F-PRIME FACTORS OF *SALMONELLA TYPHIMURIUM* AND AN INVERSION BETWEEN *S. TYPHIMURIUM* AND *ESCHERICHIA COLI*

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THE *Escherichia coli* K12 sex factor has been transmitted into *Salmonella typhimurium* LT-2, producing an F^+ strain (ZINDER 1960). Hfr donors were isolated in which the F-factor is stably attached to the chromosome and which transfer the chromosome in high frequency in a linear, oriented fashion to F^- recipients (ZINDER 1960; SANDERSON and DEMEREC 1965).

Strains of *E. coli* harboring autonomous F′ (F-prime) factors in which a portion of the terminal segment of the bacterial chromosome is attached to the F-factor have been isolated from Hfr strains (JACOB and ADELBERG 1959; ADELBERG and BURNS 1960). These F′ donors are especially valuable for transferring specific chromosomal loci between strains or species and for complementation analysis.

This paper reports the isolation and analysis of F′ factors which carry the terminal section of the chromosome of *S. typhimurium* HfrB2, including the gene loci *pyrF cysB trpABEDC*, in that order. Strains carrying the F′ factors mobilize the chromosome in the same direction as HfrB2, but unlike HfrB2, transfer *pyrF cysB trp* as proximal loci. The chromosomes of *S. typhimurium* and *E. coli* K-12, which are similar in the arrangement of most genetic loci (SANDERSON and DEMEREC 1965), differ by an inversion of the *pyrF cysB trp* region. The observation that inverted segments of the DNA can function in protein synthesis (SANDERSON 1965; BECKWITH and SIGNER 1966; BERG 1967) strongly suggests that messenger RNA can be transcribed from either of the DNA strands of the bacterial chromosome.

MATERIALS AND METHODS

The minimal medium and supplements were as described in SANDERSON and DEMEREC (1965). In transduction studies, bacteriophage P22 was used. Recipient bacteria for the transduction experiment were cultured overnight on nutrient broth on a shaker, infected with phage grown on the donor bacterium, and plated on minimal medium. The multiplicity of infection was usually five.

The recommendations of DEMEREC *et al.* (1966) are followed in nomenclature of the bacterial strains. Genotype is indicated by a three letter italicized symbol, followed by a locus designation and an isolation number, e.g., *trpA52* for a tryptophan-requiring mutant. Phenotype is indicated by the same three letter symbol, with a capital letter and not in italics, e.g., Trp. The alleles for streptomycin resistance are given serial isolation numbers as listed in Table 1. Those strains

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TABLE 1

Strains of Salmonella typhimurium *used in this study*

Stock No.*	Genotype Chromosome	Sex factor	Source
SR279	*hisD23 gal-50*	F+	ZINDER
SR315	*leu-256*	F21	ZINDER
SU64	*trpC3 cysB12*	F-	DEMEREC
SU418	*pro-26* (P22)+	HfrB2	.....
SU646	*trpD7 leu-14 gal-24*	F-	DEMEREC
SU655	*pyrF146 thi-73*	F21	.....
SU665	*hisF1009 trpA52 cysB12 metA22 xyl-1 str-201*	F-	.....
SU687	*trpA52 cysB12 pyrF146*	F-	.....
SU689	*trpA52 cysB12 pyrF146 ile-78*	F-	.....
SU698	*trpA52 cysB12 pyrF146 his-1034 str-203*	F-	.....
SU700	*hisB-1025 tre-3 str-202*	F-	.....
SU639	*his-1009 metA22 xyl-1 str-201 trpB2*	F71	.....
SU638	*his-1009 metA22 xyl-1 str-201 trpB2*	F72	.....
SU640	*his-1009 metA22 xyl-1 str-201 trpB2*	F73	.....
SU641	*his-1009 metA22 xyl-1 str-201 trpB2*	F74	.....
SU643	*his-1009 metA22 xyl-1 str-201 trpB2*	F75	.....
SU658	*trpD7 leu-14 gal-24*	F72	.....
SU694	*trpA52 cysB12 pyrF146 ile-78*	F71	.....
SU699	*trpA52 cysB12 pyrF146 ile-78*	F72	.....
SU695	*trpA52 cysB12 pyrF146 ile-78*	F73	.....
SU696	*trpA52 cysB12 pyrF146 ile-78*	F74	.....
SU697	*trpA52 cysB12 pyrF146 ile-78*	F75	.....

* All strains are *S. typhimurium* LT2, except for SU646 and SU658, which are *S. typhimurium* LT7.

for which no streptomycin allele is listed have the wild-type, or sensitive, reaction to streptomycin; this wild-type, or sensitive allele, may be listed as *str-0*. The following abbreviations will be used for genotypes: *cys*, cysteine; *gal*, galactose; *his*, histidine; *ilv*, isoleucine; *leu*, leucine; *met*, methionine; *pro*, proline; *pyr*, pyrimidine (uracil); *thi*, thiamine; *tre*, trehalose; *trp*, tryptophan; *str*, streptomycin; *xyl*, xylose.

Most of the strains used are listed in Table 1. HfrB2 (SU418) was isolated from SR279, an F+ strain of *S. typhimurium* (ZINDER 1960). The polymutant strain SU687 (*pyrF146 cysB12 trpA52*) was synthesized in the following manner. The donor strain SU655 was prepared by transmitting F21 from SR315 into *pyrF146 thi-73*. F21 carries no known chromosomal loci, but mobilizes the chromosome with *trp* near the proximal end (ZINDER 1960; SANDERSON and DEMEREC 1965). SU655 was mated with SU64 (*trpC3 cysB12* F-), and PyrF- CysB+ TrpC- recombinants were selected. One of these recombinants was transduced with P22 (H4) phage which had been grown on *cysB12 tryA52*, and PyrF- CysB- TrpA- TrpC+ recombinants were selected. This last transduction step was possible because TrpA- mutants grow on anthranilic acid, whereas TrpC- mutants do not. Further auxotrophic mutants were induced in the strain by treatment with N-methyl-N'-nitro-N-nitrosoguanidine and were selected on a partially enriched medium.

Recombinants from crosses were tested for unselected markers by transferring 40 recombinant colonies to a master plate of the selective medium, incubating, and replicating the plate to appropriate media. Fertility of the recombinants was tested by replicating onto a plate of minimal medium spread with an auxotrophic recipient, and examining for recombinants.

Mating for kinetic studies was carried out by the methods described in SANDERSON and

DEMEREC (1965), in which effective contacts between donor and recipient cells were formed on a Millipore filter. Growth of the donor strains and plate mating were prevented by omitting from the minimal medium the supplements required by the auxotrophic donor strain, and by adding streptomycin sulfate (usually 1200 μg/ml) to eliminate the Str-s donor strain.

Numerous other bacterial strains, and the P22 bacteriophage used in this study, belong to the stock collection of the late Dr. M. DEMEREC.

RESULTS

Isolation of F–trp *factors:* HfrB2 (SU418 *pro-26*), which donates *his* as a proximal locus, and *trp* as a distal locus, was mated with SU453 for 60 minutes. The mating was interrupted and the cells were plated on a medium which selected for Trp$^+$ Str-r recombinants. Because the transfer time of the entire *S. typhimurium* chromosome is about 138 minutes (SANDERSON and DEMEREC 1965), and because HfrB2 transfers *trp* distally, interruption at 60 minutes selects for recombinants which received the F–*trp* factor. Fifty-seven Trp$^+$ recombinants were tested for their capacity to transmit the *trp*$^+$ loci at high frequency, and five fertile isolates were detected.

The following properties demonstrate that these *Trp*$^+$ fertile recombinants carry F′ factors: (1) These five recombinants SU638, SU639, SU640, SU641, and SU643, are heterogenotes, segregating 1 to 5% infertile Trp$^-$ cells after overnight growth in broth. The Trp$^-$ colonies are stable, whereas Trp$^+$ colonies continue to segregate through several single colony isolations. (2) The F-prime factor is transmitted to *trp*$^-$ F$^-$ strains at a frequency of 1 to 10 recombinants per 100 donor cells. The resulting recombinants are usually fertile heterogenotes. (3) The F-prime strains are able to mobilize their chromosome in the same direction as HfrB2, but unlike HfrB2, the *trp* locus enters first rather than last.

All five F′ strains, SU639(F71), SU638(F72), SU640(F73), SU641(F74), and SU643(F75), are sister isolates from a cross of SU418 × SU453, and hence could be members of one clone, resulting all from a single event in SU418, followed by multiplication and subsequent mating of several clones with SU453. F71 is demonstrated below to carry less chromosomal material than the others, but these other four F′ factors, indistinguishable in phenotype, may be members of a single clone.

Loci carried on the F-prime factor: The five F-prime strains were crossed with SU687 (*pyrF146 cysB12 trpA52*) and Pyr$^+$, Cys$^+$, and Trp$^+$ recombinants were selected independently. In crosses with F72 to F75, regardless of the selected locus, all three loci, as well as the F-factor, were usually transmitted together. These recombinants were heterogenotes, yielding approximately 5% segregants, most of which lost the three wild-type alleles, as well as the F-factor, in a single step. F71 carries only the *trp* loci, for Trp$^+$ fertile heterogenotes were the most frequent class from crosses of F71 with SU687, and the frequency of Cys$^+$ and Pyr$^+$ recombinants was only 0.1% of the frequency of Trp$^+$ recombinants. In crosses to strains carrying mutations in each of the loci *trpA*, *trpB*, *trpC* and *trpD*, the five F′ factors were each shown to carry all of these *trp* loci. Strains carrying mutations in loci known to be in this region of the chromosome (*aroA*, *aroE*, *purB*,

pyrC, pyrD, rfcC) (SANDERSON and DEMEREC 1965), and the frequency of transfer of these loci was found to be less than one percent of that of loci known to be on the F′ factor or known to be transferred early on the chromosome of HfrB2.

Strains carrying each of the F-primes were crossed to SU700 (*his-1025 tre-3 str-202*) and a total of 32 Tre$^+$ isolates were isolated and tested for fertility. Since none transmitted *trp*$^+$ at the frequency characteristic of F′ strains, *tre* also is not carried on the F-primes.

Kinetic studies: F72 was transmitted from SU638, in which it was originally isolated, into SU646 (*trpD7 leu-14 gal-24*) to obtain a fertile Trp$^+$ strain which is His$^+$ Str-s (SU658). The entry time of F′ and chromosomal loci was studied by interrupted mating (Figure 1). The loci carried on the F′, *cysB*$^+$ and *trp*$^+$, both enter at 10 min, while the time of entry of *his*$^+$, a chromosomal locus, is 20

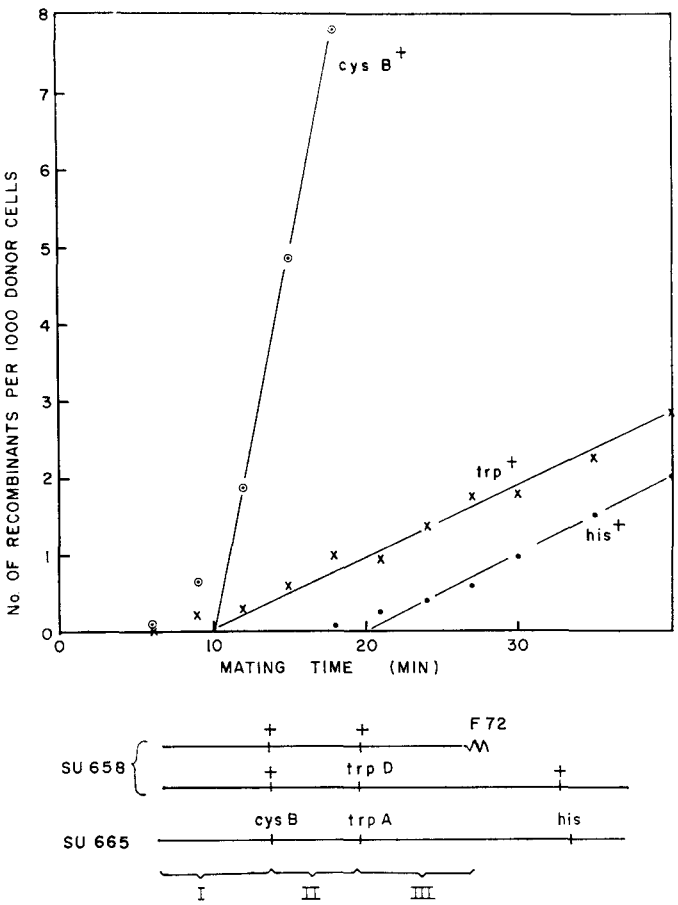


FIGURE 1.—Interrupted conjugation cross, SU658 (F72) × SU665 (F$^-$). The inferred genotype of SU658 and SU665 is shown under the graph. F72 is the upper line with the F-factor attached. SU658 is a heterogenote for *trpA* and a homogenote for *cysB* (*cysB*+ *trpA*+ F72/*cysB*+ *trpA52*).

min. The latter time is 5 min greater than the 15 min required for *his*$^+$ entry from HfrB2 (SANDERSON and DEMEREC 1965). Studies by PITTARD and ADELBERG (1964) showed that the delay of initiation of chromosome transfer in F′ strains when compared to the Hfr from which they originate is a measure of the length of the chromosomal material on the F′ factor. The length of chromosomal material on F72, therefore, requires 5 min to enter the recipient. Since the length of the *S. typhimurium* chromosome is 138 min (SANDERSON and DEMEREC 1965), this suggests that the F′ factor is 5/138 or approximately 4% of the length of the entire chromosome. However, variation in entry time for *his*$^+$ in different crosses indicates that the estimate of 5 min length may be too great.

SCAIFE and GROSS (1963) and PITTARD and ADELBERG (1964) demonstrated that chromosome transfer by F′ strains occurs as a result of a crossover between donor chromosome and the F′ factor. According to this model, recombinants for alleles from the donor chromosome are the result of two independent crossing-over events, the first in the donor, between the chromosome and the F′ factor (donor crossover) and the second in the recipient, between the transferred chromosome and the homologous region of the recipient (recipient crossover). This model was tested in the cross of SU658 (F72) × SU665 (Figure 1). Transfer may be inferred to begin from the "origin" of F72; a donor crossover in region III will transfer *cysB*$^+$ *trp*$^+$ from the donor into the recipient, while if the donor crossover is in region II *cysB*$^+$ *trpD* will be transferred. The occurrence of these two types of crossovers can be detected in this cross with F72 (Figure 1), in which the *trp* alleles are nutritionally distinguishable: The *trpA52* allele of the recipient permits growth on tryptophan or anthranilic acid, whereas the *trpD7* allele of the chromosome allows growth on tryptophan only, and the *trp*$^+$ allele of F72 permits growth on minimal medium. The His$^+$ recombinants (Figure 1) which have received the *his*$^+$ allele of the donor chromosome, were analyzed for *trp* and *cysB* alleles. According to the phenotypic analysis, of 296 His$^+$ recombinants, 3 were *trp*$^+$ (donor F′ allele), 149 were *trpD* (donor chromosome), and 144 were *trpA* (recipient chromosome). The relative frequency of these three types did not vary significantly over the period of selection from 20 to 40 minutes. Therefore, of those recombinants which received a donor *trp* allele, 3 of 152 (2%) received the donor F′ allele, whereas 149 of 152 (98%) obtained the donor-chromosome allele. Thus, when the F′ factor mobilized the donor chromosome, 98% of the donor crossovers occurred in either region I or II, while only 2% occurred in region III (see Figure 1). Considering the recipient crossover, of 296 His$^+$ recombinants, 152 (51%) integrated a donor *trp* allele, and 144 (49%) the recipient *trp* allele. These data indicate that *trp* is donated as a proximal locus to all recombinants which receive the chromosomal *his*$^+$ locus, and that in 51% of cases it is integrated into the recombinant.

The six times greater frequency of CysB$^+$ than Trp$^+$ recombinants, though both enter at 10 min after mating, occurs because the genotype of the male parent (SU658 F72) is *cysB*$^+$ *trp*$^+$ F72/*cysB*$^+$ *trpD7*. Hence recombinants which receive the *cysB*$^+$ allele of F72 or the donor chromosome are Cys$^+$, but Trp$^+$ recombinants must receive the F72 allele, not the donor-chromosome *trpD7*

allele. The suggestion that there are *trpD* recombinants, not detected in the time-of-entry test, is confirmed by the observation that among His^+ recombinants, the sum of the frequency of *trpD* and of trp^+ is about equal to $cysB^+$.

The locations of pyrF, cysB, *and* trp *on the* S. typhimurium *map:* The following data indicate that the map order is *pyrF–cysB–trpABEDC–his:*

(1) P22-mediated joint transduction studies using a wild-type donor and SU687 (*pyrF146 cysB12 trpA52*) as recipient have indicated that all three loci are carried by the same transducing fragment, and that these loci are in the order *pyrF–cysB–trpA*. (Figure 2)

(2) From the cross, SU655 (*pyrF146 thi-73* F21) × SU64 (*trpC3 cysB12*), 244 Thi^+ $CysB^+$ recombinants were isolated and analyzed. 213 were donor type, 16 were prototrophs, 2 were Trp^-, and 13 were $TrpC^-$ $PyrF^-$. According to the map order, *pyrF–cysB–trpC*, all of these recombinants could be double crossover types. However, according to the alternative order *pyrF–trpC–cysB*, the 13 $TrpC^-$ $PyrF^-$ recombinants must be quadruple crossover types; and according to the order, *cysB–pyrF–trpC*, the 16 prototrophs must be quadruple crossovers.

(3) $PyrF^+$ recombinants from the cross, SU695 (F73) × SU698, are shown in Table 2. The "+" alleles are donor F′ markers in this cross. The order, *pyrF–trpA–cysB*, is excluded, for there are 52 $PyrF^+$ $CysB^+$ $TrpA^-$ recombinants, but only 2 $PyrF^+$ $CysB^-$ $TrpA^+$. The orders, *trpA–pyrF–cysB* or *pyrF–cysB–trpA*, are both consistent with these data.

(4) *pyrF* cannot be located between *cysB* and *trp*, for deletion mutants of *cysB* and *trp* in *S. typhimurium* are not Pyr^- (MARGOLIN and BAUERLE 1966).

(5) The order, *trpOABEDC* has been established by transduction analysis,

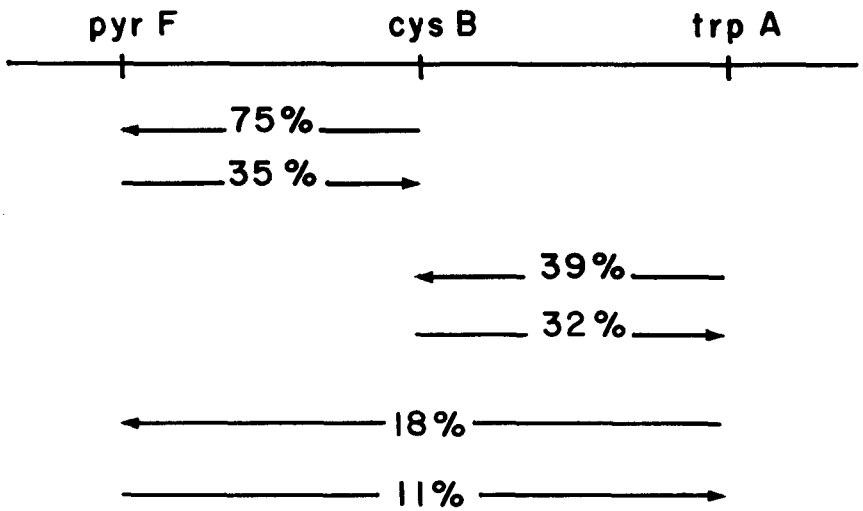


FIGURE 2.—Structure of the *pyrF–cysB–trpA* transducing fragment of bacteriophage P22. The arrowhead indicates the selected donor locus, and the percent shows the frequency of joint transduction of the other locus on the arrow, which was unselected. Samples of 400 or more transductants were analyzed to obtain each percentage. P22 phage grown on wild-type bacteria was the donor, SU687 was the recipient.

TABLE 2

Genotypes of PyrF+ Ile+ Str-r *recombinants from a cross, SU695 (F73) × SU698, for unselected markers**

Class	*pyrF*	*cysB*	*trpA*	*his*	Frequency
(1)	+	+	+	+	18
(2)	+	+	+	—	146†
(3)	+	+	—	+	3
(4)	+	+	—	—	51
(5)	+	—	—	+	0
(6)	+	—	—	—	18
(7)	+	—	+	+	0
(8)	+	—	+	—	2

* The interrupted conjugation experiment from which these recombinants are taken is shown in Figure 4A1; the genotype of the parent strains is in Figure 4A2.

† Approximately ⅓ of the recombinants of class 2 are fertile, and transfer *trp*+ and chromosomal loci to recipients. No fertile recombinants have been detected among classes 3 to 8; class 1 was not tested.

including the use of extensive numbers of overlapping deletions (BLUME and BALBINDER 1965; BAUERLE and MARGOLIN 1966; MARGOLIN and BAUERLE 1966; DEMEREC and HARTMAN 1956). The order of structural genes for enzymes with homologous functions with respect to the operator is the same in *E. coli* and *S. typhimurium*, though the letters used to designate gene loci are different.

The following data reveal the orientation of the *pyrF–cysB–trp* loci with respect to outside markers:

(1) An interrupted conjugation cross, SR315 (F21) × *cysB12 trpA3*, in which CysB+ or TrpA+ recombinants were analyzed for unselected markers, revealed the map order, *cysB–trpA–his* (SANDERSON and DEMEREC 1965). The cross, SR315 (F21) × SU698, reveals that *pyrF*, *cysB*, and *trpA* enter almost simultaneously at 8 min, while *his* enters between 15 and 20 min (Figure 3). The recombinants selected for a late mating time (30 min) were analyzed for unselected markers. Among the PyrF+ recombinants, 101/103 = 98% were Cys+, and 91/103 = 88% were Trp+. Among the Cys+ recombinants, 51/52 = 98% were Pyr+, and 47/52 = 94% were Trp+. Of 52 Trp+ recombinants, all were Pyr+ Cys+. Since SR315 donates in the order *trp–his- - -leu*, these data are in good accord with the order for SR315 entry as follows:

origin–*pyrF–cysB–trpA–his*.

(2) HfrB2 donates *his* as a proximal locus, but *pyrF*, *cysB*, and *trp* as distal loci (SANDERSON and DEMEREC 1965). The isolation from HfrB2 of F71, which carries *trp* but not *pyrF* or *cysB*, shows that *trp* must be closer to the chromosomal site of the F-factor in HfrB2, and hence closer to *his*, than is *pyrF* or *cysB*. Hence the order must be *pyrF–cysB–trp*–F– *his*.

(3) The factors F72 to F75 were transmitted into SU689 (*pyrF146 cysB12 trpA52 ile-73*) and fertile isolates were selected and mated with SU698. An interrupted mating, SU695 (F73) × SU698 (Figure 4A), shows that the three F′ loci enter at 10 min, with their order not distinguishable. The chromosomal locus

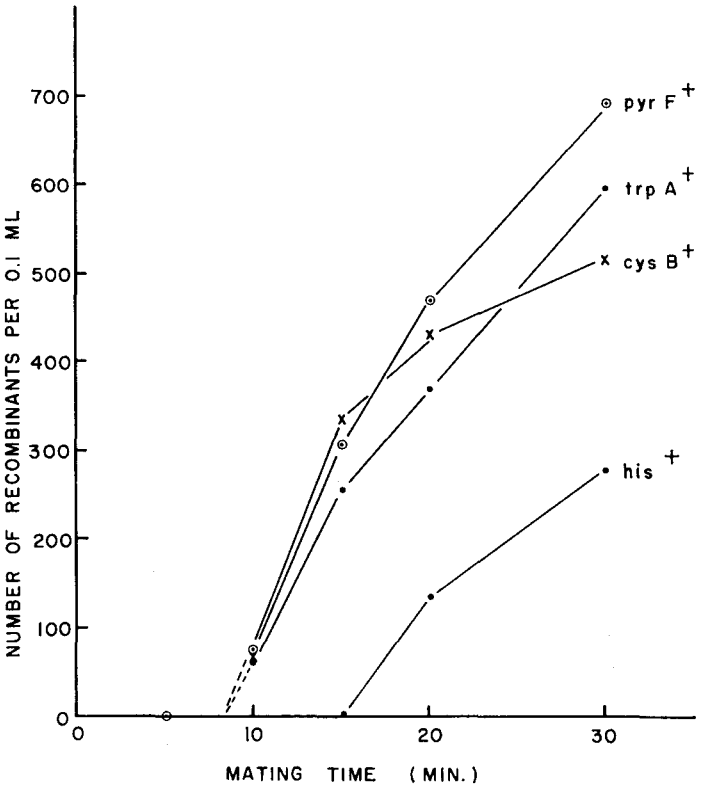


FIGURE 3.—Interrupted conjugation cross, SR315 (F21) × SU698.

his$^+$ enters at about 20 min. PITTARD and ADELBERG (1964) noted that recombinants for a chromosomal locus from a cross of F14 of *E. coli* received the proximal portion of the F′ factor more frequently than the distal portion, presumably because chromosome mobilization occurs due to crossing over from the F-prime factor to the donor chromosome. To permit similar analysis, 291 His$^+$ recombinants were tested, of which 8 were Pyr$^+$, 7 were Cys$^+$, and only 2 were Trp$^+$, indicating the number of the recombinants receiving donor F′ loci. These data suggest that the order of the loci on the F′ is *pyrF–cysB–trpA*, with the order on the chromosome, therefore, origin–*pyrF–cysB–trpA–his*, but the low frequency of transfer of F′ alleles to the recombinants makes this an inefficient method. The frequency of donor F′ *trp*$^+$ alleles among the recombinants (2 in 291) from this cross with F73 is similar to the frequency observed earlier in the cross with F72 (Figure 1).

Though His$^+$ recombinants begin to appear 10 min later than recombinants for PyrF$^+$, CysB$^+$, or Trp$^+$, the entry curve is much more steep and soon crosses the other curves (Figure 4A). This is because the only source of the normal allele of the loci *cysB* and *trp* is carried on the F′, and as in the cross, SU658 × SU665 (Figure 1), transfer of the F′ factor may be less frequent than mobilization of the chromosome. F72 and F75, transmitted into SU689 to produce donor strains

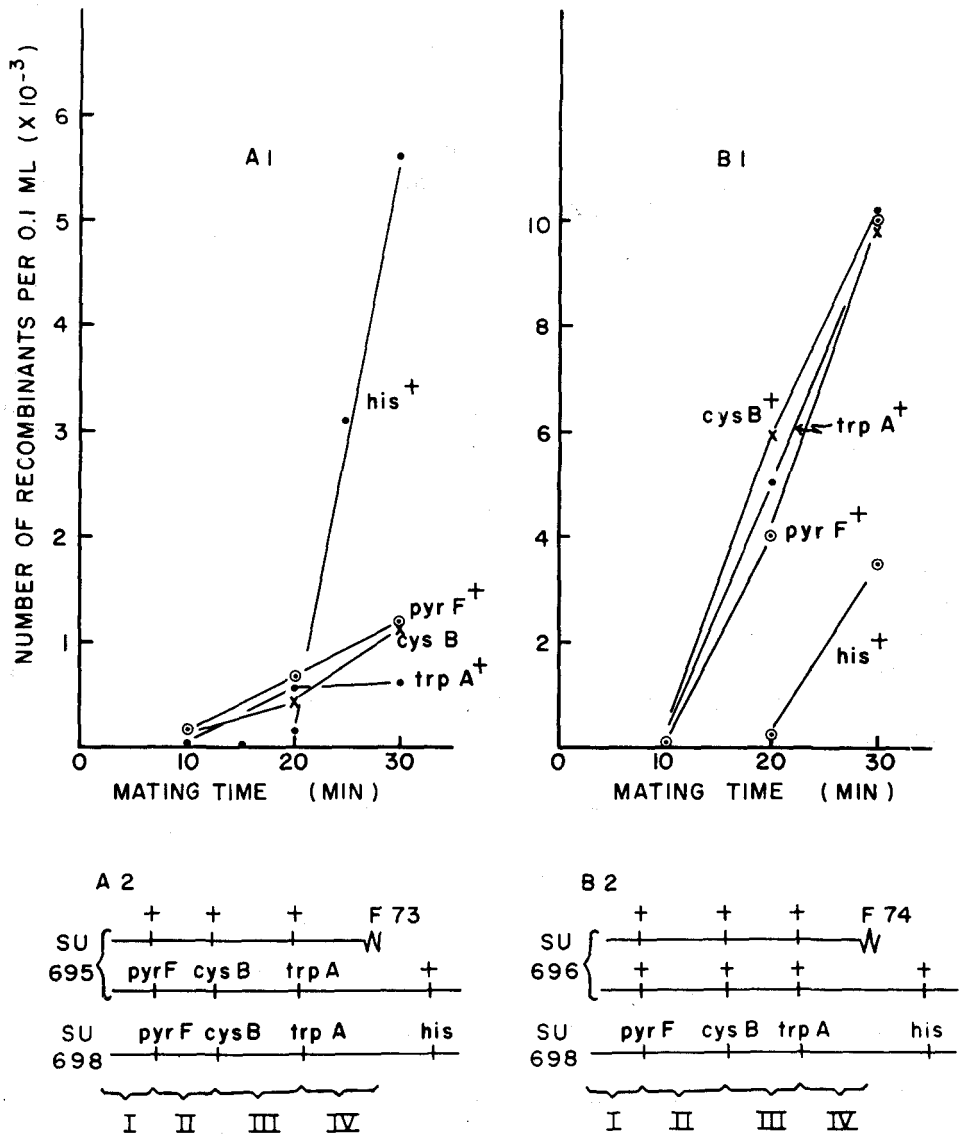


FIGURE 4.—Recombinants from interrupted conjugation crosses: A1, SU695 (F73) × SU698 (F⁻); A2, Inferred genotype of SU695 and SU698, F73 is shown as the upper line with the F-factor attached. SU695 is shown as a heterogenote for *pyrF*, *cysB* and *trpA*.—B1, SU696 (F74) × SU698 (F–); B2 Inferred genotype of SU696 and SU698, F74 is shown with the F-factor attached. SU696 is shown as a homogenote for the loci carried on F74 (see text).

SU699 and SU697, respectively, gave very similar data to those obtained with F73 (Figure 4A), but the data are not reported here. But F74 (SU696), when crossed to SU698 gave about six times as many recombinants for loci carried on the F′ than for *his* (Figure 4B). Strain SU 696, like SU699, SU695, and SU697,

should be a heterogenote, *pyrF*$^+$ *cysB*$^+$ *trpA*$^+$ F / *pyrF146 cysB12 trpA52*, but unlike the three latter strains which frequently segregate auxotrophs for these three loci, no such segregants have been detected among isolates from SU696. These findings suggest that a recombination has occurred during the isolation of SU696 by which this strain became a homogenote +++ F/ +++ for these three loci. This interpretation is strongly supported by analysis of 68 His$^+$ recombinants from this cross (Figure 4B), which showed that 32 (47%) carried the three F′ alleles, *pyrF*$^+$ *cysB*$^+$ *trpA*$^+$, for this high frequency of integration of the wild-type allele is about the same as the integration frequency of *cysB*$^+$ among the *his*$^+$ recombinants of F73 (Figure 1) in a case where *cysB*$^+$ was known to be carried on both donor F′ and donor chromosome.

Low frequency of simultaneous transfer of F′ and of chromosome: Among 587 His$^+$ recombinants which were tested, 296 from the F73 cross (Figure 1) and 291 from F72 (Figure 4A), only 5 received all their *pyrF cysB trpA* alleles from the F′ factor. All 587 recombinants were tested for fertility–i.e., for ability to transmit into suitable F$^-$ strains loci carried on the F′ and loci on the proximal region of the chromosome near *his*. None showed fertility, and it is inferred that none carried the F-factor. Among Trp$^+$ recombinants from crosses with these F′ factors, where chromosomal transfer was not selected, usually about 1/3 are fertile. These data show that chromosome donation by an F′ strain does not involve proximal transfer of the F-factor; this is expected according to the model that chromosome mobilization by an F′ is due to crossing over from the F′ to the chromosome between the origin and the F (Scaife and Gross 1963). These data also demonstrate that simultaneous donation of chromosome and intact F′ is very low, for it was not detectable among 587 recombinants for a chromosomal locus.

DISCUSSION

The circular linkage maps of *E. coli* (Taylor and Thoman 1964; Taylor and Trotter 1967) and of *S. typhimurium* (Sanderson and Demerec 1965; Sanderson 1967) are very similar in gene order. However, the two genera have now been proven to differ by an inversion, for data summarized in this paper reveal the order of loci in *S. typhimurium* to be *leu–pyrF–cysB–trpABEDC–his*, while the order in *E. coli* K-12 (Signer *et al.* 1965) is, *leu–trpEDCBA–cysB–pyrF–his*. Thus, the *pyrF–cysB–trp* region is inverted with respect to outside loci. There has been speculation that inversions might be lethal in bacteria. The bacterial genophore is a double-stranded DNA helix arranged as a closed circle (Cairns 1963). An inversion in the two stranded, anti-parallel DNA molecule will result in the DNA in the inverted segment having to switch from one strand to the other. If only one strand is transcribed into mRNA (messenger RNA) all around the chromosome, and subsequently translated into protein, then the inverted segment would not function in protein synthesis, and if essential functions are carried on this segment, the inversion would be lethal. The observation that the operons of *S. typhimurium* are polarized in both clockwise and counterclockwise directions (Sanderson 1965), however, suggests that mRNA can be transcribed from either

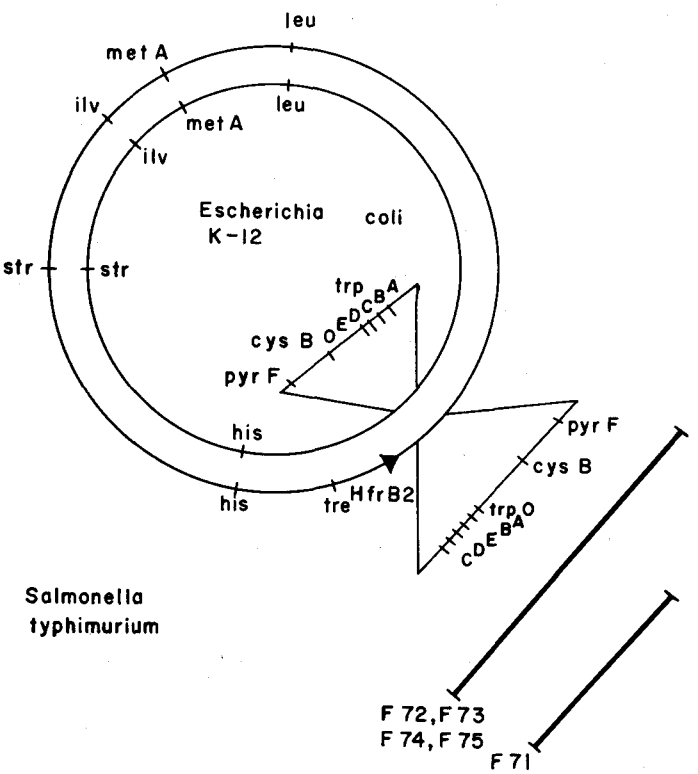
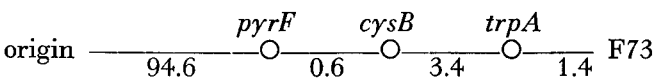


FIGURE 5.—Chromosomes of *S. typhimurium* and *E. coli* K-12, after SANDERSON (1967) and TAYLOR and TROTTER (1967). Distances on the map are not related to time of entry, or to relative physical lengths.

strand of the DNA, and further suggests that strains with inversions should be viable. It has now been proven that inverted segments of genetic material continue to function, for inversions have been reported within *E. coli* (BECKWITH and SIGNER 1966; BERG 1967), and between *E. coli* and *S. typhimurium* (this report), without loss of function in any case.

SCAIFE and GROSS (1963) presented a model for chromosome mobilization in F′ strains in which the F′ factor is transferred as a linear structure, and the mobilization of the chromosome occurs following a reciprocal genetic exchange between the F*lac* factor and the chromosome within the region of pairing. This model was confirmed by the observation that among recombinants receiving a chromosomal *pro* locus from the F′ strain, *lac*$^-$ was transmitted as a proximal locus in 85% of cases, *lac*$^+$ in 15% of cases, indicating a crossover between the point of origin on the F*lac* and the *lac* locus 15% of the time, between the *lac* locus and the F-factor 85% of the time. Similarly, PITTARD and ADELBERG (1963, 1964) noted that recombinants for a chromosomal locus from a cross of F14 of *E. coli* received the proximal portion of the F′ factor more frequently than the distal portion, presumably because chromosomal mobilization occurs due to cross-

ing over from the F′ to the donor chromosome. They also observed that this "donor crossover" occurred with equal probability per unit length along the F′ factor. The data reported in this paper may be similarly analyzed. Among 291 His$^+$ recombinants from a cross, SU695 (F73) × SU698 (Figure 4), the frequency of F′ and chromosomal loci was determined. The frequency of the F′ alleles among *his*$^+$ recombinants for each of the loci tested was as follows: *pyrF*, 8/291 = 2.7%; *cysB*, 7/291 = 2.4%; *trpA*, 2/291 = 0.7%. These numbers cannot be used directly to estimate the length of the F′ factor, because they do not take into account the "recipient crossover", necessary to integrate the donated loci into the recipient chromosome. In the cross, SU658 (F72) × SU665, the *trp* alleles of donor F′, donor chromosome, and recipient chromosome are nutritionally distinguishable. Alleles from the donor were integrated among 51% of the His$^+$ recombinants; the same general frequency was observed for donor alleles of *cysB* integrated among His$^+$ recombinants. Therefore, the transmission of *trpA*$^+$ alleles from the donor to the recipient must be about twice as frequent as their integration; hence, to obtain an accurate estimate of donor crossover frequency the percent of donor F′ loci must be multiplied by two. Based on these calculations, the genetic map of F73 based on donor crossovers is as follows:



It is not possible at this time to determine if this map of F73, based on donor crossovers, indicates physical map distances or is correlated with maps determined in other ways. Neither the distance from "origin" to *pyrF*, nor the distance from *trpA* to the F-factor, is known.

Comparison of these F′ factors with F′ factors of E. coli: The factors reported here show certain differences from the properties of most F′ factors reported from *E. coli*.

(1) Comparative frequencies of F′ and of chromosome transfer. According to Scaife and Gross (1963) the frequency of chromosome transfer is determined by the frequency of "donor crossovers" between F′ and chromosome. Confirming this prediction, Pittard and Adelberg (1964) noted that F′ strains which gave high frequency of transfer of F′ loci and low frequency of transfer of the chromosome (Type I) often changed spontaneously to a type with decreased transfer of distal F′ loci, and higher transfer of the chromosome (Type II). This change from Type I to Type II is, according to the model, to a type giving higher crossing over between F′ and chromosome. In most tests reported from *E. coli*, the frequency of transfer of F′ factors is greater than transfer of the chromosome. Scaife and Gross (1963) reported that F*lac* transfers *lac* 40 to 100 times more frequently than *pro*, an early chromosomal locus. Type I males give 3 to 10 times as much F′ transfer as chromosome transfer, but Type II males may give more chromosome transfer than transfer of distal F′ loci (Pittard and Adelberg 1963). The F′ factors of *S. typhimurium*, reported in this paper, mobilize the chromosome more frequently than they are transferred intact, and thus seem more closely

related to Type II. For example, the frequency of transfer of *trp*$^+$ from SU658 (*cysB*$^+$ *trp*$^+$F72 / *cysB*$^+$ *trp*$^-$) is only about 10% as frequent as the transfer of *cysB*$^+$, which is also transferred by chromosome mobilization (Figure 1). Among the Trp$^+$ recombinants, only about one third have received the F-factor, so we can estimate that transfer of the entire F′ factor is only about 1/30 as frequent as chromosome mobilization.

(2) Independent transfer of chromosome and F′ factor. SCAIFE and GROSS (1963) observed that a high frequency of the recombinants for *pro*$^+$, a chromosomal locus, obtained the F*lac* independently. Similarly, PITTARD and ADELBERG (1964) studied the frequency of independent transfer of the F′ factor to zygotes which received the chromosomal markers from the same F′ male, and observed that with F14, a large F′ factor, 3% of the F′ males transferred both chromosome and a second entire copy of F14 to the recipient. F′ strains carrying a smaller element, F16, gave independent transfer of F′ and chromosome as high as 80%. However, in our experiments, among 587 recombinants which integrated the chromosomal *his*$^+$ locus from the donor, (296 from F73 strains and 291 from F72 strains) no fertility was observed; thus, independent transfer of both chromosome and F′ factor occurs either extremely rarely or not at all.

These apparent differences between F72 and F73, on one hand, and F*lac*, F14 and F16, on the other, may simply be due to a very high frequency of crossing over between the chromosome of *S. typhimurium* and its F′ factors. This would produce the high ratio of chromosome mobilization to F′ transfer. The resulting low frequency of F-prime transfer is also a reasonable explanation for the failure to detect simultaneous transfer of the F′ into recombinants receiving chromosomal loci. This high frequency of crossing over might also result in genetic maps of the F′ elements, based on donor crossovers, with a disproportionate number of the crossovers in the early part of the map, next to the origin.

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SUMMARY

Five F-prime factors were isolated from HfrB2 of *Salmonella typhimurium*. F71 carries *trpOABEDC*, while F72, F73, F74, and F75 carry *pyrF–cysB–trpOABEDC*. Interrupted conjugation and P22-mediated transduction data, as well as data from the literature, reveal the map order, *leu–pyrF–cysB–trp OABEDC–his*. The order of the *pyrF–cysB–trp* loci is inverted in *Escherichia coli* K-12. Chromosome mobilization by F72 and F73 can be explained by the model of SCAIFE and GROSS (1963), in which a crossover from the F′ mobilizes the chromosome. However, the frequency of chromosome mobilization was greater than the frequency of F′ transfer by as much as 30 times in some cases, and there was no detectable simultaneous transfer of F′ and chromosome. This may be due to an unusually high frequency of crossing over between F′ and chromosome.

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