Preferential Uptake of Restriction Fragments from a Gonococcal Cryptic Plasmid by Competent *Neisseria gonorrhoeae*

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Factors involved in the specificity of DNA uptake by competent *Neisseria gonorrhoeae* were examined. Host-controlled modification did not affect uptake. Certain restriction fragments of the 4.2 kb gonococcal cryptic plasmid pFA1 and of the replicative form of the bacteriophage M13 were taken up in preference to others, independent of differences in fragment size. A 600 bp fragment from the 4.2 kb plasmid was cloned into pLES2, a gonococcal–*Escherichia coli* shuttle vector; the 600 bp fragment was taken up into a DNAase-I-resistant state in preference to the vector fragment. A second 370 bp fragment in pFA1 was also taken up preferentially. The 600 bp and 370 bp fragments share a 10 bp sequence, which is found in pFA1 only on fragments that were taken up readily. However, a fragment from M13 which was efficiently taken up did not contain this 10 bp sequence. In addition, this sequence was not sufficient to direct preferential DNA uptake by gonococci, since a recombinant plasmid containing this 10 bp sequence was not taken up appreciably better than the vector plasmid or another recombinant plasmid containing an unrelated 10 bp sequence. Sequence comparisons of the three restriction fragments which were preferentially taken up did not yield any consensus sequences greater than 7 bp. Although it is likely that efficient uptake of DNA by gonococci is determined by DNA structure, a single short sequence could not be found that accounted for specific uptake.

INTRODUCTION

Neisseria gonorrhoeae is unusual among bacterial species in that nearly all isolates constitutively express competence for genetic transformation (Biswas *et al.*, 1977; Sparling, 1966). The gonococcus is also unusual in the apparent specificity for recognition of homologous DNA during the early stages of DNA uptake by competent cells (Dougherty *et al.*, 1979); in this respect this organism resembles competent *Haemophilus influenzae*, which also exhibits preferential uptake of homologous DNA (Scocca *et al.*, 1974). In most other transformable bacteria, no specificity is exhibited during DNA uptake (Smith *et al.*, 1981).

The mechanism for specific uptake of homologous DNA by *H. influenzae* has been studied extensively (Goodgal, 1982; Smith *et al.*, 1981), and there is evidence that DNA uptake is facilitated by the presence of a specific 11 bp sequence (Danner *et al.*, 1980; Deich & Smith, 1980). The basis for specific uptake of gonococcal transforming DNA is much less well understood. Dougherty *et al.* (1979) found that a 100-fold excess of heterologous DNA did not inhibit transformation by gonococcal DNA to streptomycin resistance. Homologous and heterologous DNAs were bound by piliated competent gonococci, but only homologous DNA was taken up into a DNAase-I-resistant form (Dougherty *et al.*, 1979). Similarly, Graves *et al.* (1982*a*) showed that *Escherichia coli* DNA was unable to competitively inhibit transformation of gonococcal chromosomal DNA, or a gonococcal β -lactamase-encoding (Pc^r) plasmid, pFA10. Graves *et al.* (1982*b*) also demonstrated that gonococci selectively internalized specific

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fragments from restriction digests of pFA10. pFA10 is an 11.5 kb hybrid plasmid composed of the 7.2 kb *Pc*^r plasmid (pFA3) and the 4.2 kb gonococcal cryptic plasmid (pFA1) (Graves *et al.*, 1982*b*). Gonococci preferentially took up a 1.5 kb *Taq*I and a 3.0 kb *Msp*I fragment from pFA10 (Graves *et al.*, 1982*b*). These fragments consist of pFA1 sequences and overlap by 600 bp. Graves *et al.* (1982*b*) speculated that the pFA1 portion of pFA10 was preferred for uptake because it consisted of homologous gonococcal DNA. However, since end-labelled pFA10 restriction fragments were used as donor DNA in uptake, it was possible that additional fragments were taken up also, but not detected due to removal of the terminal ³²P label.

This paper reports the results of experiments designed to examine the question of preferential uptake of specific restriction fragments by gonococci, and to determine the structural basis for preferential uptake.

METHODS

Bacterial strains and plasmids. These are described in Table 1.

Media. Gonococci were cultured on Difco GC base agar medium plus supplements as previously described (Sarubbi *et al.*, 1974). *E. coli* strains were grown in LB medium or on LB agar (Maniatis *et al.*, 1982); ampicillin was added at 50 µg ml⁻¹ as indicated. For analysis of DNA uptake and genetic transformation of gonococci, GCB broth plus 10 mM-MgCl₂ was used, as described by Biswas *et al.* (1986*a*).

DNA purification. Plasmid DNA was isolated from gonococci as described by LeBlanc & Hassel (1976), and from *E. coli* by the alkaline lysis method of Birnboim & Doly (1979) as modified by Maniatis *et al.* (1982); the DNAs were then further purified by one or two cycles of CsCl/ethidium bromide density-gradient centrifugation (Radloff *et al.*, 1967).

Restriction endonuclease digestions. These were done according to the instructions supplied by the manufacturers (Bethesda Research Laboratories, New England Biolabs and International Biotechnologies).

Transformation. *N. gonorrhoeae* was transformed as described by Sox *et al.* (1979). *E. coli* was transformed by the calcium chloride procedure of Dagert & Ehrlich (1979) or by the method of Hanahan (1983).

Labelling of plasmid DNA. pFA10 was nick-translated using [α-³²P]dCTP (ICN Pharmaceuticals or New England Nuclear) and reagents from Bethesda Research Laboratories; reaction conditions were as recommended by the supplier. pFA1 restriction fragments were labelled by the replacement synthesis method of O'Farrell *et al.* (1980). Unincorporated [α-³²P]dCTP was removed by gel filtration on a 5 ml Sephadex G-100 (Pharmacia) column. Specific activities of the DNA ranged from 10⁶ to 10⁷ c.p.m. µg⁻¹. Oligonucleotides were end-labelled as described by Maniatis *et al.* (1982). Unincorporated [γ-³²P]ATP was removed by ethanol precipitation.

Restriction fragment uptake assay. Radioactively-labelled restriction fragments that entered into a DNAase-I-resistant state were detected by autoradiography by the method of Graves *et al.* (1982*b*). Alternatively, unlabelled fragments were used for uptake and detected by hybridization. In both assays, 1–2 µg of plasmid restriction fragments were incubated with 1 ml of pilated FA759 gonococci (approximately 5 × 10⁷ c.f.u.) in GCB broth

Table 1. *Bacterial strains and plasmids*

Strain	Plasmid content	Description (reference)
<i>N. gonorrhoeae</i>		
FA759	No autonomous plasmid	Clinical isolate (Graves <i>et al.</i> , 1982 <i>b</i>)
FA6058	pFA1, 4.2 kb gonococcal cryptic plasmid	Construction from FA759 (Biswas <i>et al.</i> , 1986 <i>b</i>)
FA675	pFA10, 11.5 kb hybrid of pFA1 and pFA3, a β-lactamase-encoding plasmid	<i>Pc</i> ^r (pFA10) (Graves <i>et al.</i> , 1982 <i>a, b</i>) transformant of FA759 (Graves <i>et al.</i> , 1982 <i>b</i>)
<i>E. coli</i>		
JM83	None	K12 strain (<i>ara</i> Δ <i>lac-pro</i> <i>strA</i> <i>thi</i> φ80 <i>dlacZ</i> ΔM15) (Vieira & Messing, 1982)
DH5α	None	F ⁻ <i>endA1</i> <i>hsdR17</i> (r _K , m _K ⁺) <i>supE44</i> <i>thi-1</i> λ ⁻ <i>recA1</i> <i>gyrA96</i> <i>relA1</i> φ80 <i>dlacZ</i> ΔM15 (Bethesda Research Laboratories)
FA6269	pLES2, 6.0 kb gonococcal- <i>E. coli</i> shuttle vector	<i>Pc</i> ^r (pLES2) (Stein <i>et al.</i> , 1983) transformant of JM83
FA6270	pSTD6, 6.6 kb recombinant of pLES2 and 600 bp <i>Hpa</i> II- <i>Taq</i> I fragment from 4.2 kb gonococcal cryptic plasmid, pJD1	Construction; <i>Pc</i> ^r (pSTD6) transformant of JM83
FA6239	pFA10	<i>Pc</i> ^r (pFA10) (Graves <i>et al.</i> , 1982 <i>a, b</i>) transformant of JM83

containing 10 mM-MgCl₂. In some experiments, nonpiliated gonococci were treated in parallel as a control. After 10 min at 37 °C, DNAase I was added to 160 µg ml⁻¹ and the cells were held on ice for 20 min. The cells were then washed three times by centrifugation in ice-cold A medium (Davis minimal medium, Difco) containing 0.25 M-NaCl. The final cell pellets were resuspended in lysis buffer (50 mM-glucose, 25 mM-Tris/HCl, pH 8.0, 10 mM-Na₂EDTA) and sodium dodecyl sulphate (SDS) was added to 1% (w/v). This mixture was incubated at 65 °C for 10 min, which was sufficient to lyse the cells. Samples were deproteinized by successive extractions in CHCl₃/phenol and CHCl₃; the nucleic acids were precipitated with ammonium acetate and ethanol. The nucleic acid precipitate was dissolved in TE buffer (10 mM-Tris/HCl, pH 8.0, and 1 mM-Na₂EDTA) for electrophoresis.

Agarose gel electrophoresis, hybridization and autoradiography. Electrophoresis of plasmid DNA was performed on horizontal agarose gels in Tris/acetate/EDTA buffer (Maniatis *et al.*, 1982). DNA recovered from gonococcal cell lysates was electrophoresed on 1–1.2% (w/v) gels and compared to ³²P-labelled standards. To detect uptake of labelled restriction fragments, the gels were fixed at room temperature in 10% (v/v) methanol and 10% (v/v) acetic acid with agitation for 10 min and blotted dry. Kodak X-AR5 film was exposed to the dried gels for 3–7 d with a Cronex Lightning-Plus intensifier screen at –70 °C. When hybridization was used to detect uptake of plasmid restriction fragments, DNA samples were transferred from agarose gels to nitrocellulose filter paper (BA85, Schleicher and Schuell) by the procedure of Smith & Summers (1980). Filters were probed and washed by standard procedures (Maniatis *et al.*, 1982). Autoradiography was performed as described above.

Uptake assays of intact pFA10. pFA10, used in uptake experiments, was isolated from *E. coli* FA6239 or *N. gonorrhoeae* FA675 and twice purified by CsCl/ethidium bromide density-gradient centrifugation. The hybridization uptake assay was done as previously described (Biswas *et al.*, 1986a).

Construction of recombinant plasmid pSTD6. Plasmid pGUT was kindly supplied by Dr M. So; it contains an approximately 600 bp insert of the *HpaII*–*TaqI* fragment (on *EcoRI* linkers) from pJD1, a gonococcal 4.2 kb cryptic plasmid (Davies & Normark, 1980; Korch *et al.*, 1985). pGUT was digested with *EcoRI* and the 600 bp cryptic plasmid fragment was purified by electroelution (Maniatis *et al.*, 1982). This fragment was ligated with *EcoRI*-cleaved pLES2, a gonococcal–*E. coli* shuttle vector (Stein *et al.*, 1983) and used to transform *E. coli* JM83.

Cloning and DNA sequencing. The 370 bp *DdeI* pFA1 fragment was electroeluted and the single-stranded ends were filled in using T4 DNA polymerase and all four deoxyribonucleotide triphosphates, as described by Maniatis *et al.* (1982). *EcoRI* linkers (Bethesda Research Laboratories) were phosphorylated using ATP and T4 polynucleotide kinase (Bethesda Research Laboratories) and were ligated to the blunt-ended *DdeI* fragment using T4 DNA ligase as recommended by the supplier. The recombinant DNA was digested with 30 units of *EcoRI*, added in two aliquots, and then electrophoresed on a 5% (w/v) polyacrylamide gel; the 370 bp band was excised and eluted (Maniatis *et al.*, 1982). *EcoRI*-cleaved replicative form M13 (mp19) was ligated to the 370 bp fragment and used to transform JM109. Phage DNA from some of the colourless plaques was purified from 1.5 ml cultures and sequenced by the chain-termination method (Sanger *et al.*, 1977). DNA sequences were compared using Pustell's Sequence Analysis programs (International Biotechnologies).

Synthesis and purification of oligonucleotides. The following oligonucleotides (obtained from Dr C. A. Hutchison) were synthesized, excised and purified as described by Hutchison *et al.* (1986).

5' GATCCGATGCTCTGTG 3' (1)

5' TCGACACAGAGCATCG 3' (2)

5' GATCCGCTTCCACATG 3' (3)

5' TCGACATGTGGAAGCG 3' (4)

The complementary oligonucleotides (1 and 2; 3 and 4) were annealed as described by Hutchison *et al.* (1986) to generate double-stranded oligonucleotide pairs that contain a *SalI* and a *BamHI* cohesive end (sequences that make up the *SalI* and *BamHI* ends are not underlined). To confirm the accuracy of the chemical synthesis, oligonucleotide-containing clones were sequenced by the chain-termination method (Sanger *et al.*, 1977).

Construction of pBR322 derivatives. The 4 kb fragment from a *BamHI*, *SalI* pBR322 digest was electroeluted as described by Maniatis *et al.* (1982) and ligated to the annealed oligonucleotides, as described by Hutchison *et al.* (1986). A 1–3 µl volume of a 1:5 dilution in TE buffer of the ligation mix was used to transform competent DH5α (Bethesda Research Laboratories). pBR322 derivatives containing oligonucleotides were identified from several ampicillin-resistant, tetracycline-sensitive transformants by hybridization of plasmids from mini-preparations to the ³²P-end-labelled homologous oligonucleotide. Hybridizations were performed at room temperature in 6 × SSC, 10% Denhardt's solution (Maniatis *et al.*, 1982), 100 µg ml⁻¹ each of yeast RNA and denatured salmon sperm DNA and 0.1% SDS. Washes were in 6 × SSC at room temperature. The 4 kb *BamHI*–*SalI* pBR322 fragment was end-repaired and blunt-end ligated (Maniatis *et al.*, 1982) to generate the 265 bp deletion derivative pBR322ΔB-S.

Scanning densitometry. An LKB model 2202 Ultrosan densitometer was used to compare intensities of bands appearing on the same autoradiographs. Bands were scanned twice at approximately one-third and two-thirds width of each band.

RESULTS

pFA10 restriction fragments are not processed during uptake

To determine whether the observations of Graves *et al.* (1982*b*) could be explained by processing of DNA fragments rather than by preferential uptake, we used a hybridization assay to detect internalized DNA which had become DNAase I resistant. When an *MspI* digest of pFA10 was used as donor DNA, the 3.0 kb *MspI* fragment was preferentially taken up (data not shown) as determined by its ability to hybridize with nick-translated pFA10. The other restriction fragments from the digest were not taken up. These data indicated that the 3.0 kb *MspI* fragment was taken up preferentially by gonococci from a mixture of pFA10 *MspI* restriction fragments, as suggested by Graves *et al.* (1982*b*). Furthermore, restriction fragments did not appear to undergo extensive processing during or immediately following uptake into a DNAase-I-resistant state.

The TaqI-MspI fragment from the cryptic plasmid is recognized for uptake

The 3.0 kb *MspI* and 1.5 kb *TaqI* pFA10 fragments, which are taken up preferentially as described by Graves *et al.* (1982*b*), overlap by approximately 600 bp. Both of these fragments lie within the pFA1 portion of pFA10 (Graves *et al.*, 1982*b*). We tested whether this common 600 bp region was specifically recognized for uptake. For this experiment we used the equivalent fragment from another cryptic plasmid isolate, pJD1, since this particular plasmid had been sequenced (Korch *et al.*, 1985). pFA1 and pJD1 are similar, based on restriction enzyme digest patterns (Davies & Normark, 1980; Foster & Foster, 1976; Korch *et al.*, 1985; and unpublished results). The 600 bp *TaqI-MspI* fragment from pJD1 was inserted on *EcoRI* linkers into pLES2, resulting in pSTD6. An *EcoRI* digest of pSTD6 was ³²P-labelled by the replacement synthesis method and exposed to competent gonococci. The 600 bp fragment was taken up in preference to the vector fragment (Fig. 1).

Identification of a second region on the cryptic plasmid which is preferentially taken up

Since fragments taken up from pFA10 were found exclusively on the pFA1 portion of pFA10, we utilized pFA1 to extend the findings of Graves *et al.* (1982*b*). We first wished to see if the 1.5 kb *TaqI* fragment would be taken up preferentially from pFA1, as it is from pFA10 (Graves *et al.*, 1982*b*). We confirmed that the 1.5 kb fragment was selectively taken up, but in addition a smaller fragment of approx. 400 bp was taken up to a lesser extent (data not shown). Since there are three pFA1 *TaqI* restriction fragments of about 400 bp, we were unable to tell which small *TaqI* fragment was taken up. We therefore examined uptake of other restriction digests of pFA1 to locate the plasmid location of this second fragment.

Two fragments were taken up from a pFA1 *DdeI* digest (Fig. 2, lanes A and B). The largest of the two *DdeI* fragments (approx. 3 kb) contains the 600 bp *TaqI-MspI* fragment, but in addition a smaller 370 bp *DdeI* fragment was taken up. Three other *DdeI* fragments were not taken up appreciably. The second, smaller *DdeI* fragment apparently was not taken up to the same extent as the larger fragment (Fig. 2, lane B). Two of four fragments from an *NciI*, *SspI* and *AvaII* triple digest of pFA1 also were preferentially taken up (Fig. 2, lanes C and D). In this experiment, the two fragments from the triple digest which were taken up (Fig. 2, lane D) migrated more slowly than the purified fragments (Fig. 2, lane C) used as standards. This occurred occasionally and may have been due to differences in the preparation of the fragments used as standards (CsCl/ethidium bromide density gradients) as compared to the DNA recovered following uptake (crude gonococcal SDS lysates). Each of these two fragments from the triple digest overlap the two *DdeI* fragments that are recognized for uptake preferentially. *HinfI* cuts pFA1 twice and both of the resulting fragments were taken up (Fig. 2, lanes E and F). The larger *HinfI*

Fig. 2. Preferential uptake of two regions of the cryptic plasmid pFA1. Lanes A, C, E, ³²P-replacement-synthesis-labelled pFA1 *DdeI* fragments (lane A), fragments from an *NciI*, *AvaII* and *SspI* pFA1 triple digest (lane C), and fragments from a *HinfI* pFA1 digest (lane E). Lanes B, D, F, DNA recovered from gonococci exposed to ³²P-labelled *DdeI* fragments (lane B), *NciI*, *AvaII* and *SspI* fragments (lane D) and *HinfI* fragments (lane F).

Fig. 1. Uptake of the 600 bp *TaqI*-*MspI* cryptic plasmid fragment in preference to the 6 kb vector pLES2. Lane A, *EcoRI*-digested pSTD6 ^{32}P -labelled by replacement synthesis. Lane B, DNA recovered from gonococci which were incubated with radiolabelled pSTD6 *EcoRI* fragments.

Fig. 3. Restriction maps of pFA1; fragments taken up preferentially by competent gonococci are shown as bold lines. Regions 1 and 2 denote portions common to all pFA1 fragments taken up. Region 1 is also referred to as the 600 bp *TaqI*–*MspI* overlap. It was unclear which of the similarly sized *TaqI* fragments (labelled with question marks) were taken up.

600 bp *TaqI*–*MspI* fragment (1590–2190)

5'AATAAACCGC GATGCTCTGT ACTCGGCATT..... 3'

370 bp *DdeI* fragment (3711–4080)

5'AGCCGTCTGT GATGCTCTGT GATGCTCTGT GCGGCTTCCT..... 3'

Fig. 4. A 10 bp sequence is common to the two cryptic plasmid fragments taken up. The underlined sequence is the 10*mer as it is found on the cryptic plasmid pJD1 (Korch *et al.*, 1985). Numbers in parentheses represent the map sites of the *TaqI*–*MspI* and *DdeI* fragments from the *AvaII* site on pJD1.

fragment contains the 600 bp *MspI*–*TaqI* overlap fragment and appears darker than the smaller *HinfI* fragment, which encompasses the 370 bp *DdeI* fragment. This suggests that the 600 bp region (designated region 1) was taken up more efficiently than the 360 bp *DdeI* fragment (region 2). These results demonstrated that there were two distinct regions present on the gonococcal cryptic plasmid which were recognized for uptake; these data are summarized in Fig. 3.

We compared sequences of the 600 bp *TaqI*–*MspI* overlap fragment in pJD1 and the 370 bp *DdeI* pJD1 fragment (Korch *et al.*, 1985); these fragments have a 10 bp sequence (5' GATGCTCTGT 3') in common which is not found elsewhere on pJD1 (Fig. 4). This sequence (denoted 10*mer) is directly repeated in tandem in the 370 bp *DdeI* fragment. We sequenced the pFA1 370 bp *DdeI* fragment and found the repeated 10*mer present on this fragment, exactly as in the published sequence for pJD1 (Korch *et al.*, 1985).

Fig. 5

Fig. 5. Uptake of radiolabelled M13 (mp19) DNA and pFA10 in the presence of competing unlabelled pFA10. Lanes A and B contain linearized pFA10 (*HincII*) and mp19 DNA (*EcoRI*), respectively. Both samples were radiolabelled by the replacement-synthesis procedure (see Methods). DNA recovered from gonococci incubated with 1 µg each of unlabelled pFA10 and radiolabelled mp19 (lane C) and pFA10 (lane D) is shown.

Fig. 6

Fig. 6. Uptake of M13 (mp19) DNA restriction fragments. Lane A contains ^{32}P -replacement-synthesis-labelled mp19 *NciI* fragments. Lane B contains DNA isolated from gonococci following exposure to mp19 *NciI* fragments.

Bacteriophage M13 (mp19) replicative-form DNA competes with pFA10 for uptake

Replicative-form DNA from the bacteriophage M13 was taken up well by gonococci (data not shown). To examine further the uptake of this heterologous DNA, we tested the ability of M13 (mp19) to compete for uptake with the hybrid plasmid pFA10. Linearized, radiolabelled M13 (mp19) DNA plus unlabelled intact pFA10 was incubated with gonococci. In a separate reaction, labelled pFA10 plus unlabelled pFA10 was also exposed to gonococci. The results indicated that M13 (mp19) DNA competed with pFA10 for uptake and was taken up almost as well (Fig. 5).

A restriction fragment from M13 (mp19) DNA is taken up preferentially

We wanted to determine if certain regions of M13 DNA were taken up selectively as they were in pFA1. Radiolabelled *NciI* restriction fragments of M13 (mp19) DNA were exposed to competent gonococci (Fig. 6). The largest *NciI* fragment from the digest was taken up well but the second fragment was taken up only slightly and there was no detectable uptake of the smallest fragment. Similarly, the largest *HaeIII* fragment, which encompasses the *NciI* fragment, was preferentially taken up from a *HaeIII* digest of M13 DNA (data not shown). The

sequence of the largest *NciI* fragment of M13 was compared to the sequences of the two regions of pJD1 (pFA1) which had been shown to be selectively taken up (see above). No consensus sequences greater than 7 bp were found among the three fragments. Additionally, the M13 *NciI* fragment contained only a 6' mer homologous to the 10*mer identified on the two pFA1 fragments. The M13 genome does contain a perfect inner 8 bp sequence of the 10*mer (5' ATGCTCTG 3') but this sequence occurs outside the largest *NciI* fragment.

*The 10*mer does not confer enhanced uptake on the pBR322 derivatives*

To determine if a pBR322 derivative containing the 10*mer was specifically recognized for uptake by gonococci, we examined uptake of three pBR322 derivatives and pFA10 in the presence of competing, unlabelled pFA10. Two of the derivatives of pBR322 served as controls: one plasmid contained a 10 bp sequence 5' GCTTCCACAT 3' (unrelated to the 10*mer) found once on the cryptic plasmid and in a region not taken up preferentially; the second plasmid contained no insert, but like the other two plasmids it lacked the *BamHI*-*SalI* 265 bp fragment of pBR322. Competing unlabelled pFA10 plus each of the radiolabelled pBR322 derivatives or pFA10 were incubated with piliated gonococci. pFA10 was added as a competitor in these experiments so that the 10*mer would be rigorously tested for its ability to confer specific uptake on pBR322. We predicted that the 10*mer would compete adequately with pFA10 if it were responsible for the preferential uptake of pFA1 (cryptic plasmid) sequences. All three pBR322 derivatives were poorly taken up (i.e. were not highly competitive with pFA10), whereas pFA10 was taken up at least 10-fold better in the presence of equal amounts of competing unlabelled pFA10. The results of scanning densitometry of two autoradiographs from these experiments were as follows (mean peak values $\pm$ SD from two or three readings): pBR322 Δ B-S, 260 ± 125 ; pBR322 Δ B-S + unrelated 10' mer, 160 ± 100 ; pBR322 Δ B-S + 10*mer, 308 ± 43 ; pFA10, 2929 ± 54 . There were some subtle differences (rarely greater than twofold) in the uptake of the pBR322 derivatives; these probably resulted from variability in recovering DNA following uptake, since similar differences were also seen between identical duplicate samples. These results showed that the 10*mer 5' GATGCTCTGT 3' was not sufficient to confer efficient uptake of pBR322.

Gonococcal DNA modification is unnecessary for efficient uptake of pFA10

Although there was selectivity in restriction-fragment uptake in the absence of any differences in DNA modification (Figs 1 and 2), we were curious whether homologous DNA modification was preferred over heterologous modification patterns. To test whether gonococcal DNA modification was required for efficient uptake, we examined the efficiency of uptake of pFA10 isolated from a gonococcal host (FA675) or *E. coli* (JM83). When pFA10 was isolated from *N. gonorrhoeae* FA675, it transformed FA759 (which is isogenic with FA675) at an efficiency of approximately 1×10^{-4} Pc^+ transformants per μ g DNA. pFA10 isolated from *E. coli* JM83 transformed FA759 at about 2×10^{-5} Pc^+ transformants per μ g DNA. This represented a fivefold decrease in transformation efficiency, probably due to gonococcal-specific restriction of the plasmid DNA obtained from the *E. coli* strain. However, there were no significant differences (Student's *t*-test, $P < 0.44$) in the DNA uptake of the two plasmid preparations. When pFA10 prepared from *N. gonorrhoeae* was used for uptake, a value of 1848 ± 165 c.p.m. (mean $\pm$ SE) was obtained for hybridization to lysates of gonococci incubated with this plasmid, compared to 1840 ± 152 c.p.m. obtained when pFA10 from *E. coli* was the donor DNA. We concluded that, although restriction of incoming plasmid DNA (prepared from *E. coli*) probably resulted in the decreased transformation efficiency, there was no effect of gonococcal-specific DNA modification on uptake of plasmid DNA.

DISCUSSION

It was reported previously that two overlapping ^{32}P -end-labelled restriction fragments consisting of gonococcal cryptic plasmid (pFA1) sequences were taken up in preference to fragments derived from the Pc^+ plasmid pFA3 (Graves *et al.*, 1982*b*). Using a different method of detection from that of Graves *et al.* (1982*b*), namely hybridization to a homologous nick-

translated probe, we have confirmed that this observation was due to preferential uptake and not due to selective processing of ends of incoming fragments.

Using a recombinant plasmid containing only the 600 bp *TaqI*–*MspI* overlap fragment, we determined that this region of pFA10 was preferentially taken up, as predicted (Graves *et al.*, 1982*b*). We also identified a second region on the cryptic plasmid which was selectively taken up. These two fragments originate from different cryptic plasmid isolates pFA1 and pJD1, but the two plasmids are identical in size and have very similar physical maps (Davies & Normark, 1980; Foster & Foster, 1976; Korch *et al.*, 1985). The 600 bp fragment in pSTD6 was derived from pJD1, and thus the sequence of this fragment is known. Our sequence results for the 370 bp *DdeI* fragment from pFA1 showed that it contains a direct repeat of a 10 bp sequence found in the 600 bp *TaqI*–*MspI* fragment, as expected based on the results published for pJD1 (Korch *et al.*, 1985). This 10 bp sequence (5' GATGCTCTGT 3') is absent from the pFA1 fragments that are not preferentially taken up by the competent gonococci. We also found a restriction fragment from the M13 genome which was selectively taken up. This fragment does not contain the pFA1 10*mer.

None of the methods discussed here enables us to differentiate with absolute certainty between selective uptake of fragments and equal uptake of all fragments with subsequent degradation of some fragments. However, degradation of selected fragments is unlikely, since we have not seen significant lower molecular mass radioactivity on gels of cell lysates following uptake. Also, we cannot formally exclude the possibility that those fragments identified as being taken up were not actually processed forms of larger fragments from the restriction digest. Since the fragments used for uptake were radiolabelled by replacement synthesis, both ends of the molecule contain extensive ³²P label. It is therefore possible that the fragments could have been processed to a smaller size and still retain ³²P label. However, using a variety of different restriction digests, our results were internally consistent as to which regions were preferred for uptake. Taken together, the results are highly unlikely to be due to selective degradation or processing of incoming fragments.

The precise mechanism of selective uptake is unclear. Korch *et al.* (1983) suggested that the extensive methylation of gonococcal DNA might contribute to its specific recognition for DNA uptake. However, we found that gonococcal DNA modification is not required for efficient uptake. In *Haemophilus*, specificity for uptake is retained when cloned *Haemophilus* DNA fragments have been propagated in *E. coli* (Chung & Goodgal, 1979; Sisco & Smith, 1979). These results indicate that preferential uptake in *N. gonorrhoeae*, like that in *Haemophilus*, does not depend on homologous DNA modification.

In *Haemophilus*, homologous DNA uptake is facilitated by presence of a specific 11 bp sequence (Danner *et al.*, 1980, 1982). We wanted to determine if the cryptic plasmid 10 bp sequence played an analogous role in DNA uptake in the gonococcus. The derivative of pBR322 containing the 10 bp sequence was taken up to essentially the same extent as either pBR322ΔB-S lacking this sequence or pBR322ΔB-S containing an unrelated 10 bp oligonucleotide. Moreover, an M13 (mp19) DNA fragment without significant homology to the 10 bp sequence was taken up efficiently, and M13 DNA fragments containing the core 8 bp of the 10*mer were not taken up as efficiently as other fragments lacking this sequence. These data indicate that the pFA1 10*mer is not sufficient to explain preferential DNA uptake by competent gonococci.

There are several possible reasons why the 10*mer did not confer increased uptake. Its presence on the two cryptic plasmid fragments which were selectively taken up may be coincidental. This argument is strengthened by the fact that the M13 DNA fragment which is selectively taken up does not contain any sequences with significant homology to this 10 bp sequence. Alternatively, we may have failed to identify auxiliary sequences which might be required for specific uptake. For instance, a putative gonococcal DNA receptor may interact with two non-contiguous sites on a DNA molecule, the 10*mer possibly being only one of those sites. An example of this type of protein–DNA interaction is seen in type 1 restriction endonucleases. These enzymes recognize two sequences of 3–4 bp that are separated by six or eight bases of random DNA sequence (Yuan, 1981). More work is required before the basis for selective DNA uptake by gonococci is understood.

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