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A Promoter Associated with the Neisserial Repeat Can Be Used To Transcribe the *uvrB* Gene from *Neisseria gonorrhoeae*

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A recombinant plasmid capable of restoring UV resistance to an *Escherichia coli uvrB* mutant was isolated from a genomic library of *Neisseria gonorrhoeae*. Sequence analysis revealed an open reading frame whose deduced amino acid sequence displayed significant similarity to those of the UvrB proteins of *E. coli*, *Micrococcus luteus*, and *Streptococcus pneumoniae*. A gonococcal *uvrB* mutant was constructed and found to be extremely sensitive to UV radiation. Transcriptional fusions between portions of the gonococcal *uvrB* upstream region and a reporter gene were used to localize promoter activity, and the transcriptional start point of the gonococcal *uvrB* gene was mapped in *E. coli* by primer extension. A corresponding σ^{70} promoter was identified within a copy of the 26-bp neisserial repeat, and this identification provided the first evidence of a promoter associated with this repetitive element in *N. gonorrhoeae*.

Neisseria gonorrhoeae is a naturally competent organism in which transformation and recombination appear to play an important role in its ability to evade the host immune response (36, 43). Although an excision repair system has been identified previously (12), *N. gonorrhoeae* is deficient in several DNA repair functions including photoreactivation (10) and error-prone repair systems (11). This absence of error-prone repair has been noticed in other highly transformable organisms such as *Haemophilus influenzae*, *Micrococcus radiodurans*, *Streptococcus pneumoniae*, *Streptococcus mutans*, and *Streptococcus sanguis* (45).

Excision repair has been extensively studied in *Escherichia coli* (50), and that system may serve as a model for a similar process in *N. gonorrhoeae*. The entire excision repair process can be divided into four main stages: incision-excision, which involves scission of a phosphodiester bond on either side of the lesion; removal of the oligomer containing the damaged nucleotide(s); repair synthesis to fill the resulting gap in the DNA; and ligation of the newly completed repair patch (39). The incision step of this process is catalyzed by the coordinated action of the UvrA, UvrB, and UvrC proteins. Specifically, this enzyme complex, termed the (A)BC excinuclease (38), incises the damaged strand at the eighth phosphodiester bond 5′ and the fourth or fifth phosphodiester bond 3′ to the lesion (41).

In *E. coli*, the *uvrA* and *uvrB* genes are part of the SOS system, a global DNA repair system that is induced by a variety of treatments that damage DNA or interrupt DNA replication (32). Expression of these damage-inducible (*din*) genes is regulated by the products of the *recA* and *lexA* genes (32, 53). The LexA protein acts as a repressor of the *din* genes by binding to a consensus sequence known as the SOS box (52). The presence of DNA damage gives rise to conformational changes in the RecA protein which mediate autocleavage of LexA and result in derepression of genes in the SOS regulon (26, 30–32).

Analogs of the *E. coli uvrB* gene have been cloned from *Micrococcus luteus* (44) and *S. pneumoniae* (46, 47). In this paper we describe the cloning, nucleotide sequence, and transcriptional analysis of the *uvrB* gene from *N. gonorrhoeae* MS11. The data provide the first evidence of a promoter associated with a 26-bp neisserial repeat (NR) sequence (15).

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MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strain used as the host for cloning experiments was DH5α (F⁻ *endA1 thi-1 hsdR17 supE44 relA1 gyrA96 recA1* Δ*lacU169* [φ80 *lacZ*ΔM15]). The strains of *E. coli* used in complementation experiments were AB1157 [*thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2 rac-hisG4*(Oc) *rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1* λ⁻] and AB1885 (*uvrB5*), a *uvrB* mutant of AB1157. The *N. gonorrhoeae* strains used in this study were MS11-A, a variant of MS11 (48), and JKD335, a *uvrB* mutant of MS11-A. The plasmids used in this study are listed in Table 1.

Media and culture conditions. *E. coli* strains were grown at 37°C in Luria-Bertani (LB) medium (40). For maintenance of plasmids, ampicillin (50 μg/ml), chloramphenicol (30 μg/ml), and tetracycline (12.5 μg/ml) were added as required. All *N. gonorrhoeae* strains were routinely cultured on GC agar base (Oxoid) enriched with 1% (vol/vol) DMIV, a modified form of Iso-Vitalex (42), and incubated at 37°C in a 5% CO_2 atmosphere. Gonococcal transformations were performed by the addition of approximately 1 μg of linearized plasmid DNA (treated with *Sss*I methylase) to a suspension of piliated *N. gonorrhoeae* MS11 cells transferred from an overnight-culture plate into GC broth supplemented with 1% (vol/vol) DMIV and 1% (vol/vol) $NaHCO_3$ as a CO_2 source. Cultures were grown for 4 h at 37°C with constant agitation and plated onto GC agar containing chloramphenicol (8 μg/ml) to select for recombinants.

Recombinant DNA techniques. All DNA manipulations were performed by following standard procedures (40). DNA sequencing was performed by using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) and analyzed with an Applied Biosystems model 373A DNA sequencing system. Sequencing reactions were performed as described by the manufacturer. Oligonucleotide primers were synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer and eluted from columns following

TABLE 1. Plasmids used in this study

Plasmid	Description	Reference or source
pSU2718/19	Cm^r cloning vectors	35
pUC18/19	Ap^r cloning vectors	37
pBR322	Ap^r Tc^r cloning vector	7
pKK232-8	Ap^r promoter selection vector	8
pJKD1249	3.8-kb partial *Sau*3AI fragment containing *uvrB* gene in pSU2718	This study
pJKD1289	Insert from pJKD1249 in pSU2719	This study
pJKD1444	3,225-bp *Ava*I-*Eco*RI fragment from pJKD1249 in pBR322	This study
pJKD1484	444-bp *Sal*I fragment from pJKD1444 replaced by P*recA*:*cat* cartridge	This study

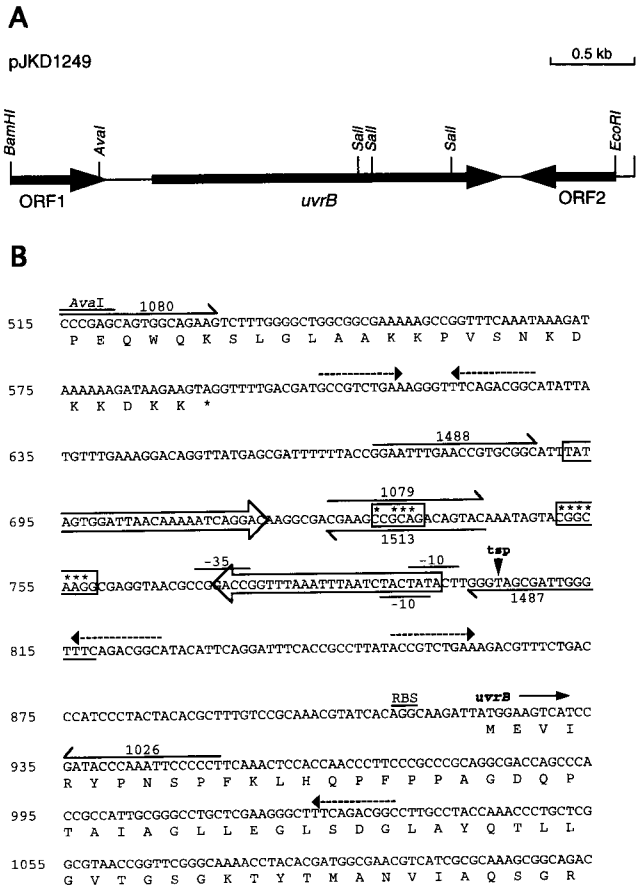
A
pJKD1249
0.5 kb
BamHI
AvaI
SalI
SalI
SalI
EcoRI
ORF1
uvrB
ORF2
B
AvaI
1080
515 CCCGAGCAGTGGCAGAAGTCTTTGGGGCTGGCGGCGAAAAAGCCGGTTTCAAATAAAGAT
P E Q W Q K S L G L A A K K P V S N K D
575 AAAAAAGATAAGAAGTAGGTTTTGACGATGCCGTCTGAAAGGGTTTCAGACGGCATATTA
K K D K K *
1488
635 TGTTTGAAAGGACAGGTTATGAGCGATTTTTTACCGGAATTTGAACCGTGCGGCATTTAT
1079
695 AGTGGATTAACAAAAATCAGGACAAGGCGACGAAGCCGCAGACAGTACAAATAGTACGGC
1513
-35
-10
tsp
755 AAGGCGAGGTAACGCCGACCGGTTTAAATTTAATCTACTATACTTGGGTAGCGATTGGG
-10
1487
815 TTTCAGACGGCATACATTCAGGATTTCACCGCCTTATACCGTCTGAAAGACGTTTCTGAC
RBS
uvrB
875 CCATCCCTACTACACGCTTTGTCCGCAAACGTATCACAGGCAAGATTATGGAAGTCATCC
M E V I
1026
935 GATACCCAAATTCCCCCTTCAAACTCCACCAACCCTTCCCGCCCGCAGGCGACCAGCCCA
R Y P N S P F K L H Q P F P P A G D Q P
995 CCGCCATTGCGGGCCTGCTCGAAGGGCTTTCAGACGGCCTTGCCTACCAAACCCTGCTCG
T A I A G L L E G L S D G L A Y Q T L L
1055 GCGTAACCGGTTCGGGCAAAACCTACACGATGGCGAACGTCATCGCGCAAAGCGGCAGAC
G V T G S G K T Y T M A N V I A Q S G R
1115 CCGCCATCATTATGGCGCACAACAAAACCCTTGCCGCCCAGCTTTATGCCGAAATGCGCG
P A I I M A H N K T L A A Q L Y A E M R
1175 AGTTTTTCCCCGAAAACGCAGTGGAATATTTCGTGTCGTATTACGACTATTACCAGCCCG
E F F P E N A V E Y F V S Y Y D Y Y Q P
1235 AAGCCTATGTGCCCAGCCGCGATTTGTTCATCGAAAAAGACAGCGCGATCAACGAACACA
E A Y V P S R D L F I E K D S A I N E H
1295 TCGAGCAGATGCGCCTTTCCGCCACCAAAAACCTGATGACGCGCGACGACGTGATTATCG
I E Q M R L S A T K N L M T R D D V I I
1355 TCGCCACCGTGTCCGCCATTTACGGTATCGGCGACCCGACCGAGTATCAACAAATGGTGC
V A T V S A I Y G I G D P T E Y Q Q M V
1415 TGTCCGTAAAAGAAGGCGACACCATCGAGCAGCGCGACATCATCGCCACGCTCGTTTCCA
L S V K E G D T I E Q R D I I A T L V S
1475 TGCAGTACGAACGCGGCGATTTGGACTTCAAACGCGGCAGCTTCCGCGTGCGCGGCGACG
M Q Y E R G D L D F K R G S F R V R G D
1535 TGATTGACGTGTACCCCGCCGAAAGCTCCGAAAACGCCTTGCGTATCAGCCTGTTTGATG
V I D V Y P A E S S E N A L R I S L F D
1595 ACGAAATCGACCGCCTCGATATGTTCGACCCGCTTTCAGGCAGCCTGCACCAGCGTGTCG
D E I D R L D M F D P L S G S L H Q R V
1655 GACGCTACACCGTCTTCCCGTCCAGCCACTACGTTACCCCGCGCGACACCGTATTGCGCG
G R Y T V F P S S H Y V T P R D T V L R
1715 CCTGCGAATCCATCAAAGAAGAATTGCGCGAACGCATCGAATTTTTCGCTCGCGAACAAC
A C E S I K E E L R E R I E F F A R E Q
1775 GCCCCGTCGAGCAACAACGCATCGAACAGCGCACCCGTTTCGACCTCGAAATGCTCTACG
R P V E Q Q R I E Q R T R F D L E M L Y
1835 AAATGGGCTTCTGCAAAGGCATTGAAAACTACTCCCGCCACTTCTCCGGTAAAAAAGAAG
E M G F C K G I E N Y S R H F S G K K E
1895 GCGAACCGCCGCCCACGCTGATGGACTACCTGCCCGACAACGCCATCATGTTCATCGACG
G E P P P T L M D Y L P D N A I M F I D
1955 AAAGCCACGTTACCGTTACCCAAATCGGCGGTATGTACAAAGGCGACGCATCGCGCAAGC
E S H V T V T Q I G G M Y K G D A S R K
2015 AAAACCTCGTGGACTACGGCTTCCGCCTGCCTTCCGCCCGCGACAACCGCCCGCTCAAAT
Q N L V D Y G F R L P S A R D N R P L K
2075 TCCACGAATTTGAAAAAGTCATGCCGCAAACCGTCTTCGTTTCCGCCACCCCCGCGAAAT
F H E F E K V M P Q T V F V S A T P A K
SalI
2135 ACGAAGAAGAACACGCCGGACAAGTCGTCGAACAAGTCGTCCGCCCCACAGGGCTGGTCG
Y E E E H A G Q V V E Q V V R P T G L V
SalI
2195 ACCCCCAAATCATCATCCGTCCCGTCGCCACCCAAGTCGACGATTTAATGAGCGAAATCA
D P Q I I I R P V A T Q V D D L M S E I
2255 ACGACCGTATTCAAAAAGGCGAACGTGTGCTCGTTACCACCCTCACCAAACGCATGGCGG
N D R I Q K G E R V L V T T L T K R M A
2315 AGCAACTCACCGACTATTACAGCGAACTCGGCATCAAAGTGCGCTACCTGCACAGCGACA
E Q L T D Y Y S E L G I K V R Y L H S D
2375 TCGACACCGTCGAGCGCGTTGAAATCATTAGAGATTTGCGGCTCGGCCTGTTTGACGTAC
I D T V E R V E I I R D L R L G L F D V
2435 TCGTTGGCATCAACCTGTTGCGCGAAGGCCTCGACATCCCCGAAGTGTCCCTCGTCGCCA
L V G I N L L R E G L D I P E V S L V A
2495 TCCTCGATGCCGACAAAGAAGGCTTCCTGCGCTCCCACCGCAGCCTGATTCAAACCATAG
I L D A D K E G F L R S H R S L I Q T I
2555 GCCGCGCCGCGCGCAACGTGAACGGCGTCGCCATCCTGTACGCCGACAAAATTACCGACT
G R A A R N V N G V A I L Y A D K I T D
SalI
2615 CCATGAAAGCCGCCGTCGACGAAACCGAACGCCGCCGCGAAAAACAGATTAAATTCAACG
S M K A A V D E T E R R R E K Q I K F N
2675 AAGAACACGGTATCGTGCCGCAGCAGATTAAAAAACAGGTCAAAGACATCATCGACGGCG
E E H G I V P Q Q I K K Q V K D I I D G
2735 TGTACCACGAAGAAGACAGTGGCAAAGGCCGTAGACAAGGCAAAAACAAGGTCAAAGTCG
V Y H E E D S G K G R R Q G K N K V K V
2795 GCGAAATCCACAACGAAGAAGACGCGATTAAAGAAATCGCCAAACTGGAAAAAGCCATGC
G E I H N E E D A I K E I A K L E K A M
2855 AGCAGGCGGCTAGGGATTTACAGTTTGAAGAAGCTGCTGTGTTGAGGGATAGGATTAGGA
Q Q A A R D L Q F E E A A V L R D R I R
2915 ATATTAAAGAGAATTTGTTGTTTGGGGCGGAGTGATTTTGAAATAGTAATCGCAGCCTGA
N I K E N L L F G A E *
2975 ATACATAGCGTGTGCGCCACTGGCGCGCGCTCATTGTGTTATCAGGAATCCTTGAAATCA
3035 TGTACGCAGCTTGCATCACACATCCTAGCTATACATAAACAACAAGGCCGTCTGAATTTT
* E I V V R A N L
3095 CAGACGGCCTTTTCCGTTTACAACTTTACTACTTATTCAATAACTACACGTGCATTCAGA
P Q V P R N N E S G V A R T F K E A Q A
3155 GGCTGTACGGGGCGGTTGTTTTCCGAACCGACGGCGCGGGTGAATTTTTCCGCTTGCGCT

FIG. 1. (A) Physical map of 3.8-kb insert of pJKD1249 showing position and orientation of gonococcal *uvrB* gene and nearby ORFs designated ORF1 and ORF2. The position of relevant restriction enzyme recognition sites is also shown. (B) Nucleotide sequence of the region 3′ to the *Ava*I site of pJKD1249 containing the gonococcal *uvrB* gene. Neisserial DNA uptake sequences are shown by dashed arrows. The 26-bp NR sequences are indicated by arrowed boxes. The transcriptional starting point (tsp) is indicated by an arrowhead, and the corresponding σ^{70} promoter sequences are overlined. The putative gearbox promoter elements are boxed, and asterisks indicate nucleotides identical to the consensus sequence (51). The deduced amino acid sequences of the *uvrB* gene and the C-terminal regions of ORF1 and ORF2 are displayed below the corresponding nucleotide sequences. RBS marks the position of a putative ribosome binding site upstream of the *uvrB* gene. Oligonucleotide primers used in this study are indicated by numbered arrows above the nucleotide sequence.

instructions specified by the manufacturer. DNA was amplified by PCR with an FTS-1 thermal sequencer (Corbett Research). The temperature program routinely used for PCR was 30 cycles (each) for 1 min at 92°C, for 1 min at 50°C, and for 1 min at 72°C followed by 1 cycle (each) for 1 min at 92°C, for 1 min at 50°C, and for 5 min at 72°C. For PCR fusion reactions, the following temperature program was used: 30 cycles (each) for 2 min at 93°C, for 2 min at 45°C, and for 7 min at 72°C. Following cloning into plasmid vectors pUC18 or pUC19, all PCR products were sequenced on both strands to confirm that no errors had occurred during amplification.

UV irradiation of bacterial strains. The sensitivity of the *E. coli* and *N. gonorrhoeae* strains to UV was assayed by determining culture viability following irradiation with a TFL-20M Hybrad "Crosslinker" transilluminator (Integrated Science Pty. Ltd.) or under a germicidal lamp (Gelman Clemco Pty. Ltd.). Quantitative measurements of bacterial survival were obtained following exposure of serially diluted stationary-phase cultures to various UV doses.

Enzyme assays. Cell extracts of the *E. coli* strains for enzyme assays were prepared from stationary-phase cultures by passage twice through a French pressure cell (Aminco, Silver Springs, Md.). The protein concentration was determined by the method of Lowry et al. (34), with bovine serum albumin as the standard. Chloramphenicol acetyltransferase (CAT) assays were performed by using a CAT enzyme-linked immunosorbent assay kit (Boehringer-Mannheim)

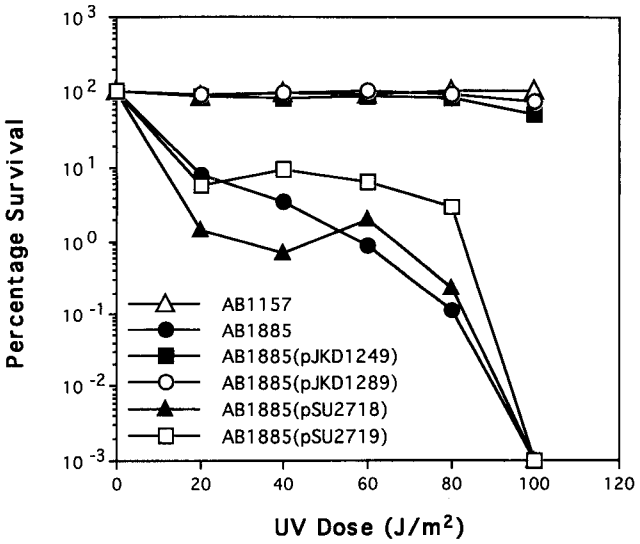

FIG. 2. Survival following UV irradiation of *E. coli* AB1157, AB1885 (a *uvrB* mutant of AB1157), and AB1885 harboring plasmid pJKD1249 or pJKD1289, which contains the gonococcal *uvrB* gene in either orientation with respect to the *lac* promoter in pSU2718 and pSU2719, respectively. AB1885 carrying vector pSU2718 or pSU2719 was included as a control. The values are the means for three independent experiments.

according to the manufacturer's instructions. Two protein extracts (10 and 50 ng of total protein) were assayed from each sample. The A_{405} for each sample was read by using a Bio-Rad model 450 microplate reader. As a control to detect plasmid-copy-number effects, β-lactamase assays were performed with the same cell extracts, according to the method of Bush and Sykes (9), and the results did not vary more than 1.5-fold for any extract.

Nucleotide sequence accession number. The nucleotide sequence reported here has been submitted to the GenBank nucleotide sequence database under accession no. U11547.

RESULTS

Cloning of *uvrB* gene from *N. gonorrhoeae* MS11 and complementation of an *E. coli uvrB* mutant. A genomic library of *N. gonorrhoeae* MS11 was constructed by partially digesting genomic DNA with *Sau*3AI and ligating fragments of 3 to 9 kb into the *Bam*HI site of the plasmid vector pSU2718. A recombinant plasmid capable of complementing the UV-sensitive phenotype of *E. coli uvrB* mutant AB1885 was isolated from this library. The complementing clone, containing an insert of approximately 3.8 kb, was designated pJKD1249 (Table 1; Fig. 1A).

The insert in pJKD1249 was also cloned into pSU2719 to generate pJKD1289 (Table 1). This allowed orientation of the insert opposite to the direction of transcription from the *lac* promoter. Both plasmids were tested for their ability to complement the UV-sensitive phenotype of the *E. coli uvrB* mutant, AB1885. Plasmids pJKD1249 and pJKD1289 were capable of restoring UV resistance in AB1885 to levels close to that observed for wild-type strain AB1157 (Fig. 2), indicating that the gonococcal *uvrB* gene is expressed from its own promoter. Deletion of the region upstream from the *Ava*I site did not affect the ability of either plasmid to complement the *E. coli uvrB* mutant (data not shown). These data suggested that the sequences downstream from the *Ava*I site are sufficient for expression of the gonococcal *uvrB* gene in *E. coli*.

Nucleotide sequence analysis of the gonococcal *uvrB* gene. The nucleotide sequence of the 3,738-bp *Bam*HI-*Eco*RI fragment of pJKD1249 was determined on both strands. The gonococcal *uvrB* open reading frame (ORF) (Fig. 1B) consists of 2,025 bp, which could potentially encode a protein of 675 amino acid residues with a molecular mass of 76.9 kDa. A putative ribosome binding site is located 8 bp upstream from the putative ATG start codon. No nucleotide sequences similar to rho-independent transcriptional terminators (13) were identified immediately downstream from the *uvrB* stop codon. In addition, no consensus sequence analogous to the LexA repressor-binding site in *E. coli* (33) or to the SOS-like (SOB) operator sequence (Cheo box) characterized in *Bacillus subtilis* (14) was detected in the *uvrB* upstream region. Such sequences were also noticeably absent from the promoter region of the gonococcal *recA* gene (18 and unpublished data), another gene that might be expected to form part of an SOS-type regulon in *N. gonorrhoeae*.

Several copies of the 10-bp neisserial DNA uptake sequence 5′-GCCGTCGAA-3′ (19) were identified within the sequenced region of pJKD1249 (Fig. 1B). Closely spaced inverted repeats containing this sequence were identified immediately downstream from the putative stop codons of ORF1 and ORF2. These inverted repeats could form stable stem-and-loop structures with ΔG values of −20.7 and −25.8 kcal/mol (ca. −86.6 and −108 kJ/mol), respectively (49), and may act as transcriptional terminators. Two copies of the 10-bp sequence separated by 28 bp were also located upstream of the gonococcal *uvrB* gene. A single copy of the DNA uptake sequence was also found at the 5′ end of the *uvrB* coding region.

Alignment of the deduced amino acid sequence of the *N. gonorrhoeae uvrB* gene with those of analogous genes from other organisms (Fig. 3) revealed extensive similarity to the UvrB proteins of *E. coli* (63.1% identity in a 672-amino-acid overlap) and *M. luteus* (54.6% identity in a 617-amino-acid overlap) and to the Uvs402 protein of *S. pneumoniae* (54.4% identity in a 667-amino-acid overlap), as calculated with the FASTA program (29).

The 3′ ends of two other ORFs were also evident within the sequenced region of pJKD1249. ORF1 was located upstream from and in the same orientation as the *uvrB* coding region (Fig. 1A). The sequenced portion of this ORF consists of 588 bp with the potential to encode 196 amino acids. Comparison of the predicted amino acid sequence of ORF1 with the primary structures of other proteins in the database revealed similarity to the *E. coli prc* gene product (55.3% identity in a 47-amino-acid overlap). This gene is involved in C-terminal processing of penicillin-binding protein 3 (23). The orientation of ORF2 is opposite that of *uvrB* (Fig. 1A). Protein database searches with the deduced amino acid sequence of the 609-bp ORF revealed similarity to several carbonic anhydrase proteins.

Construction of *uvrB* mutant of *N. gonorrhoeae* MS11. The 3,225-bp *Ava*I-*Eco*RI fragment from pJKD1249 was subcloned into the plasmid vector pBR322 to form pJKD1444 (Table 1). The 444-bp *Sal*I fragment internal to the *uvrB* coding region was subsequently replaced by the gonococcal *recA* promoter region (17a) fused to a promoterless *cat* gene. The resulting construct, pJKD1484 (Table 1), was linearized by digestion with *Pst*I and transformed into *N. gonorrhoeae* MS11. One chloramphenicol-resistant transformant, designated JKD335, was found to contain an appropriately sized insert in the *uvrB* gene by Southern hybridization analysis (data not shown). This strain was found to be extremely sensitive to UV radiation compared with the wild-type MS11 strain (Fig. 4).

Transcriptional analysis of cloned gonococcal *uvrB* gene. In order to locate the promoter of the gonococcal *uvrB* gene responsible for complementation in *E. coli*, oligonucleotide primers were designed to amplify portions of the upstream region by PCR (Fig. 1B). The amplified fragments were cloned

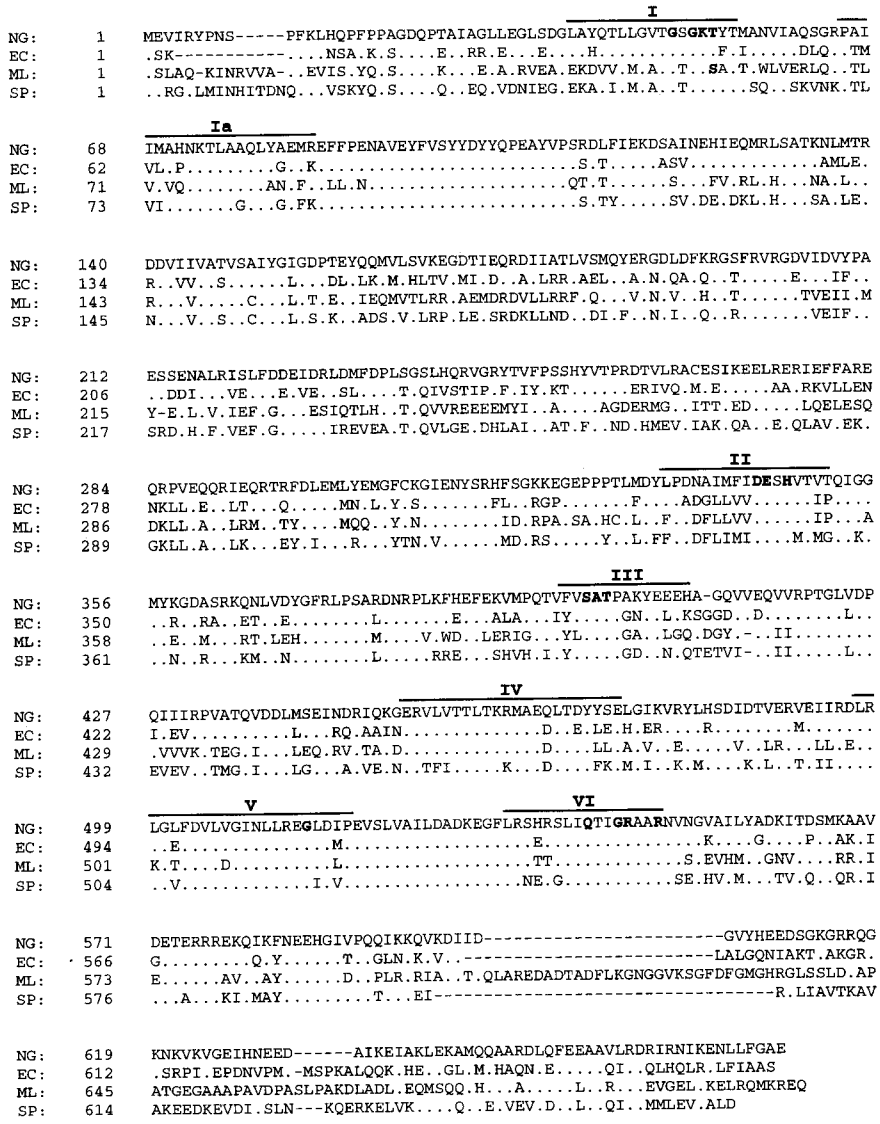

FIG. 3. Comparison between amino acid sequences of *uvrB* gene products of *N. gonorrhoeae* (NG), *E. coli* (EC) (3, 4), *M. luteus* (ML) (44), and *S. pneumoniae* (SP) (47). The amino acids are numbered according to their positions in each individual protein sequence. Dots indicate amino acid residues that are identical to those in the *N. gonorrhoeae* sequence. Dashes indicate gaps that have been introduced to optimize the sequence alignment. Conserved domains (I to VI) identified in the *E. coli* UvrB protein (21) are overlined. Consensus amino acid residues located within the conserved domains are indicated by boldface type.

into the promoter selection vector pKK232-8, and the levels of CAT were determined in cell extracts of the resulting strains. As shown in Fig. 5, strain JKD1241, which contains the 438-bp region between primer pairs 1080 and 1026, displayed levels of CAT approximately 6-fold higher than those observed for JKD1232, which contains the vector alone. A further 1.5-fold increase in CAT levels was observed for JKD1240, which contains the 228-bp region between primer combinations 1079 and 1026. When the sequence between primers 1080 and 1513 was present (JKD1403), CAT levels similar to those of the vector control were observed, indicating that this 228-bp region upstream from the *uvrB* gene does not contain a promoter that is active in *E. coli*. On the basis of this data, the promoter region of the cloned gonococcal *uvrB* gene could be localized to the sequence between primers 1079 and 1026. In order to identify the sequences responsible for the observed promoter activity, the transcriptional starting point was mapped by primer extension (data not shown). The position of this transcriptional starting point is shown in Fig. 1B. At the appropriate distance upstream of the transcriptional starting point, sequences showing similarity to the −10 and −35 regions of σ^{70} promoters were identified (Fig. 1B). One of two potential −10 sequences, TATACT, shows an identity of 5 of 6 bp to the consensus sequence TATAAT and is preceded at a distance of 18 bp by a potential −35 element (CGGACC), which has 3 of 6 bases identical to the consensus sequence TTGACA. An alternative −10 element, TACTAT, which shares 4 of 6 bases with the consensus sequence, is positioned 15 bp downstream from the −35 region. The same transcriptional starting point was mapped in strain JKD1241, which contains the entire inverted repeat, and in JKD1240, which contains only one copy of the repeat. Despite attempts to map the transcriptional starting point of *uvrB* in *N. gonorrhoeae* MS11, no primer extension product was detected. This could indicate that the gene is transcribed at very low levels or that the RNA transcript is unstable.

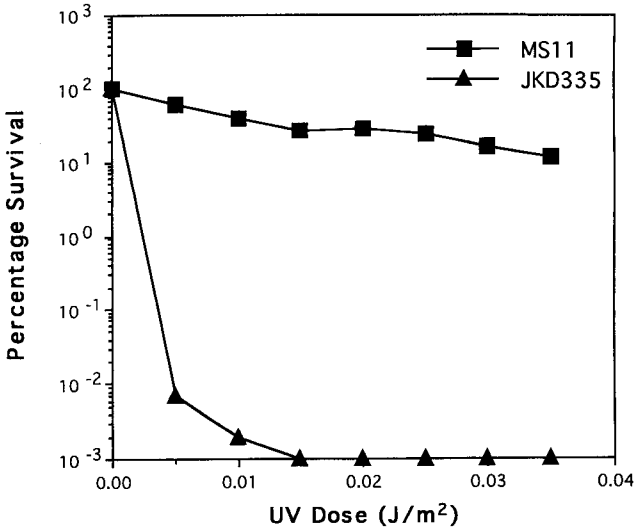

FIG. 4. Survival following UV irradiation of *N. gonorrhoeae* MS11 and JKD335 (a *uvrB* mutant of MS11). The values are the means for two independent experiments.

A characteristic feature of the region upstream of the *uvrB* gene is the presence of a structure consisting of two copies of the 26-bp NR (15) arranged in opposite orientations and separated by a conserved 54-bp intervening region, which is also found elsewhere in the gonococcal genome. It was interesting to find that the −35 and −10 promoter elements of the σ^{70} promoter identified by primer extension analysis are associated with one copy of the NR. In order to confirm that promoter activity was conferred by this repetitive sequence, the region between primers 1487 and 1488 (Fig. 1B) was cloned into pKK232-8 in both orientations. The presence of the 148-bp fragment containing the inverted repeat in the correct orientation (JKD1436) resulted in CAT levels similar to those observed for JKD1240, which possesses the region between primers 1079 and 1026 (Fig. 5). When the inverted repeat was present in the opposite orientation, CAT levels in the resulting strain (JKD1437) were reduced to those observed for the vector control. Finally, PCR fusion was used to delete the 106-bp region encompassing the 26-bp inverted repeats and the intervening region. The resulting strain, JKD1494, displayed a fourfold reduction in CAT levels compared with JKD1241, which contains the entire *uvrB* upstream region intact (Fig. 5). These results enabled the promoter region to be more precisely confined to the sequences located between primers 1079 and 1487 (Fig. 1B).

We have also noticed that in addition to the σ^{70} promoter, this 94-bp fragment contains sequences which show considerable similarity to the −10 and −35 regions of *E. coli* gearbox promoters (1, 2). The −10 region showed 7 of 8 conserved nucleotides, while 4 of 6 bases were conserved in the −35 region. Furthermore, the spacing between the two regions was maintained (Fig. 1B).

DISCUSSION

N. gonorrhoeae has been shown to possess an excision repair system that is capable of removing dimers from UV-irradiated DNA (12). In this study, the *uvrB* gene was cloned from *N. gonorrhoeae* MS11. A considerable degree of conservation is evident when the deduced amino acid sequence of the gonococcal *uvrB* gene is compared with the amino acid sequences of *uvrB* from other organisms (Fig. 3). The most extensive similarity is observed to the *E. coli* UvrB sequence, a finding which is not unexpected since both *M. luteus* and *S. pneumoniae* are gram-positive bacteria. Several highly conserved domains identified in many putative helicases, including the *E. coli* UvrB protein (21), contain consensus residues that are also present in the UvrB protein sequences of *N. gonorrhoeae*, *M. luteus*, and *S. pneumoniae*. Furthermore, the gonococcal *uvrB* gene is capable of restoring UV resistance to an *E. coli uvrB* mutant (Fig. 2), indicating that the gonococcal *uvrB* gene product is functionally similar to the *E. coli* UvrB protein. A gonococcal *uvrB* mutant was constructed and found to be highly sensitive to treatment with UV radiation (Fig. 4). This finding provides evidence for expression of the *uvrB* gene in *N. gonorrhoeae*.

The gonococcal *uvrB* gene is flanked by inverted repeats of the neisserial DNA uptake sequence. Studies have shown that

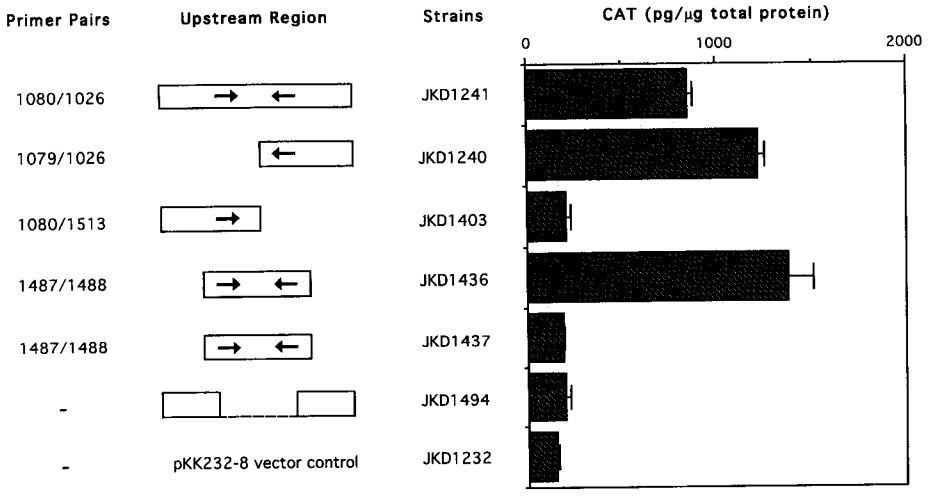

FIG. 5. Transcriptional analysis of gonococcal *uvrB* upstream region. CAT levels were determined for *E. coli* strains harboring pKK232-8 derivatives which contain segments of the *uvrB* upstream region. The upstream regions were amplified by using various combinations of primers, the precise positions of which are shown in Fig. 1B. The two copies of the NR are represented by the inverted arrows. The dashed line represents a deletion of the inverted NR from the upstream region. The CAT levels shown are the means for two independent assays. Error bars represent standard deviations.

this recognition sequence is required for the species-specific uptake of DNA in genetic transformation of *N. gonorrhoeae* (17, 19). Gonococcal DNA appears to contain on average one such sequence per kilobase (20), and this sequence has most frequently been identified as an inverted repeat in putative transcriptional terminators (19). It is possible that the inverted repeats of the uptake sequence located immediately downstream from the stop codons of ORF1 and ORF2 (Fig. 1B) may serve such a purpose. Two copies of the repeat were also identified just downstream from the mapped transcription initiation site of *uvrB* (Fig. 1B). To our knowledge, this is the first time that copies of the uptake sequence have been found between a promoter and a structural gene.

A unique 26-bp repetitive sequence is present throughout the genome of members of the genus *Neisseria* (15, 16). Approximately 20 copies of this sequence have been identified as an inverted repeat separated by a 100-bp intervening region in the genome of *N. gonorrhoeae* (15). It has been suggested that these repetitive sequences resemble transposable elements (16). An interesting observation made in this study is the presence of two 26-bp NRs, arranged in opposite orientations and separated by a 54-bp internal region in the upstream region of the gonococcal *uvrB* gene. This 106-bp sequence is almost identical to the repetitive sequence identified downstream from the structural gene for gonococcal protein III (22). Several *opa* genes from *N. gonorrhoeae* also contain an inverted NR immediately upstream of the −35 region (5).

The construction of transcriptional fusions between amplified segments of the gonococcal *uvrB* upstream region and the promoterless *cat* gene in pKK232-8 enabled initial localization of promoter activity to the 228-bp sequence between primers 1079 and 1026 (Fig. 1B). Part of the previously mentioned 26-bp inverted repeat is contained within this region. Primer extension analysis was subsequently used to determine the transcriptional starting point of the cloned gonococcal *uvrB* gene. The promoter corresponding to the mapped transcriptional starting point shows similarity to the −35 and −10 regions of σ^{70}-recognized promoters (Fig. 1B). These promoter sequences were found to be associated with one copy of the 26-bp NR identified upstream from the *uvrB* gene. Analysis of a reporter construct containing the 148-bp region between primers 1487 and 1488 confirmed that there was promoter activity present within the structure containing inverted copies of the NR.

Deletion of the 26-bp inverted repeat from the *uvrB* upstream region resulted in a significant decrease in promoter activity. On the basis of this data, the *uvrB* promoter region was more precisely localized to the 94-bp sequence between primers 1079 and 1487. Closer analysis of this region identified a sequence similar to a gearbox promoter. These promoters appear to be involved in the regulation of several genes in *E. coli* that are induced upon entry into the stationary phase (6, 27, 28). A functional gearbox promoter has recently been identified upstream from the anaerobically induced *aniA* gene of *N. gonorrhoeae* (25). We have also identified putative gearbox sequences within the intervening regions of the inverted NRs upstream from 7 of the 11 *opa* genes from *N. gonorrhoeae* MS11 (5). A comparison of these putative gearboxes with the *uvrB* sequence is shown in Fig. 6. It is possible that the potential gearbox promoter identified within the NR element upstream from the gonococcal *uvrB* gene may play a regulatory role in *E. coli* and *N. gonorrhoeae*. Experiments are currently in progress to investigate this hypothesis.

The results obtained from this study provide the first evidence of a promoter associated with the 26-bp NR. If this repetitive sequence, which is present in many copies throughout the genome of *N. gonorrhoeae* and *Neisseria meningitidis*, is indeed a transposable element, it is possible that insertion into the upstream region of a gene may result in the formation of a functional promoter. In the gonococcal *uvrB* gene, the last 2 bases in one of the potential −10 regions (TATACT) of the mapped promoter are located outside of the NR sequences. It has been shown that the first and last T residues define the −10 region of σ^{70}-dependent promoters (24). Accordingly, the formation of a functional promoter by using this particular −10 sequence would have relied on the insertion of the 26-bp inverted repeat into the *uvrB* upstream sequence such that an intact −10 element was generated at the junction point.

		-35	spacing	-10	
consensus		CTGCAA	N_{14-16}	CGGCAAGT	
uvrB	AG	CCGCAG	N_{15}	CGGCAAGG	CG
opaC	GG	CCGCAG	N_{16}	CGGCAAGG	CG
opaD,E,F,I,J,K	GG	CCGCAG	N_{15}	CGGAACCG	AT
aniA	GC	CCGCAA	N_{19}	CACCAAGT	TC

FIG. 6. Nucleotide sequence alignment showing a comparison of sequences similar to those of gearbox promoters. These sequences are located within the internal region of the NR structures upstream from the gonococcal *uvrB* gene and several *opa* genes and upstream from the gonococcal *aniA* gene. The consensus −35 and −10 regions (51) are boxed.

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