JOURNAL OF BACTERIOLOGY, Nov. 1986, p. 756–761
0021-9193/86/110756-06$02.00/0

Vol. 168, No. 2

Linearization of Donor DNA during Plasmid Transformation in *Neisseria gonorrhoeae*

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Received 7 April 1986/Accepted 14 July 1986

We examined the fate of plasmid DNA after uptake during transformation in *Neisseria gonorrhoeae*. An 11.5-kilobase plasmid, pFA10, was processed to linear double-stranded DNA during uptake by competent cells, but cleavage of pFA10 was not site specific. A minority of pFA10 entered as open circles. A 42-kilobase plasmid, pFA14, was degraded into small fragments during uptake; no intracellular circular forms of pFA14 were evident. Since pFA10 DNA linearized by a restriction enzyme was not further cut during uptake, the endonucleolytic activity associated with entry of plasmid DNA appeared to act preferentially on circular DNA. Although linear plasmid DNA was taken up into a DNase-resistant state as efficiently as circular DNA, linear plasmid DNA transformed much less efficiently than circular plasmid DNA. These data suggest that during entry transforming plasmid DNA often is processed to double-stranded linear molecules; transformants may arise when some molecules are repaired to form circles. Occasional molecules which enter as intact circles may also lead to transformants.

Transformation of *Neisseria gonorrhoeae* by plasmid DNA occurs at a low frequency relative to chromosomal DNA (4, 10, 27). Transformation of the gonococcus with either the beta-lactamase producing (Pc^r) plasmid pFA3 (7.2 kilobases [kb]) or pFA10 (11.5 kb) results in plasmid deletions in about 20% of the transformants (10, 27; data not shown). When the larger 42-kb Pc^r hybrid pFA14 is transformed into an isogenic recipient strain which lacks a homologous plasmid, Pc^r transformants are rare, and 100% of the Pc^r plasmids isolated from transformants are markedly deleted (4). Thus, deletions of incoming plasmids are common in gonococcal transformation, and the frequency of such deletions seems to increase with increasing size of the transformed plasmid.

Although pFA14 is generally quite inefficient in transformation, it efficiently transforms recipients that contain the homologous plasmid pFA2 (4). The marked increase in efficiency of transformation by pFA14 of recipients containing pFA2 was postulated to be due to marker rescue of fragmented pFA14 DNA by the resident plasmid (4), analogous to similar phenomena described in *Haemophilus influenzae* (1) and *Bacillus subtilis* (8).

Transformation of pFA3 into *Escherichia coli* does not result in plasmid deletions (27), suggesting that susceptibility to deletion is not an intrinsic property of these plasmids, but rather a result of processing by *N. gonorrhoeae* cells. Similarly, conjugal transfer of pFA3 into either gonococcal or *E. coli* recipients always produces a Pc^r plasmid identical in size to that of the donor (27). This indicates that the pathways for plasmid entry are probably different for transformation and conjugation and suggests that the deletions found in gonococcal transformants may reflect endonucleolytic attack during entry of transforming plasmid DNA (27). In this report, we present physical evidence that circular plasmid DNA is processed to linear double-stranded DNA during uptake, and that cleavage of entering plasmid DNA is not site specific. A preliminary report of these data was published recently (3).

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MATERIALS AND METHODS

Plasmids and strains. The plasmids and strains used are shown in Table 1.

Media and growth conditions. Media and growth conditions were as described previously (4). GC base broth (GCBB), made by using proteose peptone no. 3. (Difco Laboratories, Detroit, Mich.), or GC base agar (GCBA) (Difco; or GIBCO Diagnostics, Madison, Wis.) was used throughout.

Preparation of DNA. Plasmid DNA was isolated by alkali denaturation and phenol extraction of whole cell DNA, followed by one or two cycles of ethidium bromide-cesium chloride density gradient centrifugation (4). Plasmids from *E. coli* were isolated by lysing cells with alkali as described by Maniatis et al. (16). The plasmid content of transformants was examined by using crude cell lysates made by a modification of the method of Meyers et al. (18). Modifications included the addition of an equal volume of H_2O to the cleared lysate, precipitation in 0.3 M sodium acetate with 2.5 volumes of ethanol, and suspension in 10 mM Tris–1 mM EDTA, pH 8 (TE buffer).

Plasmid transformation. A detailed protocol has been described (3). Briefly, competent cells harvested from overnight growth on GCBA plates were suspended in GCBB (15 g of proteose peptone no. 3, 4 g of K_2HPO_4, 1 g of KH_2PO_4, 5 g of NaCl, and H_2O to make a liter) containing 10 mM $MgCl_2$ to yield a concentration of 10^7 to 10^8 CFU/ml. Plasmid DNA was added in a volume of 0.1 ml to the cell suspension to give the desired DNA concentration. The transformation mixture was incubated at 37°C for 30 min, and pancreatic DNase I (50 μg/ml; Worthington Diagnostics, Freehold, N.J.) was added. The mixture was immediately spread onto the surface of a GCBA plate, incubated at 37°C in the presence of CO_2 for 5 h, and overlaid with 5 ml of GCBA medium containing penicillin G to achieve the desired concentration. Recovery of maximum numbers of Pc^r transformants required the addition of the lowest drug concentration that inhibited the recipient cells; transformation frequency decreased sharply with high penicillin concentra-

TABLE 1. Plasmids and strains used in these studies

Strain	Description	Source or reference
N. gonorrhoeae		
FA559(pFA3)	pFA3 is a 7.2-kb naturally occurring gonococcal Pc^r plasmid	(10)
FA675(pFA10)	pFA10 is an 11.5-kb Pc^r plasmid, formed as a hybrid between pFA3 and the 4.2-kb gonococcal cryptic plasmid pFA1	(10)
FA867(pFA14)	pFA14 is a 42-kb Pc^r plasmid, formed as a hybrid between pFA3 and the 36-kb gonococcal conjugal plasmid pFA2	(4, 26)
FA759 (plasmid free)	Recipient for construction of FA559, FA675, and FA867	(4)
E. coli		
JM83	K-12 [ara Δ(*lac-pro*) *rpsL thi* φ80 d*lacZ*ΔM15]	D. Stein
SE5000	F^- *araD139 lacV169 rpsL relA thi recA56*	R. Taylor
FA6033	pBR322 transformant of SE5000	This work
FA6239	pFA10 transformant of JM83	This work

tions in the selective medium. With FA759 as the recipient, 2 μg of penicillin G per ml was used to select transformants. Transformants were counted after 40 h of incubation at 37°C under 5% CO_2. The production of penicillinase in the transformants was confirmed by streaking for isolated colonies onto GCBA containing 2 μg of penicillin G per ml and by use of a chromogenic cephalosporin indicator (22).

Plasmid transformation sometimes varied with the different lots of GCBA media used for growth, and also with the lots of proteose peptone no. 3 used in the GCBB transforming media. However, consistent results were obtained with the use of selected batches of GCBA and proteose peptone no. 3.

DNA uptake and reisolation of DNA. Plasmid DNA at nearly saturating concentration (0.1 to 0.25 μg/ml) (3) was added to 50 to 800 ml of GCBB plus 10 mM $MgCl_2$ containing approximately 5×10^7 CFU/ml, and the mixture was incubated for various times at 37°C. Uptake was terminated by chilling at 0°C for 30 min and adding 20 to 50 μg of pancreatic DNase I per ml. In some experiments, DNase treatment was omitted, and loosely bound DNA was partially removed by centrifuging the cells at 4°C for 10 to 15 min at 10,000 rpm in a Sorvall SS34 rotor, followed by three washes in cold GCBB medium containing 0.5 M NaCl and one wash in A medium (minimal medium A Davis; Difco). Cells were suspended in 25% sucrose–50 mM Tris hydrochloride (pH 8)–10 mM EDTA at 1/10 of the original volume. These were then lysed by the addition of 100 μg of lysozyme per ml with incubation for 20 min at 37°C, followed by the addition of 0.1 volume of 10% sodium dodecyl sulfate with gentle mixing. Next, 5 M NaCl was added to the lysate to a final concentration of 1 M and chilled overnight at 4°C. After centrifugation for 30 min at 15,000 rpm (Sorvall SS34 rotor), the supernatant was mixed with an equal volume of H_2O and extracted three times with an equal volume of chloroform-isoamyl alcohol (24:1). The aqueous phase was made 300 mM in sodium acetate and precipitated with 2.5 volume of ethanol at −20°C overnight; the precipitated DNA was suspended in TE buffer. When indicated, isolation of specific donor DNA bands from agarose gels was accomplished by electroelution as described by Maniatis et al. (16).

^{32}P labeling of plasmid DNA and blot hybridizations. DNA was nick translated by established methods (16). An average specific activity of 5×10^7 cpm per μg of DNA was obtained. Plasmid DNAs and restriction fragments were separated on submerged gels containing 0.8% agarose in a 40 mM Tris–20 mM sodium acetate–2 mM EDTA (pH 8.0) buffer or a Tris-borate-EDTA (16) buffer system. DNA separated by agarose gel electrophoresis was transferred to nitrocellulose filters (BA85; Schleicher & Schuell Co., Keene, N.H.), and filters were hybridized with ^{32}P-labeled nick-translated DNA and washed as described by Maniatis et al. (16).

Hybridization assay for comparison of plasmid uptake. Piliated FA759 gonococci (about 10^7 CFU) were suspended in 200 μl of GCBB containing 10 mM $MgCl_2$ and incubated in microtiter plates at 37°C for 20 to 30 min with 1 μg (saturating for transformation) of either linearized or circular (covalently closed circular [CCC] and open circular [OC] forms) pFA10 DNA prepared from *E. coli* FA6239. Suspensions were treated with DNase I (160 μg/ml) at 37°C for 15 min and collected onto nitrocellulose membranes on a minifold filtration apparatus (Schleicher & Schuell). This was followed by two 200-μl washes of 0.25 M NaCl in A medium. The nitrocellulose filters were treated as colony blots as described by Maniatis et al. (16) to lyse the cells and to denature and bind the liberated DNA to the membrane filter. The filters were baked for 2 h at 65°C under vacuum, soaked for 2 h in 1× Denhardt solution (16)–3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and hybridized with a saturating amount (usually 2 μg) of nick-translated pFA10 (prepared from *E. coli* FA6239) in 1× Denhardt solution–6× SSC–0.5% sodium dodecyl sulfate at 68°C. The filters were washed as described by Maniatis et al. (16). The amount of bound probe was determined for each sample by scintillation counting of the dried nitrocellulose filters in 5 ml of toluene-based scintillation cocktail. Each assay was done in triplicate.

Restriction endonuclease digestion. Restriction endonucleases were obtained from New England BioLabs, Inc. (Beverly, Mass.) or Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) and used with the procedures recommended by the suppliers.

RESULTS

Sox et al. (27) suggested that during gonococcal transformation deleted plasmids may be formed due to endonucleolytic cleavage of the transforming plasmid. We were interested in the possibility that circular plasmid DNA was linearized during uptake. To directly address this question, plasmid-free strain FA759 was transformed with unlabeled plasmid DNA, cellular DNA was extracted, and donor DNA was detected by probing with ^{32}P-labeled nick-translated donor DNA.

Plasmid pFA10 was linearized. Incubation of gonococci with pFA10 DNA showed that both competent (piliated, Pil^+) and noncompetent (nonpiliated, Pil^-) cells bound plasmid DNA in a DNase-sensitive form (Fig. 1). An additional band comigrating with linear pFA10 appeared in DNA extracted from competent cells but was nearly absent in noncompetent cells. Most DNase-resistant uptake of pFA10 by competent cells comigrated with linearized pFA10, although a minor amount of OC DNA (about 10% of total

DNase-resistant donor DNA) was also detected. In contrast, noncompetent (Pil^-) cells did not contain any detectable DNase-resistant pFA10 DNA. Since a significant amount of apparently linear pFA10 DNA was associated with competent cells even in the absence of added DNase, the linearized form of pFA10 was not an artifact due to the DNase treatment. We concluded that the majority of pFA10 DNA taken up by competent cells was converted to linear DNA. It also was evident that a high salt wash was not sufficient to remove all adsorbed DNA in the gonococci; the addition of DNase was required to remove all extracellular DNA.

Plasmid pFA14 was cleaved multiple times. Similar experiments with the 42-kb plasmid pFA14 DNA revealed that all DNA taken up by the cells into a DNase-resistant state was located at a position on the Southern blot corresponding to relatively low-molecular-weight DNA (Fig. 2). No bands were seen that corresponded to CCC, OC, or full-length linear forms of pFA14. These results suggested that pFA14 DNA was cleaved into small linear DNA fragments during uptake.

Cleavage was not site specific. The conversion of plasmid pFA10 into linear form during uptake might result either from cleavage at a specific site or from random cuts at various sites that occurred on the average only once per molecule. To distinguish between these two possibilities, we extracted DNA from cells exposed to pFA10 DNA and separated the extracted DNA on an agarose gel. The DNA region corresponding to linearized pFA10 was eluted from the agarose, digested with *AvaI* (which makes a single cut in pFA10), separated again on an agarose gel, and transferred to nitrocellulose. This Southern blot was then hybridized with labeled pBR322 (6), which shares homology with the portion of Tn*2* (ampicillin resistance transposon) found in pFA10 (23), but none with gonococcal chromosome. The autoradiogram (Fig. 3) did not show the discrete bands predicted if the gonococcal endonuclease activity that linearized pFA10 recognized a specific site; instead a smear of relatively low-molecular-weight DNA appeared. The fragmented DNA did not result from breakage during the reisolation procedure; this was shown by a control experiment where reisolated DNA not cut with *AvaI* appeared as a distinct linear-sized band on the autoradiogram (Fig. 3). These results are indicative of random (non-site-specific) cleavage of pFA10 during uptake.

Kinetics of cleavage. We examined the kinetics of plasmid cleavage by adding DNase at various times after the onset of incubation of pFA10 with competent cells (Fig. 4). Unit-length linear DNA was present at all times examined, but there was progressive degradation of plasmid DNA to lower-molecular-weight forms with increasing time of incubation. Some OC pFA10 DNA was observed at all times, but no

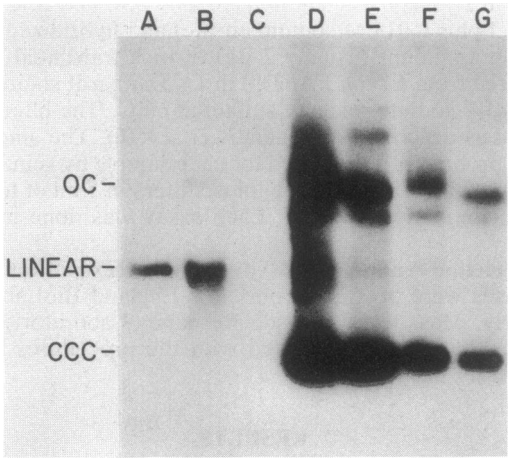

FIG. 1. Autoradiograph showing cell uptake of pFA10 DNA. The cells were suspended in 200 ml of transforming medium at a final concentration of about 5×10^7 CFU/ml and then incubated with 0.1 μg of donor DNA per ml at 37°C for 60 min. Transformation was terminated by chilling the cells in ice water. DNase (20 μg/ml) was added where indicated and incubated further in ice for an additional 30 min. The reisolated cellular DNA was dissolved in 100 μl of TE. (Each lane received the volume of extract indicated below in parenthesis.) After separation by agarose gel electrophoresis and transfer by Southern blot, DNA was detected by hybridization with ^{32}P-labeled pFA10. Control lanes: (A) cells plus 100 ng of *AvaI*-linearized plasmid DNA added during cell lysis (10 μl); (F) cells plus 200 ng of circular DNA added during cell lysis (20 μl); and (G) 200 ng of circular DNA alone (25 μl) (no cells). Lanes containing uptake of DNase-resistant DNA (DNase added): (B) competent cells (4×10^2 Pc^r transformants per ml) (20 μl); and (C) noncompetent cells (no detectable transformants) (40 μl). Lanes containing uptake of DNase-sensitive DNA (no DNase added): (D) competent cells (10 μl); and (E) noncompetent cells (10 μl). The additional minor bands are presumably multimeric forms of pFA10.

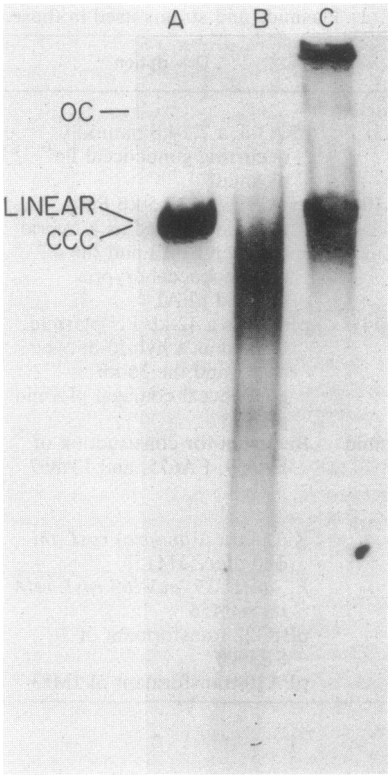

FIG. 2. Autoradiograph showing DNase-resistant uptake of pFA14 DNA. Competent cells were suspended in 50 ml of transforming medium and then incubated with 0.25 μg of donor DNA per ml at 37°C for 60 min, followed by chilling in ice water and the addition of DNase as described in Materials and Methods. The probe DNA was ^{32}P-labeled pFA14. Lanes: (A) cells plus 50 ng of *Ava*II-linearized pFA14 DNA (added during lysis); (B) DNase-resistant uptake of plasmid; and (C) cells plus 100 ng of donor plasmid DNA added during cell lysis. The band at the top of lane C is located at the origin; the identity of the DNA in this band was uncertain. The linear and CCC forms of pFA14 banded close together in ethidium bromide-stained agarose gels (not shown).

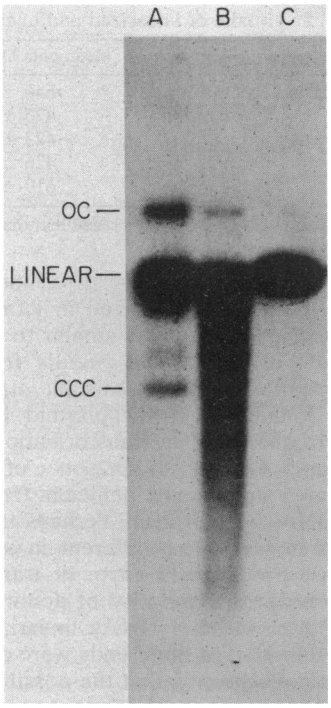

FIG. 3. Autoradiograph showing the *Ava*I cleavage pattern of linearized pFA10 DNA reisolated from competent cells. Cells were exposed to pFA10 DNA (0.2 μg/ml) in 300 ml of transforming medium for 60 min at 37°C followed by chilling in ice water; no DNase was added. Total cellular DNA was reisolated, separated on an agarose gel, and stained with ethidium bromide. The region corresponding to linear pFA10 (as judged by migration of control DNA in a separate lane) was electroeluted, coprecipitated with yeast tRNA by the addition of ethanol, and dissolved in 30 μl of 10 mM Tris hydrochloride (pH 7.4). One-half of the recovered DNA was digested with *Ava*I, and the other half was treated with buffer alone. These DNA preparations were separated on an agarose gel, transferred to a Southern blot, probed with ^{32}P-labeled pBR322 DNA prepared from *E. coli* FA6033, and autoradiographed as described in the legend to Fig. 1. Lanes: (A) reisolated pFA10 DNA incubated with buffer alone (CCC and OC forms of pFA10 were coisolated with the linearized DNA during electroelution; in the absence of added DNase, some CCC and OC DNA remained bound to the cell [Fig. 1]); (B) reisolated pFA10 DNA digested with *Ava*I; (C) 15 ng of *Ava*I-linearized pFA10 DNA.

CCC DNA was seen at any time. From these data we cannot be certain whether DNA is linearized before or shortly after entry.

Cleavage apparently is restricted to circular DNA. We also examined whether cleavage of DNA during uptake was specific to circular DNA. The donor plasmid was linearized by various restriction enzymes and exposed to competent cells for 60 min, followed by the addition of DNase I. DNA taken up by the cells migrated the same as donor DNA (Fig. 5), suggesting that linear DNA was not attacked by the enzyme that cleaves circular DNA. Note also that of the two *Hin*dIII fragments, the smaller fragment was taken up more efficiently than the larger one. This observation supports our earlier evidence of relative specificity in uptake of certain pFA10 fragments (10). The smaller *Hin*dIII fragment contains the 640-base-pair region which was proposed previously to contain an uptake site (10).

Transforming activity of OC and linear plasmids. Transformation with plasmid pFA10 was equally efficient with either CCC (1×10^{-5}) or OC (7.6×10^{-6}) DNA. Linearization of pFA10 with restriction endonucleases such as *Ava*I, for which there is a single site in the plasmid (13, 17), lowered transforming activity to 1.0×10^{-7}. Similar reductions in transforming activity occurred irrespective of the restriction endonuclease used, producing either cohesive (*Ava*I or *Pst*I) or blunt (*Hin*cII or *Pvu*II) ends (data not shown). Each of these restriction endonucleases makes only a single cut in pFA10 (13, 17; unpublished results).

Relative uptake of linear and circular pFA10. To determine whether plasmid conformation (linear versus circular DNA) affected uptake by gonococci, we utilized a hybridization assay to compare the uptake of linearized pFA10 to that of uncut pFA10. Plasmid DNA was digested with restriction endonucleases that have single recognition sites on the plasmid (*Pvu*II, *Ava*I, *Hin*cII, and *Pst*I) and that differ in the end structures they yield after digestion (blunt, 5′-protruding, or 3′-protruding ends). The uptake of linearized pFA10 was not significantly different (Student t test, $P < 0.08$) from the uptake of uncut pFA10 (Table 2). A standard analysis of variance revealed no statistically significant difference in the uptake activities of pFA10 linearized by the various single cutting enzymes ($P > 0.25$).

DISCUSSION

We present evidence that a majority of plasmid DNA molecules became linearized during gonococcal transformation. The majority of pFA10 molecules were cleaved into linear DNA, with a minority remaining as OC DNA. The 42-kb plasmid pFA14 also was converted into lower-molecular-weight linear fragments. The most likely explanation for the phenomenon is that an endonuclease(s) associated with the competent cells cleaved the donor plasmid to a linear double-stranded duplex, and that cleavage occurred more often with large plasmid molecules. The site of cleav-

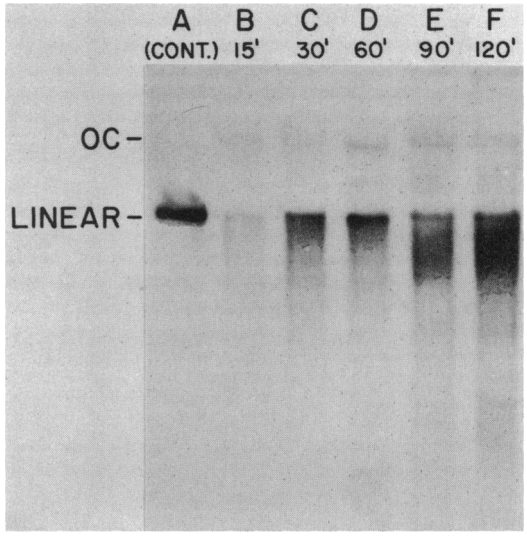

FIG. 4. Autoradiograph showing DNase-resistant pFA10 uptake as a function of minutes of incubation at 37°C before chilling in ice water. Competent cells were suspended in 800 ml of transforming medium containing 0.1 μg of pFA10 DNA per ml and incubated at 37°C. At the indicated time intervals a sample (200 ml at 15 and 30 min, 100 ml at 60, 90, and 120 min) was removed to a flask containing DNase (25 μg/ml). The sample was chilled in ice water. The remainder of the methods were as described in Materials and Methods. Lanes: (A; control) 10 ng of pFA10 DNA added during reisolation; (B through F) one-half of the reisolated DNA.

age was nonspecific, since donor pFA10 linearized in vivo was cut into a smear by the single-site enzyme *Ava*I. A lack of cleavage site specificity also was suggested by the smear of fragments isolated from competent gonococci exposed to the relatively large (42-kb) plasmid pFA14. During early stages of gonococcal transformation, most plasmid DNA was linearized. We also showed that linearized pFA10 was relatively less degraded in the first 30 to 60 min after uptake, in contrast to pFA14, suggesting that pFA10 might be placed into a protected state or site after entry. In *H. influenzae*, donor DNA remains in a protected state for some time after entry into specialized vesicles termed transformasomes (2, 12); we have no evidence as to whether competent gonococci produce structures analogous to transformasomes.

The physical detection of linearized donor plasmid DNA in recipient gonococcal cells confirms an earlier proposal from this laboratory that the donor plasmid might be fragmented during transformation (4, 27). There is abundant evidence in other bacterial transformation systems for endonucleolytic degradation of cell-associated donor DNA. For instance, in both *B. subtilis* (9) and *Streptococcus pneumoniae* (19, 25) chromosomal DNA undergoes double-strand breaks before entering the cell as single-stranded molecules. In *S. pneumoniae*, circular DNA also undergoes double-strand cleavage before entry (14). The endonuclease(s) associated with competent *B. subtilis* and *S. pneumoniae* are not site specific in their activity.

If the process of transformation with plasmid DNA requires cleavage of circular DNA to a linear form, then transformation with DNA linearized in vitro might be expected to occur with a frequency similar to that of circular DNA. Although both linear and circular forms of pFA10 were taken up into a DNase-resistant state with equal efficiency, we found that linear plasmid DNA was less efficient than circular DNA in transformation. The reasons for the discrepancy between the presence of predominantly linear plasmid after uptake and inefficient transformation of prelinearized DNA are not clear. Perhaps the linear molecules produced in vivo were different in some important ways from those produced in vitro, or transformation resulted principally from a minority of donor DNA that escaped linearization. Plasmid DNAs linearized in vitro to produce either cohesive or blunt ends were equally inactive in transformation, suggesting that the possible difference in DNA linearized in vivo was not restricted to the nature of the ends produced. In *H. influenzae* cohesive-ended linear plasmid DNA transforms well, in contrast to blunt-ended DNA, which is inactive (11, 21). In *S. pneumoniae* linear plasmid DNA is relatively inactive in transformation (24).

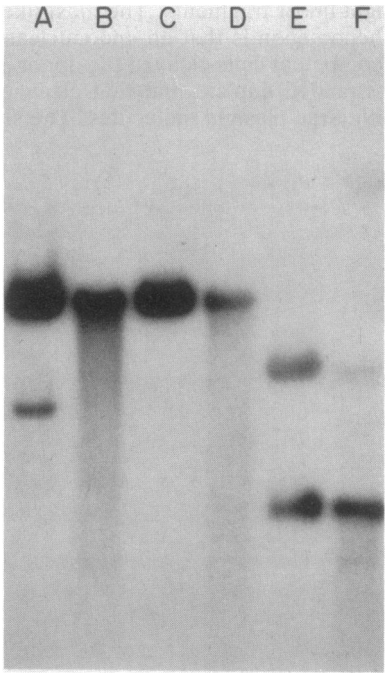

FIG. 5. Autoradiograph showing DNase-resistant uptake of pFA10 DNA linearized before uptake by restriction endonuclease digestion. Competent cells were incubated with 0.17 μg of donor DNA per ml in 100 ml of transforming medium at 37°C for 60 min. The remainder of the methods were as described in the legend to Fig. 1. Each lane received one-half of the reisolated DNA. Control lanes (linear DNA added during cell lysis): (A) 200 ng of *Pst*I-digested DNA (one site, partial digest); (C) 200 ng of *Ava*I-digested DNA (one cut); and (E) 400 ng of *Hin*dIII-digested DNA (two cuts). DNase-resistant uptake lanes: (B) *Pst*I-digested DNA; (D) *Ava*I-digested DNA; and (F) *Hin*dIII-digested DNA.

TABLE 2. Uptake of linearized and uncut pFA10

pFA10 treatment	Mean cpm hybridized[a] ± SE (*n*)
Untreated control	646 ± 87.6 (20)
*Pst*I	475 ± 101.1 (11)
*Pvu*II	423 ± 79.7 (10)
*Ava*I	426 ± 78.3 (10)
*Hin*cII	619 ± 218.8 (8)

[a] Samples which received no DNA hybridized less than 50 cpm.

A model of gonococcal transformation must explain the frequent occurrence of deletions in plasmids after transformation (4, 27). A restriction analysis of some deleted plasmids obtained by transformation with pFA3 or pFA10 revealed that the deletions occurred randomly outside the region of the selected Pc^r gene (27; unpublished results). The random cleavage of entering plasmids demonstrated here correlates well with the earlier observation that plasmids are deleted at different regions. Uptake of intact circular plasmid molecules presumably would be less likely to lead to deletions than plasmids that had been linearized during uptake. Plasmid deletions have been reported during transformation of several other organisms, including *S. cerevisiae* (7), *Streptococcus sanguis* (15), and *Pseudomonas aeruginosa* (20). The mechanisms involved in these other organisms are uncertain.

We propose the following tentative model for plasmid transformation of gonococci. Plasmid DNA rapidly binds to the gonococcal cell surface. Most circular plasmid molecules are cleaved at random, resulting in double-stranded linear molecules. It is not yet known whether the cleavage occurs before, during, or sometime after uptake. Plasmid DNA enters competent gonococcal cells as double-stranded molecules, as previously shown for chromosomal DNA (5). Subsequently, double-stranded linear molecules may be recircularized, giving rise to a plasmid replicon. During this process, some linear molecules are probably damaged, as for instance by an exonuclease or multiple endonucleolytic cleavages. After recircularization, these molecules result in deleted plasmids. Repair of linearized plasmids is inefficient, accounting in part for low efficiencies of plasmid transformation. However, if the recipient contains a plasmid homologous to the transforming plasmid, marker rescue occurs; transformation frequency is increased, and no deleted plasmids are found (4). Transformation probably also results

when circular DNA enters without undergoing double-strand cleavage; with a relatively small plasmid such as pFA10, a minority of donor DNA enters as relaxed circles.

The proposed model suggests the presence of a cell surface endonuclease, although we have no direct experimental support for the presence of such an enzyme. It may be that this putative endonuclease would have functions in the cell other than cleavage of entering transforming plasmid DNA, although we can only speculate about such functions at present. We do not believe that the postulated endonuclease is a restriction endonuclease, since the gonococcal transformation crosses presented here were isogenic.

There also is no direct experimental evidence that linearized molecules formed during uptake by competent gonococci are converted to circular molecules to produce transformants. Inferential support for this concept is provided by the study of plasmid pFA14. All detectable intracellular DNA from pFA14 was converted to less than unit-length linear DNA during entry, probably by a number of nonspecific endonucleolytic cleavage events. Although pFA14 only rarely results in transformants in the absence of a homologous recipient plasmid (4), some transformants are observed, and each is deleted (4). Thus, some linearized plasmid molecules seem to result in transformants in the absence of marker rescue.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant AI15036 from the National Institute of Allergy and Infectious Diseases.

We thank Dave Dyer and Nick Carbonetti for many helpful comments.

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