*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF221	NT	*ilvD*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF224	NT	*ilvD*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF227	NT	*ilvD*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*
KF230	NT	*ilvD*$^+$, *argH*$^+$/*ilvD16*, *metB1*, *argH1*, *his*

[a] NT, Strains do not transfer. Other donors characteristics were not tested.

[b] All strains also carry *thi-1*, *xyl-7*, *malA1*, *lacY1*, *gal-6*, *tonA1*, λ^R, λ^-.

selective media (20). The appropriate dilutions were then added to 3 ml of SA-1 (containing streptomycin in some experiments) and immediately plated on selective media. (ii) The other method used was that of De Haan and Gross (7), using the interruption and plating methods described above.

Transduction procedures. P1*kc* lysates made on haploid F14 strains, AB1206 and χ1254, were used to transduce F-merogenote markers into the F$^-$ strains, AB1450, and KF100. The recipient strains (late exponential phase, 2 × 10^9 cell/ml) and phage (multiplicity of exposure [MOE] [in all experiments analyzed, 90% of the phage were absorbed by 20 min] of 1) were incubated at 37 C for 20 min, chilled, centrifuged, resuspended in 56/2 buffer, and plated on selective media. The recipient strains carried *ilvD*, *metB*, and *argH* mutations; selection was for all three markers at once. Ilv$^+$, Met$^+$, Arg$^+$ transductants were tested for their ability to transfer F-merogenote markers by cross-streaking against *recA* strains as described above. They were further tested for their ability to transfer other F14 markers, *metE*$^+$ and *rha*$^+$, and for endogenote markers. When the F-merogenotes were characterized, they were conjugated into the *recA1* strain KF104.

AO curing of nonconjugative F14 markers. Strains carrying transduced F14 markers that could not be transferred by conjugation were grown overnight from a small inoculum in the presence of acridine orange (AO) (11) and then plated on L agar. The colonies arising were replica plated to appropriate minimal media to test for segregation of the F14 markers, *ilvD*$^+$, *metB*$^+$, and *argH*$^+$.

RESULTS

Screening of AB1206 strains from several culture collections for F14 haploid characteristics. In AB1206, which is haploid for the F14 region, the F-merogenote exhibits considerable instability, forming Hfr-like derivatives, nontransferring types, and F′ strains that only transfer segments of the F14 merogenote (2, 21, 23). Recent physical studies have given an insight into possible reasons for instability of the F14 plasmid (21). Cultures of AB1206, received from the collections of several different laboratories, were tested for the properties of AB1206 as originally described (23).

Cultures were tested by cross-streak complementation tests for the transfer of the F14 markers (Fig. 1) *ilvD*$^+$, *metB*$^+$, and *argH*$^+$ into the Rec$^+$ and *recA1* recipients, AB1472 and KF101, respectively. Two cultures of AB1206 were found to transfer F14 markers efficiently into RecA strains. They were the AB1206 strain, received from K. Brooks Low, and χ1254, an isolate from the AB1206 strain received from Norman Davidson, who had originally obtained it from the laboratory of Roy Curtiss III. These strains also transferred *metE*$^+$ and *rha*$^+$.

P1 transduction of F-merogenote markers into F$^-$ strains. The transducing efficiencies of various chromosomal markers from AB264 as the P1 donor were compared with the transducing efficiencies of the same markers from the F14 strain, using χ1254 as the P1 donor (Table 3). Genes carried on the F14 were transduced at an efficiency of about 10 to 50% of that of the same markers from the chromosome of AB264. The *trp*$^+$ gene, which is a chromosomal marker in both strains, was transduced at about the same efficiency by lysates prepared on either strain.

As Pittard and Adelberg have previously found (23), contransductional linkages were obtained with lysates made on the strains haploid for the F14 region that were not found with lysates made on the wild-type strain. In some transductants *ilvD*$^+$ was shown to be jointly inherited with both *metB*$^+$ and *argH*$^+$.

Among the Ilv$^+$,Met$^+$,Arg$^+$ contransductants, about 20% were found to be F′ donor strains. Preliminary screening tests demonstrated that these transductants transferred *ilvD*$^+$, *metB*$^+$, and *argH*$^+$ efficiently to both Rec$^+$ and *recA1* strains, indicating that these genes were carried on an F-merogenote, as they were in the P1 donor strain χ1254. The transducing efficiency of F-merogenote genes into *recA1* recipients is considerably lower than the efficiency into Rec$^+$ recipients (data not shown).

Only transductionally shortened (F-*ilv*) merogenotes (19) were obtained in *recA1* recipients, and these at a lower frequency than in Rec$^+$ recipients. At this time the significance of this observation is not known.

To obtain evidence in addition to that of Pittard and Adelberg (Bacteriol. Proc., 138, 1963) that transduction was the mode of genetic exchange, the transductional procedure was carried out in the presence of either deoxyribonuclease (100 μg/ml) or nalidixic acid (84 μg/ml), which should prevent genetic transfer by transformation or by conjugation (3, 4), respectively. The results indicated (Table 4) that neither deoxyribonuclease nor nalidixic acid had any effect on the efficiency of genetic exchange.

The transduced F-merogenotes described in this study (all of which carried *ilvD*$^+$, *metB*$^+$, and *argH*$^+$) were conjugated into, and subsequently maintained in, a *recA-1* strain, KF104. Cross-streak complementation tests were used to determine the genes carried on the F-merogenotes. These tests revealed that these strains, as well as the original transductants, transfer at a high frequency *ilvE*, *ilvD*, *ilvA*, *ilvC*, *metE*, *rha*, *metB*, and *argH* but not the chromosomal gene *rbs*, *xyl*, or *purD*. This indicates that a considerable number of the F14 genes, as well as the F factor, had been received by the primary transductants. The possibility that all of the F14 has been received could not be strongly supported by the presence of only these few markers; other methods of analysis were used to test the possibility.

TABLE 3. *Comparison of the transduction efficiency of markers from the P1 donors AB264 and χ1254*

Markers[b]	Transducing efficiency[c]	
	AB264	χ1254
trp	1.8×10^{-5}	1.2×10^{-5}
ilvE	1.5×10^{-4}	1.4×10^{-5}
ilvD	1.1×10^{-4}	9.3×10^{-6}
metE	4.6×10^{-5}	2.5×10^{-6}
rha	1.0×10^{-5}	2.5×10^{-6}
metB	1.6×10^{-5}	9.9×10^{-6}
argH	1.8×10^{-5}	9.4×10^{-6}
metB, argH	1.4×10^{-5}	9.0×10^{-6}
ilvD, argH	–[d]	6.0×10^{-7}
ilvD, metB, argH	–[d]	2.7×10^{-7}
F-*ilvD-metB-argH*	–[e]	7.0×10^{-8}

[a] This was a typical experiment that was used to measure the transducing efficiency of lysates. See text for transduction parameters. The strains used as recipients were: AB1450 (*ilvD, metB, argH*), KF2201 (*rha, metE, trp*), and AB2930 (*ilvE*).

[b] All genes are chromosomal markers in AB264. Of those tested from χ1254, only *trp* is a chromosomal marker.

[c] Number of transductants per PFU at MOE = 1.

[d] Cotransduction of these markers was less than 10^{-9}.

[e] F is not linked to *ilvD*, *metB*, and *argH* in strain AB264.

Kinetics of transfer of F-merogenotes. Since not all of the genes carried on F14 have been determined and not all of those identified are easily tested, the genetic distance between proximal and distal markers on those F-merogenotes found in the transductants was used to estimate the size of these plasmids. This genetic distance determined from differences in the time of entry of proximal and distal F14 genes was determined on both the parental F14 and the F-merogenotes found in the transductants.

(i) Kinetics of transfer of F14 from haploid strains. Interrupted mating experiments were conducted first with the original haploid F14 strains (and then with diploid strains; see below) to determine the genetic distance between proximal and distal genes of the F14 plasmid in these strains. The time of appearance in the recipient of the proximal donor marker *metB* varied from culture to culture. Figure 3 is a typical time-of-entry curve. In different matings with AB1206, *metB*$^+$ first appeared in the recipients somewhere between 15 and 19 min after the mating strains were mixed. In matings with χ1254 (Fig. 3), *metB*$^+$ was found to be transferred at between 9 and 13 min. The difference in kinetics observed in each strain, however, did not have any effect on the relative genetic distance between *metB* and *ilvD*, the proximal and distal markers. These loci con-

TABLE 4. *Effect of deoxyribonuclease (DNase) and nalidixic acid on the transduction of F14 markers*[a]

Transducing conditions	Transduction efficiency			
	ilvD	*ilvD, argH*	*ilvD, metB, argH*	F-*ilvD-metB-argH*
P1·χ1254 × AB1450 (control)	4.7×10^{-6}	4.7×10^{-7}	2.2×10^{-7}	5.5×10^{-8}
P1·χ1254 × AB1450 + DNase	6.2×10^{-6}	5.0×10^{-7}	2.7×10^{-7}	6.5×10^{-8}
P1·χ1254 × KF110 + nalidixic acid	7.1×10^{-6}	3.9×10^{-7}	2.1×10^{-7}	5.5×10^{-8}

[a] Deoxyribonuclease concentration, 100 μg/ml; nalidixic acid concentration, 84 μg/ml. See text for transduction parameters. In a parallel conjugal mating using 4×10^8 donors/ml, no recombinants were formed in the presence of nalidixic acid.

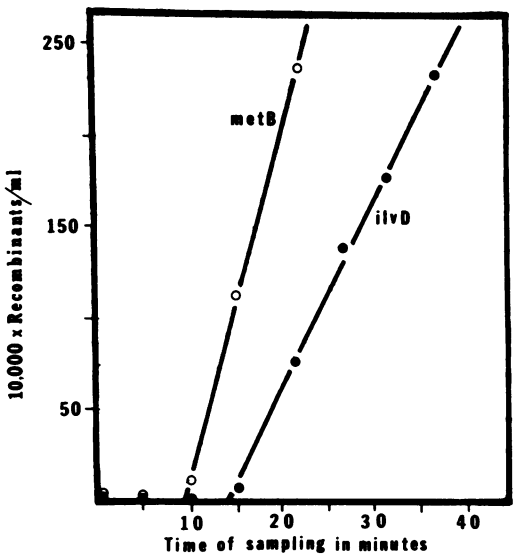

FIG. 3. *Time of entry of F14 markers from χ1254, a strain haploid for the F14 region, into KF105. Nalidixic acid was used to inhibit the donor.*

sistently exhibited a relative genetic distance of 5 ± 0.5 min. This is in agreement with the value of 5 min for AB1206 reported by Pittard et al. (24), whose curves show *metB* entering at 10 min.

(ii) Transfer kinetics of F14 from a merodiploid RecA strain. Time of entry of F14 markers from donors diploid for the parental F14 genes was of more value for comparison with those on the F-merogenotes constructed by transduction since the latter are necessarily diploid. RecA strains carrying the parental F14 were constructed, and interrupted matings were carried out. Figure 4 is a typical time-of-entry curve for these strains. As with the haploid F14 strains, these RecA F′ strains varied in the time of entry of the proximal marker. *metB*$^+$ was transferred from different donors at between 8 and 18 min. The gradient of recombination frequencies differed with each merodiploid, as did gradients obtained with the haploid F14 strains. Again, the kinetics observed in each strain had no affect on the relative genetic time of entry of *metB* and *ilvD*, which was 5 ± 0.75 min. This latter value is indistinguishable from that obtained with the donor strain haploid for F14.

(iii) Transfer kinetics of F-*ilv*'s derived from F14 by transductional shortening. If the transduced F-merogenotes to be studied carry deletions in the F14 DNA, their transfer kinetics should vary from that of wild-type F14. Transfer kinetics with strains known to carry deletions in F14 could serve for a comparison; however, strains deleted between *metB* and *ilv* are unknown to us.

Therefore, the time of entry of plasmid-carried *ilvD*$^+$ from one of the F-*ilv* merogenotes (shortened from F14 by transductions [14, 19, 23, 26]) was determined and compared with that of the undeleted F14. The *ilvD*$^+$ from AB2990 was first transferred at 4 min (Fig. 5), as was described by Pittard and Adelberg (23).

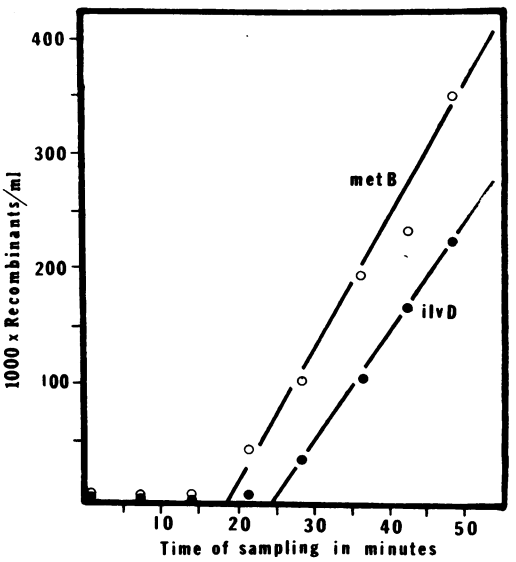

FIG. 4. *Time of entry of F14 markers from KF275, a recA$^-$ strain diploid for the F14 region, into KF105. Nalidixic acid was used to inhibit the donor.*

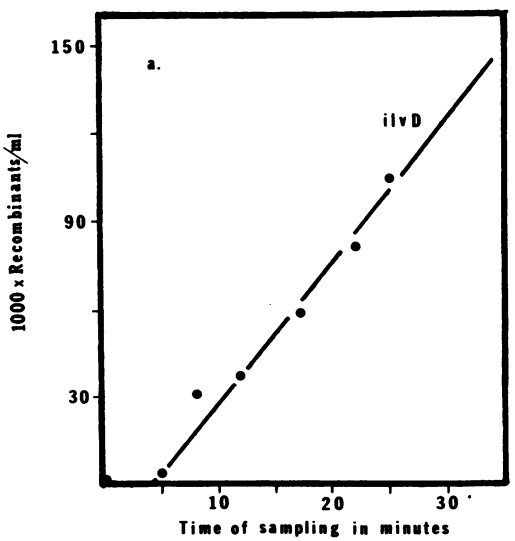

FIG. 5. *Time of entry of ilvD marker from AB2990, a recA$^-$ strain carrying F-ilvED, into AB1472. Streptomycin was used to inhibit the donor.*

Similar results were obtained with other F-*ilv*'s. The time of entry of *ilvD*$^+$ from the F-*ilv*/*recA1* strains is similar to the time of entry of *ilvD*$^+$ on F-*ilv* merogenotes carried in Rec$^+$ strains.

(iv) **Kinetics of transfer of F-merogenotes from transductants.** KF400 (Fig. 6a) is a Rec$^+$ strain carrying one of the F-merogenotes from a transductant. The *metB* gene from this strain was first transferred at 12.5 min, and *ilvD* came in 4.5 min later. A *recA* donor, KF403 (Fig. 6b), transferred the *metB* gene after 17 min. KF436 (Fig. 6c) transferred *metB* after 32 min each time it was tested. This delay of entry for the proximal marker occurred whether the donor was grown in minimal media (as were all donors tested) or in L broth a short time before mating. The reason for this delay in the entry of the proximal marker, demonstrated by several strains, has not been determined as yet. The interesting observation is that, with the F-merogenotes tested, *ilvD* consistently followed the entry of the proximal marker (*metB*) by 4.5 to 5.5 min; this is about the same genetic distance as seen with parental F14. The interrupted mating curves presented in Fig. 6 are representative of those obtained with 17 independently isolated transduced F-merogenotes from several different transductions using four different P1 lysates.

Characterization of transductants that did not transfer F-merogenote markers. (i) Segregation of F14 markers in transductants. The above analysis provided some evidence toward the nature of some of the transductants that could conjugally transfer all of the selected genes. However, many transductants could not transfer these genes in mating experiments and so could not be genetically analyzed by interrupted mating. Spontaneous segregation of F14 markers was used as a first indication as to whether these selected markers were integrated or were replicating autonomously. It can be seen (Table 2) that transductants that did not transfer merogenote markers were of two genotypes, those that had received *ilvD*$^+$, *metB*$^+$, and *argH*$^+$ and those that had received *ilvD*$^+$ and *argH*$^+$. Spontaneous segregation of F14 markers after 12 h in L broth occurred at a high frequency in some strains and not at all in others. Strain KF217 did not lose any of the F14 markers spontaneously, whereas 27% of the KF209 colonies, arising on L agar after 12 generations in broth, had segregated one or more of the plasmid markers (Table 5).

Representatives of three classes from the above segregation analysis (nonsegregating, moderately segregating, and highly segregating) were then grown in the presence of AO to follow segregation of the F14 markers under curing conditions (Tables 6 and 7). A wide variety of segregation patterns and segregation frequencies of these classes was observed. Some F-merogenote markers were segregated independently of other F-merogenote markers. KF201, for example, segregated six different phenotypes after being grown in the presence of AO. This strain could be cured of *ilvD*$^+$, *metB*$^+$, and *argH*$^+$ together: 43% of the colonies arising had lost these markers after 15 generations (overnight) in AO. On the other hand, about 38% of the colonies had retained all three markers. KF217, in which spontaneous loss of

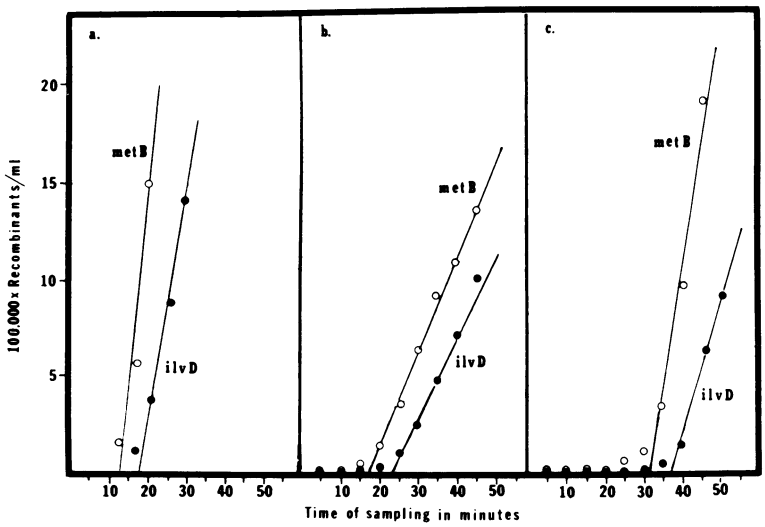

FIG. 6. *Time of entry of F14 markers carried on transduced F14-like plasmids. (a) KF400 (Rec$^+$) × KF105; (b) KF403 (RecA$^-$) × KF105; (c) KF436 (RecA$^-$) × KF105. Nalidixic acid was used to inhibit the donor.*

these F14 markers was not detected, did show a low level of segregation of these markers when grown in AO.

Colonies from representative genotypes of strains that had been grown in the presence of AO were again subjected to growth in AO to determine whether *ilvD*$^+$, *metB*$^+$, or *argH*$^+$ was stable (integrated?) or was still curable (on a plasmid?). The results indicated (Table 8) that isolates which had retained single or closely linked markers (such as *metB*$^+$ and *argH*$^+$) when grown in AO the first time did not segregate these markers when subjected to the dye the second time. Further, some isolates that had retained unusual transduction linkages (*ilvD* and *argH; ilvD, metB,* and *argH)* after the first exposure to AO either retained these genotypes after the second exposure to the curing agent or segregated the plasmid genes only at a low level. Conversely, one isolate of KF201 that had retained *ilvD, metB,* and *argH* after growth in AO the first time lost all three markers at a high frequency after growth in the dye the second time (94% of colonies arising after overnight growth in AO). In this strain no single marker was lost independently of the other markers with which it had been cotransduced. One isolate of KF213 had retained *ilvD*$^+$ and *metB*$^+$ after the first treatment with AO. When exposed to the dye a second time, this isolate lost *ilvD*$^+$ independent of *metB*$^+$ at a segregation frequency of 46% (Table 8). Although interpretation of these curing patterns is difficult, they seem to indicate that the transduced genes are often carried on autonomously replicating elements. Some of the regions on these nontransferring plasmids may undergo recombination, or the entire plasmid may insert into the chromosome.

(ii) Transductional analysis of linkage in nontransferring transductants. Analysis of linkage between F14 genes and chromosomal genes was carried out to study some of the properties of the transductants that had received proximal and distal F14 markers but that could not transfer these genes in conjugation. This analysis would show whether the transduced genes had been inserted as part of a plasmid or had exchanged with the chromosomal genes in the region homologous to the F14 segments. Transductional linkage in the P1 recipient between P1 donor *ilvD* and recipient *rbs* or donor *argH* and recipient *purD* would indicate exchange or insertion of F14 genes near the normal site on the chromosome (Fig. 1). P1*vir* lysates were made on selected strains that segregated F14 markers at either high frequencies (KF209, KF201) or low frequencies (KF217, KF227, KF230). Analyses of these strains, using KF102 and KF109 as recipients, are shown in Table 9 and 10, respectively.

Transductional analysis did show linkages in some strains between *ilvD*$^+$, *argH*$^+$, and *metB*$^+$

TABLE 5. *Spontaneous segregation of F-merogenate markers in nontransferring strains*[a]

Strain	No. of isolates tested	Segregation of F14 markers (%)
KF201	30	10.0
KF205	23	4.3
KF209	48	27.1
KF213	45	6.7
KF217	23	0.0
KF220	30	10.0
KF221	36	22.2
KF224	44	4.7
KF227	51	3.9
KF230	27	7.4

[a] Each strain was grown overnight in L broth through 12 to 13 generations and plated on L agar. Plates were replicated on synthetic media to test for segregation of F14 markers: *ilvD, metB,* or *argH*. Percent segregation of F14 markers includes the segregation of one or more markers.

TABLE 7. *Phenotypes of unfertile transductants (Ilv*$^+$*, Arg*$^+$*) obtained after growth in AO*[a]

Strain	No. of each phenotype after exposure to curing agent			
	IlvD$^-$ ArgH$^-$	IlvD$^-$ ArgH$^+$	IlvD$^+$ ArgH$^-$	IlvD$^+$ ArgH$^+$
KF227	60	3	10	134
KF230	5	1	0	62

[a] For experimental procedure, see text.

TABLE 6. *Phenotypes of unfertile transductants (Ilv*$^+$*, Met*$^+$*, Arg*$^+$*) obtained after growth in AO*[a]

Strain	No. of each phenotype after exposure to curing agent							
	IlvD$^-$ MetB$^-$ ArgH$^-$	IlvD$^-$ MetB$^-$ ArgH$^+$	IlvD$^-$ MetB$^+$ ArgH$^-$	IlvD$^-$ MetB$^+$ ArgH$^+$	IlvD$^+$ MetB$^-$ ArgH$^-$	IlvD$^+$ MetB$^-$ ArgH$^+$	IlvD$^+$ MetB$^+$ ArgH$^-$	IlvD$^+$ MetB$^+$ ArgH$^+$
KF201	62	2	11	11	2	0	0	56
KF209	42	1	5	18	1	0	0	100
KF213	15	2	40	5	0	0	1	62
KF217	2	0	0	2	0	0	0	87

[a] For experimental procedure, see text.

(all F14 genes), in others between *ilvD*$^+$ and *rbs*$^+$ (F14 and chromosomal genes, respectively) (Table 9), and in still others between *argH*$^+$ and *purD*$^+$ (F14 and chromosomal genes, respectively) (Table 10). The first type was the linkage found in the original transductants (Table 3) and indicated a conservation of that sequence of genes. The latter two would be expected if the appropriate F14 markers had integrated at legitimate chromosomal regions.

Analysis of these nontransferring transductants also revealed unexpected linkages between chromosomal genes (either *rbs* or *purD*) and both proximal (*metB* and *argH*) and distal (*ilvD*) F14 genes: *rbs*$^+$ with *ilvD*$^+$ and *argH*$^+$; *rbs*$^+$ with *ilvD*$^+$, *argH*$^+$, and *metB*$^+$; and *purD*$^+$ with *ilvD*$^+$ and *argH*$^+$. These last three linkages indicate close proximity between markers that are widely separated in wild-type chromosomes.

DISCUSSION

In *E. coli* K-12, genetic markers that are very closely linked in conjugal crosses can be contransduced by the bacteriophage P1 (3). The frequency of cotransduction decreases sharply as the genetic distance between markers increases. The genetic and physical limits of cotransduction are determined by the size of the DNA packaged by P1, specifically 100 ± 0.5 kb pairs (6×10^7 daltons) or just under 2.0 min of the *E. coli* K-12 chromosome (12, 14, 32).

These limits seem to be exceeded by P1 transducing particles in lysates made from the haploid F14 strain AB1206: the F14 markers *ilvD*$^+$, *metB*$^+$, and *argH*$^+$ appear to be cotransduced since they are coinherited in appropriate cultures infected with these lysates. The longest molecular distance on the circular molecule of F14 (Fig. 2) between *ilv* and *arg* is 188 kb. The

TABLE 8. *Phenotypes of isolates obtained after growth in AO of strains that had been previously exposed to AO*[a]

Parent strain	Phenotypes of isolates after first exposure to AO	No. of each phenotype after exposure to curing agent							
		IlvD$^-$ ArgH$^-$ MetB$^+$	IlvD$^-$ ArgH$^+$ MetB$^+$	IlvD$^+$ ArgH$^-$ MetB$^+$	IlvD$^+$ ArgH$^+$ MetB$^+$	IlvD$^-$ ArgH$^-$ MetB$^-$	IlvD$^-$ ArgH$^+$ MetB$^-$	IlvD$^+$ ArgH$^-$ MetB$^-$	IlvD$^+$ ArgH$^+$ MetB$^-$
KF201	IlvD$^+$, MetB$^+$, ArgH$^+$	0	0	0	6	100	0	0	0
KF217	IlvD$^+$, MetB$^+$, ArgH$^+$	0	0	0	104	2	0	0	0
KF213	IlvD$^+$, MetB$^+$, ArgH$^-$	42	—[b]	48	—	0	—	0	—
KF230	IlvD$^+$, MetB$^-$, ArgH$^+$	—	—	—	—	0	0	0	116
KF201	IlvD$^-$, MetB$^+$, ArgH$^+$	0	74	—	—	0	0	—	—
KF217	IlvD$^-$, MetB$^+$, ArgH$^+$	0	116	—	—	0	0	—	—
KF201	IlvD$^-$, MetB$^+$, ArgH$^-$	80	—	—	—	0	—	—	—
KF201	IlvD$^+$, MetB$^-$, ArgH$^-$	—	—	—	—	0	—	116	—

[a] The experimental procedure is the same as the one followed in previous AO curing.
[b] Phenotype given did not apply to isolate tested.

TABLE 9. *Genetic analysis of nontransferring strains carrying F14-derived genes*[a]

Mating pairs	No. of transductants with each phenotype							
	IlvD$^+$ ArgH$^-$ MetB$^-$ Rbs$^-$	IlvD$^+$ ArgH$^-$ MetB$^+$ Rbs$^-$	IlvD$^+$ ArgH$^+$ MetB$^-$ Rbs$^-$	IlvD$^+$ ArgH$^+$ MetB$^+$ Rbs$^-$	IlvD$^+$ ArgH$^-$ MetB$^-$ Rbs$^+$	IlvD$^+$ ArgH$^-$ MetB$^+$ Rbs$^+$	IlvD$^+$ ArgH$^+$ MetB$^-$ Rbs$^+$	IlvD$^+$ ArgH$^+$ MetB$^+$ Rbs$^+$
A. P1*vir*·KF201 × KF102	34	2	16	9	22	0	2	1
B. P1*vir*·KF201 × KF102	—[b]	—	93	0	—	—	1	0
C. P1*vir*·KF209 × KF102	114	3	21	10	19	0	1	0
D. P1*vir*·KF209 × KF102	—	—	45	4	—	—	3	0
E. P1*vir*·KF217 × KF102	76	1	18	9	59	0	4	0
F. P1*vir*·KF217 × KF102	—	—	59	14	—	—	3	0
G. P1*vir*·KF227 × KF102	123	—	44	—	25	—	7	—
H. P1*vir*·KF227 × KF102	—	—	40	—	—	—	0	—
I. P1*vir*·KF230 × KF102	45	—	10	—	4	—	3	—
J. P1*vir*·KF230 × KF102	—	—	40	—	—	—	0	—

[a] In transductions A, C, E, G, and I, the selected marker was IlvD. In transductions B, D, F, H, and J, the selected markers were IlvD and ArgH.
[b] Phenotype did not apply to these transductants.

TABLE 10. *Genetic analysis of nontransferring strains carrying F14-derived genes*[a]

Mating pairs	No. of transductants with each phenotype							
	ArgH⁺ IlvD⁻ MetB⁻ PurD⁻	ArgH⁺ IlvD⁻ MetB⁺ PurD⁻	ArgH⁺ IlvD⁺ MetB⁻ PurD⁻	ArgH⁺ IlvD⁺ MetB⁺ PurD⁻	ArgH⁺ IlvD⁻ MetB⁻ PurD⁺	ArgH⁺ IlvD⁻ MetB⁺ PurD⁺	ArgH⁺ IlvD⁺ MetB⁻ PurD⁺	ArgH⁺ IlvD⁺ MetB⁺ PurD⁺
A. P1*vir*·KF201 × KF109	88	92	38	20	2	0	0	0
B. P1*vir*·KF201 × KF109	—[b]	—	65	29	—	—	0	0
C. P1*vir*·KF209 × KF109	82	71	26	18	3	0	0	0
D. P1*vir*·KF209 × KF109	—	—	77	28	—	—	0	0
E. P1*vir*·KF217 × KF109	87	65	37	21	9	0	1	0
F. P1*vir*·KF217 × KF109	—	—	81	45	—	—	2	0
G. P1*vir*·KF227 × KF109	119	—	82	—	0	—	0	—
H. P1*vir*·KF227 × KF109	—	—	96	—	—	—	0	—
I. P1*vir*·KF230 × KF109	125	—	76	—	0	—	0	—
J. P1*vir*·KF230 × KF109	—	—	96	—	—	—	0	—

[a] In transductions A, C, E, G, and I, the selected marker was ArgH. In transductions B, D, F, H, and J, the selected markers were IlvD and ArgH.

[b] Phenotype did not apply to these transductants.

shortest distance (including the sex factor) is 123 kb (Fig. 2, Table 11). Thus, cotransduction of even the *ilv* and *arg* genes from F14 appears to be beyond the traditionally accepted capabilities of phage P1. Many of the transductants selected for by these three markers are also capable of conjugal transfer of these genes, as well as *ilvEDAOC*, *metE*⁺, and *rha*⁺, all of which are carried on F14. Thus, a considerable amount of the parental F14 appears to be present in these transductants: the essential genes of the sex factor and at least nine genes from the proximal, middle, and distal sections of the F-merogenote.

The ability to transfer these transduced genes into RecA recipients indicates that they are carried in the primary transductant on an F-merogenote rather than on an Hfr chromosome. The logical question, then, concerns the size of this transduced F-merogenote, relative to the parental F14; specifically, is the whole F14 present in the transductant? The F14 is 311 kb in molecular size (Table 11). One cannot, for practical reasons, determine whether every gene carried on F14 is present on the F-merogenote in the transductant (as mentioned earlier, not all genes have been identified, and many of those identified are not easily selected for in conjugation). A more simple question to ask is whether the F-merogenote in the transductant consists of less genetic material than the F14. The genetic distance between a proximal gene (*metB*) and a distal gene (*ilvD*) was used as such a measure. Transfer kinetics of these genes by the parental F14 and by the F-merogenote that had been transduced were compared. If the region between *metB* and *ilvD* on the bacterial segment had undergone extensive deletion at some stage in the transductional process, then the genetic distance between these markers would be shorter on the F-merogenote in the transductant. The genetic distance between *metB* and *ilvD* on the F14, as determined by time-of-entry curves, was 4.5 to 5.5 min and was indistinguishable from that observed with any of the several F-merogenotes from the independently isolated transductants.

TABLE 11. *Molecular size of segments on F14*[a]

Segment	Molecular size (kb) of segments
Complete F14	311.2
F DNA	100.2
Bacterial DNA	211.0
ilvD-metB	161.0 and 150.2[b]
ilvD-argH	188.1 and 123.1
ilvD-metE	38.0 and 273.0
metE-argH	150.0 and 161.0
rha-argH	62.0 and 249.0
rha-ilvD	126.0 and 185.0
Segregated circle	216.7
F DNA	5.7
Bacterial DNA	211.0
ilvD-metB	161.0 and 55.7[b]
ilvD-argH	188.1 and 28.6
ilvD-metE	38.0 and 179.0
metE-argH	150.0 and 66.0
rha-argH	62.0 and 155.0
rha-ilvD	126.0 and 91.0

[a] Note: 46.5 kb = 1.0 min of the *E. coli* K-12 chromosome. Data were produced by a combination of P1 transductional experiments, and information on the location of these genes was from Ohtsubo et al. (21), Sharp et al. (28), and Taylor and Trotter (33).

[b] Longest and shortest molecular distance around circular plasmid between genes.

We know of no F-merogenote deleted between *metB* and *ilvD* with which to compare the data presented. Indications that interrupted matings can detect extensive deletions can, however, be seen using an F-merogenote derived from F14 but deleted for all genes except those closely linked to F, namely, the *ilv* genes. The *ilv* genes are transferred very early by the F-*ilv* (at 5 min) compared with the large F-merogenote (at 14 to 39 min). As mentioned earlier, F-merogenotes found in the transductants transfer at least nine genes carried on F14. Within the limitations of interruped mating data, there is no evidence of large deletions between *metB* and *ilv* in any of the F-merogenotes found in the transductants. However, until more definative proof is obtained by means of heteroduplex homology (21) with parental F14, these F-merogenotes can only be said to be "F14-like."

The earliest transfer of the *metB* from haploid F14 strains varied from 9 to 19 min in the several interrupted mating experiments. Transfer of *metB* from various RecA strains diploid for the F14 region varied from 8 to 18 min in various matings. The time of entry of this marker from RecA strains carrying the F-merogenote from transduced strains varied from 17 to 35 min. The reason for the delay in initiation of transfer is not known at this time but might be of interest if it is related to the molecular nature of these F-merogenotes. The role of the RecA function in the transfer delay is not supported since the shortened F-*ilv* merogenote transferred *ilv* genes at the same time from Rec$^+$ and RecA strains.

Some of the transductants that coinherited the distal and proximal merogenote markers could not conjugally transfer these markers. Some of these transductants segregated some or all of the F14 markers spontaneously at low frequencies. When grown in the presence of AO, the frequency of segregated markers increased in some cases as much as four- to five-fold. The joint loss of these characters indicates that these markers are located on autonomously replicating plasmids, most likely derived from the F14 but which have lost the ability to transfer.

Among those transductants that were subjected to AO curing, some transductants showed very low segregation frequencies of F14 markers. Further, when these were subjected to AO a second time, most isolates retained those same merogenote markers that were retained during the first exposure to the dye, as they would if these merogenote markers had integrated.

Cotransductional analysis of transductants carrying nontransmissible F14 markers showed that some of these F-merogenote markers were indeed linked to endogenote chromosomal markers. But some of the transductants showed close linkages between markers that are normally too far apart to be cotransduced by P1, e.g., *ilvD* linked to *argH* and *purD*, and *metB* and *argH* linked to *rbs*. Although such events would be rare, this observation suggests how certain translocations might occur. The mechanism generating these unusual linkages is unknown at this time, but various models are being tested.

Recent physical studies of the F14 reveal that there are three direct repeats in its nucleotide base sequence. One of these, a direct repeat of the sequence 2.8 to 8.5 F, is thought to be responsible for a frequent reciprocal crossover that results in the formation of two smaller circular molecules. One of these circles consists of the complete F factor, and the other consists of the complete bacterial sequence of the F14 plus 1 copy of the 2.8 to 8.5 F direct repeat (21) (Fig. 7). Thus, the molecular distance between any two markers has been shortened by the deletion of F DNA (Table 11). The shortest molecular distance between *ilvD* and *metB* on the circle deleted of F is now 56 kb, instead of 150 kb. Lee et al. (14) have proposed that it is from this segregated circular molecule, deleted for F DNA, that the cotransduction of *ilvD*$^+$, *metB*$^+$, and *argH*$^+$ occurs. This model was proposed by Lee et al. after they obtained evidence demonstrating that all F-*ilv*'s made by transductional shortening of F14 (19, 26) were of the same length as the P1 genome (100 ± 5 kb). They reasoned that P1 should be able to pack-

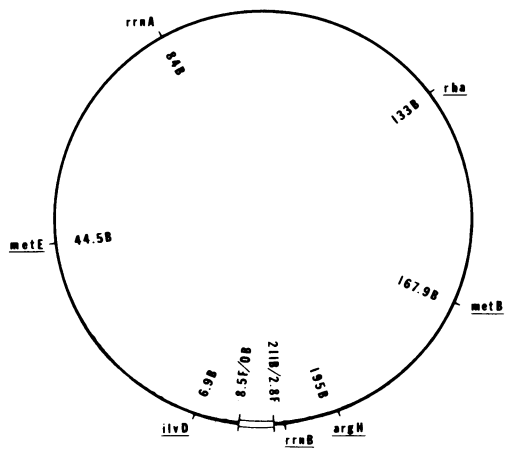

FIG. 7. *Fine-structure map of segregated circle formed by the reciprocal crossover at the 2.8 F-8.5 B junction on the F14. Modified from reference 16.*

age a 100-kb segment from the large segregated circle, which could include *ilv*, *metB*, and *argH*.

Although some *ilv*$^+$,*argH*$^+$,*metB*$^+$ transductants may arise by this means, this mechanism, without modifications, does not provide for many of the observation reported here. Specifically, some transductants can transfer *ilv*, *metB*, and *argH*, an activity requiring most of the F genes that are between *arg* and *ilv* on the F14. Further, many transductants transfer *rha*$^+$, a gene carried near the middle of F14. Many transductants that did not transfer these genes did segregate them. It seems likely that these genes are carried on a replicon which is self-replicating because of F genes carried on the plasmid. Alternately, certain P1 genes might provide the replication functions if a P1-*ilv*,*arg*,*met* plasmid had formed (17, 25, 27). Such a plasmid would likely have to form in the recipient since it would be too large for a normal P1 headful, as were several P1*dlac* (25) and P1*pro* (27). However, P1 DNA may not be able to integrate regions other than *lac-pro*. Heteroduplex analysis of transductionally shortened F-*ilv*'s showed that these plasmids did not carry and P1 DNA (14). The presence of P1 functions (phage production, immunity, restriction) has been examined and is found in some but not all transductants. Heteroduplex analysis of these nontransferring plasmids would be necessary for certain characterization.

In the model of Lee el al. (14), *ilv* and *arg* are carried on a "P1-genome size" linear segment of DNA. This could account for the initial "coinheritance" of *ilv* and *arg* in some strains. Two recombination events (with the *ilv* and *arg* regions) would generate Ilv$^+$,Arg$^+$ transductants. In the resulting strain, however, linkage of *ilv* and *arg* would no longer exist. The *ilv* genes would not recombine with the *arg-purD* region, and the *argH* locus would not recombine in the *rbs-ilv* region at the frequencies observed.

If linear segments from the circles of DNA segregating from F14 as described by Lee et al. (14) could recircularize once they are inside the recipient cell, small circles might then insert into the vegetative chromosome by a single crossover. The latter could occur by a legitimate crossover between the exogenote and endogenote *ilv* or *arg* region. Random insertion of these circles into the endogenote region corresponding to the F14 bacterial sequence (Fig. 8) would complicate the analysis of the gene sequence in these unusual linkage strains.

Pittard and Adelberg (Bacteriol. Proc., p. 138, 1963) destroyed the ability of the P1 lysate to cotransfer these three genes by treating the

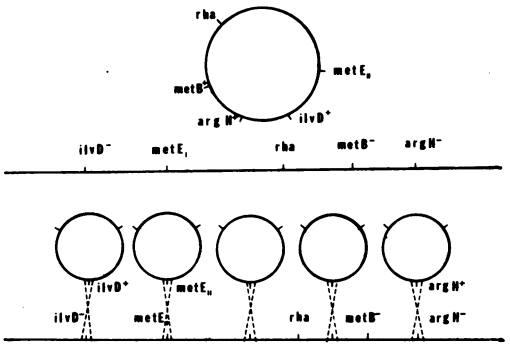

FIG. 8. *Illustration of multiple integration sites of a plasmid carrying transduced merogenote sequence.*

P1·AB1206 lysate with P1 antiserum. They also found that the activity sedimented with the P1 PFU.

Transductions carried out after treatment of the P1 lysate with deoxyribonuclease did not alter the efficiency of "cotransduction" of *ilvD*, *metB*, and *argH*. In these experiments, no transformation of any kind had occurred; thus, the data had no positive control. Transformation of the same recipient for His$^+$ was sucessful after calcium shock, but Ilv$^+$ was not transformed with DNA form AB1206. We were unable to transform any of three F-merogenotes into this strain under these conditions. These experiments are being repeated with a known transformable strain of *E. coli*. Thus far, transformation as the mode of genetic transfer of F14 has not been supported.

No colony-forming units were present in the chloroform-sterilized P1 lysates. Further, the presence of nalidixic acid in the mating mixture did not reduce the frequency of cotransfer of *ilvD*$^+$, *metB*$^+$, and *argH*$^+$ either before or after plating on selective media. Conjugation as the primary mechanism of transfer is not supported. Nalidixic acid did not affect transducing efficiency for the three markers.

Since the F14 is presumably too large (311 kb) to be transduced, one model that had been considered involves reassembly, by mechanisms suggested by Rae and Stodolsky (25), of several F14 segments resulting from multiple transfers into the same cell. Transduction of the F DNA (100.2 kb) segregated from F14 is possible but, theoretically, not the 211-kb circle (21) as one piece; two or more segments would be necessary. If the transduction is carried out using a multiplicity of infection of 1, the efficiency of transducing the chromosomal *ilv* gene is about 10^{-4}/PFU (Table 2); the efficiency of transducing one particular 2-min (100 kb) segment, including the *ilv* gene, would be consid-

erably lower. We have found the chromosomal *ilv-metE* segment (about 1 min, 46 kb) to be cotransduced at an efficiency of about 5×10^{-5}/PFU at MOE = 1. The efficiency of transducing the *ilv* genes from F14 is 10^{-5} (Table 2); the efficiency of cotransduction of the F14 *ilv-metE* segment would be lower than *ilv* alone. If we assume that the efficiency of cotransducing 2-min segments of the F14 was about the same as the *ilv-metE* region and if this efficiency were even as high at 10^{-5}/PFU, then, among 10^9 PFU (the number of phage used as MOE = 1), 10^4 transducing particles would carry any one of those 2-min segments from the F14. Since each transducing particle can carry about 100 kb, it would take at least three phages to transduce the whole F14 (311 kb). From these numbers, a first estimate of the probability of three phages carrying any three F14 segments infecting the same cell is on the order of 10^{-15}. We have found an efficiency of 7×10^{-8} for coinheritance of all three selected genes (as well as *metE*$^+$ and *rha*$^+$) plus F DNA (Table 3).

Poisson probability of multiple infection producing an F14 can be determined. The number of phages among 10^9 PFU that are carrying any of the three F14 segments is 3×10^4 phages or less. The multiplicity of infection with respect to these three transducing particles on 1×10^9 recipient cells is 3×10^{-5}. If three particles are required for the transduction of the F14, then, according to Poisson distribution, the probability of any one cell being infected by any three particles of this group all at once is 2.2×10^{-14}. It is obvious that the probability of a cell receiving three different F14 segments is lower than that of a cell receiving any random 2-min segment of F14. As mentioned above, efficiency of the lysates used in this study for "cotransducing" *ilvD*$^+$, *metB*$^+$, *argH*$^+$, and F was found to be on the order of 7×10^{-8} (Table 3). This value is over a million times higher than the predicted probability of any one cell being infected by any three transducing particles, each carrying a random 100-kb segment of the F14. Harriman (9) suggests that there may be regions on the *E. coli* K-12 chromosome that may be more preferentially packaged. If this were true for F14 fragments, the probability of transducing several segments into one cell could be a little higher, but it seems unlikely that it would raise this several log cycles. Multiple infection by independent, unaggregated transducing particles seems unsupported. In preliminary experiments a curve relating multiplicity of infection and frequency of three-marker cotransduction (25) is linear with a slope of near one (unpublished data), suggesting that one particle (or one aggregate) is sufficient.

In the few transductions carried out in RecA recipients, only shortened merogenotes (F-*ilv*) have been found. This suggests the possibility that some kind of recombination event is required for the apparent cotransduction of the whole F14. However, the greatly reduced frequency of all types of transductants (including shortened merogenotes) in RecA recipients makes analysis of the alternatives very difficult.

The process of genetic exchange observed in this study involves the phage P1. As has been discussed above, random multiple infection by three separate transducing particles has been calculated to be too rare an event to explain this phenomenon. Alternate explanations have been considered. (i) The phage normally has an incompletely filled head (unlikely since most virions do not take negative stain [34]) and, as a rare event, is capable of packaging a large extensively folded and condensed plasmid DNA molecule. (ii) A giant phage particle (such as those found in T4 lysates [6, 8]) may be formed by P1 around DNA molecules with certain unknown properties that may be found on F14. Arguing against these possibilities is the failure of others to transduce hybrid P1 plasmids larger than the P1 genome (P1*dl* and P1*pro* [25, 27]). If, however, either of the above particles exists, it should be detectable by appropriate centrifugational analysis. The ability to transduce F14-like plasmids (but not *ilv*$^+$ alone or *ilv*$^+$, *arg*$^+$, *met*$^+$) was lost each time CsCl density gradient analyses were attempted. Several studies on CsCl gradient analysis have reported 30 to 40% loss of input PFU (12, 13). P1 transducing particles carrying the F14 (if they exist) may be among these sensitive particles. Rosner (27) was also unable to detect large particles on the basis of density in CsCl gradients.

A third explanation considered was that aggregates of transducing particles carrying adjacent segments of the F14 (packaged sequentially, by the headful, in transducing particles within one donor) might be responsible for the apparent cotransduction of the F14. Each of the members of the aggregate transducing particles would inject each of the various sections of the F14 into a host, where upon the segments would be somehow reassembled. If these aggregates were held together by calcium ions (13), then transfer of the transducing lysate into calcium-free CsCl should cause a disassembly of the aggregates. Total chelation of Ca^{2+} by ethylenediaminetetraacetate did lead to a loss of ability in the lysates to transduce any gene, since Ca^{2+} is required for P1 adsorption (15). Readdition of calcium ion restored the ability to transduce both chromosomal genes and F14-

like plasmids (unpublished data). Thus, we have no explanation at this time for the loss of ability to transduce F14-like plasmids in CsCl. Other kinds of centrifugational analysis are being carried out.

To summarize, transductants that have inherited a genetic element resembling the F14 have been described. No indication as to how they have arisen has been obtained. There is as yet no evidence supporting the role of large or very dense P1 particles. Probability argues against multiple infection, and the linear relationship between numbers of particles used and F14-like transductants obtained favors the role of a single unit: a virion or an aggregate. But removal and replacement of calcium did not prevent the appearance of F14-like transductants as would be expected of calcium ion-dependent aggregates.

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