Identification, Characterization, and Variable Expression of a Naturally Occurring Inhibitor Protein of IS1106 Transposase in Clinical Isolates of Neisseria meningitidis

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Transposition plays a role in the epidemiology and pathogenesis of Neisseria meningitidis. Insertion sequences are involved in reversible capsulation and insertional inactivation of virulence genes encoding outer membrane proteins. In this study, we have investigated and identified one way in which transposon IS1106 controls its own activity. We have characterized a naturally occurring protein (Tip) that inhibits the transposase. The inhibitor protein is a truncated version of the IS1106 transposase lacking the NH2-terminal DNA binding sequence, and it regulates transposition by competing with the transposase for binding to the outside ends of IS1106, as shown by gel shift and in vitro transposition assays. IS1106Tip mRNA is variably expressed among serogroup B meningococcal clinical isolates, and it is absent in most collection strains belonging to hypervirulent lineages.

Many studies have pointed out the importance of mobile genetic elements in microbial pathogenesis and adaptation to changing environmental conditions. Virulence genes of pathogenic bacteria, which code for toxins, adhesins, invasins, capsules, pili, resistance determinants, or other virulence factors, may be located on transmissible genetic elements such as transposons, plasmids, or bacteriophages (16, 50). Insertion sequence (IS) elements are known to be involved in microevolution of bacterial genomes by several mechanisms (1, 32). (i) In the chromosomes of both gram-negative and gram-positive bacteria, virulence genes are often clustered in “virulence blocks” or “pathogenicity islands,” surrounded by IS elements that promote their transposition and lead to changes in virulence in the course of evolution. Pathogenicity islands are invariably found in pathogenic strains of a given species but are either absent or rarely present in nonpathogenic variants of the same species (12, 20, 21, 23, 30, 33, 34). (ii) In addition, two copies of certain IS elements flanking a DNA segment are able to act in concert, mobilizing the intervening region. (iii) Programmed insertion and excision of IS elements and of invertible DNA sequences may control the expression of several virulence factors by a mechanism of phase variation (24, 58). (iv) “Jumping” of DNA sequences (transposition) and subsequent recombination events may also cause gene activation (5) and antigenic variation of virulence factors, leading to the emergence of novel pathogenic variants (48). (v) IS-related DNA rearrangements do occur in resting bacterial cultures and confer plasticity on the genome under conditions of nutritional deprivation, thereby playing an adaptive role (1, 3, 18, 35, 36).

With the development of studies of the mechanisms of bacterial pathogenesis and the advent of whole-genome sequencing technologies in recent years, the finding of association between IS elements and pathogenic and virulence functions has become increasingly evident. Such associations have been observed in a variety of animal pathogens (6, 9, 14, 17, 31, 52).

Whole-genome sequence analysis and subtractive hybridization procedures have led to the identification of putative islands of horizontally transferred DNA into the genomes of serogroup B and serogroup A meningococci (42, 55). Several of these regions encode proteins that are specific to the pathogenic Neisseria species and may have a role in virulence. These regions do not have the classical characteristics of pathogenicity islands. Several, however, have a particularly low G+C content and are associated with transposase and integrase genes, suggesting that at some time in the genetic history of these species, the regions were the results of recombination events with DNAs from other species. One of these regions in a serogroup A strain, characterized by a significantly low G+C content and containing open reading frames (ORFs) with no homology to genes in databases, is flanked by several copies of IS1106 and a copy of IS50 (42).

Transposition plays a role in the epidemiology and pathogenesis of Neisseria meningitidis. IS1301 is involved in reversible capsulation by insertion into and excision from the siaA gene locus in serogroup B meningococci (24). This transposo-
able element is also responsible for insertional inactivation of the \( \text{porA} \) gene encoding the class 1 outer membrane protein, which is considered to function as a porin and invasin (57) in several serogroup B and C meningococcal isolates (38). In addition, analysis of the nucleotide sequence of the chromosomal region downstream of the \( \text{porA} \) gene has revealed the presence of a rearranged copy of the IS1106 element in carrier strains/isolates but not in invasive meningococcal strains/isolates of serogroup B, type 15, subtype 16 (B15:P1.16) (26).

IS1106 is an IS present in multiple copies in all of the meningococcal strains so far examined (39, 40) belonging to the IS5 group of the IS4 family of transposable elements (46, 26, 32). It was the first IS to be characterized in \( N. \text{meningitidis} \) and has been used as a DNA probe in phylogenetic and epidemiological analyses (25, 39) and to develop rapid, specific, and sensitive PCR-based tests for the diagnosis of meningococcal disease (40).

In this study, we have investigated the regulation of transposase activity of the element IS1106 in clinical isolates of \( N. \text{meningitidis} \) and characterized a naturally occurring inhibitor protein (IS1106Tip) of the transposase of IS1106 (IS1106T) by using biochemical and genetic approaches.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The meningococcal strains used in this study are listed in Table 1. Invasive strains/isolates were derived from a collection of strains isolated during outbreaks of epidemic disease that have occurred in different places in Italy and France during the last 20 years. The serotypes and subserotypes of these strains are shown in Table 1. A total of 24 carrier strains/isolates were enrolled in this study. These strains were sampled from the nasopharynges of different healthy subjects at the time of their military enlistment in the course of a routine screening program for the surveillance of meningococcal disease. The carrier strains/isolates used in this study were sampled in different geographical areas in Italy and France. Two strains, BL9513 and BF9513, were isolated, respectively, from the cerebrospinal fluid of a single sick subject in France. All meningococcal strains were isolated during outbreaks of epidemic disease that have occurred in different geographical areas in Italy and France. Two strains, BL9513 and BF9513, were isolated, respectively, from the cerebrospinal fluid of a single sick subject in France. All meningococcal strains were cul-

Transformations of meningococci. Transformations were performed as previously described (19) by using 500 ng of chromosomal DNA extracted from rifampin-resistant derivatives of strains BL85 and BF52 (Table 1). A recipient strain was BP25. Transformants were selected on GC agar base supplemented with rifampin (36 µg/ml) or erythromycin (7 µg/ml).

Plasmids and cloning procedures. To obtain plasmid pUCIS1106:ermC, a 1,245-bp-long PCR-derived EcoRV fragment spanning an entire IS1106 element and flanking direct repeats (GGTC) was cloned into the \( HincII \) site of plasmid pUC19. The oligonucleotides used to amplify the IS1106 DNA sequence were 5'-TAA GATATCGTCGACGGTCGAGACCTTTGCAAAATTCCCCAAAATC-3' and 5'-CTTCTCCCGAGATGCGCCTTTCCGAGACCTTTGCAAAATTCCCCAAAATC-3' (the EcoRV sites are underlined). The template DNA was derived from strain BL85. The resulting plasmid, pUC1106, was linearized at the unique \( Clai \) site within the IS1106T element, and a 1,573-bp Clai-AscI fragment containing the ermC gene was inserted.

To construct plasmids pETI106T and pETI106TTip, genomic regions containing the entire or 5'-end-truncated transposase were amplified, respectively, by using oligonucleotides 5'-TAA TGGAGGCGATCATGACCATTTCTTCCGGCAAACCGGC-3' and 5'-CACCTTTGCGAGATGCGCCTTTCCGAGACCTTTGCAAAATTCCCCAAAATC-3' (for IS1106T) and 5'-CCGTGGTCCGAGACCATTTCTTCTTCCGGCAAACCGGC-3' (for IS1106TTip) (the underlined sequences are CATATG for NdeI sites and GGATCC for BamHI sites). The template DNAs were derived from strain BL85 (for IS1106T) or BL9513 (for IS1106TTip) by PCR using the genomic DNA derived from strain BS849 as the template. The oligonucleotides used as primers (5'-ATGGACGAGATGCGCCTTTCCGAGACCTTTGCAAAATTCCCCAAAATC-3' and 5'-TCCCGGAGACCTTTCTTCCGGCAAACCGGC-3') were designed to amplify a region of 1,126 bp on the basis of the nucleotide sequence of IS1106 shown in Fig. 1 (from nucleotide 68 to nucleotide 1194). The IS1106-specific probe used in the S1 mapping experiment shown in Fig. 5 was obtained with oligonucleotides 5'-AATAATGTAGTTCTTCTGTCGCTTGTC-3' and 5'-ACATCGCTTCAATCGGTTGCTTGTT CGACATCAC-3' (from nucleotide 456 to nucleotide 482 of Fig. 4B) and genomic DNA derived from strain BF18 used as a template. The amplification reactions consisted of 30 cycles including 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 1 to 2 min of extension at 72°C. They were carried out in a Perkin-Elmer Cetus DNA Thermal Cycler 480. 5'-end labeling was performed with the T4 polynucleotide kinase and [\( \gamma \text{P} \)]-ATP (5,000 Ci mmol\(^{-1}\)). In North-
Materials and Methods. Other, not belonging to lineage 3, ET-5, or ET-37

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\[ ^a \text{NST, not serotypeable.} \]
\[ ^b \text{Assignment of isolates to hypervirulent lineages was done as detailed in Materials and Methods. Other, not belonging to lineage 3, ET-5, or ET-37 complex, IV-1 cluster.} \]

\[ ^c \text{IS1106Tip mRNAs were detected by S1 nuclease protection experiments. +, presence of specific transcripts; –, absence of detectable transcripts; ±, barely detectable transcripts; NA, not analyzed.} \]
measure of IS1106::ermC transposition. Each value is the mean of at least five independent experiments. The variation within one set of assays was usually less than twofold.

Cell extract preparation and protein purification. Crude (S30) extracts were prepared from E. coli BL21 ΔDE3 cells transfected with plasmid pET15b, pET1106T, pET1106Tip, pT7-7, or pT71106Tip grown to early logarithmic phase and induced with isopropyl-β-D-thiogalactopyranoside (IPTG) for 2 h at 37°C or not induced. Cells were mechanically broken with a French press in a buffer containing 20 mM HEPES (pH 8.2), 100 mM KCl, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, and 20% glycerol. Induction of E. coli cells transfected with pT71106Tip resulted in the appearance of polypeptides with an apparent molecular mass of 16,000 Da as determined by SDS-polyacrylamide gel electrophoresis. Histidine-tagged IS1106T and IS1106Tip were partially purified by the rapid affinity purification protocol in accordance with the Novagen pET system manual.

DNA gel mobility shift assay. The double-stranded probe used in the gel mobility shift experiments was obtained by annealing the complementary 5'-end-labeled oligonucleotides 5'-GGTCGAGACCTTTGCAAAATTCC-3' and 5'-GGAATTTTGCAAAGGTCTCGACC-3' spanning the 19-bp sequence of the left arm of IS1106. The annealing reaction was carried out at 65°C for 1 h.

The gel mobility shift assay was performed by mixing (at 24°C) purified proteins, double-stranded labeled probe, unlabeled competitor DNA, and buffer in a total volume of 20 μl. The final buffer contained 20 mM HEPES, 40 mM KCl, 4% Ficoll, 5 mM spermidine, 0.25 μg of poly(dI-dC) μl⁻¹. The protein concentrations ranged between 2.5 and 25 ng μl⁻¹, and the concentration of the probe was 1 ng μl⁻¹ (20,000 cpm). Unlabeled competitor was added in 5- to 50-fold excess.
RESULTS

Structure and nucleotide sequence of the N. meningitidis IS1106 element and analysis of transposase-specific transcripts in meningococcal strains. The IS1106 element located within a complex repetitive region downstream of porA is a rearranged element in which a transposon-like repetitive element (also known as a small repetitive element [SRE]) (10) interrupts the region encoding the amino terminus of the putative IS1106T protein (26). By PCR using two oligonucleotides complementary to the IS1106 inverted-repeat sequences (IRs), we isolated a wild-type element from serogroup B strain BL859 (Fig. 1A). The element has a length of 1,207 bp, is flanked by 19-bp-long IRs (a left IR [IRL] and a right IR [IRR]), and encodes a putative 38,505-Da peptide showing extensive homology to the 5A transposase protein of IS5 (27, 8) (Fig. 1B). Analysis of the DNA sequence revealed the presence of several putative control elements upstream of the start codon of the transposase: (i) a 14-bp-long perfect IR located 2 bp downstream from IRL, (ii) a repeated motif resembling a 38,505-Da peptide showing extensive homology to the 5A transposase protein of IS5 (27, 8) (Fig. 1B), and (iii) putative −10 and −35 promoter sequences (Fig. 1A).

We next analyzed transposon-specific transcripts in different serogroup B meningococcal strains by Northern blotting. Total RNAs extracted from either “carrier strain” or “invasive strain” isolates were probed with an IS1106T-specific probe. A specific transcript of about 1,200 nucleotides was detected only in carrier strain isolates (Fig. 2A, lanes 1 to 4) and not in invasive strain isolates (Fig. 2A, lanes 5 to 8). We performed an S1 mapping experiment to define the ends of this transcript (Fig. 2B). After treatment with S1 nuclease, the amounts of full-length protected hybrids were very low in all of the strains tested and detectable only after overexposure of the autoradiogram. On the contrary, a shorter hybrid was present in carrier strain isolates (Fig. 2A, lanes 1 to 4) and not in invasive strains/isolates (Fig. 2A, lanes 5 to 8). The 5′ end corresponded to nucleotide 700 of the IS1106 sequence (Fig. 1A).

Isolation of a transcriptionally active, 5′-end-truncated IS1106 element from the genome of a carrier strain/isolate. The script mapping data led us to hypothesize the existence of a transcriptionally active, rearranged version of the IS1106 element in the meningococcal genome. The sequence of the IS1106 element in the genome of serogroup B carrier strain/isolate BF18 was determined (Fig. 3). Inspection of the nucleotide sequence revealed the presence of a rearranged IS1106 element located downstream of an IS1106-like element (32) (Fig. 3A). Alignment of the rearranged IS1106 sequence with that of the wild type (Fig. 1A) revealed that the element is truncated at the 5′ end and located immediately downstream from a neisserial SRE (Fig. 3B).

The putative start site(s) of the 5′-end-truncated IS1106-specific transcript was determined by S1 nuclease mapping (Fig. 4A). The analysis revealed two major transcripts whose 5′ ends mapped within the SRE located upstream of the rearranged IS1106 element (Fig. 3B). These transcripts could be detected only in carrier strains/isolates BF10 and BF18 (Fig. 2B). After treatment with S1 nuclease, the reaction products were resolved on a 5% polyacrylamide-urea denaturing gel. The sizes of the protected hybrids (arrows) were determined by running in parallel a sequencing reaction ladder (G and A, lanes 1 and 2) of the same DNA fragment used as a probe. The bar indicates the relative migration of the probe.
isolates (data not shown). To seek evidence that the 5'-end truncation could be impaired by a point mutation(s). Analysis of the nucleotide sequence of strain BS849 revealed four nucleotide substitutions in the putative promoter region in both strains, mapping 12, 24, 82, and 86 nucleotides upstream of the transcription start site.

In the rearranged element, an ORF initiates at the rare codon for leucine TTG (15) (nucleotide 140), possibly encoding a 14,870-Da peptide corresponding to the carboxy-terminal half of the putative wild-type IS1106Tip. This ORF is found downstream from a neisserial SRE. Two copies of neisserial repetitive sequence RS3 are arranged in tandem downstream from the truncated element. The bent arrow indicates the transcription start point of the IS1106Tip mRNA. Asterisks mark the positions of a putative gearbox promoter sequence (5'-CACCAAGT-3'). Four nucleotide substitutions (indicated above the nucleotide sequence of BF18) were found in invasive strains/isolates BL859 and BL892 and map 12, 24, 82, and 86 nucleotides upstream of the transcription start site. Amino acid residues that are different from the deduced sequence of IS1106Tip are underlined.

Analysis of IS1106Tip mRNA and distribution of IS1106 in clinical isolates of N. meningitidis. We next investigated the presence of the IS1106Tip mRNA in clinical isolates of meningococci by S1 mapping. The meningococcal strains were isolated in different regions of Italy and France over the last 10 years. The genetic relationships among 50 clinical isolates and meningococci by S1 mapping. The meningococcal strains were isolated in different regions of Italy and France over the last 10 years. The genetic relationships among 50 clinical isolates and meningococci by S1 mapping. The meningococcal strains were isolated in different regions of Italy and France over the last 10 years. The genetic relationships among 50 clinical isolates and meningococci by S1 mapping.

In most cases, lack of IS1106Tip expression was associated with the same point mutations previously mapped (Fig. 3A). However, in strains of the ET-5 complex, the IS1106Tip gene could not be amplified by PCR, suggesting that a rearrangement or deletion had occurred (data not shown).
results are shown in Fig. 6B, any band in the autoradiograph might be interpreted as an insertion of a single copy of either an intact or a truncated IS1106 element. The results demonstrate that IS1106 is present in multiple copies in the genomes of the meningococcal isolates, ranging from about 5 or 6 to more than 15 or 16. The copy number did not correlate with the expression of IS1106 Tip. However, in phylogenetically related strains that do not express IS1106Tip, a high heterogeneity of the insertion pattern was observed. For instance, strains BL847 and BL859, which did not express IS1106 Tip although both belonging to lineage 3 (Fig. 5), appeared to be unrelated on the basis of the IS1106 transposition pattern. A similar heterogeneity was observed in several strains of the ET-5 complex, for instance, BL899 and BL937. By contrast, all of the examined strains of the ET-37 complex that express IS1106Tip exhibited very similar transposition patterns. This suggested that IS1106Tip might act as a negative modulator of IS1106 transposition.

Effects of IS1106Tip expression on in vitro transposition of an IS1106:ermC' element. To investigate the possibility that IS1106Tip might function as a repressor of IS1106T, an in vitro transposition assay was developed. Histidine-tagged IS1106T or IS1106Tip overexpressed in E. coli BL21 ADE3 cells (Fig. 7A, lanes 4 and 6) was partially purified and variously mixed with tester DNA. A modified version of IS1106, IS1106:ermC', was engineered by inserting the ermC' gene conferring resistance to erythromycin into the gene for IS1106T. The assay measured the movement of the transposase-defective element from a donor plasmid to target rifampin-resistant N. meningitidis chromosomal DNA in the presence of different amounts of partially purified IS1106T and IS1106Tip. The target DNA was used to transform a sensitive N. meningitidis strain to erythromycin or rifampin resistance. Transposition events were scored as the recovery of erythromycin-resistant host cells after natural transformation. As the in vitro treatment was expected to affect the transforming ability of the target DNA, values were normalized with transformation efficiencies to rifampin resistance. Therefore, the ratio of the total number of erythromycin-resistant transformants to the number of rifampin-resistant transformants was taken as a measure of IS1106:ermC' transposition. The data demonstrate that IS1106T was able to activate transposition of the transposase-defective element in trans and that transposition efficiencies strongly decreased in the presence of IS1106Tip. The extent of inhibition was dependent on the ratio of IS1106T to IS1106Tip (Table 2). In particular, at ratios of 50:1, 10:1, and 2:1 (amounts of IS1106T to amounts of IS1106Tip), the frequencies of transposition events decreased about 5-, 15-, and 50-fold, respectively.

Binding of IS1106Tip to the IR of IS1106. To elucidate the mechanism of inhibition of IS1106 transposition by the truncated transposase, we investigated the ability of IS1106Tip to interfere with binding of the full-length transposase to IS1106 termini (IS1106IR). Incubation of partially purified IS1106T with 5'-end-labeled double-stranded oligonucleotides corresponding to the IS1106 IR led to the appearance of a retarded complex I (Fig. 7B, lane 2) specifically titrated out by excess cold probe (Fig. 7B, lanes 3 and 4). When the IS1106 IR was incubated with IS1106Tip, two specific major DNA-protein complexes were detected: a faster-migrating one (complex II)
and a much more abundant complex that migrated considerably more slowly (complex III) (Fig. 7B, lanes 5 to 7). Significantly, the amount of complex III was much greater than that of the IS1106 IR-IS1106T complex (Fig. 7B, lane 2), although IS1106Tip was used at a concentration 10-fold lower than that of IS1106T. This finding indicated that the truncated transposase was able to bind the IS1106 IR more efficiently than was the full-length transposase. This result was confirmed when the probe was incubated in the presence of both IS1106T and IS1106Tip at different relative ratios (Fig. 7B, lanes 8 to 10). Formation of complex I was substantially inhibited when IS1106Tip and IS1106T were used at a 1:10 ratio (Fig. 7B, lane 10).

**DISCUSSION**

Transposons have evolved various regulatory mechanisms that limit their movement and the accompanying mutagenic effect within the host cell. Several of these mechanisms are general and involve transcriptional repressors and translational inhibitors (antisense RNA). Others are more specific and include (i) sequestration of translation initiation signals, (ii) pro-
FIG. 6. Southern blot analysis of IS1106 insertions in the genomes of meningococcal strains. (A) Physical and genetic map of an IS1106 element. The positions of the ClaI and HindIII sites are indicated. The dashed bar below the map corresponds to the 322-bp-long fragment used as a probe in the Southern blot experiment shown in panel B. (B) Total DNAs derived from the meningococcal strains indicated above the panels were digested with EcoRI and HindIII and hybridized to the 332-bp 32P-labeled DNA fragment corresponding to the 5'-proximal one-third of the IS1106 sequence (A). The IS1106 sequence contains a unique HindIII site mapping downstream of the probe but does not contain any EcoRI site (A). Therefore, any band in the autoradiograph might be interpreted as an insertion of a single copy of either an intact or a truncated IS1106 element. The probe we used minimized the detection of rearranged elements because truncation of IS1106 occurred mostly at the 5'-proximal end (data not shown). The relative migrations of molecular size markers (sizes are in base pairs) are shown beside each panel. In addition to the names of the strains, their assignment to hypervirulent lineages is also indicated (lineage 3, ET-5, ET-37, IV-1, and other).
grammed translational frameshifting, (iii) coupling of translation termination, transposase binding and transposon activity, (iv) impinging transcription from an outside promoter and/or from within the element, (v) transposase stability, (vi) activity in cis of transposase (32). In this paper, we have characterized the wild-type IS1106 element and a novel mechanism by which this transposon controls its own activity.

Four copies of the wild-type element and several rearranged copies are found in the genome of serogroup A strain Z2491 (Sanger Centre database) (41). Computer sequence analysis of the regions flanking the IS1106 copies in strain Z2491 did not reveal a consensus target site. However, copies of repetitive sequence RS3 are located downstream of several IS1106 elements in the genome of Z2491, suggesting that the primary sequence and/or the architecture of the RS3 or RS3-like sequence may be involved in target site selection and possibly in the orientation of insertion. Interestingly, a repeated motif resembling part of the core sequence of the neisserial RS3 repeat is located upstream to the start codon of the transposase gene and partially overlaps the IRL (Fig. 1A). The analysis of the nucleotide sequence of this region also revealed several putative control elements: −10 and −35 promoter elements and a 14-bp-long perfect IR located in the space between the IRL and the start codon of the transposase gene.

Because formation of this palindromic structure at the level of RNA is predicted to sequester the ribosomal binding site, one may speculate that the 14-bp-long IR protects IS1106 from activation by impinging transcription following insertion into highly expressed genes. A similar control mechanism regulates the activity of other transposable elements (32).

Transcriptional mapping analysis has demonstrated the presence, in several meningococcal strains, of an IS1106-specific transcript corresponding to a 5′-end truncated transposase mRNA (Fig. 2). The sequence analysis of the genomic region encoding the truncated transposase (IS1106Tip) revealed the presence of a 5′-end truncated IS1106 element downstream from a neisserial SRE (10) (Fig. 3). SREs have been associated with transposition in neisseriae. It is therefore reasonable that the genomic rearrangement leading to the 5′-end-truncated transposase gene has been promoted by insertion of the SRE into an IS1106 element. Transcription of the IS1106Tip gene starts within the SRE (Fig. 3 and 4A). Transcription from SREs has been reported for other meningococcal genes, including uvrB (2) and dgr (7). No canonical σ70-dependent promoter consensus sequence is detectable in the SRE upstream of the truncated transposase gene. However, a putative gearbox promoter sequence (5′-CACCAAGT-3′) is present a few nucleotides upstream of the transcript start site (Fig. 3). Gearbox promoters appear to be involved in the regulation of several genes in E. coli that are induced upon entry into the stationary phase (4, 28, 29). In N. gonorrhoeae, putative gearbox sequences have been identified within the SRE upstream of uvrB and of the 11 opa genes from strain MS11 (2). IS1106Tip mRNA is variably expressed among meningococcal clinical isolates (Table 1). The absence of IS1106Tip-specific transcripts is associated either with mutations in the putative promoter region located within the SRE or with rearrangement/deletion of the gene (in strains of the ET-5 complex).

The results of the in vitro transposition assay demonstrated that IS1106Tip might act as a negative modulator of IS1106 transposition (Table 2). Incomplete transposase peptides contribute to repression of transposition by different mechanisms: (i) binding to an IR, leading to either repression of the transposase pIRL promoter or competition with the transposase for binding to the ends of the element, and (ii) generation of nonproductive heteromultimers with full-length transposase peptides (32). The results of the DNA band shift assays dem-
onstrate that IS1106Tip was able to bind the IS1106 IR more efficiently than the full-length transposase (Fig. 7). This finding was quite surprising, as the DNA binding domains involve N-terminal regions in many transposases (32). IS1106Tip lacks the N-terminal half of IS1106T, including part of the DDE motif (Fig. 1B). The analysis of the amino acid sequence of IS1106T by a computer program for prediction of helix-turn-helix DNA binding motifs using the algorithm of Dodd and Egan (available at http://npsa-pbil.ibcp.fr/) indicated a unique sequence at the C-terminal starting from amino acid 276 to amino acid 297 (Fig. 1B), albeit with a low score (0.84). This sequence is conserved in IS1106Tip (Fig. 3B). In the DNA band shift assays, IS1106Tip generates two complexes (II and III) when mixed with IS1106IR (Fig. 7). We are currently investigating the nature of complex III (Fig. 7). It is possible that it is formed by multimers of IS1106Tip with a molecule(s) of IS1106 IR. Competition with the transposase for binding to the ends of the element and formation of multimers may account for the ability of IS1106Tip to act as a negative modulator of IS1106 transposition.

Lack of IS1106Tip and, possibly, hypertransposition may contribute to plasticity of the meningococcal genomes in several pathogenic clones, playing an adaptive role and leading to changes in virulence in the course of evolution. For instance, it has been proposed that IS1106-mediated transposition and recombination may be involved in genetic instability at the porA locus, thereby influencing antigenic variation of this important surface antigen (26). This hypothesis is further supported by computer sequence analysis of the meningococcal genome (serogroup A strain Z2491). The IS1106 elements are located close to genes encoding virulence factors and subjected to genetic variation, including rfbAB, encoding the lactoferrin receptor (43, 44), and frpC, a meningococcal-specific gene absent in gonococci (as well as porA) (11) and coding for an iron-regulated protein related to the RTX family of cytotoxins (56).

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