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Kinetic Analysis of the Products of Donor Deoxyribonucleate in Transformed Cells of *Bacillus subtilis*

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This paper describes the major transmutations of donor deoxyribonucleic acid (DNA) after uptake by competent *Bacillus subtilis* cells. Kinetic experiments confirm that after exposure to competent cells, donor DNA is converted to double-stranded fragments (DSF) which can be isolated as early as 30 s from the beginning of the reaction. At this time, DSF represent the only identifiable product of donor origin. After 1 to 2 min, DSF are converted to deoxyribonuclease-resistant forms, identified as single-stranded DNA fragments (SSF). SSF are intermediates in the transformation process leading to the formation of donor-recipient complex. This component makes its appearance between 2 to 4 min from the beginning of the transformation process. All the donor-recipient complexes found at the end of the reaction can be accounted for quantitatively by the DSF and the SSF found in the initial stages of transformation. A quantitative discussion of the transformation process is included.

At early times after uptake of ^{3}H-thymidine-labeled transforming deoxyribonucleic acid (DNA) by competent cultures of *Bacillus subtilis*, Dubnau and Cirigliano (11) were able to identify three radioactive products derived entirely from donor DNA: (i) acid-soluble material identified as 5'thymidine monophosphate (TMP); (ii) double-stranded DNA fragments (DSF) with a molecular weight of about 9×10^6; (iii) single-stranded DNA fragments (SSF) of lower molecular weight: 10^5 to 2×10^5. Upon further incubation of the transformation mixture, the donor DNA radioactivity appeared in association with the recipient genome in donor-recipient complex (DRC). The formation of DRC was closely followed in time by an increase in the donor and recombinant transforming activity in lysates prepared from transformed cells (14, 25). In *B. subtilis* single-stranded donor DNA is integrated (2, 5, 7, 14), single-stranded DNA is capable of transforming competent cells (9, 24), and only one strand from each donor molecule is integrated (23). In *Pneumococcus* approximately one-half of the donor DNA is degraded while the remainder appears as single-stranded DNA and is probably used for recombination (18, 21). It has been suggested (18) that one donor strand from a given double-stranded molecule is degraded exonucleolytically while the other is made available for integration. The production of SSF and 5' TMP from transforming DNA in *B. subtilis* was consistent with this model. However, the low molecular weight of SSF made the latter a poor candidate for an intermediate on the pathway of integration, since the size of the integrated donor moiety was estimated as 2.8×10^6 daltons (13). A modified DNA extraction procedure (10, 22) has permitted the recovery of higher-molecular-weight SSF. This has encouraged us to perform a kinetic analysis of the macromolecular products of donor DNA in transformed cells. Previous results had shown that DSF, the first detectable product of donor DNA, is a precursor of DRC (12). The present work demonstrates that SSF is derived from DSF, and is itself a precursor of DRC.

MATERIALS AND METHODS

B. subtilis strains. The bacterial strains used were derivatives of *B. subtilis* 168. Strain BD170 (*thr-5 trp-2*) was used as the recipient for the transformation experiments, while BD204 (*hisB2 thyA thyB*) was used for the preparation of donor DNA.

Preparation of competent cells and transforming DNA. Competent cells were prepared as described

previously (14). To prepare labeled DNA, Spizizen medium (1), containing 0.5% glucose, 0.03% casein hydrolysate, 50 μg of tryptophan per ml, 50 μg of histidine per ml, and 50 μg of thymidine per ml, was inoculated with *B. subtilis* BD204 and incubated overnight at 30 C. The overnight culture was centrifuged, washed, and used to inoculate 150 ml of the same medium containing 5 mCi of thymidine-*methyl*-^3H and 300 μg of cold thymidine as carrier. The culture was incubated at 37 C with shaking, until the end of exponential growth. The cells were centrifuged and then washed and suspended in 5 ml of lysis medium (0.1 M sodium versenate plus 0.05 M NaCl, pH 6.9). The cell suspension was treated with 500 μg of lysosyme per ml at 37 C for 15 to 20 min. Lysis was completed by the addition of 0.1% sodium dodecyl sulfate. Pronase (Calbiochem) was added to a concentration of 1 mg/ml, and the lysate was incubated at 48 C for 2 h. The lysate was then gently shaken with an equal volume of redistilled phenol saturated with 0.015 M NaCl plus 0.0015 M sodium citrate (0.1 × SSC) and adjusted to pH 7.0 to 8.0 with 1 M NaOH. After centrifugation, the aqueous layer was removed, and the DNA was precipitated by the addition of two volumes of ethanol. The precipitated DNA was spooled on a glass rod and then redissolved in 0.1 × SSC. Ribonucleic acid was removed by incubation at 37 C for 30 min with pancreatic ribonuclease (100 μg/ml) and takadiastase T_1 (10 U/ml) (Worthington). The DNA was precipitated two additional times with ethanol and finally dissolved in 0.1 × SSC. To avoid contamination the DNA solution was kept over chloroform. The specific activity of the DNA prepared in this way was 0.4 × 10^6 counts per min per μg.

For the preparation of heavy DNA, BD204 was grown in the deuterium medium described by Bodmer and Schildkraut (6) with ^3H-thymine as label. Since growth in the deuterium medium is poor, the cells were harvested at the early stationary phase. The extraction of the DNA was the same as described for ^3H-labeled DNA. The specific activity of the ^3H, ^2H-labeled BD204 DNA was 0.5 × 10^6 counts per min per μg.

Transformation and DNA extraction. Frozen competent BD170 cells were quickly thawed and incubated for 3 min at 37 C. To begin the transformation reaction, ^3H-DNA was added and transformation was interrupted at different times as indicated in the text. The chilled cell samples were washed twice with 0.1 M ethylenediaminetetraacetate (EDTA) plus 0.15 M NaCl. The cell samples were suspended in half their original volume in 0.1 M NaCl plus 0.001 M EDTA, pH 6.9. Samples were removed to determine radioactive uptake, and DNA extraction was carried out by the method of Piechowska and Fox (10, 22). Lysozyme was added to 10 mg/ml. After incubation at 0 C for 40 min with four 1-min interruptions at 37 C, 3 drops of Sarkosyl NL-30 were added, and the suspension was gently mixed and heated at 70 C for 20 min until total clearing occurred. Pronase (1 mg/ml) was added, and the preparation was dialyzed overnight at 37 C against 0.15 M NaCl plus 0.1 M EDTA, pH 8.5. The next day dialysis was continued for 2 h against 0.15 M NaCl plus 0.001 M EDTA, pH 8.5, and the samples were then treated with 0.2 mg of additional Pronase per ml for 2 h at 37 C. The samples were adjusted to their original volumes, and solid NaCl was added to a concentration of 4 M. The samples were again heated at 70 C for 20 min and subjected to CsCl density gradient centrifugation, as described below. The CsCl gradients were all loaded with DNA corresponding to the same initial volume of cells.

CsCl density gradient centrifugation at pH 11.1. The CsCl gradients were prepared by mixing 4.45 g of CsCl with 3.5 ml containing the DNA samples and 0.4 ml of 0.4 M K_2HPO_4 buffer, pH 11.1. A 15-μg amount of heat-denatured *Escherichia coli* carrier DNA was added to decrease the loss of single-stranded material. The final refractive index of the solution was adjusted to 1.4025 to 1.4030. Gradients were centrifuged in polyallomer tubes topped with mineral oil, in a Ti 50 angle rotor at 42,000 rpm at 25 C for about 42 h. The tubes were punctured, and 4-drop fractions were collected onto Whatman GFA filters. The filters were washed twice with ice-cold 5% trichloroacetic acid and twice with 95% ethanol, dried and counted in toluene containing 5 g of 2,5-diphenyloxazole per liter. Beckman LS 200B scintillation spectrometers were used.

The use of heavy ^3H-labeled DNA as a donor allows the differentiation on CsCl gradients between different DNA products of donor origin: DSF, SSF, and DRC. DSF and SSF band in CsCl at the density of heavy native and denatured DNA, respectively. DRC is a heteroduplex structure (14) with a relatively small heavy donor moiety, associated with the much longer DNA from the recipient genome; therefore its density is essentially that of native light *B. subtilis* DNA. Thus, the integration of donor DNA can be followed by monitoring the shift of the ^3H-label to the density of light, native DNA.

CsCl density gradient centrifugation at pH 8. The pH 8.2 CsCl sample mixtures contained 10^{-2} M tris(hydroxymethyl)aminomethane, pH 8.2, 10^{-3} M EDTA, and 1 ml of cell lysate. Solid CsCl was added to bring the refractive index to $\rho = 1.4015$. Centrifugation was carried out at 28 C in nitrocellulose tubes topped with mineral oil in a Ti 50.1 rotor at 40,000 rpm at 25 C for 65 h. Five-drop fractions were collected onto Whatman GFA filters. Work-up of samples was carried out as with pH 11.2 CsCl gradients. DRC in Fig. 1 was estimated as the total trichloroacetic acid-precipitable radioactivity banding at the density of heavy light DNA.

Sucrose gradient sedimentation analysis. The DNA samples were dialyzed against 0.001 M EDTA (pH 6.9), and then layered on 4.2-ml, 5 to 20% linear sucrose gradients containing 1 M NaCl and 0.05 M potassium phosphate (pH 7.0), resting on an 0.8-ml cushion of 60% sucrose. The gradients were centrifuged at 44,000 rpm at 20 C for 110 min in an SW50.1 rotor. The fractions (about 0.22 ml) were collected on GFB filters and washed with trichloroacetic acid and ethanol, as described above. To measure molecular weights, the DNA samples were cosedimented with coliphage T7 DNA. The relationship of Burgi and Hershey (8) was used to calculate the

molecular weight, with the T7 DNA molecular weight regarded as 25.3×10^6 (3).

Materials. Methyl-labeled ^{14}C and ^{3}H-thymidine were obtained from the New England Nuclear Corp. Deuterium oxide was obtained from Bio-Rad, and deuterated sugars and amino acids were from Merck, Sharpe & Dohme of Canada. CsCl was purchased from Harshaw.

RESULTS

Competent cells were transformed at 37 C with $^{3}H, ^{2}H$-BD204 DNA. The reaction was interrupted at different times by the addition of deoxyribonuclease (DNase) (50 μg/ml). Samples were plated for Trp^+ transformants, DNA was extracted, and DRC complex formation was estimated on pH 8.0 CsCl gradients as described in Materials and Methods. Figure 1 shows the time course of appearance of Trp^+ transformants in DNase-interrupted transformation and the total radioactivity banding at the position of DRC in CsCl gradients. When the data were extrapolated to zero transformation, the yield of Trp^+ transformants showed a lag of about 2 min, in agreement with previous results (12, 19). The appearance of DRC, however, was delayed by an additional 1 min. This suggested that a DNase-resistant intermediate, other than DRC, was formed beginning at about 2 min.

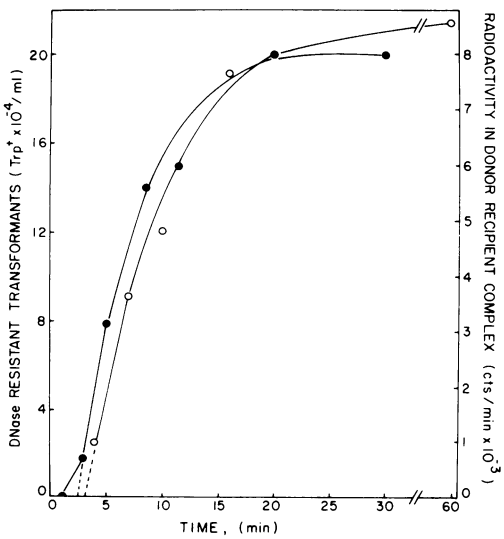

FIG. 1. *Competent cells were transformed with $^{3}H, ^{2}H$-BD204 DNA. At the indicated times samples of the transformation mixture were removed, and the reaction was interrupted by the addition of DNase (50 μg/ml). Samples were plated for Trp^+ transformants (●). Additional samples were washed, lysates were prepared, and the radioactivity in DRC was determined on CsCl pH 8.2 gradients* (○), *as described in Materials and Methods.*

To investigate the nature of this postulated intermediate, competent cells were transformed at 37 C with ^{3}H-thymine-labeled BD204 DNA. Samples containing equal volumes of the transformation mixture were withdrawn at 30 s, 2.5 min, 5 min, 8 min, 15 min, and 75 min. The cell samples were chilled and washed, and DNA was extracted as described in Materials and Methods. The macromolecular components were resolved by isopycnic centrifugation in CsCl at pH 11.1 (Fig. 2).

The ^{3}H-DNA in the 30-s sample formed a band at the density of native DNA. The appearance of SSF was evident in the 2.5-, 5-, and 8-min samples. At 75 min all the ^{3}H-DNA was found at the position of native DNA although some unexplained loss of radioactivity is obvious in this sample. Neutral sucrose gradient sedimentation profiles of the 30-s and 75-min samples revealed that all the radioactive material present at 30 s sedimented at the position corresponding to the molecular weight of DSF as expected from previous results (11). In the 75-min sample however, the ^{3}H-DNA was pelleted on the 60% sucrose shelf. We have shown previously that this rapidly sedimenting material is a complex of donor and recipient DNA (DRC) and that it contains most of the donor and all of the recombinant transforming activity of the lysates (14).

The appearance of SSF was evident by 2.5 min after initiation of uptake (Fig. 2) and was preceded by the appearance of DSF. SSF could, therefore, have arisen either directly from donor DNA or from DSF. To distinguish between these possibilities, it is necessary to stop the uptake of exogenous DNA at an early stage of the transformation process, ideally when the only product of donor origin found associated with the cell is DSF. To this end, prewarmed competent cells were mixed with $^{3}H, ^{2}H$-BD204 DNA at a concentration of 2 μg/ml, and the mixture was incubated at 37 C for 30 s. At this time a sample was removed, diluted 10-fold into chilled medium containing 200 μg of salmon sperm DNA per ml and Vortex mixed for 30 s. At the same time the rest of the reaction mixture was Vortex mixed for 30 s, diluted 10-fold into warm competence medium containing 200 μg of salmon sperm DNA per ml, and incubated further at 37 C. These manipulations were accomplished within 1 min and 15 s. Additional samples were withdrawn from the diluted transforming mixture at 2, 4, 6, 8, 12, and 75 min after the initial exposure to DNA. The dilution of the cells into a 1,000-fold excess

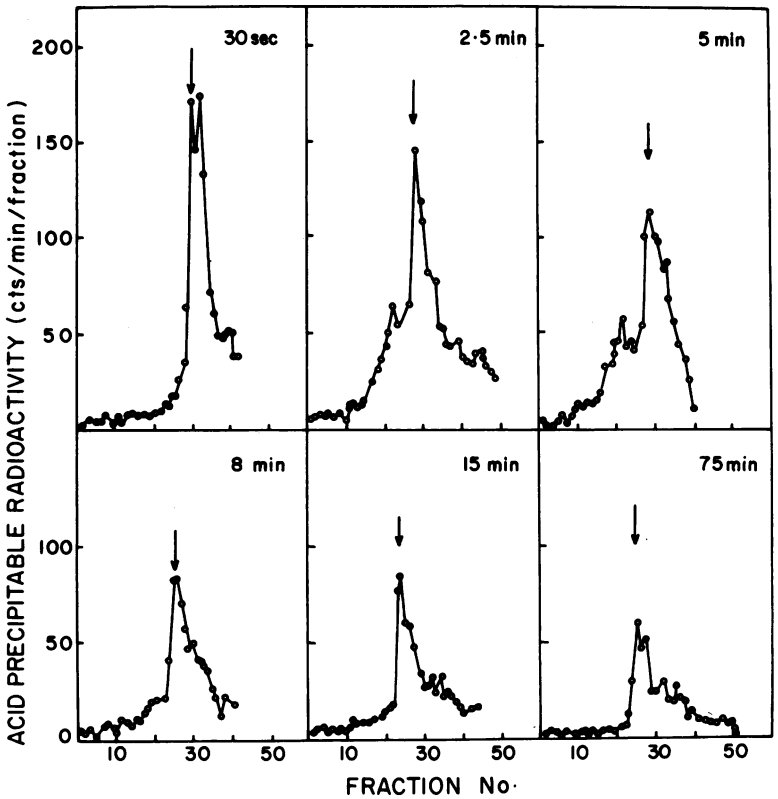

FIG. 2. *Kinetic analysis of the products of donor DNA in transformed cells. Prewarmed, competent cells were mixed with 20 μg of ^{3}H-BD204 DNA per ml. At the indicated times a sample was removed and washed, and DNA was extracted, as described in Materials and Methods. Samples (0.4 ml) of the lysates were centrifuged in pH 11.1 CsCl density gradients. The position of native DNA determined by the appearance of viscosity is indicated by the arrow.*

of salmon sperm DNA prevents new attachment of donor DNA to the cell (12, 15), while the Vortex mixing serves to remove DNA already attached which has not yet been converted to DSF (12). Thus, the analysis of the DNA samples after different times of incubation at 37 C would describe the further transmutations of donor DNA accumulated in the first 30 s as DSF. The use of heavy ^{3}H-labeled DNA allows the differentiation among DRC, DSF, and SSF. DSF and SSF, entirely of donor origin, have the density in CsCl of double- and single-stranded heavy DNA, respectively. DRC, as explained in Materials and Methods, will have the density of light native DNA. The cell samples, treated as described above, were washed, and DNA was extracted and resolved in pH 11.1 CsCl gradients (Fig. 3). The 30-s sample gave a symmetrical peak at the position of heavy donor DNA, as expected for DSF. In the 2-min sample there was a substantial decline in the amount of this material. At the same time a new band appeared at the density of denatured, heavy DNA (SSF). Radioactivity was apparent at the light, native buoyant density in the 4-min sample. This material, which corresponds to DRC, steadily increased in amount in the latter samples. The 75-min sample still contained some residual counts banding mainly at the density of DSF. This phenomenon has been observed a number of times with several samples of competent cells. (The data presented in Fig. 3 are summarized in Fig. 4 and in Table 1.) In the 30-s sample all of the trichloroacetic acid-precipitable counts were in the form of DSF. This macromolecular species declined very rapidly and then leveled off at about 12% of the initial value. The appearance of SSF began shortly after that of DSF and corresponded in time to the decrease observed in DSF. The radioactivity in DSF plus twice the radioactivity in SSF and DRC was approximately constant after 4 min. An increase in this computed total is observed before 4 to 6 min, indicating

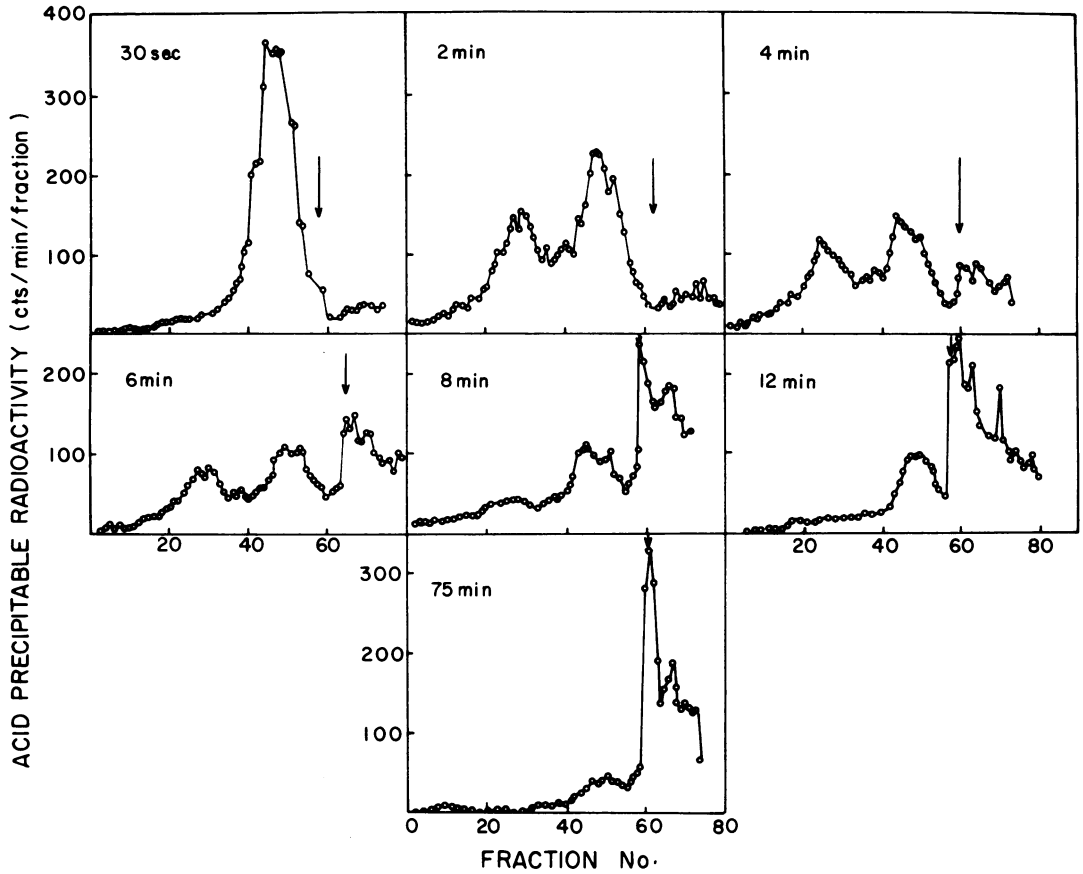

FIG. 3. *Kinetic analysis of the products of donor DNA in transformed cells. The distribution of acid-precipitable radioactivity is determined after pH 11.1 CsCl density gradient centrifugation of DNA samples extracted from B. subtilis cultures following transformation with ^{3}H, ^{2}H-labeled DNA, as described in the text. The position of light, native DNA determined by the appearance of viscosity is indicated by the arrow.*

that uptake continued beyond 30 s. By 2 min, however, (Table 1) close to 90% of the maximal uptake has occurred. SSF never rose above one-half the initial level of DSF. These numerical relationships would hold if SSF were an intermediate in a reaction that leads to the formation of the DRC and were derived from DSF by the destruction of one strand of each DSF molecule. DRC was detectable by 4 min after the initial exposure to transforming DNA and reached a plateau about 12 min after the beginning of the transformation process. Only half of the radioactivity in DSF is available for integration since only one strand is integrated from each donor molecule (23). On this basis, DSF cannot be the only precursor of DRC, since the amount of DSF present at 2, 4, and 6 min cannot account for the subsequent increase in DRC. The amount of SSF in the 2-min sample was also insufficient to account for all the DRC in the 75-min sample. However, if DSF is a precursor of SSF in the reaction DSF $\rightarrow$ SSF + 5'-mononucleotides, and if SSF is a precursor of DRC, then half of DSF plus the SSF in the 2-min samples should be equal to the amount of DRC found at the end of the reaction. Our data are in reasonable agreement with this model, although some of the quantitative aspects are obscured by the increase in total counts per minute mentioned above.

This model also requires that at least 50% of the DNA taken up by the competent cells be degraded. Additional degradation would depend on the efficiency of integration of the single-stranded fragments. Figure 4b shows the total directly determined trichloroacetic acid-precipitable radioactivity in the washed cell samples of this same experiment. The peak of uptake was not found at 30 s, but in the 2-min sample, indicating again that some uptake

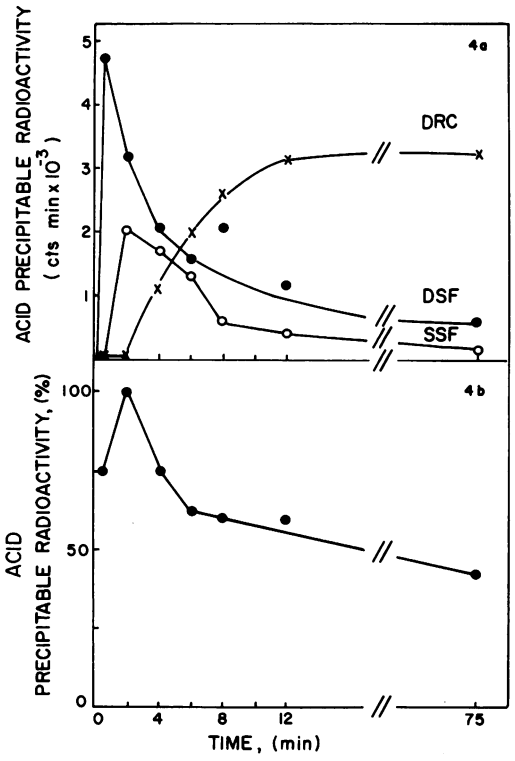

FIG. 4. *(a) Radioactivity in donor recipient complex (×), DSF (●), and SSF (○), in lysates resolved in the CsCl pH 11.1 gradients, as described in the text. The data were taken from Fig. 3. (b) Trichloroacetic acid-precipitable radioactivity after uptake of ²H, ³H-BD204 DNA, expressed as percentage of the 2-min value. A competent culture was incubated at 37 C for 30 s with ²H, ³H-BD204 DNA. The uptake was arrested by Vortex mixing the reaction mixture for 30 s and diluting the cells into prewarmed SP11 medium containing 20 μg of salmon sperm DNA per ml. At the indicated times samples were removed, washed, and assayed for trichloroacetic acid-precipitable radioactivity, DSF, SSF, and DRC.*

continued in this experiment beyond 30 s. If the 2-min value was taken at 100%, the total trichloroacetic acid-precipitable counts were reduced to 42% at 75 min. From the data in Table 1 we calculate that roughly 37 to 44% of the total counts per minute appear in DRC since the most extreme values of the ratio DRC/total computed counts per minute are 3,142/8,443 and 3,200/7,238.

A more direct experiment, based on the differential sensitivity of DSF and of SSF to pancreatic DNase, reveals the role of SSF as an intermediate in transformation and as a precursor of DRC formation. Competent cells were mixed with ³H, ²H-labeled *B. subtilis* DNA and

TABLE 1. *Analysis of pulse-transformation experiment*[a]

Time (min)	Radioactivity (counts/min)			
	DSF	SSF	DRC	DSF + 2 (SSF + DRC)
0.5	4,750	0	0	4,750
2	3,177	2,030	0	7,237
4	2,045	1,719	1,105	7,693
6	1,598	1,303	1,982	8,168
8	2,063	551	2,639	8,443
12	1,197	383	3,142	8,247
75	598	120	3,200	7,238

[a] Data of Fig. 3.

incubated at 37 C for 3 min. At that time two samples were withdrawn and chilled, one of which was treated with 50 μg of pancreatic DNase per ml. At the same time, 50 μg of pancreatic DNase per ml was added to the rest of the DNA-cell mixture, and incubation was continued at 37 C. Additional samples were withdrawn 6 and 45 min after the initial exposure to DNA. The cells were washed and DNA was extracted as described above. The different macromolecular components were resolved in pH 11.1 CsCl gradients (Fig. 5).

The addition of DNase not only terminated further uptake of exogenous DNA by the competent cells, but also degraded the DSF accumulated by the cells prior to the addition of DNase. A comparison of the gradients of the 3-min samples with and without DNase treatment led to the following observations. (i) Addition of the DNase after 3 min of uptake reduced the trichloroacetic acid-precipitable wash-resistant radioactivity by 78%. (ii) At least the bulk of the DNase-sensitive radioactivity appeared at the position of heavy, native donor DNA (DSF). (iii) The amount of radioactivity banding at the position of heavy, denatured DNA (SSF) in the two gradients was essentially the same. The recovery of acid-precipitable radioactivity from the gradients in the DNase-treated and untreated samples was 90 and 95%, respectively. In the 6-min sample the DNA banding at the position of SSF was significantly reduced, while radioactivity appeared in a new band corresponding to the density of the native DNA (DRC). This movement of radioactivity from the heavy, denatured position (SSF) to the native, light density (DRC) was essentially completed in the 45-min sample where all the radioactivity banded in the position of DRC.

Although acid-soluble radioactive material is

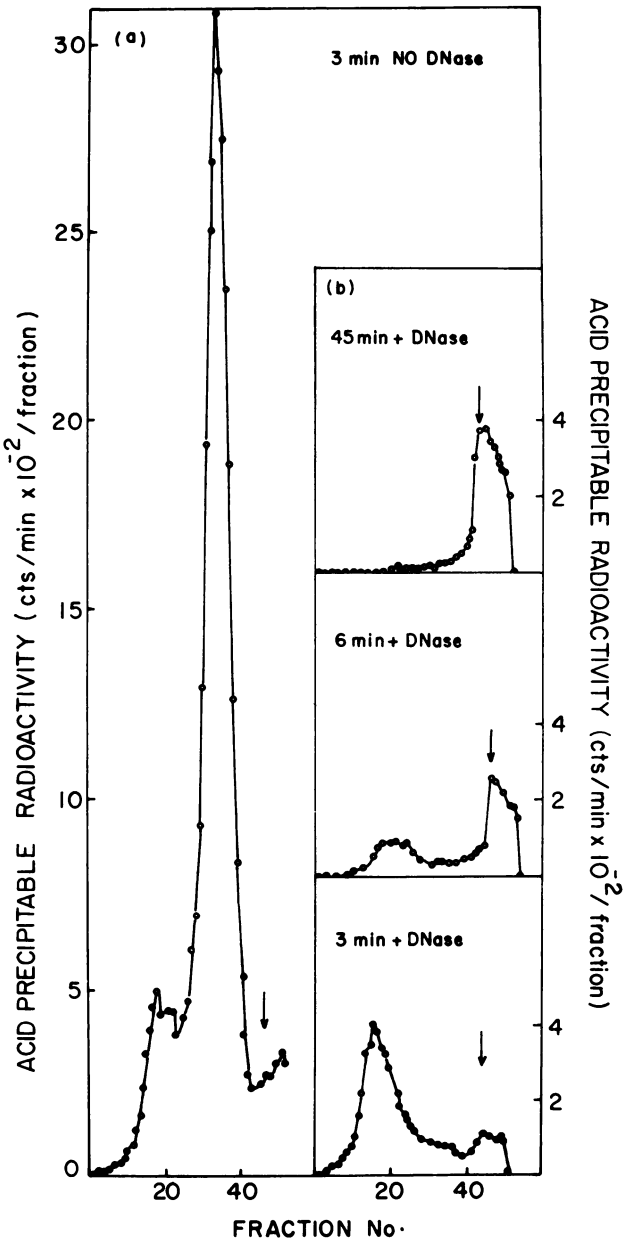

FIG. 5. *Competent cells were transformed with ^{2}H, ^{3}H-BD204 DNA. After 3 min a sample was removed and washed (a). The rest of the reaction mixture was further incubated in the presence of 50 μg of DNase per ml. Samples were removed at the indicated times (b), and DNA was extracted as described in Materials and Methods. Samples (0.6 ml) of the treated lysates were centrifuged in pH 11.1 CsCl density gradients. The position of native light DNA determined by the appearance of viscosity is indicated by the arrow.*

present in the reaction mixture, this material is not reutilized by the competent cells to any detectable extent (11). Since SSF was the only identifiable macromolecular product of donor DNA found in the 3-min DNase-treated sample, we can conclude that this material was the precursor of the DRC found in the 45-min sample. Furthermore, enough SSF is found in the 3-min sample to account for all the DRC present at 45 min.

DISCUSSION

The use of an improved method of DNA extraction (10, 22) has enabled us to add to our previous description of the major transmutations of donor DNA leading to the formation of DRC.

Exposure of competent *B. subtilis* cells to transforming DNA gives rise to a cell-DNA complex resistant to washing and dilution. In this complex the attachment of the DNA occurs with the donor DNA occupying a spatially extended configuration still sensitive to hydrodynamic shear (12). Complex formation occurs with immeasurable delay under usual conditions, since the wash-resistant transformation in *B. subtilis* proceeds linearly from time zero (12, 19). This first cell-DNA complex can be formed at more than one point on a given DNA molecule, possibly at the ends (17).

The development of resistance to shear corresponds in time to the endonucleolytic cleavage across both strands of the donor molecule which gives rise to DSF having an initial molecular weight of about 9×10^6 (11, 12). This first intermediate in the transformation process is probably produced and accumulated outside the cell permeability barrier since it is accessible to extracellular nuclease action (Fig. 5) (11). Our data show that DSF can be isolated as early as 30 s after initial exposure of the competent cells to transforming DNA (Fig. 2 and reference 11). Moreover, at this early time it represents the only identifiable form of donor DNA. Levine and Strauss (19) observed that the appearance of DNase-resistant transformants began after a lag of 1 to 1.5 min. Under our conditions this lag was about 2 min in length (Fig. 1). The fact that at 30 s the only identifiable product is DSF, which we have shown to be DNase-sensitive, is in agreement with these results. The 1- to 2-min lag in DNase-interrupted transformation probably corresponds to the time necessary to convert this first intermediate to a DNase-insensitive form. That this form is not DRC is suggested by the kinetic results showing that DRC formation begins at about 3 min (Fig. 1).

The appearance of SSF at 37 C begins between 0.5 and 2 min after exposure to transforming DNA (Fig. 3), which suggests that this is the second postulated intermediate, since it is possible to isolate SSF in the presence of DNase (10, 22). Our data strongly suggest that SSF is formed from DSF (Fig. 3 and 4). However, the mechanism of this conversion can only be postulated on the basis of circumstantial evidence. The appearance of SSF corresponds in time to the appearance of acid-soluble radioactivity (5′-mononucleotides) derived from donor DNA and their release into the medium (11). This suggests the possibility that a cell envelope-associated exonuclease hydrolyzes one strand of the DSF releasing the products into the medium, while transferring the sister strand (SSF) to a DNase-inaccessible location, possibly the cell membrane. A similar model has been suggested in *Pneumococcus* (18). Morrison (20) identified an EDTA-sensitive step before donor DNA becomes resistant to DNAse. Thus, either DSF or SSF formation, or both, may depend on the presence of divalent cations. The further elucidation of this problem depends on the isolation of the appropriate nucleases and of mutants lacking in these functions. Such efforts are now in progress in our laboratory.

The resistance of SSF to DNase may be relative, since Dubnau and Cirigliano (11) were able to render 95% of the donor DNA acid soluble by the combined use of both micrococcal nuclease and spleen phosphodiesterase, each at a concentration of 100 μg/ml. It is possible, therefore, that SSF is only partially protected from exogenous DNase.

The appearance of DRC follows very closely the disappearance of SSF in CsCl density gradients. At 37 C the transfer of ^{3}H-label from the heavy, denatured to the light density begins about 3 min after the beginning of the transformation process (Fig. 1 and 3). From previous data we know that at least two major steps are included in the formation of the DRC (14, 25). The first is the formation of an "open" DRC in which the donor moiety is attached to the recipient DNA by noncovalent interactions. This open complex has low transforming activity for donor markers, and the covalent association of donor and recipient DNA is concomitant with the recovery of the donor and the appearance of recombinant marker transforming activity (14).

Quantitatively, all of the estimated DRC at the end of transformation (Fig. 4 and 5) can be accounted for by the DSF and the SSF found in the initial stages of transformation. As shown above, and previously reported (12), about 40% of the material present as DSF is eventually integrated. If transformation were completely efficient, this value would be 50%. Our data do not allow us to determine whether this loss of material occurs during the conversion of DSF to SSF, during the production of DRC from SSF, or during both processes. The data shown in Fig. 5 allow us to calculate that 72% of the SSF found in the 3-min sample was finally converted

to DRC, in reasonable agreement with the finding noted above that about 40% of the donor DNA initially taken up resists degradation during transformation and is eventually found in DRC, corresponding to 80% on a single-strand basis. This implies that the loss of material occurs during DRC production from SSF. The incubation in DNase could, in fact, have partially degraded some of the available SSF, thus artifactually reducing the final amount of DRC formed. Alternatively, loss of donor material might depend on the initial number of single-stranded nicks in the donor DNA. Nicked DSF would give rise to small fragments, the integration of which would require many synapsis events, rendering the entire process less efficient. This model is at least qualitatively in accord with published data correlating the efficiency of transformation with the single-strand size of the donor DNA (4, 16).

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LITERATURE CITED

1. Anagnostopoulos, C., and J. Spizizen. 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81**:741-746.
2. Ayad, S. R., and G. R. Barker. 1969. The integration of donor and recipient DNA during transformation of *Bacillus subtilis*. Biochem. J. **113**:167-174.
3. Bancroft, F. C., and D. Freifelder. 1970. Molecular weights of coliphages and coliphage DNA. I. Measurement of the molecular weight of bacteriophage T_7 by high-speed equilibrium centrifugation. J. Mol. Biol. **54**:537-546.
4. Bodmer, W. F. 1966. Integration of deoxyribonuclease-treated DNA in *Bacillus subtilis* transformation. J. Gen. Physiol. **49**(no. 6, part 2):233-258.
5. Bodmer, W. F., and A. T. Ganesan. 1964. Biochemical and genetic studies of integration and recombination in *Bacillus subtilis* transformation. Genetics **50**:717-738.
6. Bodmer, W. F., and C. Schildkraut. 1964. The preparation and characterization of $N^{15}H^2H^3$-labeled DNA from *Bacillus subtilis* and *Escherichia coli* phage T_2. Anal. Biochem. **8**:229-243.
7. Bresler, S. E., R. A. Kreneva, V. V. Kushev, and M. I. Mosevitskii. 1964. Molecular mechanism of genetic recombination in bacterial transformation. Z. Vererbungsl. **95**:288-297.
8. Burgi, E., and A. D. Hershey. 1963. Sedimentation rate as a measure of molecular weight of DNA. Biophys. J. **3**:309-321.
9. Chilton, M. D., and B. Hall. 1968. Transforming activity in single-stranded DNA from *Bacillus subtilis*. J. Mol. Biol. **34**:439-451.
10. Davidoff-Abelson, R., and D. Dubnau. 1973. Conditions affecting the isolation from transformed cells of *Bacillus subtilis* of high-molecular-weight single-stranded deoxyribonucleic acid of donor origin. J. Bacteriol. **116**:146-153.
11. Dubnau, D., and C. Cirigliano. 1972. Fate of transforming DNA following uptake by competent *Bacillus subtilis*. III. Formation and properties of products isolated from transformed cells which are derived entirely from donor DNA. J. Mol. Biol. **64**:9-29.
12. Dubnau, D., and C. Cirigliano. 1972. Fate of transforming DNA following uptake by competent *Bacillus subtilis*. IV. The endwise attachment and uptake of transforming DNA. J. Mol. Biol. **64**:31-46.
13. Dubnau, D., and C. Cirigliano. 1972. Fate of transforming deoxyribonucleic acid after uptake by competent *Bacillus subtilis*: size and distribution of the integrated donor segments. J. Bacteriol. **111**:488-494.
14. Dubnau, D., and R. Davidoff-Abelson. 1971. Fate of transforming DNA following uptake by competent *Bacillus subtilis*. I. Formation and properties of the donor-recipient complex. J. Mol. Biol. **56**:209-221.
15. Gabor, M., and R. D. Hotchkiss. 1966. Manifestation of linear organization in molecules of pneumococcal transforming DNA. Proc. Nat. Acad. Sci. U.S.A. **56**:1441-1448.
16. Hutchinson, F., and H. B. Hales. 1970. Mechanism of the sensitization of bacterial transforming DNA to ultraviolet light by the incorporation of 5-bromouracil. J. Mol. Biol. **50**:59-69.
17. Kelly, M. S. 1967. The causes of instability of linkage in transformation of *Bacillus subtilis*. Mol. Gen. Genet. **99**:350-361.
18. Lacks, S., B. Greenberg, and K. Carlson. 1967. Fate of donor DNA in pneumococcal transformation. J. Mol. Biol. **29**:327-347.
19. Levine, J. S., and N. Strauss. 1965. Lag period characterizing the entry of transforming deoxyribonucleic acid into *Bacillus subtilis*. J. Bacteriol. **89**:281-287.
20. Morrison, D. A. 1971. Early intermediate state of transforming deoxyribonucleic acid during uptake by *Bacillus subtilis*. J. Bacteriol. **108**:38-44.
21. Morrison, D. A., and W. R. Guild. 1972. Transformation and deoxyribonucleic acid size: extent of degradation on entry varies with size of donor. J. Bacteriol. **112**:1157-1168.
22. Piechowska, M., and M. S. Fox. 1971. Fate of transforming deoxyribonucleate in *Bacillus subtilis*. J. Bacteriol. **108**:680-689.
23. Strauss, N. 1970. Transformation of *Bacillus subtilis* using hybrid DNA molecules constructed by annealing resolved complementary strands. Genetics **66**:583-593.
24. Tevethia, M. J., and M. Mandel. 1970. Nature of the ethylenediaminetetraacetic acid requirement for transformation of *Bacillus subtilis* with single-stranded deoxyribonucleic acid. J. Bacteriol. **101**:844-850.
25. Venema, G., R. H. Pritchard, and T. Venema-Schröder. 1965. Properties of newly introduced transforming deoxyribonucleic acid in *Bacillus subtilis*. J. Bacteriol. **90**:343-346.