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Entry of Double-Stranded Deoxyribonucleic Acid During Transformation of *Neisseria gonorrhoeae*

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The state of donor deoxyribonucleic acid after entry into competent cells was examined by assaying the transformed cell lysates for donor-marker transforming activity and density of donor deoxyribonucleic acid in CsCl gradients. The experiments showed that deoxyribonucleic acid entered in native, double-stranded form.

The mechanism of entry of DNA in transformation of gonococci is understood poorly. In *Bacillus subtilis* and *Pneumococcus* spp., transforming DNA loses its biological activity immediately after absorption by the recipient cell (eclipse), but this activity reappears after a few minutes (2, 12). The transient loss of donor DNA activity in extracts of transformed cells is due to conversion of duplex donor DNA to high-molecular-weight but biologically inactive single-stranded DNA (5, 8). Neither the eclipse phenomenon nor free-single stranded DNA was detected in the transformation of *Haemophilus influenzae* (7, 11, 13). We reported earlier our preliminary findings that failed to demonstrate an eclipse in gonococci (10). We now directly confirm our previous inference, that gonococcal DNA enters in double-stranded form, by showing that all detectable donor DNA sediments as double strands immediately after uptake.

The gonococcal strains studied were FA19 (wild type), FA286 (FA19 *rif-1*), FA287 (FA19 *str-7*), F62, FA607 (F62 *str-7*), and FA617 (F62 *rif-6*). Labeling of DNA with [^{32}P]orthophosphoric acid (carrier free) was achieved in a phosphate-depleted 3% protease peptone medium. This medium was made in the following way. A 100-ml amount of a solution containing 6 g of peptone, 2 g of NaCl, and 2 ml of 1 M $CaCl_2$ was heated at 70°C for 10 min followed by rapid chilling, and the $CaPO_4$ precipitate was removed by centrifugation. Subsequently, it was autoclaved, adjusted to pH 7.4 with NaOH, and filter sterilized. Before use, this medium was diluted with an equal volume of sterile distilled water buffered by the addition of 1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid to a final concentration of 20 mM, and 2% (vol/vol) Kellogg supplement I and 0.2% (vol/vol) Kellogg supplement II (4) were added. The DNA was prepared as described by Marmur (6). A specific activity of 1×10^5 to 5×10^5 cpm/μg of DNA was obtained by using 10 to 50 μCi of [^{32}P]-orthophosphoric acid per ml.

The fate of DNA immediately after uptake was studied by brief exposure of competent cells to DNA followed by DNase treatment, washing, cell lysis, and assaying for either the biological activity of donor and recipient DNA in transformation of a second recipient or the physical properties of labeled donor DNA in a CsCl density gradient. Initial transformation was carried out by exposing competent colony type 1 cells (9) (3×10^7 to 10×10^7 colony-forming units per ml) suspended in GCB broth (4) containing 10 mM $MgCl_2$ to either ^{32}P-labeled DNA (0.3 to 0.7 μg/ml) or to 1 μg of unlabeled DNA per ml at 37°C (1). After 3 to 5 min, 10 to 25 μg of pancreatic DNase I per ml was added to terminate uptake. At 2 min after adding DNase (zero time) and at subsequent time intervals, samples were taken by pipetting into an equal volume of ice-cold GCB broth. All subsequent procedures were at 0 to 4°C until the completion of lysis. The samples used for assaying transforming activities were washed once in GCB broth containing 0.5 M NaCl, followed by two more washes with 0.15 M NaCl-0.015 M sodium citrate (SSC). Finally, the cells were resuspended in SSC and lysed by the addition of 0.1% Sarkosyl. Lysates were either diluted 100-fold in SSC or were dialyzed against SSC and diluted at least 10-fold in SSC before being used to transform FA19 or F62 by a standard transformation procedure (1). The transformants were selected for donor as well as recipient markers by the use of appropriate antibiotics (1).

In experiments using ^{32}P-labeled DNA to study the physical properties of donor DNA, the transformed cells were washed once with GCB broth supplemented with 0.5 M NaCl, washed twice with Davis minimal salt solution (Difco), and finally were suspended in 0.9 ml of SSC containing 500 μg of lysozyme per ml. After 30

min, 0.1 ml of 10% Sarkosyl was added and incubated until complete lysis occurred. ^{14}C- or ^{3}H-labeled reference gonococcal DNA that had been heat denatured was added to each lysate. The lysates were diluted in 0.15 M NaCl, and solid CsCl was added to produce a final refractive index of 1.401. The precipitate obtained at this stage was removed by low-speed centrifugation. Samples of 5 to 6 ml of supernatant CsCl solution were transferred to polyallomer tubes, which were overlaid with paraffin oil, and were centrifuged in a Beckman type 65 rotor at 45,000 rpm for 64 h at 15°C. At the end of the run, fractions of 0.1 ml (5 drops) were collected in scintillation vials by piercing the bottom of the centrifuge tube. Radioactivity was assayed by use of a scintillant consisting of 0.25 g of 1,4-bis-(5-phenyloxazolyl)benzene, 8.25 g of 2,5-diphenyloxazole, 500 ml of Triton X-100, and 1 liter of toluene in a Packard liquid scintillation spectrometer. Under the conditions used, there was less than 0.1% spillover of either ^{3}H or ^{14}C counts into the ^{32}P window.

The biological fate of donor DNA as a function of time after uptake is shown in Table 1. During the 30 min after zero time, there was little or no loss of donor-transforming activity as evidenced by the unchanged ratio of donor *rif-1* to recipient *str-7* marker activity. Since heat-denatured single-stranded gonococcal DNA is about 100-fold less active than native double-stranded DNA (data not shown), we concluded that most donor DNA was not converted to single strands after uptake. These results are similar to our earlier preliminary results with different markers in the donor and recipient (10).

TABLE 1. *Transforming activities in lysates as a function of time after uptake of rif-1 DNA by str-7 cells*[a]

Reisolation time (min)	Transformants/ml		*rif-1/str-7* ratio (10^{-2})
	rif-1 (10^{2})	*str-7* (10^{4})	
0	6.1	2.9	2.1
2.5	3.8	2.1	1.8
5	5.6	5.1	1.1
7.5	5.5	2.7	2.0
10	3.4	2.0	1.7
15	3.0	2.7	1.1
30	4.4	3.1	1.4

[a] FA286 DNA (*rif-1*) (2 μg/ml) was used to transform 10^{8} colony-forming units of FA287 cells (*str-7*) per ml for 5 min at 37°C in GCB broth containing 1 mM $CaCl_2$. (Similar results were attained with 10 mM $MgCl_2$.) Immediately thereafter, DNase (10 μg/ml) was added to terminate the transformation. Beginning 2 min after the addition of DNase (zero time), 40-ml cell samples were removed at periodic intervals and placed in chilled GCB broth, washed, and lysed as described in the text. Diluted lysates were used to transform *str*$^{+}$ *rif*$^{+}$ FA19 cells (6×10^{7} colony-forming units per ml). Selection for the donor *rif-1* marker was made with 10 μg of rifampin per ml; selection for the recipient *str-7* marker was made with 200 μg of streptomycin per ml. Results shown are the mean of three experiments.

The physical state of DNA that had just entered recipient cells was investigated by isopycnic centrifugation of samples from cells that had been exposed to donor DNA. Figure 1 shows the sedimentation profile of a sample removed 5 min after initiation of DNA uptake. ^{32}P-labeled donor DNA reisolated from transformed cells banded at the same density as the reference ^{14}C-labeled native double-stranded DNA. Since no ^{32}P peak was observed at the position of ^{14}C-labeled single-stranded DNA, we concluded that single-stranded donor material was not present in an appreciable amount in transformed cells. (Identical results were obtained by using ^{3}H-labeled reference DNA or no added reference DNA.) Similar sedimentation patterns were obtained in extracts that were allowed to incubate for 10 to 15 min after initiation of DNA uptake.

Our experiments do not disprove the existence of small amounts of single-stranded donor DNA during transformation; indeed, it is likely that

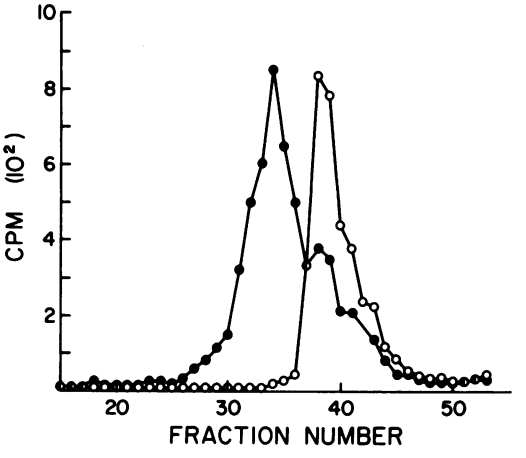

FIG. 1. *CsCl equilibrium centrifugation of transforming DNA immediately after uptake by a competent population of N. gonorrhoeae. FA617 cells (10^{8} colony-forming units per ml, 100 ml) were treated at 37°C for 5 min with ^{32}P-labeled FA607 DNA (0.6 μg/ml, 1.3×10^{5} cpm/μg). After an additional 2 min of incubation with 10 μg of pancreatic DNase I per ml, cells were chilled, washed, and lysed in 1 ml of SSC as described in the text. A mixture of one part native DNA and nine parts heat-denatured ^{14}C-labeled reference DNA prepared from F62 was added to the lysate. Cleared lysate (6 ml) in neutral CsCl was centrifuged at 45,000 rpm for 64 h. Density increases from right to left. Symbols: ○, ^{32}P; ●, ^{14}C.*

some donor DNA is converted at least transiently to single strands before formation of stable recombinants. However, observation of the retention of transforming activity of donor DNA and the co-sedimentation of donor and native DNA suggests that the bulk of donor DNA in *Neisseria gonorrhoeae* enters the cell in double-stranded form. This is similar to the situation in *Neisseria meningitidis* and *H. influenzae* in which free single-stranded DNA could not be detected in lysates of transformed cells (3, 7, 11), but it is unlike the extensively studied pneumococcal system in which DNA is taken up as single strands (5).

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