JOURNAL OF BACTERIOLOGY, July 1975, p. 1-6
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Vol. 123, No. 1
Printed in U.S.A.

Conservation of *Salmonella typhimurium* Deoxyribonucleic Acid by Chromosomal Insertion in a Partially Diploid *Escherichia coli* Hybrid

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Received for publication 25 February 1975

A partially diploid *Escherichia coli* hybrid recovered from mating with a *Salmonella typhimurium* donor was converted to an Hfr strain, designated WR2080, as a means to examine the manner in which the added *Salmonella* genetic material was conserved in it. The *Salmonella* $argH^+$, $metB^+$, and rha^+ alleles contained as supernumerary genes in WR2080 were transferred together to *E. coli* recipients in interrupted mating experiments approximately 25 min after initial parental contact; transfer of the allelic *E. coli* genes by a haploid Hfr of the same transfer orientation occurred between 23.5 min ($argH^+$) and 25 min (rha^+) after initial contact. Entry of the *E. coli* ilv^+ marker of WR2080 in these experiments occurred at 29.5 min, 1.5 min later than the entry time of this marker from the haploid *E. coli* Hfr. When unselected inheritance of the recessive *E. coli* $argH^-$ and rha^- alleles of WR2080 was examined among ilv^+ selected *E. coli* recipients in which unselected inheritance of the *Salmonella* donor genes was shown to be low (8%), inheritance of $argH^-$ was only 7%, whereas 51% inherited the neighboring rha^- gene. In a comparative cross employing a haploid *E. coli* Hfr, in which *rha* inheritance was similar at 56%, *argH* inheritance was 41%. It was concluded that the *Salmonella* genes contained in WR2080 were conserved on a genetic segment about 1.5 min in length chromosomally inserted near the allelic *E. coli* genes, thus creating a duplication on that region within the hybrid chromosome.

In a previous study (7) we examined the nature of partially diploid *Escherichia coli* hybrids formed in matings with a *Salmonella typhimurium* Hfr strain. We identified three types of partially diploid hybrids, differing with respect to the manner in which the *Salmonella* chromosomal fragments were conserved in them. The first type consisted of F′ strains in which the *Salmonella* genetic material was maintained extrachromosomally by association with a donor-derived F factor, as part of a functional F-merogenote. Such hybrids were lysed by the male-specific phage R-17, and were able to transfer their inherited *Salmonella* genes at high frequency in conjugation experiments. Diploid hybrids of the second type were not sensitive to R-17, nor were they able to transfer the *Salmonella* genes. Nevertheless, they responded as males when tested with the female-specific phage ϕII, and the *Salmonella* genes were conserved in them, extrachromosomally, as parts of covalently closed circular (CCC) molecules of deoxyribonucleic acid (DNA); these circular elements were considered to be defective F-merogenotes unable to direct synthesis of the F-pili upon which male-phage sensitivity and conjugal transmissibility depend. In diploid hybrids of the third type, which comprised the majority of those examined, the *Salmonella* DNA was conserved in some manner which involved neither association with F nor assumption of the CCC configuration. It was with a diploid hybrid of this latter type that we carried out the experiments detailed in the present communication. Our findings indicated that the added *Salmonella* DNA in this hybrid is not maintained extrachromosomally, but is conserved instead as a genetic segment inserted within the hybrid chromosome.

MATERIALS AND METHODS

Organisms. With the exception of the hybrid Hfr WR2080, the bacterial strains employed are described in Table 1. Transfer orientations of the Hfr strains listed in this table, as well as of the hybrid Hfr WR2080, are shown in Fig. 1. The characteristics of WR2080 are detailed in the Results section. To avoid possible confusion, we have used only the *E. coli* gene designations throughout the presentation, and thus refer to a homologous *Salmonella* gene by the same designation given its *E. coli* allele. For the sake of propriety, however, we point out here that the *Salmonella* genes which we refer to as *argB* and *argH*

TABLE 1. *Pertinent characteristics of the bacterial strains*

Strain	Description	Relevant genotype
WR2004	*E. coli* Hfr	*metB*
WR2005	*E. coli* Hfr	*purE*
WR2017	*E. coli* Hfr	*metB, argH, thiA, his, strA*
WR3026	*E. coli* F⁻	*ilv, metB, argH, thiA, his, xyl, malA, strA*
WR3029	*E. coli* F⁻	*ilv, metB, argH, thiA, his, xyl, rha, malA, strA*
WR3040	*E. coli* F⁻	*ilv, trp, xyl, rha, strA*
WR3041	*E. coli* F⁻	*ilv, trp, xyl, strA*
WR3051	*E. coli* F⁻	*proA, thr, leu, argB, thiA, his, strA*
WR4016	*S. typhimurium* Hfr	*serA*

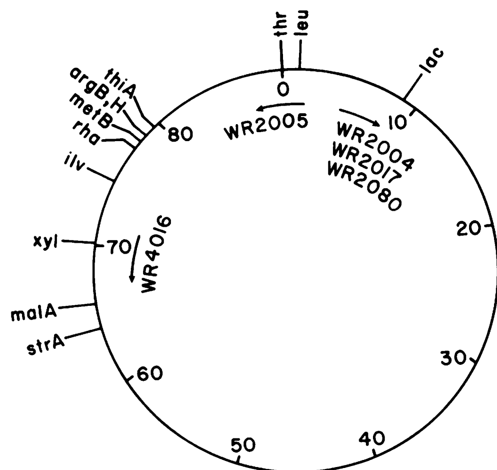

FIG. 1. *Chromosome map of E. coli K-12 showing the positions of relevant genetic loci and the transfer orientations of the Hfr strains employed. Time units are those of Taylor and Trotter (13).*

are, in the *Salmonella* nomenclature (12), referred to as *argC* and *argF*, respectively.

Noninterrupted mating and unselected marker analysis. As described previously (8), bacterial matings were carried out in Penassay broth for 2 h at 37 C after which the mating suspensions were diluted appropriately and plated on minimal agar selective medium. Hybrids were purified by streaking on minimal medium of the same composition as that used for their selection. Unselected inheritance of auxotrophic markers was scored by determining the ability of the hybrids to grow on minimal medium lacking the amino acid or vitamin in question. The ability to ferment a carbohydrate was scored on MacConkey agar indicator medium containing 1% of that carbohydrate. This medium was used also to identify partially diploid hybrids by their segregation of fermenting and nonfermenting clones as described previously (1).

Interrupted mating procedure. The procedure employed for interrupted matings was similar, essentially, to that described by de Haan and Gross (5). Prewarmed donor (5×10^8 cells/ml) and recipient (2×10^9 cells/ml) suspensions in Penassay broth were mixed with gentle agitation at 37 C for 5 min. This mating mixture was then diluted 1:200 into prewarmed liquid minimal medium and kept at 37 C without agitation for the duration of the experiment. At 3-min intervals, 0.5-ml samples of the minimal mating suspension were added to tubes containing 0.5 ml of Penassay broth, subjected to vigorous agitation for 60 s by a Vortex Jr. mixer, and plated (0.1-ml/plate) on appropriate selective media containing 625 μg of streptomycin sulfate per ml. The numbers of exconjugants found in successive plated samples plotted against the times of plating revealed the time of entry for the marker selected.

Bacteriophage testing. The procedures for testing sensitivity to the male-specific bacteriophage R-17 (3, 11), and for determining the male or female response of a hybrid to the female-specific bacteriophage φII (4), were described in our previous communication (7).

Examination for the presence of CCC DNA. Examination of hybrids for the possible presence of CCC DNA was accomplished by the dye-buoyant density centrifugation procedure of Bazaral and Helinski (2).

RESULTS

Construction of an Hfr derivative of a partially diploid S. typhimurium × E. coli hybrid. In previous crosses (7) between *S. typhimurium* Hfr WR4016 and the *E. coli* K-12 recipient strain WR3026, in which hybrids receiving an early transferred donor marker (xyl^+) were selected, approximately 40% of the *E. coli* exconjugants examined exhibited the segregation behavior of partially diploid hybrids. About 25% of those partially diploid hybrids were found to conserve the added *Salmonella* DNA as part of an F-merogenote. In some cases these F-merogenotes were functional, i.e., transmissible by conjugation, and in others they were not. Most of the partially diploid hybrids, however, were seen to conserve the added *Salmonella* genes in some manner which did not involve association with F. Nevertheless, because of the instability of the sex factor of *S. typhimurium* Hfr WR4016, many of the diploid *E. coli* hybrids in which conservation of the added *Salmonella* genes did not involve F did contain a superinfecting F factor.

For the purpose of the present study, it was necessary to obtain a partially diploid *E. coli* hybrid in which the added *Salmonella* genes were not conserved by association with F and in which no superinfecting F factor was present. It was desired also that the *Salmonella* genetic segment contained in this hybrid should bear

those markers which would allow the best determination of its length, with reference to the available markers of the recipient. To accomplish these ends, we first mated *S. typhimurium* Hfr WR4016 with *E. coli* WR3029, a Rha$^-$ derivative of WR3026, selecting for those hybrids receiving the *argH*$^+$ allele of the donor. We then tested these Arg$^+$ hybrids for sensitivity to the male-specific phage R-17, a procedure which eliminated from consideration (because of the indication of F-infection) the majority of the hybrids examined. Among the remainder, whose resistance to R-17 suggested that they had escaped F-infection, we narrowed further the scope of our consideration to those which also had acquired the donor *rha*$^+$ allele (for use as an indicator of diploidy), but not the adjacent (see Fig. 1) donor *ilv*$^+$ allele (to help define the limits of the *Salmonella* gene segment and to allow the subsequent introduction of a positive *E. coli* allele at this locus). We then searched among these hybrids for any which showed evidence of diploidy, as indicated by their segregation (on MacConkey rhamnose agar), of daughter cells from which the *Salmonella rha*$^+$ allele had been lost.

We selected for further study a partially diploid *E. coli* hybrid containing the *Salmonella* alleles *rha*$^+$, *argH*$^+$, *metB*$^+$, and *thiA*$^+$. This hybrid responded as a female when tested with the female-specific phage ϕII. It was examined by dye-buoyant density centrifugation for the possible presence in it of CCC DNA, and none was detected. Loss of the *Salmonella rha*$^+$ marker from this hybrid occurred spontaneously at low frequency, generating one segregant per 500 to 1,000 cells. Examination of Rha$^-$ segregants of this hybrid revealed that they had lost also the *Salmonella* genes *argH*$^+$ and *metB*$^+$. None of the segregants, however, were observed to lose the *thiA*$^+$ gene.

Anticipating the conversion of this partially diploid hybrid to an Hfr, we desired two additional allelic alterations, namely, its conversion from streptomycin resistance to streptomycin sensitivity, and the substitution of a positive *E. coli* allele at its *ilv* locus. The hybrid was mated, therefore, with the streptomycin sensitive *E. coli* Hfr WR2005, without streptomycin counter selection, and exconjugants were selected for receipt of the donor *malA*$^+$ allele. An exconjugant was chosen which had received, in addition to the selected *malA*$^+$ character, the *strA-s* and *ilv*$^+$ alleles of the donor. The responses of this exconjugant to R-17 and ϕII remained those of a female, and again, no CCC DNA was detected in it. It segregated Rha$^-$ daughter cells at a low frequency which, as before, had lost also the *Salmonella argH*$^+$ and *metB*$^+$ alleles. Again, all segregants remained Thi$^+$. Conversion of this exconjugant to an Hfr was then accomplished by the F-linked terminal marker (in this case *lac*$^+$) selection procedure (6) using the *E. coli* Hfr WR2017. The new Hfr generated by this procedure was designated WR2080. As did each of its predecessors, WR2080 segregated daughter cells exhibiting en bloc loss of the *Salmonella rha*$^+$, *metB*$^+$, and *argH*$^+$ alleles. As before, all segregants remained Thi$^+$.

Interrupted mating experiments with the partially diploid E. coli Hfr WR2080. In noninterrupted matings between the diploid *E. coli* Hfr WR2080 and *E. coli* recipient WR3029, the *Salmonella rha*$^+$ and *argH*$^+$ markers of the donor were transferred at approximately the same frequency (2×10^{-3}), and the transfer frequency of its *E. coli ilv*$^+$ marker was somewhat lower at 5×10^{-4}. In interrupted mating experiments performed with this pair, the following entry times were determined for the *Salmonella* markers of WR2080: *rha*$^+$, 24.7 min; *metB*$^+$, 24.8 min; *argH*$^+$, 25.3 min. The *thiA* allele of WR3029 proved unsuitable for use in these experiments, and no entry time could be determined for this donor marker. Entry time of the *E. coli ilv*$^+$ allele of WR2080 was 29.5 min. In comparative experiments using the same recipient in interrupted matings with the normal haploid *E. coli* Hfr WR2004, which has the same transfer orientation as WR2080, we recorded the following marker entry times: *argH*$^+$, 23.5 min; *rha*$^+$, 25.0 min; *ilv*$^+$, 28.0 min. The comparison of the marker entry times of this donor with those of WR2080 is shown in Table 2.

To reassure ourselves that the later entry times of the *argH*$^+$ and *ilv*$^+$ markers of WR2080 were not due to a difference in the injection speed of its chromosome from that of WR2004, we employed both Hfr strains individually in interrupted matings with the *E. coli* recipient WR3051 to examine the entry of a marker located proximal to the *argH* locus in the transfer order. The results of these experiments also are shown in Table 2. Entry of the *thr*$^+$-*leu*$^+$ (joint selection) alleles of Hfr WR2080 occurred at the expected time of 12 min, the same time as obtained for WR2004, thus indicating that there was no difference between these Hfr strains with respect to the speed of chromosome injection. Nevertheless, whereas entry of the *argB*$^+$ marker of WR2004 occurred at the expected time of 23.5 min (the *argB* and *argH* loci are inseparable by interrupted mating), entry of the *argB*$^+$ allele of WR2080 did not occur until 25.1

TABLE 2. *Comparative marker entry times (min) for the partially diploid E. coli hybrid HFR WR2080 and the similarly oriented haploid E. coli HFR WR2004*[a]

Interrupted mating pairs	Entry time (min)					
	thr$^+$-*leu*$^+$	*argB*$^+$	*argH*$^+$	*metB*$^+$	*rha*$^+$	*ilv*$^+$
WR2080 × WR3029			25.3	24.8	24.7	29.5
WR2004 × WR3029			23.5		25.0	28.0
WR2080 × WR3051	12.0	25.1				
WR2004 × WR3051	12.0	23.5				

[a] The *argB*$^+$, *argH*$^+$, *metB*$^+$, and *rha*$^+$ markers of WR2080 are derived from *S. typhimurium*.

min. The entry of the *Salmonella* alleles of WR2080 near the 25 min mark, coupled with the 1.5 min delay in the entry of its *E. coli ilv*$^+$ allele (29.5 min versus 28.0 min for WR2004) is consistent with the view that a segment bearing the *Salmonella* genes is inserted in the WR2080 chromosome near the region occupied by the allelic *E. coli* genes.

Inheritance of unselected markers derived from Hfr WR2080. We examined 200 of the earliest-appearing *argH*$^+$-selected WR3029 exconjugants formed in the interrupted matings with the diploid Hfr WR2080 for unselected inheritance of *Salmonella metB*$^+$ and *rha*$^+$ alleles; all were found to have inherited both of these alleles in addition to the selected *argH*$^+$ gene. We examined also 200 of the earliest appearing *rha*$^+$-selected WR3029 exconjugants formed in those interrupted matings for unselected inheritance of the *Salmonella metB*$^+$ and *argH*$^+$ alleles. Again, all had inherited both of these *Salmonella* alleles in addition to the selected *rha*$^+$ gene. Finally, we examined 100 *ilv*$^+$-selected exconjugants from a WR2080 × WR3029 cross for unselected inheritance of the *Salmonella rha*$^+$, *metB*$^+$, and *argH*$^+$ alleles. There were 42 exconjugants in which unselected inheritance of *Salmonella* alleles occurred and each of these 42 acquired all three *Salmonella* markers.

The inseparability of the *Salmonella* alleles in these experiments contrasts with the behavior of their *E. coli* counterparts when transferred by a haploid *E. coli* Hfr. An example of the normal inheritance picture is seen in the cross between the haploid *E. coli* Hfr WR2017 and the *E. coli* recipient WR3040 shown in Table 3. Examination of 100 WR3040 exconjugants selected for receipt of the donor *ilv*$^+$ marker shows that although the *rha*$^+$, *metB*$^-$, and *argH*$^-$ donor alleles were most frequently inherited en bloc, separation of one or more of these markers did occur in 17 of the 56 hybrids in which their unselected inheritance was observed.

Unselected inheritance of the recessive *E. coli* alleles of WR2080 was examined in a mating with the *E. coli* recipient WR3041, a Rha$^+$ derivative of WR3040. The inheritance pattern of the *E. coli rha*$^-$, *metB*$^-$, and *argH*$^-$ alleles among 100 *ilv*$^+$-selected WR3041 exconjugants, shown in Table 4 differs significantly from that seen in the haploid Hfr WR2017 × WR3040 cross (compare Table 3). Although unselected inheritance of the *E. coli rha*$^-$ marker of WR2080 by 51 WR3041 exconjugants was similar to the 56% inheritance of the *rha*$^+$ marker of WR2017, there was a noticeable reduction in the inheritance of the *E. coli metB*$^-$ marker of WR2080, compared with the WR2017 cross, and the WR2080 *E. coli argH*$^-$ marker was inherited by only 5 exconjugants, as opposed to 41 which received *argH*$^+$ unselected from WR2017.

It should be pointed out here that WR3040 and its derivative, WR3041, differ from WR3026 and its derivative, WR3029, in their behavior as recipients of *Salmonella* DNA. Whereas WR3026 and WR3029 are good acceptors of *Salmonella* genes (recall the 42% unselected en bloc inheritance of the *Salmonella rha*$^+$, *metB*$^+$, and *argH*$^+$ alleles by WR3029), WR3040 and WR3041 are not. As seen in the cross between WR2080 and WR3040 (Table 5), in which inheritance of the *Salmonella rha*$^+$ allele can be scored, only 8 of 100 *ilv*$^+$-selected WR3040 exconjugants inherited this *Salmonella* marker. Inheritance of the *E. coli metB*$^-$ and *argH*$^-$

TABLE 3. *Unselected marker inheritance among 100 ilv*$^+$*-selected E. coli WR3040 exconjugants from a cross with haploid E. coli HFR WR2017*[a]

Hybrid class	No.	Summation of marker inheritance	No.
ilv$^+$	44	*rha*$^+$	56
ilv$^+$ *rha*$^+$ *metB*$^-$ *argH*$^-$	39	*metB*$^-$	45
ilv$^+$ *rha*$^+$	9	*argH*$^-$	41
ilv$^+$ *rha*$^+$ *metB*$^-$	6		
ilv$^+$ *rha*$^+$ *argH*$^-$	2		

[a] Markers listed are those inherited from the Hfr.

alleles in this cross was similar to that observed with WR3041 (compare Table 4) with 29 exconjugants expressing the *E. coli metB⁻* donor allele, and only 7 expressing the *E. coli argH⁻* donor allele. We believe that the low unselected inheritance of the *E. coli argH⁻* allele of WR2080 in these crosses is indicative of its proximity to the inserted *Salmonella* genetic segment (Fig. 2) whose inheritance by WR3041 occurs at about the same low frequency.

In the mating of WR2080 with both WR3040 and WR3041, the *ilv⁻*-selected exconjugants also were examined for possible inheritance of the *E. coli thiA⁻* allele originally present in the WR3029 strain from which WR2080 was derived. None of the 200 exconjugants examined was observed to express this allele. In view of the fact that no Thi⁻ segregants were ever observed from WR2080 or any of its predecessors, it might be presumed that, as the consequence of genetic recombination, the original *E. coli thiA⁻* allele no longer exists in WR2080.

DISCUSSION

We conclude that the *Salmonella* chromosomal segment bearing the *rha⁺*, *metB⁺*, and *argH⁺* alleles is inserted in the chromosome of WR2080 near, and to the right of (see Fig. 2),

TABLE 4. *Unselected inheritance of the recessive E. coli alleles of the partially diploid E. coli hybrid HFR WR2080 among 100 ilv⁻ selected E. coli WR3041 exconjugants*[a]

Hybrid class	No.	Summation of marker inheritance	No.
ilv⁺	48	*rha⁻*	51
ilv⁺ rha⁻ metB⁻	26	*metB⁻*	30
ilv⁺ rha⁻	21	*argH⁻*	5
ilv⁺ rha⁻ metB⁻ argH⁻	4		
ilv⁺ argH⁻	1		

[a] Markers listed are those inherited from the Hfr.

TABLE 5. *Unselected inheritance of dominant Salmonella and recessive E. coli alleles of the partially diploid E. coli hybrid HFR WR2080 among 100 ilv⁺-selected E. coli WR3040 exconjugants*[a]

Hybrid class	No.	Summation of marker inheritance	No.
ilv⁺	63	*metB⁻*	29
ilv⁺ metB⁻	22	*argH⁻*	7
ilv⁺ rha⁺	8		
ilv⁺ metB⁻ argH⁻	7		

[a] Markers listed are those inherited from the Hfr. The *rha⁺* marker is a *Salmonella* allele, the others are *E. coli* alleles.

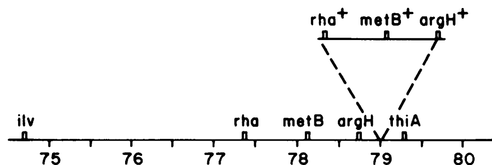

FIG. 2. *Approximate location of the chromosomally inserted Salmonella genetic segment in E. coli hybrid WR2080 relative to the original sites of its argH and thiA loci. Time units are those of Taylor and Trotter (13). Assuming identity in the lengths of Salmonella and E. coli chromosomes in this area, the 1.5-min later interrupted mating entry time of the E. coli ilv⁺ marker of WR2080 (which represents the additional time required for entry of the Salmonella genes) suggests that the Salmonella rha⁺ and argH⁺ markers very nearly define the extremities of the inserted Salmonella DNA segment. Although depicted here in the normal order, it is not known whether the Salmonella genes are inserted in this manner or in the reverse order (see text).*

the original site of the *argH* locus, thus creating a duplication of this region within the hybrid chromosome. This arrangement is indicated both by the interrupted mating entry times for the *Salmonella* alleles near the 25 min mark, and by the abnormally low unselected inheritance of the *E. coli argH⁻* marker of WR2080 in *E. coli* recipients that exhibit a similar difficulty inheriting *Salmonella* genes. Also, it is consistent with the observation that entry of the *E. coli ilv⁺* allele of WR2080, which is situated distal to the proposed insertion site, was delayed 1.5 min, whereas entry of *E. coli* markers located proximal to the insertion site in this Hfr occurred at the expected time.

Although a somewhat earlier entry time was determined for the *Salmonella rha⁺* marker (24.7 min) than for the *Salmonella argH⁺* marker (25.3 min), we do not think that this difference is great enough to be considered indicative of an inverted gene order within the inserted segment. Our opinion is that these different time determinations probably represent variations of a single time of entry which is the same for all of the *Salmonella* markers. In no instance during these studies was any of the *Salmonella* alleles inherited separately, and it is not unreasonable to suppose that recovery of hybrids expressing them might require entry and conservation of the entire *Salmonella* genetic segment. If this is indeed the case, and if the lengths of the *Salmonella* and *E. coli* chromosomes are as we believe identical in this region (1.4 min between *argH* and *rha*), then the minimum estimate of the common entry time for the three *Salmonella* markers would be 24.9

min. Regardless of whether or not this interpretation of the data is correct, however, we do not believe that any conclusions can be drawn as to the order of the inserted *Salmonella* genes.

In our previous study (7) of the partially diploid hybrids formed in this mating system, we observed that about 25% of those examined conserved the *Salmonella* DNA extrachromosomally in the CCC configuration. Maintenance of the added DNA in that configuration involved, in every instance, association with at least some part of the sex factor F. The majority of the partially diploid hybrids examined, however, were seen to conserve the added *Salmonella* DNA in some manner which did not involve association with F, or assumption of the CCC configuration. Examination of one such hybrid in the present study showed that its *Salmonella* DNA is not maintained extrachromosomally, but is conserved instead as an insertion within the hybrid chromosome. Although none of these observations rules out the possibility that some hybrids might conserve the *Salmonella* DNA extrachromosomally in a form other than CCC, we think it more likely that, within this mating system, all partially diploid hybrids in which F is not involved in maintaining a CCC exogenote conserve the added DNA by chromosomal insertion.

In an earlier study (9) involving *S. typhosa* as the recipient of *E. coli* genes, we found that the added *E. coli* DNA in some partially diploid hybrids was maintained extrachromosomally in the CCC configuration. The diploid hybrids in which this DNA form was detected, however, constituted a minority of those examined; the manner of conservation of the added *E. coli* DNA in the majority of the hybrids was not determined. Subsequently, in a report concerning *S. typhimurium* as the recipient of *E. coli* genes, Mojica-a and Middleton (10), interpreting data from their transduction studies, concluded that some partially diploid hybrids maintained the added genetic material extrachromosomally, whereas others conserved it by chromosomal integration (insertion). The partially diploid *S. typhimurium* hybrids which they classified as conserving the added *E. coli* DNA by insertion constituted over 70% of those examined. In retrospect, we think it very likely that the added *E. coli* DNA in those *S. typhosa* hybrids in which we detected no CCC material was conserved by chromosomal insertion.

From all considerations, it appears to us that chromosomal insertion probably is the general mechanism by which partially diploid hybrids are formed in both *E. coli* Hfr $\times$ *Salmonella* F^- and *Salmonella* Hfr $\times$ *E. coli* F^- mating systems. Extrachromosomal conservation of added DNA in the CCC configuration occurs, we believe, only in those special circumstances in which F or possibly some other replicator, chances to be present and active in maintaining that configuration. In the presently studied *S. typhimurium* $\times$ *E. coli* system, the only replicator observed to be active in this manner was F.

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