Vegetative Replication and Transfer Replication of Deoxyribonucleic Acid in Temperature-Sensitive Mutants of Escherichia coli K-12

M. G. MARINUS AND E. A. ADELBERG

Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut 06510

Received for publication 16 July 1970

Crosses were carried out at 34 C and 42 C between eight pairs of isogenic strains of Escherichia coli K-12. The donor and recipient of each pair carried the same mutation for temperature-sensitive deoxyribonucleic acid (DNA) synthesis; they differed only in the presence of F-lac in the donor and a spectinomycin-resistance marker in the recipient. A different temperature-sensitive mutation was present in each of the eight pairs, the eight temperature-sensitive mutations being located in at least two different genes. In all eight pairs, the transfer of F-lac occurred at high and equal rates at 34 C and 42 C, although vegetative DNA replication at 42 C was approximately 10^-4 of that at 34 C. The transfer of F-lac at 42 C was accompanied in seven of the eight crosses by an equivalent amount of DNA synthesis in excess of that observed in the unmated controls. The DNA synthesized during transfer at 42 C was characterized by equilibrium centrifugation in cesium chloride and by its sedimentation velocity in sucrose gradients. It was found to have a density and a molecular weight characteristic of F-lac DNA. A small proportion of the material labeled during transfer was recovered in the form of covalently closed DNA. It is concluded that vegetative replication of the chromosome and transfer replication of F are separate processes, the former requiring at least two gene products which are nonessential for the latter.

During conjugation in Escherichia coli K-12, genetic material is transferred from donor to recipient in which it is subsequently expressed. Cells are able to donate deoxyribonucleic acid (DNA) only if they carry a sex factor, such as the fertility factor (F). It has recently been shown that during conjugation only one strand of the sex factor DNA is transferred from donor to recipient, in which it acquires a complementary strand; the strand not transferred to the recipient remains in the donor in which it also acquires a complement (D. Vapnek and W. D. Rupp, J. Mol. Biol., in press). These results show that DNA replication occurs in both parents during mating; they do not, however, indicate whether such replication is required, either in the donor or in the recipient, for transfer of the single strand to occur.

Many investigations have attempted to answer this question by selectively inhibiting DNA replication in either or both of the parents by the use of chemical inhibitors, or—in the case of certain temperature-sensitive mutants—by the use of high temperatures. (For a review of these experiments, see reference 8.) The results to date fall into two categories: those in which transfer appeared to be arrested when DNA synthesis was inhibited, and those in which transfer continued. To the first category belong the experiments with nalidixic acid (1, 4, 6, 10) and Bonhoeffer's experiments with certain temperature-sensitive mutants which do not synthesize DNA at 42 C (2, 3). Nalidixic acid appears to inhibit transfer when applied to mating couples containing nalidixic-sensitive donor cells; no effect is observed when the donor cells are resistant to the drug, whether or not DNA synthesis is inhibited in the recipient. The results, however, do not establish that replication and transfer share the same enzymatic machinery in the donor; the processes may, instead, be independent, being inhibited by nalidixic acid because they have in common double-stranded DNA as substrate. Although the binding of nalidixic acid to DNA has not been detected (5), the possibility remains that the drug does act in this way and thus in-

---

1 Present address: Botanical Laboratory, Free University, De Boelelaan 1087, Amsterdam, Netherlands.
hibits all processes for which DNA is the substrate.

Bonhoeffer's experiments (2, 3) showed that transfer (as measured either by recombinant formation or by the expression of the transferred lacZ gene in the zygote) was inhibited at 42°C when the recipient, but not the donor, was temperature-sensitive for DNA synthesis. Other workers, however, have not been able to confirm these results; they suggest that DNA synthesis in the recipient is not essential for the transfer of DNA but rather for posttransfer events in the zygote which are essential for recombination or for gene expression (6, 8).

The second category of results—those in which DNA transfer continued when DNA replication was blocked in one or both parents—have also been equivocal. The majority of these experiments have involved the use of mutants with temperature-sensitive DNA synthesis. In a typical experiment, a wild-type donor was mated with a temperature-sensitive recipient (or vice versa), and transfer was observed to take place normally at the nonpermissive temperature. In one case (6), transfer at the nonpermissive temperature was observed when two different temperature-sensitive mutants were mated with each other.

Such experiments, however, may be interpreted in two ways: either the gene product which is inactivated at high temperature is essential for vegetative DNA replication but not for DNA transfer; or it is essential for both processes, but can be supplied to the transfer machinery by the other mating partner. To eliminate the second of these possibilities, we have carried out a series of matings in each of which the donor and the recipient carried the same temperature-sensitive mutation. If, in such a mating, DNA transfer is inhibited at the nonpermissive temperature, the normal product of the mutant gene would appear to be essential for transfer and for vegetative replication. If DNA transfer is not inhibited, one of two conditions might obtain. (i) The amount of DNA transferred might exceed the amount of replication observed during the transfer period. In this case, the replication which Vapnek and Rupp (in press) showed to be associated with transfer would appear to be a secondary consequence, rather than an essential feature, of the transfer process. The enzymes of transfer would act to unwind and segregate the two strands of F DNA into the donor and recipient cells, without simultaneously synthesizing their complements. (ii) The amount of DNA transferred might coincide with the amount of DNA replicated in excess over the amount replicated in the unmated controls. In this case, transfer and replication would once again be associated, but vegetative replication and transfer replication would be shown to be different processes, to the extent that a particular gene product would be essential for one but not for the other.

In the present work, we measured DNA replication and transfer in eight independently isolated temperature-sensitive (DNA) mutants, by using isogenic crosses as discussed above.

**MATERIALS AND METHODS**

**Organisms.** Parental strains carrying the temperature-sensitive mutations tsm-1 through tsm-7 were provided by F. Bonhoeffer and correspond to his isolation numbers DNAK500, 454, 308, 43, 313, 399 and 7a, respectively. These strains were all derived by him from strain CR34 of *E. coli* K-12. The parental strain carrying tsm-8 was provided by W. Fangman, and corresponds to his strain no. FA-22 (9). All these strains synthesize DNA at 34°C but not at 42°C. The strains used for the present experiments are described in Tables 1 and 2; donors were derived from the parental tsm-F strains by the introduction of F42 (F-lac), and recipients by the selection of spectinomycin-resistant mutants.

**Media and reagents.** Minimal medium 56/2 is a half-strength preparation of medium 56 (15). When spectinomycin was used, it was incorporated at a final concentration of 100 µg/ml. Sugars were incorporated at a final concentration of 0.2%. 3H-thymidine (6.7 Ci/mM) was purchased from New England Nuclear Corp., Sarkosyl NL-97 (sodium lauryl sarcosinate) from Geigy Chemical Co. and cesium chloride from Penn Rare Metals.

**Mating and labeling conditions.** Organisms were grown in minimal medium 56/2 supplemented with 1.0% Casamino Acids (Difco), 2 µg of thymidine per ml, 200 µg of DL-threonine per ml, 50 µg of DL-leucine per ml, and 0.2% glucose. Cultures were incubated with shaking at 34°C. When the cell concentration reached 10^9 to 2 × 10^9/ml, the cultures were divided in two, centrifuged, and washed twice with supplement 56/2 0.2% medium minus thymidine. Cells to be mated at 34°C were suspended at the original cell density in minimal medium supplemented as described above, whereas those to be mated at 42°C were suspended in minimal medium similarly supplemented except that the thymidine concentration was reduced to 0.1 µg/ml. (This reduction in nonradioactive thymidine concentration was employed to increase the incorporation at 42°C of radioactive thymidine which was added to the mixture before mixing donor and recipient cultures.)

The suspensions were incubated for 10 min at 34 or 42°C, respectively, and then equal numbers of males and females were mixed to give a final volume of 3.0 ml containing 10^9 to 2 × 10^9 cells/ml. The mating flasks contained 3H-thymidine at a final specific activity of 3.0 µCi/µg (34°C) and 600 µCi/µg (42°C). Males and females, preincubated for 10 min at 42°C, were also incubated separately at 42°C in the presence of 3H-thymidine (unmated controls). Incubation was carried out for 60 min, at the end of which the cells were chilled in an ice bath. At this time, the unmated control suspensions were mixed.
Measurement of DNA replication. Samples from the mating mixtures and controls were subjected to lysis and centrifugation in alkaline CsCl as described below, and the amount of DNA replication in each case was determined by summing the label detected in the fractions representing the DNA peak.

Measurement of genetic transfer. Samples from the mating mixtures and controls were diluted appropriately in 56/2, vortexed for 1 min, and plated on minimal agar supplemented so as to select for Lac\(^+\) spectinomycin-resistant hybrids. The plates were incubated at 34 C for 3 to 4 days.

Lysis procedure. The lysis procedure was carried out at 0 C by the method of Vapnek and Rupp (in press). After 5 min at 0 C, 2.8 ml of each cell suspension was centrifuged and washed twice with minimal medium (56/2). The cells were resuspended in 1.0 ml of 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.0) containing 10% sucrose; 0.2 ml of a lysozyme solution (5 mg/ml) and then 0.4 ml of 0.25 M ethylenediaminetetraacetic acid (EDTA) (pH 8.0) were then added. After 5 min of further incubation at 0 C, 0.4 ml of a 2% solution of Sarkosyl was added to lyse the spheroplasts formed by the lysozyme-EDTA treatment.

Centrifugation in alkaline cesium chloride. A sample of each lysate was added to an alkaline CsCl solution (pH 12.0) containing \(^{3}P\) lambda DNA (kindly provided by W. D. Rupp). The density of the solution was adjusted to 1.700 g/cm\(^3\), and the solution was then centrifuged at 20 C for 16 hr at 36,000 rev/min in an SW-50.1 rotor or at 40,000 rev/min in an SW50L rotor. Twenty-five-drop fractions were collected by puncturing the bottom of the centrifuge tube, and the amount of label in each fraction was determined as described in the following section.

Sedimentation in alkaline sucrose. The CsCl fractions containing the highest radioactivity were transferred to a dialysis bag and dialyzed in the cold against 0.01 M Tris-0.001 M EDTA (pH 8.0) to remove the CsCl. Samples from each dialysis bag were then subjected to sedimentation in alkaline sucrose as described by Freifelder (11). A 0.1-ml sample was layered onto a 5-ml linear, 5 to 20% sucrose gradient containing 0.3 M NaOH, 0.7 M NaCl, and 0.001 M EDTA. The gradient was centrifuged for 60 min at 40 C at either 36,000 rev/min in an SW-50.1 rotor or at 40,000 rev/min in an SW-50L rotor. A 0.1-ml portion from the cell lysate, prepared as described in "lysis procedure" above, was also layered onto a similar gradient and centrifuged for 20 min at the same speed. This gradient was used to detect covalently closed DNA (Vapnek and Rupp, in press).

The bottom of the centrifuge tube was punctured and 10-drop fractions collected directly onto Whatman 3MM filter paper discs. The discs were washed three times with 5% trichloroacetic acid, once with ethanol-ether (1:3), and once with ether. Each wash lasted 15 min. The discs were air dried and placed in scintillation vials containing 5 ml of scintillation fluor [3.5 g of 2,5-diphenyloxazole and 0.09 g of 1,4-bis-(2-[4-methyl-5-phenyloxazolyl])benzene per liter of toluene]. The vials were counted in a Beckman LS-250 scintillation counter.

RESULTS

Genetic transfer and DNA replication. Eight isogenic crosses were carried out. In each case, the donor and recipient were identical temperature-sensitive strains except that the donor carried F42 (an F-lac), and the recipient was spectinomycin-resistant (Spc\(^R\)). The matings were carried out at two temperatures (see Materials and Methods), and Lac\(^+\) Spc\(^R\) hybrids were selected at 34 C. Measurements of DNA replication in the mating mixtures and in the controls were also carried out as described above.

The results are shown in Table 3. The data in columns 2 and 3 show that F-lac transfer at 42 C was only slightly lower than at 34 C. When corrected for viability, which was two to three times lower at 42 C, this difference vanishes entirely. Since, in each cross, 10\(^8\) to 2 x 10\(^8\) donors were mixed with 10\(^8\) to 2 x 10\(^8\) recipients per ml, the yields of F-lac hybrids ranged from 1.5 to 100% of the mating couples.

The amount of replication in the mating mix-
The table below shows DNA transfer and replication at two temperatures in isogenic mating mixtures.

<table>
<thead>
<tr>
<th>tsm allele</th>
<th>F-lac spΔ hybrids/ml</th>
<th>14H counts per min in DNA per ml of cell suspension (60-min incubation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34 C</td>
<td>42 C</td>
</tr>
<tr>
<td>tsm-1</td>
<td>100 × 10^4</td>
<td>59 × 10^4</td>
</tr>
<tr>
<td>tsm-2</td>
<td>170 × 10^4</td>
<td>24 × 10^4</td>
</tr>
<tr>
<td>tsm-3</td>
<td>12 × 10^4</td>
<td>8.7 × 10^4</td>
</tr>
<tr>
<td>tsm-4</td>
<td>14 × 10^4</td>
<td>5.0 × 10^4</td>
</tr>
<tr>
<td>tsm-5</td>
<td>13 × 10^4</td>
<td>3.0 × 10^4</td>
</tr>
<tr>
<td>tsm-6</td>
<td>25 × 10^4</td>
<td>13 × 10^4</td>
</tr>
<tr>
<td>tsm-7</td>
<td>19 × 10^4</td>
<td>8.4 × 10^4</td>
</tr>
<tr>
<td>tsm-8</td>
<td>85 × 10^4</td>
<td>53 × 10^4</td>
</tr>
</tbody>
</table>

* The observed values were multiplied by 200 to normalize for 14H-thymidine specific activity.
* Counts per minute in the mating mixture at 42 C (column 6) minus counts per minute in the unmated controls at 42 C (column 5).

1. The calculated number of counts per minute is based on the following considerations. (i) Each donor cell transfers one F-lac with a molecular weight of 55 × 10^6 daltons, representing 9 × 10^4 daltons or 1.5 × 10^-11 μg of thymidine. (ii) The labeled thymidine used for the 42 C matings had a specific activity of 600 μCi/μg, and the counting efficiency in our experiments was 5%. Under these conditions, 1 μg of thymidine produces 66 × 10^4 counts/min. Each F-lac transferred, if accompanied by the synthesis of two complementary strands, should thus produce an incorporation of (1.5 × 10^-11μg) × (66 × 10^4 counts per min per μg) = 1.0 × 10^5 counts/min.

* The data at 34 and 42 C, as well as in the unmated control cultures at 42 C, is shown in columns 4, 5, and 6. The data may be summarized as follows. (i) With the exception of the two leaky strains carrying tsm-3 and tsm-6, the amount of replication in the unmated control was approximately 10^-4 of that occurring in the mating mixtures at 34 C (compare columns 4 and 5). (ii) With two exceptions, the amount of replication in the mating mixture at 42 C exceeded the amount in the unmated control at 42 C by a factor of three-fold or higher (compare columns 5 and 6). The exceptions were the two leaky strains, in which the difference was only 1.5-fold. (iii) With one exception, the excess amount of replication observed varied between 45 and 200% of the amount calculated for the synthesis of two complementary strands per F-lac transferred (compare columns 7 and 8). The one exception was the cross involving tsm-8, in which the observed amount of excess replication was only 18% of the calculated amount.

Characterization of the DNA synthesized by transfer replication. Portions of the mating mixtures and unmated controls were subjected to lysis and centrifuged in alkaline CsCl. The results were similar for all eight crosses; typical data from one cross (tsm-4) are presented in Fig. 1. As shown in Fig. 1, all of the recovered counts banded in a sharp zone with a density equivalent to that of the lambda DNA used as a marker. The recovery of label was 90 to 99% for the 34 C mating mixtures, and 30 to 60% for the 42 C mating mixtures and unmated controls. It was not determined where the balance of the input counts had been incorporated.

The peak fractions containing radioactivity from the CsCl centrifugation were pooled and subjected to sedimentation in alkaline sucrose gradients as described in Materials and Methods. Again, the results were the same for all eight crosses, the data for tsm-4 being presented in Fig. 2. Figure 2 shows that the DNA synthesized during transfer sedimented faster than the marker lambda DNA. The molecular weights, calculated from the equation of Studier (17), were approximately 10^8 daltons for the DNA synthesized at 34 C and 5 × 10^6 daltons for the DNA synthesized at 42 C.

Recovery of covalently closed DNA. Samples from the cell lysates were also centrifuged directly on alkaline sucrose gradients without prior purification in CsCl gradients. The results (Fig. 3) show a minor peak at fraction 9 in the material taken from the mating mixtures, but none in the material from the unmated control. This minor peak presumably represents covalently closed DNA (Vapnek and Rupp, in press). The amount of label found in the minor peak for the 42 C mating mixture was 4 to 5% for the tsm-4 and tsm-7 crosses. For the other crosses at 42 C, the percentages were much lower (<0.1%); none was detected in the tsm-3 and tsm-6 crosses.

Rough mapping of the tsm mutations. Several
Hfr H, that is, in the interval 79 to 87 min on the E. coli K-12 genetic map (18). This agrees with the preliminary location assigned to tsms by Hirota, Ryter, and Jacob (13), and with the more precise location of 80 to 82 min determined by Moody and Lukin (16). The location of tsms has not been determined, but it does not lie in the same interval as the mutations listed above. The location of tsm-8 was shown by Fangman and Novick (9) to be closely linked to the str locus, which is situated at minute 64. Thus, the mutations used in this work represent at least two different loci.

**DISCUSSION**

The use of isogenic pairs of temperature-sensitive mutants, as in the crosses described donors with different points of origin were mated with tsms recipients and selected recombinants were scored for the presence of the tsms allele. The results show that tsms-2, 4, 5, 6, and 7 are located between the origins of Hfr J4 and

![Fig. 1. Cesium chloride centrifugation of 3H-DNA recovered from cell lysates. (A) From the mating mixture at 34 C. (B) From the mating mixture at 42 C (○) and the unmated control (○). Centrifugation was for 16 hr at 40,000 rev/min at 20 C. Details of the mating and preparation of the lysates are described in the text. The banding position of lambda DNA is indicated by the arrow.](image)

![Fig. 2. Zone sedimentation in alkaline sucrose of 3H-DNA after purification by cesium chloride centrifugation. (A) From the mating mixture at 34 C. (B) From the mating mixture at 42 C (○) and the unmated control (○). Centrifugation was for 60 min at 40,000 rev/min at 20 C. The sedimentation position of lambda DNA is also shown.](image)
above, provides an unequivocal test of the essentiality for DNA transfer of the thermolabile gene product in each case. This system has the further advantage that the background of replication in the unmated controls at 42 C is sufficiently low to permit a direct measurement of the replication associated with F-lac transfer.

The results with eight different isogenic pairs (representing at least two genetic loci) show that F-lac is transferred, with concomitant DNA replication, at a temperature which almost completely inhibits vegetative DNA synthesis in the same cells. It can be concluded that vegetative replication and transfer replication are separate processes, the former requiring the action of at least two gene products which are not required by the latter. The gene products involved have not yet been identified.

In a separate experiment, we have measured the amount and the molecular weight of the DNA replicated at 42 C in unmated cultures of tsm-4 F-lac males and tsm-4 females. The amount of replication and the molecular weight of the replicated DNA were identical in the two cultures, suggesting that F-lac is not replicated at 42 C in the unmated male cells. If so, vegetative F-lac replication resembles vegetative chromosomal replication, rather than transfer replication, in its dependency on certain gene products.

The amount of DNA replication in seven of the eight mating mixtures at 42 C exceeded that of the unmated controls by amounts ranging from 45 to 200% of that calculated for the synthesis of two complementary strands per F-lac transferred. Given the experimental error in these measurements, such results are compatible with the synthesis of either one or two complementary strands.

A distinction between vegetative replication and transfer replication was also suggested by Bresler, Lanov, and Lukjaniec-Blinkova (6), based on crosses between an Hfr and an F-strain carrying different temperature-sensitive mutations. They observed a threefold increase in DNA replication in their mating mixture over that in the unmated controls at 42 C. Their results, however, might have reflected complementation between the different mutants used; furthermore, their system did not permit a genetic estimation of the amount of DNA transferred during the mating.

The mutation used in one of our isogenic pairs, tsm-4, is the one reported by Bonhoeffer to prevent transfer at 42 C when present in the recipient (2, 3). We have confirmed Bonhoeffer's observation that the yield of recombinants is sharply depressed when this strain is mated with Hfr H at 42 C; however, the positive results with F-lac donors (reference 5 and this study) coupled with those of Curtiss (8) and of Moody and Lukin (16) with different Hfr strains, indicate that at 42 C tsm-4 interferes with processes taking place in the zygote, rather than with transfer itself.

We have characterized the DNA synthesized during F-lac transfer by centrifugation in CsCl and by its sedimentation velocity in sucrose gradients. The replicated DNA had the same density as λ DNA, as expected for F-lac. At 34 C, the replicated DNA for mated cells had a molecular weight of 10^8 daltons. At 42 C, the replicated DNA from unmated cells had a molecular weight of 5 x 10^7 to 6 x 10^7 daltons; this difference may reflect a limited degradation of the DNA at 42 C, as reported for a tsm-4 strain by Buttin and Wright (7). The bulk of the

![Graph](http://jb.asm.org/Downloaded_from)
labeled DNA synthesized in the mating mixture at 42°C, which presumably is F-lac DNA, had a molecular weight of 5 × 10^7 to 6 × 10^7 daltons. This value may be compared to the molecular weight of F-lac DNA, which has been determined by Freifelder and Freifelder to be 5.5 ± 0.8 × 10^7 daltons (12).

ACKNOWLEDGMENTS

We thank F. Bonhoeffer and W. C. Fangman for supplying the temperature-sensitive strains, W. Dean Rupp for encouragement and advice, and N. Rosenbaum for excellent technical assistance.

During part of this study, M. G. Marinus was the recipient of a University Grants Committee of New Zealand postdoctoral fellowship. This work was supported by grant GB8062 from the National Science Foundation.

LITERATURE CITED