Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6982–6986, September 1988
Microbiology

Identification and arrangement of the DNA sequence recognized in specific transformation of *Neisseria gonorrhoeae*

(stem-loop structure/transcriptional terminator sequences/palindromes)

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Communicated by Hamilton O. Smith, May 6, 1988 (received for review March 18, 1988)

ABSTRACT **DNA segments from *Neisseria gonorrhoeae*, cloned and propagated in *Escherichia coli*, were tested for the ability to competitively inhibit gonococcal transformation. The nucleotide sequences of active segments were determined and compared; these sequences contained the sequence 5′ GCCGTCTGAA 3′ in common. Subcloning studies confirmed the identity of this sequence as the gonococcal DNA recognition site. The three instances of the recognition sequence isolated from *N. gonorrhoeae* chromosomal DNA contain the sequence in the immediate neighborhood of its inverted repeat. Because a single copy of the sequence functions as a recognition site, the inverted duplication is not required for specific binding. The dyad symmetric arrangements of the chromosomal recognition sequences may form stable stem-loop structures that can function as terminators or attenuators of transcription. These inverted repeats are located at the boundaries of long open reading frames. The recognition sequence also constitutes part of two other probable terminators of gonococcal genes. We conclude that the signal for recognition of transforming DNA by gonococci is a frequent component of transcriptional terminator sequences. This regulatory function might account for the origin and maintenance of recognition sequences in the chromosomes of Gram-negative transformable bacteria.**

In *Neisseria gonorrhoeae*, transformation is the principal natural process mediating the transfer of chromosomal genes (1). This genetic exchange process is specific; only DNA from members of the genus Neisseria is bound by competent gonococci (2–4). This specificity is similar to that seen in *Haemophilus influenzae* transformation (5). However, *H. influenzae* and *N. gonorrhoeae* do not recognize the same sequences (3). DNA uptake by *H. influenzae* involves recognition of specific 9- to 11-base-pair (bp) sequences on the external DNA by surface components of the competent cell (6–8); these sites occur frequently in *Haemophilus* DNA and infrequently in DNA from other genera. Because either cloned (6) or synthetic (9) recognition sites are fully active in *H. influenzae*, specific methylation plays no role in recognition.

Earlier studies of gonococcal DNA recognition employed the common small cryptic plasmid of *N. gonorrhoeae* (10). This plasmid contains at least one recognition site (4, 11), but the exact site was not identifiable (11).

To determine the DNA component recognized in gonococcal transformation, we prepared recombinant plasmids containing unselected fragments of the *N. gonorrhoeae* chromosome and screened the plasmid DNA for the ability to competitively inhibit gonococcal transformation. This assay is applicable to numbers of candidate clones, does not require recovery of intact fragments, and allows estimation of the affinity of interaction between DNA and competent gonococci. The nucleotide sequences of active DNA fragments were determined‡ and compared. The results showed that DNA segments that specifically interact with competent gonococci contain a specific short DNA sequence, which is the recognition site. This assignment also accounts for the results of earlier studies (4, 11). The arrangement of the recognition sequences isolated in our studies suggests that they frequently form parts of certain gonococcal transcriptional regulatory sequences.

MATERIALS AND METHODS

Materials. Restriction endonucleases and other DNA modifying enzymes were from either New England Biolabs or Bethesda Research Laboratories and used as recommended. NACS (nucleic acid chromatography system) columns were from Bethesda Research Laboratories.

Bacterial Strains and Plasmids. *N. gonorrhoeae* FA19 (pFA102) (wild type) and FA130 (streptomycin resistant) were from P. F. Sparling (University of North Carolina, Chapel Hill). *Escherichia coli* HB101 and pBR322 were used for cloning. The sequence of pJD1 from *N. gonorrhoeae* 82409/55(pJD1) has been reported (12); pFA102 from *N. gonorrhoeae* FA19 has a restriction map identical to pJD1.

Growth of Bacteria. *N. gonorrhoeae* strains were grown and maintained as described (3, 13). *E. coli* HB101 was grown in LB medium with antibiotics as appropriate (14). For preparation of pFA102, *N. gonorrhoeae* FA19 (pFA102) was grown in liquid GC medium (Difco) with 1% IsoVitaleX (Baltimore Biological Laboratory Microbiology System) and 24 mM $NaHCO_3$. Transformation of *E. coli* HB101 was by the method of Mandel and Higa (15).

Purification of DNA. Chromosomal DNA from *N. gonorrhoeae* was prepared and purified as described (13). Plasmids were prepared by published methods (16, 17). Restriction fragments were separated by electrophoresis on low-gelation-temperature agarose; segments containing the desired fragments were excised and melted, and the DNA was purified using NACS columns. DNA for competition assays was centrifuged to equilibrium on cesium chloride ethidium bromide density gradients. Ethidium bromide was removed by exhaustive extraction with aqueous isobutanol. Plasmids used in competition assays were converted to linear form by digestion with *Ssp* I or *Eco*RI followed by heating at 65° for 30 min. DNA concentrations were determined using diphenylamine (18), with deoxyadenosine as standard.

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‡The sequences reported in this paper are being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03842, J03843, and J03844).

Nucleotide Sequences. The sequences of both DNA strands were determined by chemical cleavage (19) at least twice on independent DNA preparations.

Assay for Competitive Inhibition of Transformation. Suspensions of competent *N. gonorrhoeae* FA19 were prepared and stored at −80°C. Experiments were done with preparations stored for 5 days or less; transformability remained constant for this period. Competing DNA at the indicated concentrations was mixed with DNA from strain FA130 (final concentration, 2 μg/ml) in 2% brain heart infusion (Difco) containing IsoVitaleX and 8 mM $MgCl_2$, and competent cells were added. After incubation for 30 min at 37°C, DNase I was added to stop the reaction (3). Transformants were determined by duplicate platings on GC agar containing streptomycin (2) and were counted after 72 hr. Dilutions were adjusted to give between 150 and 400 transformants per plate in the absence of competing DNA.

Construction of Plasmids Containing Gonococcal DNA Fragments. Purified DNA from *N. gonorrhoeae* FA19 was digested with *Eco*RI and *Hin*dIII, and fragments of 1–2 kilobases (kb) were recovered and ligated with pBR322, which had been digested with *Eco*RI and *Hin*dIII. Transformants were isolated, and their plasmids were analyzed by restriction mapping to verify the presence of inserted DNA. Three plasmids, designated pGCU1, pGCU2, and pGCU3, were active in inhibiting transformation; the restriction maps of the inserts are shown in Fig. 1.

Construction of Subclones. The 1.4-kb gonococcal insert in pGCU1 was subcloned by digesting pGCU1 DNA with *Eco*RI and *Rsa* I, and the products were recovered. The 800-bp *Eco*RI–*Rsa* I fragment was ligated with the 3847-bp *Eco*RI–*Sca* I fragment of pBR322. The smaller fragments were ligated with pBR322, which had been digested with *Sca* I. Plasmids were isolated from transformant clones and identified by restriction mapping. The three plasmids containing subfragments of the pGCU1 insert, designated pGCU11, pGCU12, and pGCU13, are shown in Fig. 1*A*.

The 1.6-kb gonococcal DNA segment of pGCU2 was subdivided by digesting pGCU2 DNA with *Eco*RI and *Rsa* I. The electrophoretically purified *Rsa* I fragments were ligated with pBR322, which had been digested with *Sca* I; the 325-bp *Eco*RI–*Rsa* I fragment was ligated with the 3847-bp *Eco*RI–*Sca* I fragment of pBR322. Many plasmids obtained from this cloning contained multiple inserts. pGCU21 contains the 325-bp *Eco*RI–*Rsa* I fragment in the *Eco*RI–*Sca* I site of pBR322. pGCU22 contains a single copy of the 670-bp *Rsa* I fragment, and pGCU23 contains two copies of this fragment. pGCU24 contains the 250-bp *Rsa* I fragment. pGCU25 contains two copies of the 400-bp *Rsa* I fragment as well as the 325-bp *Eco*RI–*Rsa* I fragment. These plasmids were sufficient to locate the active DNA segment from pGCU2 unambiguously. Maps of the inserts in these plasmids are shown in Fig. 1*B*.

The 1.2-kb gonococcal DNA segment of pGCU3 was subdivided by digesting pGCU3 DNA with *Eco*RI, *Hin*cII, and *Hin*dIII. The products were separated and purified. The 450-bp *Eco*RI–*Hin*cII fragment was ligated with the 2.3-kb *Eco*RI–*Pvu* II fragment of pBR322, whereas the 750-bp *Hin*cII–*Hin*dIII fragment was ligated to the 2.3-kb *Hin*dIII–*Pvu* II fragment of pBR322. Plasmids were isolated and characterized; the restriction maps are shown in Fig. 1*C*. pGCU31 contained the 450-bp *Eco*RI–*Hin*cII fragment, and pGCU32 contained the 750-bp *Hin*dIII–*Hin*cII fragment.

Construction of pGCU11 Subclones. pGCU11 was digested with *Sau*3AI, and the products were separated by electrophoresis. A 300-bp band containing a 303-bp fragment from the insert and a 317-bp vector segment, and a 958-bp fragment that contains 349 bp of pBR322 DNA, were each recovered and purified. The unpaired ends of the 958-bp fragment were filled in by use of the large fragment of *E. coli* DNA polymerase I and ligated to pBR322, which had been digested with *Sca* I and dephosphorylated with *E. coli* alkaline phosphatase. Transformants were isolated and characterized; the plasmid pGCU112 containing the 958-bp fragment was obtained. The 300-bp fragment mixture was digested with *Alu* I and *Hae* III. The products were separated by electrophoresis, and the 92-bp *Alu* I–*Hae* III fragment was recovered, purified, and ligated to pBR322, which had been digested with *Sca* I. The resulting plasmid, pGCU111, was isolated from a transformant clone and characterized.

Construction of pFA102 Subclones. pFA102 (4.2 kb) was digested with *Hin*dIII and *Xmn* I. The unresolved digestion products were ligated either with pBR322 that had been digested with *Hin*dIII and *Nae* I or with pBR322 that had been digested with *Eco*RV and dephosphorylated with *E. coli* alkaline phosphatase. Three plasmids were isolated: pGCU4 (4.4 kb), which contains the 1.3-kb *Xmn* I–*Hin*dIII fragment; pGCU5 (4.75 kb), containing the 1.65-kb *Hin*dIII–*Xmn* I fragment; and pGCU6 (5.6 kb), containing the 1.25-kb *Xmn* I–*Xmn* I fragment.

RESULTS

Identification of the Recognition Sequence. Plasmids containing random restriction fragments from the gonococcal chromosome were constructed, and purified linear plasmid

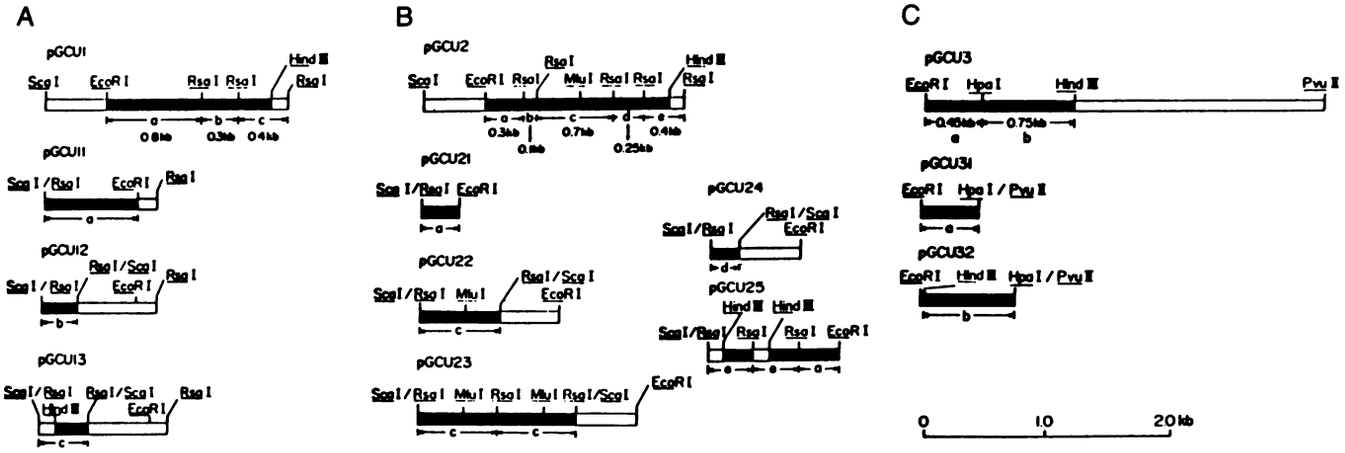

FIG. 1. Restriction maps and subdivision of gonococcal DNA inserts. Fragments of chromosomal DNA from *N. gonorrhoeae* FA19 were inserted into pBR322 as described. The restriction maps of the three plasmids that inhibited transformation are presented, together with the methods used to subdivide the gonococcal inserts. Solid bars indicate DNA derived from *N. gonorrhoeae*, and open bars indicate DNA derived from the vector. (*A*) pGCU11 and plasmids derived from it; (*B*) pGCU2 and derivatives; and (*C*) pGCU3 and derivatives.

DNA was assayed for competitive inhibition of transformation. Data were analyzed using double-reciprocal plots (6), in which the slope of the line provides an estimate of the relative affinity of the test DNA for the transforming DNA receptor. A slope of one indicates that the test DNA is equivalent to transforming DNA, and slopes greater or less than one indicate higher or lower affinity, respectively.

Candidate plasmids were constructed as described, and twelve of these were tested for inhibition of gonococcal transformation. Reciprocal plots of results for active and inactive plasmids, the vector, and gonococcal DNA are shown in Fig. 2; the slopes determined for the active plasmids are summarized in Table 1. DNA from these plasmids, pGCU1, pGCU2, and pGCU3, competitively inhibited transformation. DNA from the remaining nine plasmids, pGC1–pGC9, did not inhibit transformation significantly (data not shown). None of the cloned DNA fragments inhibited to the same degree as chromosomal DNA; positive plasmid DNA preparations gave slope values between 0.15 and 0.4, whereas inactive plasmid DNA gave slopes between 0 and 0.05. Restriction maps of the active plasmids are shown in Fig. 1.

The Role of DNA Methylation. Methylated residues have been suggested as sites for gonococcal DNA recognition (20); this possibility was examined using the plasmid pFA102, which contains a recognition site (4). Subclones of pFA102 were prepared as described. The parent plasmid and the three derivative plasmids were tested for competition. Both pFA102 prepared from *N. gonorrhoeae* and one subclone of this plasmid (pGCU5) prepared from *E. coli* competitively inhibited gonococcal transformation. The two slopes were indistinguishable and ≈0.15. The ability of pFA102 to compete was associated with the 1.65-kb *Hin*dIII–*Xmn* I segment in pGCU5, and the degree of competition was the same irrespective of whether the DNA was isolated from *N. gonorrhoeae* or propagated in *E. coli*. These results quantitatively confirm the conclusions reached using fragment-uptake assays; DNA modification does not contribute to the specific interaction between *N. gonorrhoeae* and transforming DNA (11).

Identification of the Gonococcal Uptake Sequence. To obtain shorter active segments for sequencing, the three active DNA segments from pGCU1, -2, and -3 were each subcloned into pBR322 as described. The results of assays using these plasmids are summarized in Table 1. In each case, the original activity was associated with a single subfragment. The active segment of pGCU1 was located on the 0.8-kb fragment in pGCU11, the active portion of pGCU2 was associated with the 0.7-kb segment common to pGCU22 and pGCU23, and the active region of pGCU3 was present on the 0.7-kb fragment in pGCU32.

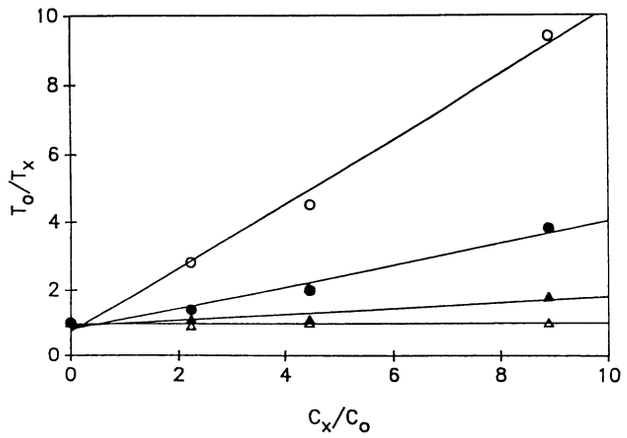

FIG. 2. Competitive inhibition of transformation by hybrid plasmids. Streptomycin-resistant transformants were measured as a function of the relative concentrations of transforming DNA and competing DNA. The data were plotted according to Sisco and Smith (6); C_o, transforming DNA concentration; C_x, concentration of competing DNA; T_o, transformants in the presence of transforming DNA only; T_x, transformants in the presence of added competing DNA. ○, DNA from *N. gonorrhoeae* FA19 (slope ≈1); ●, pGCU1 DNA; ▲, pGC1 DNA; △, pBR322 DNA.

Table 1. Competitive inhibition of transformation by plasmid DNA containing inserts of gonococcal origin

Plasmid	Size, kb	Slope
pGCU1	1.4	0.32
pGCU11	0.8	0.40
pGCU12	0.3	<0.05
pGCU13	0.3	<0.05
pGCU111	0.09	0.42
pGCU112	0.8	<0.05
pGCU2	1.65	0.20
pGCU21	0.3	<0.05
pGCU22	0.7	0.22
pGCU23*	1.4	0.32
pGCU24	0.25	<0.05
pGCU25*	1.1	<0.05
pGCU3	1.2	0.12
pGCU31	0.45	<0.05
pGCU32	0.75	0.17
pFA102	4.2	0.17
pGCU4	1.3	<0.05
pGCU5	1.65	0.15
pGCU6	1.25	<0.05

Plasmid constructions and assays are described in text. The slope values are the averages of at least four independent determinations. Sizes are of the inserts only.
*Contains multiple inserts.

The nucleotide sequences of the active segments from pGCU11, pGCU22, and pGCU3 were determined and compared; the sequences are shown in Fig. 3. The only sequence >5 bp in length common to the active segments in pGCU1, pGCU2, and pGCU3, and the active region of pFA102, was contained within the 10-bp sequence 5′ GCCGTCTGAA 3′. Active derivatives of pGCU1, with the highest relative affinity for competent cells, contain two copies of this sequence arranged as a tandem inverted repeat. Derivatives of pFA102 contain one 10-mer beginning at residue 1889 and an 8-bp site at position 3714 (12). These two sites account for the uptake of specific restriction fragments; fragments containing the 10-mer interact strongly, and those containing the 8-mer interact weakly with competent cells (11). Active plasmids derived from pGCU2 contain one complete 10-mer and one site containing a single mismatch. Clones derived from pGCU3 contain three sites in which nine of ten residues match the above sequence. The reduced affinity of shorter matching sequences for the transforming DNA receptor is similar to observations in the *H. influenzae* system (8).

Confirmation of the identification of this sequence as the gonococcal recognition site was obtained by subcloning pGCU11. pGCU112, which contains the first 609 bp of the insert from pGCU11, was completely inactive, whereas pGCU111, which contains a 93-bp segment with two copies of the 10-mer, gave a slope value of 0.42, indistinguishable from the value of 0.40 obtained with the parent plasmid. The presence of this short sequence containing the 10-mers accounts for the specific interaction of all the derivatives of pGCU1 with competent *N. gonorrhoeae*; the results are summarized in Table 1.

pGCU11

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  1  GAATTCCCCG AACTCGAACC CATCGCCAAA GACCGCCTGC ACATCTTCAG CAAACCGATG CAGCTGGTTA CCGAAAAAGG TAAGGAAAAC ATGATTCAAC
101  GCGGCACATA CAACTACCAA TACCGCAGCA ACCGTCCCGT CAAAGACGGC AGCTACCTCA TTACCGCCGA ATATCAGCCT ACTTTCCGGT CAAAAAACAA
201  AGCAGGCTGG AAACAGGCTG GCATCAAAGA AATGCCTGAC GCAAGCTATT GCGAACAAAC CCGTATGTTC GGATTTTTCA TTGTCAACGT GGAATACGAA
301  GCGCGACACC GCCATCATCA CCAAACCGGT CGGACAAAAC TTGGAAATCG TTCCCGCTGG ACAATCCCGC CAACATTCAC GTGGGCGAAC GCTTCAAAGT
401  CCGCGTTCTG TTCCGTGGCG AACCGCTGCC CAATGCCACC GTTACCGCTA CATTTGACGG CTTCGACACC AGCGACCGCA GCAAAACGCA CAAAACCGAA
501  GCCCAAGCCT TCTCCGACAC CACCGACGGC AAAGGCGAAG TGGACATCAT CCCCTTGCGC CAAGGCTTTT GGAAAGCGAG TGTCGAATAC AAAGCCGATT
601  TCCCCGATCA AAGCCTGTGC CAAAAACAGG CGAACTACAC AACTTTAAcc ttccaaatcg gccattctca ccattaattc cgtccaaaca aaaatGCCGT
701  CTGAAatggt TTCAGACGGC atcctttgtt caaacatcaa taccagccgc gcagctcatc gctttttcaa cacggcggat actcatcata taagacgcgg
801  tac
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pGCU22

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  1  GTACGCGGTC GGAATACTGA TAACGAGGTT TCTTTGTGAT GATTTTGCTG GACACGAATG TGATTTCCGA ACCTTTGCGC CCTCCAATGA ACGTGTGGTG
101  GCATGGTTGG ATAGTTTGAT ATTGGAAGAT GTGTATTTGT CTGCCATTAC TGTTGCAGAA TTGCGTTTGG GTGTGGCGTT GTTGCTCAAT GGCAAGAAAA
201  AGAATGTGCT GCACGAACGT TTGGAACAAT CCATTTTGCC TTTATTTGCG GGGCGGATTC TGCCTTTTGA TGAACCGGTT GCCGCAATCT ATGCGCAAAT
301  TCGTTCCTAT GCCAAAACAC ATGGCAAAGA GATTGCTGCC GCAGACGGCT ATATTGCCGC CACTGCAAAA CAGCACAGTT TGACAGTTGC TACGCGTGAT
401  ACCGGCTCAT TTTTTGCGGC CGATGTCGCG GTGTCAATCC GTGGCACGAT TAAAAGACGC TTTTAAgcag ccttaatgtc aggctgccta agcgtcttag
501  cattccgttt cattatttat ttttgcgcgt tcaggctgct tttctgGCCG TCTGAAgccc tgttttcggg tTTCAGACGG tatttttgcg aacaactttt
601  tatgccttgt tctttttggc gcatttttcc cgccatcagt tcgtgcaggg ttttcttcgc cggtttcatc ggtac
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pGCU3

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  1  AAGCTTTGGA ATTGGATAAC CTGAGGTGGC GAAAGCGACT TTGGTGTCTG CCGAAGCACG TAAAGAATCA CGCGGTGCGC ACGCTTCAGA CGACCATCCT
101  GAGCGCGATG ACGAAAATTG GATGAaacac accctgtatc attcggacac caatacctta tcttacaaac cggtacacac cgagcctttg agcgtggaat
201  acatcaaacc ggccaaacgc gtttattgat gcgtttTTCA GACGGtcttc gcctcaaagG TCGTCTGAAa cctaaccata cccacattga actgcttgaa
301  tttataatac aaaatcattg gggcagttgA TGAGAAAAGG AACACTTCTC ATGGAAAAAA TGAGTTTTGA AATTTACCGT TACAACCCGG ACGTTGATGC
401  CAAGCCTTAT ATGCAGCGTT ACGAGTTGGA ATTGGAACCG ACCGACGTGA AACTTTTGGA CGCCTGAAAG CACAAGACGA TACCTTGTCT
501  TTCCGCCGCT CCTGCCGCGA AGGGATTTGC GGACCGGACG GTATGAACAT CAACGGCAAA AACGGCTTGG CGTGTCTGAC CGATTTACGC AGCTTGAAAC
601  AGCCTGTCAA AATCCGCCCG CTGCCCGGTC TGCCCGTCAT CCGCGACCTG ATTGTGGATA TGACCCAGTT CTTCAAACAA TACCATTCCG TCAAACCTTA
701  TGTTGTTAAC GACAATCCGA TTGGTGCGGA CAAAGAGCGT CTGCAAACTC AGGAAGAGCG CAAAGAGTTG GATGGTTTGT ACGAATGTAT TTTGTGCGCT
801  GTTCGACCGC CTGTCCGGTC ATTCTGGTGG AATCCCGACA AATTCGTCGG TCCGTCCGGC TTGCTGAACG CCTACCGCTT CATTGCGGAC AGCCGAGATA
901  CCATCACTAA TGAGCGTTTG GATAATTTGA ACGATCCGTA CCGTTTGTTC CGCTGCCACA CCATTATGAA CTGCGTAGAC GTATGCCCCA AACACTTGAA
1001  TCCGACCCGG GCCATCGGTA AGATTAAAGA GATTATGTTG AAACGGGTTG TTTAAgaaat gatggttttt gacgacattg ccaaacggaa aatccctttt
1101  caaacccgcc ggggattctt ggaactagat ttaatcttcg gcaggtttat ggaaaaagaa ttc
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FIG. 3. Nucleotide sequences of the cloned gonococcal DNA inserts. Uppercase letters are the sequences corresponding to open reading frames; uppercase underlined sequences are of the proposed uptake sites.

Arrangement of Uptake Sites. The three isolates of gonococcal recognition sequences each contained the sites as parts of inverted duplications. However, the data with pFA102 and derivatives showed that a single 10-bp site is sufficient for inhibition of transformation. The arrangement and sequences of the inverted repeats are similar to terminators and attenuators of bacterial transcription (21, 22), and the calculated free energies of formation of stem-loop structures from these inverted repeats are quite favorable, ranging from -10 to -27 kcal/mol (1 cal = 4.184 J) (23). Runs of thymine residues are present immediately downstream from the dyad symmetry elements in the pGCU11 and pGCU22 sequences, but are not present in pGCU3. These runs are present in most, but not all, *E. coli* terminators (22), and their length is related to the efficiency of termination (24).

To function as transcriptional signals, these inverted repeats should be located outside of sequences encoding polypeptide chains. To test this hypothesis, the DNA sequences of the gonococcal inserts were checked for the presence of open reading frames; the results are shown in Fig. 3. Each inverted repeat was located immediately downstream from a termination codon, which ended an open reading frame. In pGCU3, the inverted repeat separates a short 3′ portion of an open reading frame from a complete long open reading frame. The locations of the inverted repeats containing the 10-mer are consistent with a transcriptional regulatory function.

Additionally, the transcriptional terminators associated with the *N. gonorrhoeae* MS11 IgA1 protease-encoding gene (25) and two variants of the *opaE1* gene (26) each contain at least one copy of the 10-mer in the stem portion of the proposed terminators. These sequences are also shown in Table 2.

DISCUSSION

The specific interaction between transforming DNA and recipient cells requires the recognition of either a specific nucleotide sequence or a specific DNA modification that must be present at high frequency on active DNA and rare or absent from most other DNA molecules (5). In *H. influenzae*, recognition of a short sequence is responsible for specificity (6–9). For *N. gonorrhoeae*, in which the DNA is methylated extensively, recognition has been proposed to involve interaction with modified residues (12). However, our results using DNA derived from the cryptic plasmid of *N. gonorrhoeae*, together with earlier results (11), clearly eliminate this possibility. Therefore, *N. gonorrhoeae*, like *H. influenzae*, must recognize a DNA sequence during transformation.

Table 2. Comparison of palindromic uptake sequences with presumed gonococcal terminators

DNA source	Sequence
pGCU11	aaacaaaaaGCCGTCTGAAatggtTTCAGACGGCatcctttgttc
pGCU22	gcttttctgGCCGTCTGAAgccctgttttcgggtTTCAGACGGta
pGCU3	tggttaggtTTCAGACGGtcttcgcctcaaagGTCGTCTGAAaaa
opaE1	gataccgatGCCGTCTGAAccTTCAGACGGCtttttgat
IgA protease-encoding gene	aaataaaatGCCGTCTGAActcaagcTTCgGACGGCatttttatc

The sequences of the uptake sites of pGCU11, pGCU22, and pGCU3 are from Fig. 3; the *opaE1* terminator sequence is from ref. 25, and that of the IgA protease terminator is from ref. 26. Recognition sequences are in uppercase, and inverted repeats are underlined.

To identify this sequence, we cloned several active DNA segments from the *N. gonorrhoeae* chromosome. Active segments were identified by their capacity to competitively inhibit gonococcal transformation. Subcloning showed that in every case specific inhibition was associated with a specific restriction fragment, again indicating that a distinct site was recognized. The sequences of these four independently derived active DNA segments were determined and compared. The only common sequence longer than 5 bp was contained within the 10-bp sequence 5′ GCCGTCTGAA 3′. This sequence is unrelated to the short repetitive sequence identified in pathogenic *Neisseria* DNA (27). The presence of either terminal or internal mismatches in an uptake sequence reduced the apparent affinity between DNA and competent gonococci. This is similar to the behavior of shorter recognition sites in the *H. influenzae* system (8). None of the constructions in this study inhibited transformation to the same degree as gonococcal chromosomal DNA. This reduced affinity appears to be the consequence of the lower density of sites relative to nonspecific DNA in our clones; the evidence for this conclusion will be reported elsewhere (S.D.G. and J.J.S., unpublished work). Identification of the 10-mer as the gonococcal recognition sequence was confirmed by inserting a 93-bp fragment containing two copies of the site into pBR322. This was sufficient to convert an inactive DNA molecule to one that competitively inhibited gonococcal transformation.

It is intriguing that the recognition sites originating from the gonococcal chromosome were arranged as inverted repeats. These interrupted dyad symmetry elements may form stable stem-loop structures similar to terminators or attenuators of transcription (21, 22). Each repeated recognition sequence was located near the end of a long open reading frame. Furthermore, the proposed terminators for the gonococcal *opaE1* and IgA protease genes each contain two copies of the 10-mer in the stem portion of the stem-loop structure (25, 26). In these two cases, the sequence of interest was isolated without reference to the transformation properties of the DNA. However, the inverted repeats proposed as terminating transcription of surface proteins of *N. gonorrhoeae* do not contain recognition sequences (28, 29). Therefore terminators containing the uptake sequence may represent a specific functional class of these regulatory elements. Interestingly, the sequence does not occur in any *E. coli* transcriptional terminator sequences (22).

The occurrence of gonococcal uptake sequences within putative transcriptional terminators has a number of important implications. (*i*) It suggests that uptake sequences originated as regulatory signals and that their role in DNA recognition has evolved to take advantage of their frequent occurrence. Single recognition sites might then evolve secondarily as a consequence of transformation-related recognition. (*ii*) The regulatory role of uptake sequences in the genome allows multiple copies to be accommodated without unduly constraining coding regions. (*iii*) It can account for the genus specificity that characterizes the recognition of transforming DNA. If the detailed sequences of transcriptional terminators are shared by members of a genus, then the recognition of transforming DNA would also cross species boundaries—but not those between genera. (*iv*) A transformation system that recognizes sequences at transcriptional boundaries would have the useful property of interacting with noncoding DNA sequences. If DNA processing during transformation were confined to the point of interaction with the receptor, then recognition of a terminator sequence would tend to preserve the informational content of transforming DNA during entry.

To summarize, we have identified the DNA recognition sequence active in specific transformation of *N. gonorrhoeae*. The uptake sequence was frequently located in the stem structure of probable terminators of transcription, suggesting that recognition sites may have evolved and be, in part, maintained as constituents of these regulatory structures. Whether these sequences impede transcription and demarcate functional transcriptional units remains to be established.

We thank M. Hauser, S. Krag, H. O. Smith, and E. Lattman for their advice and comments, and K. L. Burnstein for communicating results prior to publication. Supported by U.S. Public Health Service Grant PO1 AI 16969.

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