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Genome plasticity in *Neisseria gonorrhoeae*

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Abstract

The pathogenic *Neisseria* have exploited the processes of horizontal DNA transfer and genetic recombination as mechanisms for the generation of extensive protein variation and modulation of gene expression. Localized recombinations have been well documented in members of multigene families as have alterations in short repetitive sequences. Here we report an analysis of the chromosomal structure of a defined lineage of *Neisseria gonorrhoeae* strain MS11 pilin variants. This study reveals the occurrence of large rearrangements, including the amplification of a 26 kb region and an inversion involving more than a third of the chromosome. Additionally, a restriction site polymorphism that correlates with pilin expression has been observed. These findings highlight the flexibility of the gonococcal genome.

Keywords: *Neisseria*; Chromosome; Rearrangement; Inversion

1. Introduction

Neisseria gonorrhoeae, the Gram-negative diplococcus causing gonorrhoea in humans, is well-known for its ability to undergo genetic recombinations, leading to exceptional diversity in the expression of cell-surface molecules [1,2]. The gonococci are naturally competent for DNA uptake [3], which may serve as a means for the exchange of genetic information between cells. Rearrangements involving members of multi-gene families result in new combinations of variable sequences within these loci, enabling a population to produce a large repertoire of variant proteins that are not only antigenically and immunologically distinct, but may also have altered functional properties. Transformation-mediated recombinations can also lead to changes in single-copy genes [4,5]. In addition to the larger regions involved in recombinations, localized genetic changes modulate expression of specific proteins: short repetitive regions are subject to alterations in the number of repeats, allowing a fine-tuning of gene expression [1].

Despite its relatively small size (approx. 2.3 Mb [6,7]), the gonococcal genome contains several multi-copy gene families. In some cases, the sequence homology extends well beyond the coding regions, perhaps reflecting the duplication of a larger region. A short repetitive sequence and a multi-copy transposon-like element have also been identified [8]. Other duplications have been observed to occur in vitro; initial attempts to mutagenize the *pilA* locus led to heterodiploidy of the region [9].

Physical and genetic maps have been obtained for

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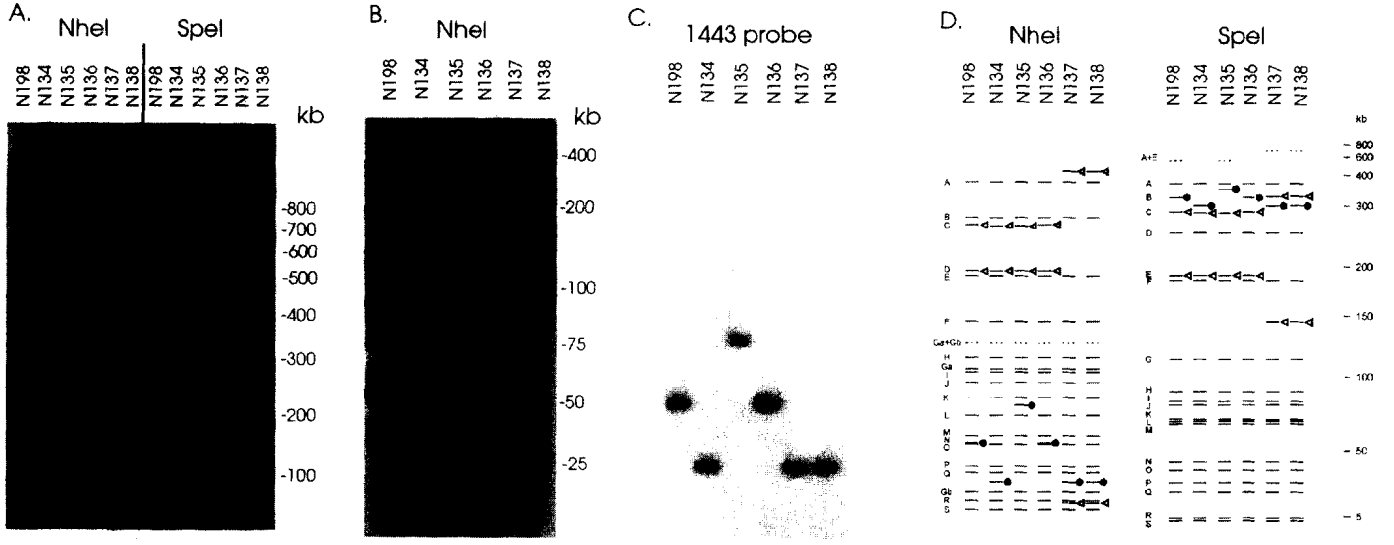

Fig. 1. Restriction digestion analysis of *N. gonorrhoeae* pilin variants. Genomic DNAs were digested with *NheI* or *SpeI* and analyzed by pulsed field gel electrophoresis under conditions optimized for separation of (A) larger fragments or (B) smaller fragments. (C) Southern blot analysis of fragment nN. The gel of panel B was transferred to nitrocellulose and hybridized to an oligonucleotide probe specific for the 1443 bp repeat. (D) Composite drawing of the ethidium bromide stained pulsed field gels. Fragments are labeled according to Bihlmaier et al. [6]; partial digestion products are indicated by dotted lines. Circles and arrowheads are explained in the text.

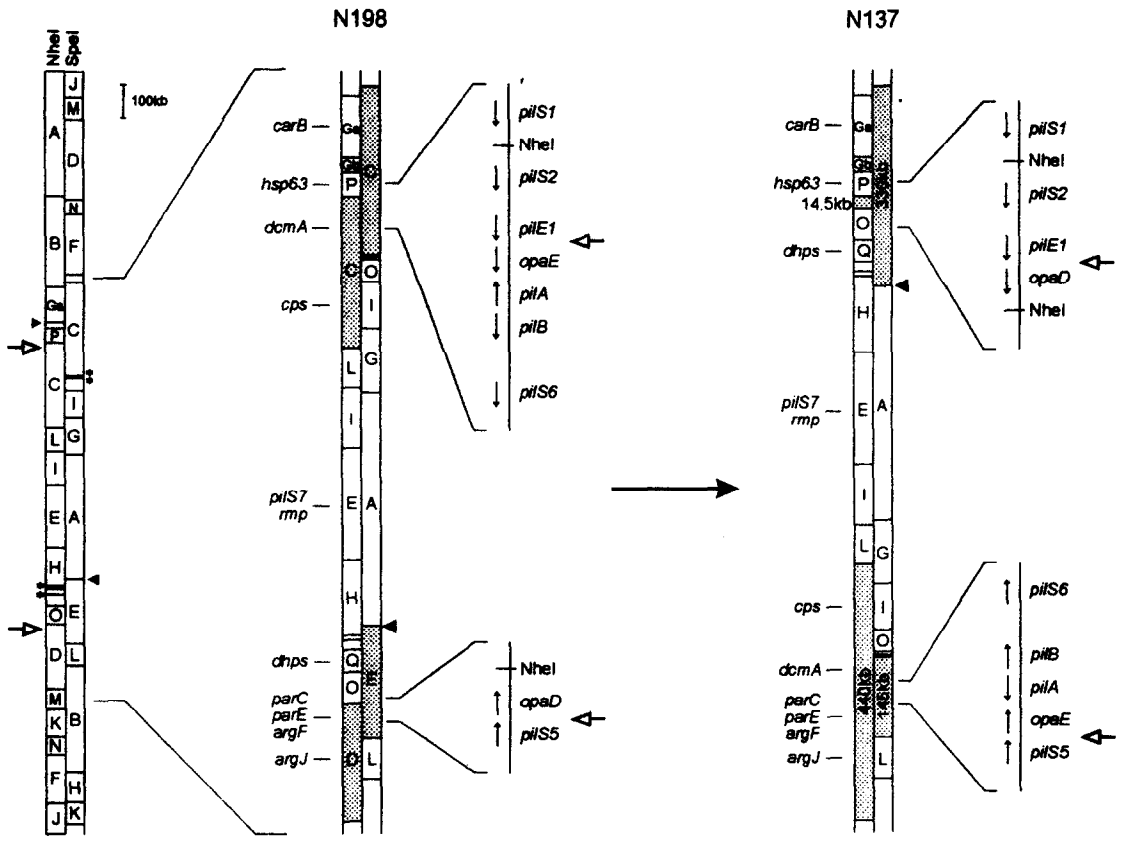

Fig. 2. Chromosomal inversion in variant N137. The physical map of variant N198 [6] is compared to that of N137. Arrows define the limits of the inversion; restriction fragments involved are indicated by shading. Arrowheads indicate semi-resistant restriction sites; restriction fragments whose locations remain tentative are marked with asterisks.

two gonococcal strains, FA1090 [7] and MS11-N198 [6]. The maps are similar, but do show, in addition to restriction site polymorphisms, significant differences in the locations of certain genes. Additionally, the MS11-N198 chromosome (over 2.3 Mb) is slightly larger than that of FA1090 (approx. 2.2 Mb). Differences have also been observed within strains. The original MS11 isolate (MS11-A) contains two *pilE* loci [10]; this arrangement is unstable in vitro, one of the loci being usually deleted upon passage. Other differences are seen in the number of *opa* loci present in MS11 variants [11,6]. Furthermore, a comparison of the gonococcal genetic maps with those of *N. meningitidis* strains B1940 [12] and Z2491 [13] reveals a series of complex rearrangements between the two species.

These observations suggest *N. gonorrhoeae* has a relatively flexible chromosome. We have used a defined lineage of pilin variants to investigate the stability of the gonococcal genome in vitro. Restriction analyses of *Nhe*I, *Spe*I and double-digested chromosomal DNA revealed several changes in banding pattern among the different variants. These changes reflect a region of expansion/contraction, a large chromosomal inversion and a restriction site polymorphism.

2. Materials and methods

2.1. Bacterial strains

N198 [6] is a *recA* derivative of *N. gonorrhoeae* strain MS11-A, deposited in the American Type Cul-

ture Collection (ATCC 49759). The lineage of pilin variants, N134, N135, N136, N137 and N138, was derived from MS11-A and has been described by Haas et al. [14].

2.2. Molecular biological techniques

Preparation of genomic DNA, restriction digestion, pulse field gel electrophoresis and Southern hybridizations were performed according to Bihlmaier et al. [6].

3. Results

3.1. MS11 pilin variants have different restriction patterns

*Nhe*I and *Spe*I, which had been employed in the construction of the MS11-N198 chromosomal map [6], were used to analyze a series of gonococcal pilin variants descending from MS11-A [14]. Initial experiments revealed numerous alterations in the restriction patterns (Fig. 1), three of which were readily interpretable. In N198, both the nC and sC bands and the nJ and sK bands are slightly larger than their counterparts in the other variants. The nC and sC size difference of ~0.4 kb is due to the presence of *pilE2*, a locus found in the parental MS11-A and in the isogenic *recA* derivative, N198, but deleted in N134 and therefore not present in the subsequent variants [15]. The slight difference in size (800 bp) of nJ and sK results from the C19a *recA* mutation in N198; the other variants contain the wild-type *recA* gene. Likewise, the slight increase in size of nC and sC in variant N136 results from the ~0.4 kb increase in the *pilE* locus due to the overlength L-pilin gene in this variant [16]. Other changes can be seen with nC, nD and nN and sB, sC, sE and the sA+sE partial digestion product. These changes are more complex, and were analyzed by Southern hybridizations, as described below.

3.2. Fragment nN/sB undergoes discrete size variations

A 55 kb fragment, nN, is present in *Nhe*I digests of N198 and N136, but not in the other variants (Fig. 1D, circles). Instead, novel fragments appear; in N134, N137 and N138 a band of 29 kb can be seen, while an 81 kb band is found in N135. Both the *aroA* locus and the 1443 bp repeat have been mapped to fragment nN in N198 [6]. Hybridization with an oligonucleotide probe specific for the 1443 bp repeat (Fig. 1C) and with the cloned *aroA* gene (data not shown) demonstrated that the 29 kb fragment of N134, N137 and N138 and the 81 kb fragment of N135 correspond to the 55 kb nN fragment of N198 and N136. In each case, the fragment expands or contracts in increments of 26 kb. The nN

Table 1
Southern hybridization analysis of varikbants N198 and N137

Locus	*Nhe*I		*Spe*I	
	N198	N137	N198	N137
carB	nGa, nGa+nGb	nGa, nGa+nGb	sC	330 kb, sA+330 kb
hsp63	nP	nP	sC	330 kb, sA+330 kb
pilE1	nC	14.5 kb	sC	330 kb, sA+330 kb
pilA,B	nC	440 kb	sC	145 kb
dcmA	nC	440 kb	sC	145 kb
cps	nC	440 kb	sI	sI
rmp	nE	nE	sA, sA+sE	sA, sA+330 kb
dhps	nQ	nQ	sE, sA+sE	330 kb, sA+330 kb
opaD	nD	14.5 kb	sE, sA+sE	330 kb, sA+330 kb
pilS5	nD	440 kb	sE, sA+sE	145 kb
parC,E	nD	440 kb	sE, sA+sE	145 kb
argF	nD	440 kb	sE, sA+sE	145 kb
argJ	nD	440 kb	sL	sL

*Nhe*I and *Spe*I fragments hybridizing to the particular probe used are indicated for variants N198 and N137.

fragment is located within the *Spe*I fragment sB; corresponding size alterations of 26 kb increments are evident in this fragment (Fig. 1D, circles).

The nature of the size alterations in fragment nN is unknown, but the fact that these changes occur in discrete 26 kb increments suggests that a region of this size is able to undergo amplification and condensation. The most simple explanation is that a 26 kb element is present in a single copy in variants N134, N137 and N138, in duplicate in variants N198 and N136, and in triplicate in variant N135. As the size of nN in variants N134, N137 and N138 is only 29 kb, the amplifiable region must encompass nearly the entirety of this fragment; the flanking fragments, nF and nK, do not undergo size alterations. It is of interest to note that the gonococcal 1443 bp repeat, a transposon-like element of unknown function [8], maps to fragment nN, and thus it is tempting to speculate that this element may be involved in the size alterations.

3.3. Chromosomal inversion in variant N137

Comparison of the restriction patterns of the six variants revealed a major alteration occurred between variants N136 and N137 (Fig. 1D, arrowheads). In *Nhe*I digests, fragments nC (261 kb) and nD (196 kb) were lost, while two new bands of approx. 440 and 14.5 kb could be observed. Because of the semi-resistant cleavage site between fragments sA and sE [6], the *Spe*I pattern is more complex. However, it is evident that neither fragment sC (288 kb) nor sE (191 kb) is present in N137, where novel bands of 330 and 145 kb appear. In addition, the sA+sE partial digestion product (565 kb), seen in N198 and N135, but not in N134 or N136, is replaced with a larger fragment (705 kb) in N137; the complete digestion product, sA (374 kb), does not change in size. The altered restriction pattern was inherited in the daughter variant, N138.

The conserved size changes of the fragments involved (nC/sC and nD/sE) suggested an inversion occurred involving these regions (Fig. 2). From size calculations, it could be deduced that the crossover occurred in nC, close to nP, and in nD, close to nO. Inversion involving these regions would result in a large *Nhe*I fragment, encompassing most of nC and nD, and a small *Nhe*I fragment, containing the remainder. Rearrangement in these two regions would also lead to changes in the size of both sC and sE, which in turn alters the size of the partial digestion product, sA+sE. Novel *Spe*I fragments of 145 and 330 kb are consistent with this interpretation, as is the 705 kb size of the partial digestion product [sA (374 kb)+330 kb]. Furthermore, in two-dimensional reciprocal gels of the N137 chromosome, it could be demonstrated that *Nhe*I fragments nE, nGa+nGb, nH, nO and nP are contained within the large *Spe*I partial digestion product, and that *Spe*I fragments sI, sL, sO and the 145 kb fragment are contained within the 440 kb *Nhe*I fragment (data not shown; in these experiments, fragments smaller than 36 kb were beyond the limits of resolution).

To further analyze the rearrangement, Southern hybridizations were performed with N198 and N137, using probes specific for the fragments involved or for flanking fragments. These results are summarized in Table 1, and are consistent with a large inversion having occurred, with the cross-over located within 14.5 kb from the nP-nC and the nO-nD sites, within nC/sC and nD/sE. Thus *pilA*, *pilB* and *dcmA*, found in the nC/sC fragment in N198 and *pilS5*, *parC*, *parE* and *argF*, found in the nD/sE fragment in N198 all map to the 440 kb *Nhe*I fragment and the 145 kb *Spe*I fragment in N137.

Both of the regions involved in the inversion contain *pil* and *opa* loci [6]; in N136, the nC/sC fragment contains the closely linked *pilE1* and *opaE*, as well as *pilS2* and *pilS6*, while the linked *pilS5* and *opaD* [17] are located in the nD/sE fragment. To investigate the possibility of recombination involving the *pil-opa* loci, hybridizations were performed using *pil-* and *opa*-specific oligonucleotide probes (Table 1). In N198, the *pilE* probe hybridized to nC and the *pilS5* and *opaD* probes hybridized to nD. In contrast, in N137, *opaD* and *pilE* have identical hybridization patterns, mapping to the 14.5 kb *Nhe*I fragment, while the *pilS5* probe hybridized to the 440 kb *Nhe*I fragment. As *opaE* does not contain unique sequences [11], no probe was available to examine definitively this locus. Nevertheless, the data suggest the inversion breakpoints are located within *pilE1-opaE* and *pilS5-opaD*, leading to a *pilE1-opaD* linkage and a *pilS5-opaE* linkage (Fig. 2). The cross-over must have occurred upstream of the *opa* semi-variable domain, as the *opaD* probe hybridizes in this

region. Likewise, the extent of homology between *pilE1* and *pilS5*, starting at codon 44 of the mature protein, defines the 5′ limit for recombination. The distance between these regions in *pilE1* and *opaE* is less than 1 kb.

Localized RecA-dependent recombination events involving members of the *pil* and *opa* multi-gene families have been well documented in *N. gonorrhoeae*. However, the large chromosomal inversion occurring in variant N137 between the *pilE1*, *opaE* and *pilS5*, *opaD* loci is the first known example of a major chromosomal rearrangement in this species. One consequence of the inversion is the uncoupling of the regulatory genes, *pilA* and *pilB*, from the expressed *pilE* locus. That variant N137 and its progeny N138 exhibit no detectable alterations in pilin expression or degree of piliation (data not shown) demonstrates that physical coupling of the regulatory genes to *pilE* is not essential.

3.4. SpeI polymorphism

A semi-resistant *Spe*I cleavage site had been previously identified between fragments sA and sE [6]. In Fig. 1, it can be seen that the susceptibility of this site to digestion varies in the different variants. In N134 and N136, virtually complete digestion was obtained at this site. In contrast, in N198, N135, N137 and N138, digestion was very poor, and the large partial digestion product (Fig. 1D, dotted lines) remained refractory to digestion. In N137 and N138, this band is larger (705 kb) than that seen in N198 and N135 (565 kb), due to the inversion involving fragment sE (see above). Southern hybridizations using probes specific for this region have confirmed that the 705 kb band derives from a partial digestion of fragments sA (374 kb) and the 330 kb *Spe*I fragment resulting from the inversion (Table 1). Resistance of the sA-sE site to digestion correlates with pilus production in these six variants, however, examination of additional pilin variants and mutants (data not shown) indicates that, although strong, the correlation is not absolute.

The fact that this site digests well in some variants but poorly in others suggests that digestion may be related to gene expression in this region. The physical basis for the resistance to digestion in certain variants is unknown. Although *Spe*I has no known site preference or sensitivity to methylation, these possibilities cannot be ruled out at present. Another possibility could be a localized rearrangement involving the *Spe*I site; the site may be present in one configuration but absent in the other. In this case, the degree of digestion in a given DNA preparation might reflect the proportion of the bacterial population in the configuration amenable to digestion. Any alterations in this region must be conservative or below the limits of detection (approx. 500 bp), as the *Nhe*I fragment, nH, which spans the semi-resistant *Spe*I site, does not show detectable alterations in size.

3.5. Chromosomal flexibility

Our analysis of a defined lineage of *N. gonorrhoeae* pilin variants indicates this organism possesses a highly flexible genome. Recombination events among members of the multi-gene *pil* and *opa* families have been well-documented [18,19] and horizontal gene transfer is proposed to occur in vivo [4,5]. Localized genetic changes involving alterations in the number of short repeated sequences have been observed in several genes [20–24]. We now demonstrate the occurrence of larger rearrangements, including chromosomal inversion and amplification of a 26 kb unit. Furthermore, we identify an *Spe*I restriction site polymorphism that shows correlation with pilus production.

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