CHROMOSOME TRANSFER KINETICS OF SALMONELLA HFR STRAINS

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ABSTRACT

Johnson, E. M. (Walter Reed Army Institute of Research, Washington, D.C.), Stanley Falkow, and L. S. Baron. Chromosome transfer kinetics of Salmonella Hfr strains. J. Bacteriol. 88:395-400. 1964.—The kinetics of chromosome transfer of an Hfr strain of Salmonella typhosa and an Hfr strain of S. typhimurium were examined in interrupted matings with multiply auxotrophic S. typhimurium recipients. The S. typhosa Hfr, TD-7, was found to transfer the pro-A, met-A, arg (A, C, F, or H), and ile markers at 8, 32, 36, and 51 min, respectively, after contact with the recipient strain. Comparison of these entry times with those of the analogous Escherichia coli Hfr P4X-6 for the same markers showed the gene order to be identical. However, the TD-7 entry times were considerably extended over those of P4X-6, which transfers these markers to E. coli F- strains at, respectively, 5, 20, 22.5, and 28 min. A similar extension of the entry times was noted with the S. typhimurium Hfr, SR-305, which transfers the markers in the reverse order, ile-met-A-pro-A, at 3 to 4, 18, and 46 min, respectively. Examination of P4X-6/Salmonella Hfr entry time ratios showed them to be constant at 0.63 for the earlier markers transferred by both TD-7 and SR-305. These data suggest that the physical length of the Salmonella chromosome is the same as that of E. coli, and that the rate of chromosome transfer of the Salmonella Hfr strains to S. typhimurium recipients is only 0.63 that of P4X-6 to E. coli F- strains under the same physical conditions.

The study of sexual recombination in Salmonella originated with the demonstration of chromosomal transfer by an Hfr strain of Escherichia coli K-12 to a fortuitously isolated strain of S. typhimurium, which behaved in this cross as a genetic recipient (Baron, Carey, and Spilman, 1958). A similar intergeneric cross was employed by Zinder (1960a) to establish the existence of a gross chromosomal homology between S. typhimurium and E. coli, as well as an identical gene order in each species for five studied genetic determinants. These findings, based on recombination frequencies and linkage analysis, have been confirmed and extended in intergeneric matings by Miyake (1962) and by Falkow, Rownd, and Baron (1962). Salmonella Hfr strains have been isolated after infection with the sex factor, F, of E. coli K-12 (Zinder, 1960b; Mäkelä, 1963), and as a consequence of K-12 Hfr hybridization with selection for a terminal genetic marker linked to F (Miyake, 1962). It has thus become possible to analyze the recombination process entirely within Salmonella. One such analysis, carried out within S. abony by Mäkelä (1963), has established that S. abony Hfr strains exhibit the oriented chromosome transfer that characterizes K-12 Hfr donors, and a gene order identical to K-12 for seven studied markers. While this determination was based on recombination frequencies, it was also indicated that interrupted mating experiments supported the recombination data, although entry times for the markers were not presented. In the present communication, the kinetics of chromosome transfer of an S. typhosa Hfr and an S. typhimurium Hfr are examined in interrupted matings with multiply auxotrophic S. typhimurium recipients.

MATERIALS AND METHODS

Organisms. The bacterial strains employed in this study are described in Table 1. The genetic markers in these strains, referred to as pro, met, and arg, map at the loci for pro-A, met-A, and arg-A, -C, -F, or -H. As far as is known, only one locus has been mapped for the ile marker. The Hfr strains P4X-6, SC-19, and TD-7 all transfer their chromosomal genes in the order pro-met-arg-ile, with lac as the terminal genetic marker linked to the sex factor. SR-305 transfers its chromosome in the opposite order, with ile as
the lead marker, followed in order by arg, met, and pro.

**Media.** The minimal agar selective medium was described in a previous communication (Johnson, Falkow, and Baron, 1964). Glucose added at a concentration of 0.4% served as the carbon source. Amino acids were added at a concentration of 25 µg/ml, and selection of prototrophic recombinants was accomplished by omission of a given amino acid. Streptomycin sulfate at a concentration of 625 µg/ml served as a counter-selective agent.

**Technique of genetic crosses.** Genetic crosses were performed in the manner previously described by Johnson et al. (1964), with the exception that the mating suspensions were incubated at 37°C for 2.5 hr instead of 2 hr. Recombination frequencies were expressed as the number of recombinants per donor cell.

**Interrupted mating procedure.** Interrupted mating experiments with the Salmonella Hfr strains were carried out as described by de Haan and Gross (1962), with the following modifications. Mid-log phase donor and recipient suspensions in Penassay Broth were mixed at time zero to give a donor concentration of about 2 × 10⁸ cells per ml and a recipient concentration of about 5 × 10⁶ cells per ml. The mating mixture was kept at a barely perceptible degree of agitation in a water bath (37°C) by means of a Burrel wrist-action shaker for a period of 5 min. The cells were then diluted gently into prewarmed liquid minimal medium unsupplemented except for 0.4% glucose, and kept at 37°C without agitation; the amount of dilution varied with the mating system and the selected genetic marker. Thus, in the TD-7 × 08R-1Sr cross a 1:20 dilution was found to give satisfactory results for both the pro and met markers, whereas with 74R-1Sr as the recipient the dilutions were 1:20 for pro, 1:12 for arg, and 1:4 for ile. In the SR-305 × 08R-1Sr interrupted matings, a 1:6 dilution was made for ile, and 1:4 dilutions were made for met and pro. Samples of the diluted mating mixture were taken at 5-min intervals, subjected to vigorous agitation (blended) for 100 sec by a Vortex Jr. mixer to break up the mating pairs, and plated on selective media (0.1 ml per plate) along with 0.1 ml of Penassay Broth.

The P4X-6 × AB1133 interrupted matings were carried out in the same manner, except that the donor concentration employed was about

<table>
<thead>
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<th>TABLE 1. Characteristics of the bacterial strains*</th>
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<tr>
<td><strong>Strain</strong></td>
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<td>------------</td>
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<tr>
<td><em>Salmonella typhimurium</em></td>
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<tr>
<td>Recipient strains</td>
</tr>
<tr>
<td>08R-1Sr</td>
</tr>
<tr>
<td>74R-1Sr</td>
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<tr>
<td>Hfr strains</td>
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<tr>
<td>SC-19</td>
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<td>SR-305</td>
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<tr>
<td><em>S. typhosa</em></td>
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<tr>
<td>Recipient strain</td>
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<tr>
<td>643</td>
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<tr>
<td>Hfr strain</td>
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<tr>
<td>TD-7</td>
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<tr>
<td><em>Escherichia coli K-12</em></td>
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<td>F⁻ strain</td>
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<td>AB1133</td>
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<tr>
<td>Hfr strain</td>
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<tr>
<td>P4X-6</td>
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*Abbreviations and symbols: pro, proline; met, methionine; arg, arginine; ile, isoleucine; his, histidine; thr, threonine; cys, cystine; try, tryptophan; leu, leucine; lac, lactose; ara, arabinose; rha, rhamnose; xyl, xylose; fuc, fucose; str, streptomycin; R, resistant; S, sensitive; +, utilized; -, not utilized.*
2 \times 10^6 \text{ cells per ml, and the dilution into minimal medium was 1:500. Dilutions of this magnitude were not possible with the Salmonella systems because of the lower number of recombinants produced. However, since only the entry times were desired in these experiments, it was not imperative that further effective contacts be prevented after the initial 5 min in broth.}

**Results**

*Derivation of the S. typhosa Hfr.* The S. typhosa Hfr, TD-7, was derived from S. typhosa strain 643 by the method of F-linked terminal marker hybridization (Jacob and Wollman, 1957). The donor employed for this hybridization was S. typhimurium Hfr SC-19. SC-19 is itself a hybrid of an LT-7 mutator strain of S. typhimurium and the E. coli Hfr P4X-6, containing the terminal lactose utilization (lac+) gene of P4X-6 linked to the sex factor (Miyake, 1962). Selection for lac+ in the SC-19 \times 643 cross results in receipt of the closely linked sex factor in a certain percentage of the lac+ hybrids, which thereby become Hfr donors. Thus, TD-7 possesses the lac+ marker and F, and has the transfer orientation of SC-19 and P4X-6; i.e., pro is injected as the lead marker and lac+ is terminal.

Besides the F-lac region, the extent of E. coli deoxyribonucleic acid (DNA) substituted in SC-19, if any, is not known. It is likewise unknown whether any such DNA, if present, would have been resubstituted in TD-7. Most probably, however, the extent of such resubstitution, if it occurred, would be slight. The difficulty of integration of E. coli genetic material in Salmonella was observed previously by Falkow et al. (1962), who pointed out that transfer of more than 12% of E. coli material usually results in the production of unstable partial diploids. In any case, with the exception of its lactose utilization and donor capacities, TD-7 is indistinguishable by antigenic and biochemical tests from the parent 643 strain. Further, as will be shown subsequently, its chromosome transfer kinetics are similar to those of SR-305, which was isolated after infection with the E. coli sex factor (Zinder, 1960) without the contingent substitution of chromosomal genes.

The donor capabilities of TD-7 were determined by matings with the S. typhimurium LT-7 strains 08R-15 and 74R-15. The recombination frequencies are presented in Table 2, along with the comparative frequencies of SC-19, SR-305, and P4X-6. As noted previously by Makelä (1963) in the S. abony system, all crosses exhibit a transfer gradient for the genetic markers, indicating that their order is the same in the S. typhosa and S. typhimurium donors as it is in E. coli K-12 strains.

**Chromosome transfer kinetics of TD-7, P4X-6, and SR-305.** The kinetics of chromosome transfer of TD-7 in interrupted matings with 08R-15 and 74R-15 are shown in Fig. 1. The entry times for the pro, met, arg, and ile markers are, respectively, 8, 32, 36, and 51 min. Thus, they confirm the identity of gene order for these markers in Escherichia and Salmonella. On the other hand, it will be noted that the magnitudes of these entry times differ considerably from those obtained with the analogous E. coli Hfr P4X-6. The transfer kinetics of P4X-6 in the cross with AB1133 for the pro and arg markers are shown in Fig. 2, and indicate entry times of 5 and 22.5 min, respectively. These times for pro

| Table 2. Recombination frequencies of the Hfr strains employed* |
|---------------------|-------------------|-----------------|-----------------|-----------------|-----------------|
| **Cross**           | **Selected marker**| **pro**         | **met**         | **arg**         | **ile**         | **his**         |
| S. typhosa TD-7 \times S. typhimurium 08R-15 \times S. typhimurium |    | 2 \times 10^{-4} | 3 \times 10^{-4} |             | 1 \times 10^{-6} |             |
| S. typhosa TD-7 \times S. typhimurium 74R-15 \times S. typhimurium |    | 9 \times 10^{-4} |             | 1 \times 10^{-4} | 9 \times 10^{-4} | 2 \times 10^{-4} |
| S. typhosa TD-7 \times E. coli AB1133 |    | 3 \times 10^{-4} |             | <10^{-7}        |             |             |
| S. typhimurium SC-19 \times S. typhimurium 74R-15 |    | 3 \times 10^{-4} |             | 4 \times 10^{-4} | 1 \times 10^{-4} | 5 \times 10^{-4} |
| E. coli P4X-6 \times E. coli AB1133 |    | 2 \times 10^{-4} |             | 3 \times 10^{-4} |             |             |
| S. typhimurium SR-305 \times S. typhimurium 08R-15 |    | 7 \times 10^{-4} |             | 4 \times 10^{-4} |             | 7 \times 10^{-4} |

* Recombination frequencies are expressed as the number of recombinants per donor cell.
and arg are in excellent agreement with those observed by A. L. Taylor (personal communication), who further indicates that P4X-6 entry times for the met and ile markers would be 20 and 28 min, respectively. A comparison of the TD-7 entry times with those of P4X-6 is shown in Table 3.

To establish that the later entry times of TD-7 were not the result of any anomalies stemming from its derivation by terminal marker hybridization, interrupted matings were performed with SR-305, using 08R-1Sp as the recipient. By virtue of its isolation after F infection, rather than by hybridization for a terminal marker, SR-305 may safely be considered to contain only the sex factor as DNA of E. coli origin. The kinetics of chromosome transfer in the SR-305 × 08R-1Sp cross are shown in Fig. 3. Although in this cross it was necessary to subtract a certain

<table>
<thead>
<tr>
<th>Strain</th>
<th>pro</th>
<th>met</th>
<th>arg</th>
<th>ile</th>
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<tbody>
<tr>
<td>P4X-6</td>
<td>5</td>
<td>20</td>
<td>22.5</td>
<td>28</td>
</tr>
<tr>
<td>TD-7</td>
<td>8</td>
<td>32</td>
<td>36</td>
<td>51</td>
</tr>
<tr>
<td>Ratio (P4X-6/TD-7)</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
<td>0.55</td>
</tr>
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</table>

*The entry times, in minutes, for TD-7 were determined by interrupted matings with 08R-1Sp and 74R-1Sp. P4X-6 entry times for the pro and arg markers were determined by interrupted matings with AB1133; those for met and ile were obtained from A. L. Taylor (personal communication).

**FIG. 1.** Kinetics of chromosome transfer of TD-7 to (A) 08R-1Sp and (B) 74R-1Sp. In (A) the mating mixture is diluted 1:20 into minimal medium, prior to blending, for both markers; in (B) it is diluted 1:20 for pro, 1:12 for arg, and 1:4 for ile. After blending, samples are plated at 0.1 ml per plate.

**FIG. 2.** Kinetics of chromosome transfer of P4X-6 to AB1135. The mating mixture is diluted 1:600 into minimal medium, prior to blending, for both markers. After blending, the arg sample is plated at 0.1 ml per plate; the pro sample is further diluted 1:8 in Penassay Broth and then plated at 0.1 ml per plate.
background count, the point of inflection above background was clear enough to determine entry
times of approximately 3 to 4 min for ile, 18 min for met, and about 46 min for pro. It is evident
that the extended entry times are not peculiar to TD-7, but characterize as well the transfer ki-
etics of SR-305.

Discussion

In view of the previously cited demonstrations of genetic homology between Salmonella and
Escherichia (Zinder, 1960a; Falkow et al., 1962), and the confirmation in this study of gene order
identity, the proposition that the later entry times of the Salmonella Hfr strains reflect a
longer physical length of their chromosomes seems untenable. Delay in pair formation or in
initiation of transfer is likewise excluded as an explanation. If this were the case, a comparison
of the TD-7 entry times with those of P4X-6 should have shown, for each marker, the same
3-min offset observed for the lead marker, pro. What is seen, however, is a proportional increase
in the TD-7 entry times, such that the ratio P4X-6/TD-7 for the pro, met, and arg times is,
in each case, 0.63 (see Table 3). If the physical lengths of the Escherichia and Salmonella chromo-
somes are assumed to be identical, then it would appear that the transfer of the TD-7 chromosome
to S. typhimurium occurs at a rate which is only
0.63 that of the P4X-6 transfer to E. coli F−
strains, under the same physical conditions.
Conversely, the existence of this constant ratio is,
in itself, strong evidence that the physical
lengths of the Salmonella and Escherichia chromo-
somes are the same.

The possibility that the 0.55 ratio for the ile
marker might indicate an additional length of chromosome between arg and ile is contradicted
by the data from the SR-305 cross. If the P4X-6
interval between met and ile is 8 min, then the
14-min interval between these markers observed
with SR-305 is about what would be predicted
by the 0.63 ratio. On the other hand, the met-
pro interval of 28 min, as measured by SR-305,
is 4 min longer than the TD-7 measurement for
this segment. It thus appears that the entry
times measured for the most distal markers
transferred by each Hfr are about 4 to 6 min
later than the 0.63 ratio would predict. This
might be due either to a variation in the rate of
transfer or to the inability of the technique to
provide accurate times for late markers. In this re-
gard, K. E. Sanderson (personal communication),
who has studied the transfer kinetics of S. typhi-
murium Hfr strains over a more extensive area
of the chromosome, and whose entry times for
proximal Hfr markers are in close agreement with
ours, has noted a considerable lengthening of
entry times for the distal markers. However,
with his system, in which the mating bacteria
are impinged on Millipore filters prior to inter-
ruption of mating, the initially observed E. coli-
S. typhimurium entry time ratio appears to be
maintained longer (i.e., for more distal markers)
than it is in ours. Therefore, it is likely that the
variations for the late markers which we have
observed in the present study reflect the limita-
tion of the technique employed, rather than a
variation in the initial transfer rate of the Hfr
strains.

The present data suggest that the K-12 sex
factor, when integrated in a Salmonella, is un-
able to mobilize the chromosome of that or-
ganism with the same degree of efficiency that it
exhibits in mobilizing the E. coli chromosome,
under identical experimental conditions. Whether,
under these circumstances, this stems from a
deficiency of the transferred sex factor itself, or
from species differences which might affect the
conjugal process and interfere with the rate of
transfer, is not known. The former proposition
would seem the more attractive and, in addition,
the more amenable to analysis.

The behavior of the E. coli sex factor in a
Shigella Hfr strain was recently analyzed by
Schneider and Falkow (J. Bacteriol. in press),
who observed that the entry time curves for all
markers transferred by this donor exhibit a low
initial slope followed by a steeper secondary
slope. Although the entry times, as determined
from the initial slope, are identical to those of
P4X-6, these authors note that the time of
plateau, i.e., when the maximal number of zygotes
have received a given marker, occurs 2.5 times
later than with P4X-6. They considered, there-
fore, that this may represent a heterogeneity in
the donor population with respect to initiation
of transfer, or to the rate of transfer, or to both.
In the present study, no comparable initial slope
was detected with Salmonella donors. However,
it is entirely possible that, within the limits of
the system with which we worked, a small per-
centage of the Salmonella Hfr population whose
transfer rate might equal that of E. coli would
not have been detected.
ACKNOWLEDGEMENTS

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LITERATURE CITED


