Fate of Transforming Deoxyribonucleate in *Bacillus subtilis*

MIROSŁAWA PIECHOWSKA* AND MAURICE S. FOX

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received for publication 12 July 1971

The majority of donor deoxyribonucleate (DNA) at early stages after uptake was found in a complex with a cell component which changes its buoyant behavior on equilibrium density gradients. Analysis of the recipient cell lysates, after treatment to dissociate the complex, showed about two-thirds of the donor molecules in denatured form and the rest associated with recipient DNA. Incubation of cells after DNA uptake leads to the disappearance of denatured donor DNA and to the increase of donor label associated with recipient DNA. Some characteristics of a component from intact cells or spheroplasts with affinity for denatured *Bacillus subtilis* DNA are described.

Earlier studies on the fate of donor deoxyribonucleate (DNA) in *Bacillus subtilis* by different investigators, using physical methods based on fractionation on CsCl gradients, have some contrasting results. At early stages after DNA uptake, nonintegrated donor molecules were found in native as well as denatured form by Bodmer and Ganesan (3), and in native form only by Pene and Romig (14) and Ayad and Barker (2). The existence of native or a native-like form of unintegrated DNA was difficult to reconcile with a transient loss of donor DNA transforming activity ("eclipse") between uptake and integration, observed by Venema et al. in biological experiments with *B. subtilis* (16, 17). This prompted us to undertake a further physical study in the hope that the determination of the state of transforming DNA, at early times after uptake, might improve our understanding of the possible events preceding its integration into the *B. subtilis* recipient chromosome. Special attention was devoted to the preparation of samples for analysis on CsCl or sucrose gradients, which involved treatments permitting total recovery of DNA from cells of the recipient culture.

**MATERIALS AND METHODS**

Strains. *B. subtilis* 168 (thy⁻ trp⁻) was used as the recipient and *B. subtilis* 168 (thy⁻ trp⁺) as the donor of the transforming DNA. *Salmonella typhimurium* (thy⁻) DB25 (obtained from D. Botstein, Massachusetts Institute of Technology) was the source of DNA used as position marker on CsCl gradients.

Transforming DNA. *B. subtilis* 168 thy⁻ bacteria from an overnight culture in Penassay Broth (Difco Antibiotic Medium No. 3) at 37 °C were centrifuged, washed, and suspended with a 10-fold dilution in Laird's growth medium 1 (C. D. Laird, Ph.D. thesis, Stanford University, Palo Alto, Calif., 1966; cited by F. H. Cahn and M. S. Fox in reference 6) supplemented with 3 μg of thymine per ml. After one optical density (OD) doubling at 37 °C (about 5 to 5.5 hr), the culture was diluted four times with identical preheated medium. Tritiated thymine (thymine-methyl-³H) from New England Nuclear Corp., Boston, Mass.) was added to a final activity of 1.15 Ci/mmole of thymine, and incubation was continued to the end of the logarithmic growth phase. Heavy tritiated DNA was isolated from bacteria grown in a deuterium oxide medium (99.9% D₂O, Bio-Rad Laboratories, Richmond, Calif.) containing a deuterated amino acid mixture and a deuterated sugar mixture (Merck Sharp and Dohme, Canada) approximating the composition of Laird's growth medium 1 (6). The medium was inoculated with bacteria that had been adapted by prior growth to the heavy medium. Labeled bacteria were harvested, washed twice with 0.15 M NaCl–0.1 M ethylenediaminetetraacetate (EDTA) at pH 8.3, resuspended in saline-EDTA, incubated with 250 μg of lysozyme per ml at 37 °C for 20 min, and lysed with 0.2% Sarkosyl (Geigy Industrial Chemicals, Ardsley, N.Y.). The lysate was incubated at 37 °C with 200 μg of boiled ribonuclease per ml (Worthington Biochemical Corp., Freehold, N.J.) and 20 units of T, ribonuclease per ml (Calbiochem, Los Angeles, Calif.) and digested with 1 mg of Pronase per ml (Calbiochem). DNA was isolated by isopycnic separation on a CsCl gradient. The fractions containing the DNA were pooled and dialyzed against a solution of 0.015 M NaCl plus 0.0015 M sodium citrate (SSC) at pH 7.0. DNA concentration was determined by the diphenylamine reaction (5). Heavy tritiated DNA used for experiments presented in this report had a specific activity of 3.3 × 10⁸ counts.

1 Present address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.
per min per μg of DNA; in general, 0.1 to 0.2 mg of DNA was obtained from 100 ml of culture.

For a position marker, heavy, 14C-S. typhimurium DB25 DNA was isolated from bacteria cultivated on TGC 20 medium (4) without casein hydrolysate, supplemented with 14C-thymine to 15 μCi per 4.5 μg of thymine per ml of medium. (Thymine-2-'4C from New England Nuclear Corp., Boston, Mass., was used.) N2H4, Cl, deuterium oxide, and deuterated glucose were used in the medium. The method of isolation was identical to that described above for transforming B. subtilis DNA.

Competent cultures. Competent cultures were prepared from a 12- to 13-hr culture of B. subtilis 168 (thy-'trp') grown in Penassay Broth (Difco Antibiotic Medium No. 3) at 37 C with shaking. The bacteria were centrifuged at 9,000 × g in a Sorvall SS34 rotor for 10 min and washed once by suspension and centrifugation in Laird's growth medium 1, supplemented with vitamin free Casamino Acids (Difco) to 0.04% and thymine to 8 μg/ml. The packed cells were resuspended in the supplemented medium 1, with a dilution of 10- or 15-fold and incubated with shaking at 37 C. After 2.5 hr of incubation (twofold increase of OD), 14C-thymine was added [methyl-14C](thymine from New England Nuclear Corp., Boston, Mass.) to a final concentration of 0.05 μCi per 2 μg of thymine per ml of medium, and incubation was continued to the end of the log phase. Dimethyl sulfoxide was added to 5% by volume, and portions of the culture were frozen in ethyl alcohol dry ice. The cultures were stored at −80 C and could be used reproducibly for 5 months as an inoculum for the final growth stage. A sample of the frozen culture was quickly thawed at 37 C and diluted fivefold into Laird's growth medium 2 (C. D. Laird, Ph.D. thesis, Stanford Univ., Palo Alto, Calif., 1966; cited by F. H. Cahn and M. S. Fox in reference 6) supplemented with 14C-thymine and cold thymine to a concentration of 0.05 μCi per 2 μg of thymine per ml of medium. The culture was shaken at 30 C for 2.5 to 3.5 hr until it reached maximal competence, as determined from previous tests (6). At this time, MgCl2 was added to a final concentration of 0.02 μM, and the culture was used for experiments on DNA uptake. The density of the recipient culture was 2 × 108 cells/ml. Transforming DNA was added to a concentration of 1 μg/ml, and the culture was incubated with shaking at 30 C for 15 min. DNA exposure was terminated with 25 μg/ml deoxyribonuclease (deoxyribonuclease I, Worthington Biochemical Corp., Freehold, N.J.) and 0.5 min of incubation at 37 C. In some experiments, longer incubation at 37 C was used (see detailed explanation in Results). After DNA exposure, the cells were chilled, centrifuged, washed twice with 0.15 M NaCl-0.1 M EDTA (pH 8.5), and resuspended in 0.15 M NaCl-0.001 M EDTA (pH 8.5) in one-fourth the volume of the recipient culture.

Lysates. Lysates were obtained by addition of lysozyme to 5 or 10 mg/ml and 30 to 40 min of storage at 2 C (interrupted by four 1-min heatings at 37 C) followed by addition of Sarkosyl to 1% and warming to 10 C for 1 min. Lysates were fractionated on a CsCl gradient directly or after different supplementary treatments described below. Fractionation on CsCl gradients was always preceded by two dialyses: one against 0.15 M NaCl-0.1 M EDTA (pH 8.5), and the second against 0.15 M NaCl-10-3 M EDTA (pH 8.0).

CsCl gradients. CsCl gradients were prepared by mixing 3.384 g of CsCl (Harshaw Chemical Co., Cleveland, Ohio), with 2.6 ml of 0.07 M phosphate buffer (pH 11.2) or 2.6 ml of 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.0) and 0.6 ml of the dialyzed cell lysate (corresponding to a 2-ml sample of the recipient culture) or isolated DNA in 0.15 M NaCl-10-4 M EDTA (pH 8.0). A small volume (2 to 5 μl) of a suitable reference DNA was added to the sample. The final refractive index (n25°) of the solution was 1.4034, corresponding to a density of ρ25° = 1.740. The pH of the simulated final system was correspondingly 11.2 or 8.0, as measured with a general-purpose Beckman combination electrode. No salt correction was made for these pH readings, but they may be regarded as reproducible instrument readings (18). Gradients were centrifuged, in polystyrene tubes topped with mineral oil, for 4 hr at 38,000 rpm in an SW39 rotor at 16 C. The bottom of the tube was pierced, and 3-drop fractions were collected on 25-mm filter paper discs (Schleicher and Schuell, Keene, N.H.). About 80 fractions were obtained. Radioactivity was measured after precipitation of DNA on the filters with cold 5% trichloroacetic acid, followed by washing with cold absolute ethanol and drying. Recoveries from fractions that were rerun and from the experiment described in Fig. 3 were from 90 to 100%. Less than 1% of the centrifuged trichloroacetic acid precipitable radioactivity could be detected on the centrifuge tubes after collection in any of the experiments.

Sucrose gradients. Analyses on sucrose gradients were done by layering 0.3 ml (corresponding to a 1-ml sample of the recipient culture) of the cell lysate on 4.6 ml of a 5 to 20% (w/v) linear sucrose gradient in 0.02 M Tris-10-3 M EDTA-0.1% Sarkosyl (pH 8) in a nitrocellulose tube, and centrifuging the sample in a Spinclo SW39 rotor for 100 min at 35,000 rpm at 22 C. The tube was pierced and 3-drop fractions were collected on filter paper discs. Radioactivity was counted after trichloroacetic acid precipitation, as described for samples collected from a CsCl gradient.

Thermal denaturation. DNA solutions at a concentration of 10 μg/ml in 0.015 M NaCl-0.0015 M sodium citrate were denatured by heating 0.5-ml samples at 100 C for 6 min and then rapidly cooling them in ice water.

Radioactive counting was done on a scintillation counter (Nuclear-Chicago Mark I) with 42 ml of liquifluor (New England Nuclear Corp., Boston, Mass.) per liter of toluene as the scintillation solution.

RESULTS

State of donor DNA at an early stage after its uptake. Preliminary experiments were carried out with donor DNA labeled with 3H-thymine and recipient cells labeled with 14C-thymine. Half a minute after DNA uptake, cells were collected, lysed, and analyzed on a CsCl gradient at pH 11. The recipient DNA was found at the position expected for B. subtilis DNA, but the donor DNA floated to the top of the gradient or ex-
tended from the top to the position characteristic of *B. subtilis* DNA. The distribution of the donor DNA on the gradient depended on the method of preparation of the lysate, or the pH of the gradient, or both.

The difference in state of donor and recipient DNA was confirmed by a detailed study in which deuterated 3H-donor DNA was used. The relative positions of native and denatured "heavy" 3H-donor and "light" 14C-recipient *B. subtilis* DNA, versus "heavy" *Salmonella* DNA used as a position marker, on gradients at pH 8 and pH 11.2, are shown in Fig. 1A and 1B. On both gradients, deuterated denatured DNA bands are on the "heavy" side, and deuterated native DNA is on the "light" side of the marker. At pH 8 the peak of denatured, deuterated molecules is separated by 3 fractions and at pH 11.2 by 15 fractions from the marker, which is compatible with the behavior of denatured DNA on an alkaline CsCl gradient, at pH 11.2 (18). The cell lysates prepared from the recipient culture, incubated for 0.5 min at 37 °C after termination of exposure to deuterated 3H-donor DNA, were fractionated on a CsCl gradient at pH 11.2 and pH 8, with addition of a "heavy" *Salmonella* DNA position marker. Figure 2 shows sharp bands of the recipient DNA and broad, heterogeneous bands of donor DNA. All samples of cell lysates were digested with Pronase before fractionation on the basis of results of preliminary experiments showing that this treatment shifts the donor molecules away from the top of the gradient. The influence of Pronase may also be appreciated by a comparison of results obtained with samples digested once (Fig. 2A) and a second time (Fig. 2B) with the enzyme. In both samples, donor DNA extends from the position of native "light" to that of denatured "heavy" DNA on the CsCl gradient at pH 11.2, but the increase in the amount of donor DNA found at positions heavier than that expected of heavy native DNA, due to the more extensive digestion with Pronase, is apparent. A similar change was observed when digestion with lipase preceded the treatment with Pronase (Fig. 2C). Even after this combined treatment, the amount of donor DNA found at the position characteristic of denatured "heavy" molecules on the gradient at pH 8 is strikingly lower than at pH 11.2 (Fig. 2C and 2D). At pH 8, most of the donor DNA is found at densities lower than that of "light" recipient DNA. The occurrence of donor DNA on non-characteristic densities and in extended bands on CsCl gradients suggests that it is strongly complexed with some cell components that must be dissociated if the secondary structure of the DNA is to be determined by density gradient behavior. After heating of the cell lysates at 70 °C in high salt (4 M NaCl) with detergent (1% Sarkosyl) and analysis on CsCl gradients, the donor DNA banded at characteristic densities. This treatment, based on one of the oldest methods of DNA purification (7) and some newer observations of bacterial cell membrane properties (8), was chosen because of the suggestive evidence for DNA-protein (possibly lipid) complexes and their stability under conditions of centrifugation.

![Figure 1: Relative buoyant densities on a CsCl gradient, at pH 8 (A) and pH 11.2 (B), of 3H-labeled native (NH) and denatured (DH) heavy Bacillus subtilis DNA (O) and of 14C-labeled heavy Salmonella DNA (S) (Δ), which served as a marker. The expected location of native, light, *B. subtilis* DNA (NL) is indicated by the arrows.](http://jb.asm.org/)
FIG. 2. Influence of Pronase and lipase treatments, and of pH of the CsCl gradient, on the density of \(^{3}H\)-heavy donor DNA in lysates of recipient cells after uptake of \(^{3}H\)-heavy donor DNA. (A) Cell lysate digested with 1 mg of Pronase per ml during overnight dialysis at 37 to 42°C against 0.15 M NaCl-0.1 M EDTA (pH 8.5), followed by dialysis against 0.15 M NaCl-10\(^{-4}\) M EDTA (pH 8.0); pH of gradient, 11.2; 0.25-ml lysate. (B) As in (A), but 0.2 mg of Pronase per ml added after dialysis and incubation continued for 2 hr at 37°C; pH of gradient, 11.2; 0.75-ml lysate. (C) As in B, but each Pronase treatment was preceded by treatment with 100 µg of lipase per ml (wheat-germ, Calbiochem) for 20 min at 37°C, pH 8.5 before dialysis, pH 8.0 after dialysis; pH of gradient, 11.2; 0.5-ml lysate. (D) Lysate prepared as for C, but pH of gradient, 8; 0.5-ml lysate. \(^{14}C\)-recipient and (S) Salmonella marker DNA (Δ); \(^{3}H\)-donor heavy DNA (○). Expected position of denatured heavy donor DNA (DH) is indicated by the arrows.

shows about 70% of donor DNA forming a sharp band at the position characteristic of denatured “heavy” DNA; the rest is associated with native “light” recipient molecules (Fig. 3A and 3B). The absence of native donor DNA in these lysates is particularly evident from the pH 11.2 gradient. The separation of donor DNA bands can be increased by a slight modification of the procedure, consisting in the twofold heating of the cell lysate and an additional Pronase treatment (Fig. 3C and 3D).

Sucrose gradients. Cell lysates were analyzed on sucrose gradients to determine whether the donor DNA fraction, showing noncharacteristic density on a CsCl gradient, was associated with recipient DNA but dissociated at high salt concentrations. The recipient DNA present in a cell lysate carefully prepared to avoid exposure to shearing forces sediments more rapidly than the highly purified donor DNA. Therefore an association of donor with recipient DNA might be revealed by their cosedimentation on a sucrose
Fig. 3. Liberation of denatured donor DNA from cell lysate after heavy $^3$H-DNA uptake. (A) NaCl to 4 M concentration was added to cell lysate containing 1% sarcosyl. Sample was heated at 70°C for 20 min and fractionated on a CsCl gradient at pH 8. (B) Same sample as for A, fractionated at pH 11.2. (C) Lysate heated at 70°C for 20 min and digested with 1 mg of Pronase per ml during overnight dialysis against saline-EDTA at 37 to 42°C. Pronase to 0.2 mg/ml was added, incubation at 37°C was continued for 2 hr. NaCl was added to 4 M, and the sample was heated at 70°C for 20 min and fractionated on CsCl gradient at pH 8. (D) Same sample as for C, but on CsCl gradient at pH 11.2 $^3$H-donor, heavy DNA (○); $^{14}$C-recipient, light DNA and (S) Salmonella DNA position marker (Δ).

Gradient. The recipient cell lysate was prepared after exposure to heavy donor DNA and digested with lipase and Pronase under conditions giving DNA with noncharacteristic density (procedure as described for Fig. 2D). Fractionation on sucrose gradients was carried out under conditions in which DNA with a molecular weight of 50 $\times$ 10$^6$ should band in the middle of the gradient. The result (Fig. 4A) shows three bands of donor DNA: (i) containing about 64% of the label as
slowly sedimenting DNA, practically free of recipient label; (ii) 24% as a fast sedimenting band found at the same position as the recipient molecules, and (iii) the remaining 12% of the donor label cosedimenting with a portion of the recipient DNA which may be in nonlysed cells or bound to cell membrane fragments. Bands i and ii were collected and analyzed on a CsCl gradient at pH 8 (Fig. 4B and 4C). Band i was broad and heterogeneous and extended from the top of the gradient to the position of "heavy" B. subtilis DNA (compare with Fig. 2D). Band ii formed one sharp peak composed of "heavy" donor DNA label associated with "light" recipient molecules. This fraction did not contain the DNA with noncharacteristic density.

These results suggest that the donor DNA found in the complex was not associated with recipient DNA.

Reconstruction experiments. A set of experiments was carried out to examine the relationship between the observed cell component-donor DNA complex and the transformation process. Native or denatured 3H-DNA (light) treated with lysozyme and Pronase under conditions leading to the appearance of the DNA with noncharacteristic density (as described for Fig. 2A) formed bands at their characteristic densities on CsCl gradients. Native 3H-DNA added to the recipient cell suspension of a competent culture or their spheroplasts just before lysis (Fig. 5A and 5B) similarly exhibited behavior characteristic of native DNA. On the other hand, denatured 3H-DNA added to the cells (Fig. 5C) or spheroplasts (Fig. 5D) at low temperature (2°C) before completion of lysis with detergent (samples treated by the procedure described for Fig. 2A) bands at densities that are not characteristic of denatured DNA. The results of fractionation on CsCl gradients are shown in Fig. 5C and 5D. The 3H-DNA appears in positions extending from the top of the gradient to the position of the Salmonella DNA marker, with little, if any, of the material appearing at the expected density for denatured DNA.

These observations clearly indicate the existence of a cell component or components with surprising affinity for denatured DNA. The differences in the shape of DNA bands observed in experiments with intact cells and lysozyme-treated cells might suggest that a cell wall component could be involved in the complex.

Fate of the donor DNA fraction which may be released as denatured DNA molecules. Lysates were prepared from samples of transformed bacteria that had been allowed 0.5, 4, and 20 min of incubation at 37°C after termination of DNA exposure and treated to obtain the optimum resolution, i.e., heated Sarkosyl at high salt concentration and digested with Pronase (as described for Fig. 3C). The analysis was carried out on CsCl gradients at pH 11.2. In the 0.5-min sample, about 30% of the donor label was found
The fate of donor DNA was further examined by isolating fractions in the density region of the recipient DNA and fractionating them a second time on CsCl gradients, after shearing to reduce the molecular weight of the DNA. A cell lysate from a recipient culture, incubated at 37°C for 0.5 min after 3H-heavy DNA uptake, was prepared in the manner described for Fig. 6 and fractionated on a CsCl gradient at pH 11.2. The band in the region of the recipient label was collected, dialyzed against 0.15 M NaCl-0.015 M sodium citrate (SSC), concentrated in a flash evaporator, and dialyzed again against SSC. Glycerol to a concentration of 30% was added, and the sample was sheared in an ice bath to reduce the molecular weight to 4 x 10^4, dialyzed against 0.1 x SSC, and run on CsCl gradients before and after thermal denaturation (Fig. 7A and B). A second sample of the same culture was incubated for an additional 20 min at 37°C after DNA uptake. This sample was lysed and treated similarly (Fig. 7C and D). As in the previous experiment, the 20-min incubation resulted in a substantial increase, about 2.2-fold, of donor label associated with DNA of the recipient bacteria. In the gradients of native DNA, the donor label appears at a density position significantly heavier than that of the recipient DNA. The gradients of denatured DNA show that the donor DNA is heterogeneous in density and that most of it is substantially heavier than the recipient DNA. These observations suggest that most of the donor label that becomes associated with

Sarkosyl, and Pronase, as described for Fig. 2B, and fractionated on a CsCl gradient. (B) As in A, but native 3H-DNA was added to the spheroplasts at 2°C. (C) As in A, but denatured 3H-DNA was used in place of native DNA. (D) As in B, but with denatured 3H-DNA added to the spheroplasts. 3H-transforming DNA (O); 14C-DNA from the recipient culture and (S) Salmonella DNA marker (△). Expected position of the 3H-denatured light DNA (DL) is indicated by arrows.

Fig. 5. Distribution on a pH 11.2 CsCl gradient of native and denatured DNA added to a suspension of cells or spheroplasts prepared from a recipient culture. (A) Native, light 3H-transforming DNA (0.016 μg) was added at 2°C to 0.5 ml of a cell suspension in saline-EDTA. The sample was treated with lysozyme, at the recipient "light" DNA peak, the remainder forming one band at the density characteristic of denatured "heavy" DNA (Fig. 6). Longer incubation caused the disappearance of single-stranded donor DNA and a simultaneous increase in the amount of donor label in the recipient peak. The ratio of the donor to the recipient label found in the light peak increased from 0.3 in the 0.5-min sample (Fig. 6A) to 0.61 in the 4-min sample (Fig. 6B) and up to 0.65 in the 20-min sample (Fig. 6C). The overall ratio of 3H/14C in the 0.5-min sample was 1.2, so that after 20 min of incubation at 37°C, about half of the donor label is lost, presumably as trichloroacetic acid-soluble fragments arising from DNA that appeared in the single-strand peak.
recipient DNA during incubation of the transformed bacteria results from incorporation of long, continuous polynucleotide chains. These polynucleotide chains appear to have been drawn from the pool of material that is largely unassociated with the DNA of the recipient bacteria (Fig. 4) and can be released as single-strand DNA from the 0.5-min extract. Little of the donor label that is associated with the recipient DNA appears to originate from reincorporation of hydrolysis products of donor DNA.

**DISCUSSION**

The bulk of the donor DNA found in cell lysates at early stages after having been fixed does not appear to be associated with the DNA of the recipient bacteria. This major component, as much as two-thirds of the macromolecular label, can be separated from the recipient DNA by zone sedimentation and is complexed with one or more cell components. The complex survives the concentrations of CsCl and the high pH present in our equilibrium gradients and results in an altered characteristic buoyant density of the DNA. Although the complex is somewhat sensitive to Pronase digestion, the characteristic density of the DNA is manifested after heating to 70 C at high salt concentrations. After this treatment, the donor DNA assumes the characteristic density of denatured DNA. Reconstruction experiments show that such complexes need have nothing to do with transformation, but appear to be characteristic of denatured DNA present in solution at the time of cell lysis. The simplest interpretation would be that, as in pneumococcal transformation (10, 11, 13), some of the donor DNA is present as single-stranded material and that, on extraction from *B. subtilis*, it appears in the form of the complex.

About one-third of the newly introduced donor DNA cosediments with and bands at a buoyant density very nearly that of the DNA of the recipient bacteria and presumably represents material that has been integrated. There appears to be little or no physical material resembling native donor DNA present in any of the lysates.

On further incubation of the transformed bacteria, the complexed material which appears as denatured DNA (heating to 70 C at high salt) disappears, and about half of its label appears at densities very nearly that of the DNA of

---

Fig. 6. Fate of transforming DNA after uptake by recipient culture. CsCl (pH 11.2) density-gradient analysis of the lysates of cells incubated for 0.5 min (A), 4 min (B), and 20 min (C) at 37 C after 15-min uptake at 30 C of heavy 3H-transforming DNA. Preparation of lysates as described for Fig. 3C. 3H-donor heavy DNA (O); 14C-recipient light DNA and (S) Salmonella marker DNA (Δ).
the recipient bacteria. After shearing to a molecular weight of $4 \times 10^8$ and denaturation, most of the label resides in molecules that are more than half heavy, suggesting integration in poly nucleotide reaches of about $10^8$ daltons. It appears that about half of the material that in early lysates can be released as denatured DNA can become integrated into the recipient DNA; the remainder appears to be lost by hydrolysis.

The pathway of integration of transforming DNA remains elusive. Donor DNA which, on extraction, is found as a complex between a cell component(s) and single-stranded DNA later becomes associated with the DNA of the recipient bacteria. Although such complexes are demonstrable without the intervention of transformation, their existence might, among many possibilities, suggest some similarity with the T4 gene 32 product, a protein which binds preferentially to denatured DNA (1), catalyzes denaturation of DNA, and may play a role in T4 recombination (15). It remains possible that a preliminary interaction between donor and recipient DNA molecules occurs with complicity of a protein as a mediator between double-stranded recipient and donor molecules or double-stranded recipient and single-stranded donor DNA, leading to the formation of a DNA/protein/DNA complex.

ACKNOWLEDGMENTS
We thank David Dubnau for his suggestion of the reconstruction experiment.

This investigation was supported by Public Health Service grant AI-05388-08 from the National Institute of Allergy and Infectious Diseases to M. S. Fox. M. Piechowska was supported by National Institutes of Health Biophysics training grant 5TI GM00778-13.

LITERATURE CITED