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The Physical Map of the Chromosome of a Serogroup A Strain of *Neisseria meningitidis* Shows Complex Rearrangements Relative to the Chromosomes of the Two Mapped Strains of the Closely Related Species *N. gonorrhoeae*

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A physical map of the chromosome of *N. meningitidis* Z2491 (serogroup A, subgroup IV-1) has been constructed. Z2491 DNA was digested with *Nhe*I, *Spe*I, *Sgf*I, *Pac*I, *Bgl*II, or *Pme*I, resulting in a limited number of fragments that were resolved by contour-clamped homogeneous electric field (CHEF) electrophoresis. The estimated genome size for this strain was 2,226 kb. To construct the map, probes corresponding to single-copy genes or sequences were used on Southern blots of chromosomal DNA digested with the different mapping enzymes and subjected to CHEF electrophoresis. By determining which fragments from different digests hybridized to each specific probe, it was possible to walk back and forth between digests to form a circular macrorestriction map. The intervals between mapped restriction sites range from 10 to 143 kb in size. A total of 117 markers have been placed on the map; 75 represent identified genes, with the remaining markers defined by anonymous cloned fragments of neisserial DNA. Comparison of the arrangement of genetic loci in Z2491 with that in gonococcal strain FA1090, for which a physical map was previously constructed, revealed complex genomic rearrangements between the two strains. Although gene order is generally conserved over much of the chromosome, a region of approximately 500 kb shows translocation and/or inversion of multiple blocks of markers between the two strains. Even within the relatively conserved portions of the maps, several genetic markers are in different positions in Z2491 and FA1090.

The genus *Neisseria* contains two pathogenic species, *Neisseria gonorrhoeae* and *N. meningitidis*, which are associated with distinct clinical spectra of disease. *N. gonorrhoeae* is the causative agent of the sexually transmitted disease gonorrhea. The majority of gonococcal infections remain confined to the mucosa of the urogenital tract, with invasion of the bloodstream occurring only rarely. When such invasion occurs, the infection is seldom lethal. Meningococci can colonize the nasopharynx asymptomatically and are considered part of the normal flora. However, invasion of the bloodstream does occur, and such infections are fulminant and life-threatening. Meningococci can also invade the central nervous system, causing meningitis (36). Despite the differences in infections caused by these neisserial species, they share many characteristics. Overall DNA homology between the two species is high, with reassociation of gonococcal and meningococcal DNA of up to 77% under conditions in which reassociation of DNA from different meningococcal strains is 90% (31). Interspecies sequence similarity increases to 98% or more when housekeeping genes are compared (95). Characterizing similarities and differences in the genomes of gonococci and meningococci should help in identifying new genes or gene products that affect virulence in each species.

Both gonococci and meningococci possess numerous multigene families and repetitive sequence elements, with the copy number usually lower in the meningococcal chromosome than in that of the gonococcus. Recombination among different gene copies contributes to the antigenic variability of surface components, such as pili and Opa (opacity) outer membrane proteins, in both species (51, 64). Variability of these multigene families, as well as of other genes, is also increased by horizontal genetic exchange within and between *Neisseria* populations. *Neisseria* spp. are naturally competent for genetic transformation (12), and genetic exchange occurs between pathogenic and commensal *Neisseria* species, between gonococci and meningococci, and among meningococcal strains (28, 33, 37, 47, 55, 70, 82, 95). The impact of frequent recombination and horizontal genetic exchange events on the organization of the gonococcal and meningococcal chromosomes is not known.

The goals of understanding gene regulation, the genetic basis of pathogenesis, or molecular evolution of *Neisseria* species will be facilitated by genomic maps showing the arrangement of genetic loci on the gonococcal and meningococcal chromosomes. Physical (macrorestriction) maps have been constructed for gonococcal strains FA1090 (23, 26) and MS11 (11). The chromosomes of the two strains are circular and are approximately 2.2 Mb in size. The locations of most of the genes that have been mapped in both strains are the same (within the limits of resolution of the maps), and many restriction sites for the enzymes used in mapping are conserved. However, no information about chromosome organization in *N. meningitidis* has been available. An *Nhe*I macrorestriction map of a serogroup B strain was constructed by using two-dimensional pulsed-field gel electrophoresis (PFGE) techniques, but no genetic markers were located on the map (7). In this study, we have constructed a macrorestriction map of the chromosome of a serogroup A strain of *N. meningitidis*, determined the locations of 117 markers on the map, and compared

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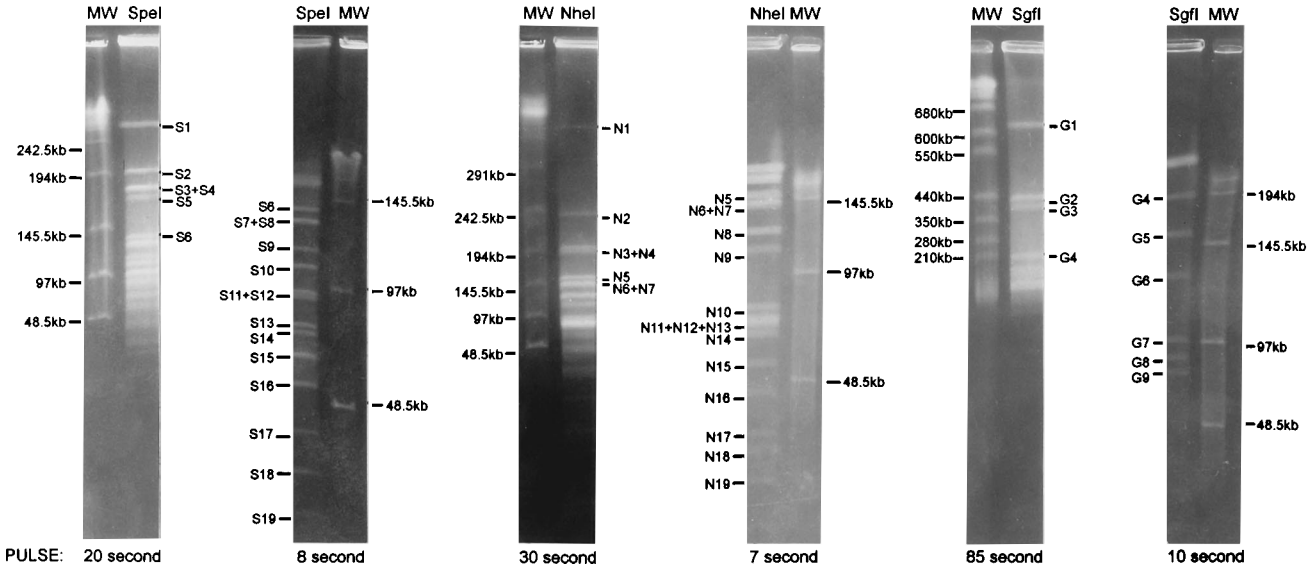

FIG. 1. CHEF electrophoresis of *Spe*I, *Nhe*I, and *Sgf*I digests of strain Z2491 DNA. DNA in agarose blocks was digested with the enzyme indicated above each panel and subjected to CHEF electrophoresis at the pulse times indicated below the panels. Lanes MW, size standards. All of the size standards were lambda concatemers except those for the *Sgf*I digest with an 85-s pulse, which were MegaBase I DNA standards (BRL).

the arrangement of those loci with that in gonococcal strain FA1090.

MATERIALS AND METHODS

Bacterial strains. *N. meningitidis* Z2491, also published as strain C751, is a serogroup A strain (subgroup IV-1) isolated in The Gambia in 1983 (2, 21). This strain contains three *opa* loci (37) and produces class 1 pilin. Meningococci were grown in GC broth or on GC agar (Difco Laboratories) with the supplements described by Kellogg et al. (40) at 37°C in a 5% CO_2 atmosphere. *Escherichia coli* strains were grown on Luria-Bertani agar (65).

DNA preparation and restriction digestions. Methods for preparation of meningococcal DNA in agarose blocks were the same as previously described for *N. gonorrhoeae* (26). Blocks were digested with 10 U of enzyme at 37°C in 120 μl of 1× buffer. *Bgl*II, *Nhe*I, or *Spe*I (GIBCO BRL) or *Pme*I (New England BioLabs) digestions were done in KGB (88) as previously described (26). Digestions with *Sgf*I (Promega) or *Pac*I (New England BioLabs) were done in the buffers provided by the manufacturers. Digested blocks were melted at 75°C, loaded in a 1% agarose gel (15 by 15 cm) containing either individual wells or one large well extending the width of the gel, allowed to solidify, and subjected to PFGE.

Electrophoresis. PFGE was performed in a contour-clamped homogeneous electric field (CHEF) apparatus with a hexagonal electrode array (17). Electrophoresis was done at 115 V and 12 to 15°C in 0.5× TBE buffer (65) for 40 to 44 h, with pulse times ranging from 3 to 85 s. Power was supplied from an EC570 power supply (E-C Corp.) or from a custom power supply constructed as previously described (25). Conventional gels (0.7% agarose, 1× TAE buffer [65]) were used to resolve fragments of <10 kb, using cesium chloride-banded DNA (73).

DNA transfer and hybridization. Procedures for nicking DNA with UV light, transferring DNA fragments to supported nitrocellulose filters (Schleicher & Schuell, Inc.), and cross-linking the DNA to the filters were described previously (26). Filters from gels with one continuous sample well were cut into narrow strips, which were hybridized with different probes. One strip from each gel was probed with radiolabeled Z2491 chromosomal DNA to highlight every DNA fragment. The autoradiograph of this strip was used as a reference to identify bands on autoradiographs of the remaining strips from that gel after hybridization with individual probes. When necessary, probe-hybridizing fragments were identified unambiguously by reprobing individual strips with radiolabeled chromosomal DNA to mark all of the fragments.

Oligonucleotide probes were synthesized with an ABI 394 RNA/DNA Synthesizer and were end labeled with ^{32}P (65). Oligonucleotides PorA (5′ CCG GCTGTTGTCGGCAAGCCCGG 3′), PorB (5′ CCTCGGTAACGGCCTGA AAGCC 3′), and 896 (5′ ATTCAAAACCCAAGCCGGCA 3′) were based on published sequences of the *porA* (class 1) (6), *porB* (class 3) (13), and *opc* (54) genes, respectively. The sequences of the remaining oligonucleotides are given in the references cited below. Hybridization and washing temperatures for end-labeled probes varied as follows for the different oligonucleotides: 45°C for 896 and Cys1C (91); 55°C for PorA, PorB, and Rmp (26); and 60°C for GyrB (26) and Az-2 (89). The sequences and hybridization temperatures for the probes for hypervariable regions of *opa* genes were as described previously (37). Hybridization and washing procedures for oligonucleotide probes were as described previously (26).

Probes specific for some genes were generated by using DNA amplimers derived from the nucleotide sequence of each gene in a PCR. Amplimers Po and Pc3 were used for the amplification of the 16S *rrn* genes as described by Enright et al. (27). Amplification of the *ctrA* gene was performed as described by Frosch et al. (29).

DNA fragments and PCR products were labeled with ^{32}P by random priming using an oligonucleotide-labeling kit (Pharmacia), with hybridization and washing conditions as described previously (26), or by enhanced chemiluminescence (ECL) direct nucleic acid labeling (Amersham). Probes labeled with the ECL kit were hybridized at 42°C in Amersham hybridization buffer plus 0.5 M NaCl. The filters were washed twice for 20 min in 6 M urea–0.4% sodium dodecyl sulfate–0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (65) at 42°C and twice for 5 min in 2× SSC at room temperature. Signal generation and detection were performed according to the manufacturer's protocol.

Densitometry. Photographic negatives were scanned and DNA fragments were sized as previously described (26). Molecular weight standards were lambda concatemers (16), high-molecular-weight DNA markers (Bethesda Research Laboratories, Inc. [GIBCO BRL]), MegaBase I DNA standard (GIBCO BRL), and lambda DNA digested with *Hin*dIII.

λgt11 cloning. Restricted Z2491 DNA was loaded in a 1% SeaPlaque GTG agarose (FMC Corp.) gel and subjected to PFGE. Appropriate fragments were cut from the gel, and the gel slices were washed three times for 15 min in TE (65). DNA fragments were digested in the gel slice with 100 U of *Eco*RI (GIBCO BRL) at 37°C for 2 h. DNA was eluted from gel slices by using Qiaex (Qiagen) according to the manufacturer's protocol. *Eco*RI fragments were cloned into λgt11 (92), and PCR products were generated as described previously (26).

RESULTS

N. meningitidis Z2491 (also designated C751) is a well-characterized serogroup A, subgroup IV-1 strain isolated during an epidemic of meningococcal meningitis in The Gambia in 1983 (1, 2, 21). In order to construct a macrorestriction map of the strain Z2491 chromosome, it was necessary to identify restriction endonucleases that cleaved the chromosome into a limited number of large DNA fragments. The similarity in G+C content (50%) and the high level of homology of gonococcal and meningococcal DNA led us to test the restriction endonucleases used in constructing the physical map for gonococcal strain FA1090: *Nhe*I, *Spe*I, *Bgl*II, and *Pac*I (26). Two additional restriction endonucleases with 8-bp recognition sequences, *Pme*I and *Sgf*I, were also used. DNA fragments resulting from

TABLE 1. Fragment sizes of Z2491 DNA after digestion with various restriction endonucleases[a]

Fragment	Size (kb) after digestion with:					
	*Nhe*I	*Spe*I	*Sgf*I	*Bgl*II	*Pac*I	*Pme*I
1	378	320	596	380	265	482
2	246	204	411	304	238	389
3	202	180[b]	374	210[b]	230	174
4	195	180[b]	194	210[b]	181	160
5	153	173	153	132	149	145
6	143[b]	138	129	120	145	117[b]
7	143[b]	130[b]	97	110	137	117[b]
8	125	130[b]	87	81[b]	114[b]	85
9	114	116	79	81[b]	114[b]	80
10	89	109	18	70	114[b]	60
11	82[b]	99[b]		51[b]	94	51[b]
12	82[b]	99[b]		51[b]	74	51[b]
13	82[b]	88		43	71	51[b]
14	74	84		41	68	44
15	59	75		38	63	38[b]
16	42	62		32	54	38[b]
17	25	35		29[b]	49	34
18	18	20		29[b]	29	26
19	11	13		19	24	21
20	7	7		16	21	13
21	4	5		15	12	11
22				12[b]	9	9
23				12[b]		
24				9		
25				6		
26				2		
Total	2,274	2,267	2,138	2,103	2,255	2,196

[a] Each value is the average of 2 to 11 independent determinations for different gels, except for *Nhe*I fragment 21, *Spe*I fragment 21, *Bgl*II fragments 14 to 18, and *Pac*I fragments 21 and 22, for which single gels were analyzed. The sums of the fragment sizes for *Nhe*I, *Spe*I, and *Sgf*I are more reliable than those for *Bgl*II, *Pac*I, and *Pme*I. Because not all of the *Bgl*II, *Pac*I, and *Pme*I fragments have been mapped, it is possible that there are additional undetected doublets or triplets in those digests.

[b] Doublet or triplet that was not resolved under any electrophoresis conditions used.

digestion of Z2491 DNA with these enzymes were resolved by CHEF electrophoresis (Fig. 1).

*Nhe*I, *Spe*I, and *Sgf*I were the most useful for map construction, cleaving the Z2491 chromosome into 21, 21, and 10 fragments, respectively. *Bgl*II, *Pac*I, and *Pme*I cleaved the chromosome into 26, 22, and 22 fragments, respectively, with many of the fragments being <100 kb (Table 1). Most of the digests contained doublets or triplets, defined as restriction fragments that were the same or very nearly the same size and were not resolvable by any electrophoresis conditions we used. The existence of some of these comigrating fragments was revealed by densitometry of photographic negatives of ethidium bromide-stained gels. In other cases, we detected the doublets or triplets in constructing the macrorestriction map, when probes for single-copy genes located in different portions of the map hybridized to a band that appeared to contain a single fragment.

Several classes of plasmids have been identified in *N. meningitidis* (63). To determine if any of the fragments identified on the CHEF gels resulted from the linearization of plasmid DNA, we prepared genomic DNAs of strain Z2491 and gonococcal strain FA1090 from cells embedded in agarose blocks and subjected these preparations to conventional electrophoresis. Under these conditions, plasmid DNA separates from uncut chromosomal DNA and can be visualized on the ethidium bromide-stained gel. The 4.2-kb cryptic plasmid of strain FA1090 was visible by this method, but we detected no plasmids from Z2491 (data not shown).

Because we have mapped all of the *Sgf*I fragments and all of the *Spe*I and *Nhe*I fragments except S21 (5 kb) and N18 (18 kb), as described below, we are confident that no additional unrecognized comigrating fragments were generated in these digests. For the *Bgl*II, *Pac*I, and *Pme*I digests, we mapped only the larger fragments, using information from these digests to confirm overlaps between DNA fragments generated with different restriction enzymes and to order markers within the overlaps. It is likely that there are undetected doublets or triplets among the smaller *Bgl*II, *Pac*I, and *Pme*I fragments, making the use of these digests to estimate genome size less reliable. The sums of the fragment sizes for *Nhe*I, *Spe*I, and *Sgf*I are 2,274, 2,267, and 2,138 kb, respectively, yielding an estimated genome size for strain Z2491 of 2,226 kb.

Construction of the macrorestriction map. In previous mapping studies with gonococcal strain FA1090, the presence of numerous repeated genes and sequences in the genome led to an approach using single-copy genes or sequences to probe Southern blots of chromosomal DNA digested with different restriction enzymes and subjected to CHEF electrophoresis. By determining which fragments from different digests hybridized to a specific probe and thus how the fragments overlapped, it was possible to walk back and forth between digests to form a complete macrorestriction map (26). To construct the macrorestriction map of meningococcal strain Z2491, we used the same repeat-avoiding strategy.

Z2491 DNA was digested with the different mapping enzymes and separated by CHEF electrophoresis; Southern blots were probed with a collection of probes for single-copy genes or sequences. These included cloned neisserial genes from previous studies in our laboratory, cloned genes obtained from other investigators, PCR products generated by using published DNA sequences of neisserial genes, anonymous clones of Z2491 DNA in λgt11, and anonymous clones of gonococcal strain FA1090 DNA that we produced in our previous mapping studies. Tables 2 and 3 list the gene-specific and anonymous probes, respectively, and the restriction fragments to which they hybridized. This collection contained probes specific for all of the fragments in *Sgf*I, *Spe*I, and *Nhe*I digests except for *Spe*I fragment S21 (5 kb) and *Nhe*I fragment N18 (18 kb). Because of the uncertainty in identifying the smallest *Bgl*II, *Pac*I, and *Pme*I fragments, only the larger fragments from these digests (B12, P18, M13, and larger) were included in the analysis.

Analysis of double digests by Southern blotting with appropriate probes or by examining ethidium bromide-stained gels allowed us to determine whether a particular fragment in one digest contained sites for another enzyme and, thus, how the maps for the different enzymes were aligned relative to each other. The restriction fragments completely contained within an overlapping fragment from a second digest were as follows: N19 within G8; G10 within N8; P2, S11, and S12 within N1; S16 within N2; S9 within N4; S15 and S19 within N5; N15 and N20 within S1; N12, N17, and N19 within S2; N7 within S4; N11 within S7 and P4; N21 within S10; S11, S12, and S14 within B1; S13 and S19 within B3; and S16 and B12 within M5. Fragments containing a recognition site for one or more of the other mapping enzymes were as follows: S13, S14, S18, B8, B9, B12, and M5 contained *Nhe*I sites; N3, N8, N9, N10, B2, B4, B7, B9, B10, B12, and P4 contained *Spe*I sites; S1, S2, S3, S4, S5, S7, S8, P1, P2, and P4 contained *Bgl*II sites; S1, S2, S5, N2, N3, N5, N8, N16, B1, B3, B4, and B6 contained *Pme*I sites; N8, N10, N15, S1, S6, and S16 contained *Sgf*I sites; and S1, S2, S3, S4, and S5 contained *Pac*I sites.

TABLE 2. Cloned gene probes and fragments to which they hybridized

Gene	Gene product or function and probe	Reference or source	Hybridizing fragment					
			*Nhe*I	*Spe*I	*Bgl*II	*Pac*I	*Pme*I	*Sgf*I
aniA	Anaerobically induced protein Pan1; pKS4.21	38	N16	S5	B4	P11	M11	ND[a]
argF	Ornithine transcarbamoylase; pRGF1	48	N2	S16	B6	P15	M5	ND
argJ	Ornithine acetyltransferase; pRGE172	49	N1	S12	B1	<P10[b]	ND	ND
asd	Aspartate semialdehyde dehydrogenase (*N. meningitidis*); pNM1	34	N1	S11	B1	ND	M3	ND
carA	Carbamoylphosphate synthetase; pFL50[c]	60	N1	S8	ND	P2	M4	ND
carB	Carbamoylphosphate synthetase; pFL35	60	N1	S8	<B12	ND	ND	ND
ctrA	Capsule outer membrane transport (*N. meningitidis*)[c]	29	N21	S10	B1	P1	M7	ND
dcmA	M.*Ngo*PI methylase; pLV111	75	N1	S11	B1	ND	M3	G7
dcmB	M.*Ngo*PII methylase; pLV155	76	N5	S15	B3	ND	<M13	ND
dcmD	M.R.*Ngo*MI methylase and restriction enzyme; pCBB49.1	32	N9	S1	B8	P16	M1	ND
dcmE	M.*Ngo*BIII; pMMB3	32	N11	S7	B2	P4	M1	G1
dhp	Dihydropteroate synthase (*N. meningitidis*); pNMBT5701	43	N6	S5	B4	P8	M8	G4
fbp	37-kDa iron-repressible OMP[c,d]	C. Cornelissen	N7	S4	<B12	ND	M2	ND
frpA, *frpC*	RTX-like toxin; pUNCH210[e]	78, 79	N4	S3	B2	ND	ND	ND
			N7	S4	ND	ND	ND	ND
frpB	70-kDa iron-repressible OMP; pUNCH325	9	N1	S12	B1	<P10	ND	G6
fur	Ferric uptake regulator; pUNCH601	77	N5	S15	B3	P9	M12	ND
galE	UDP-galactose-4-epimerase; pGALE5	D. Stein	N8	S14	B1	P1	M7	G2
gyrA[f]	DNA gyrase subunit; pGC.P11[c]	8	N4	S17	B2	P13	M1	ND
gyrB	DNA gyrase subunit; GyrB[g]	71	N5	S15	B3	ND	ND	ND
himA[f]	Integration host factor subunit; pGC.P13[c]	R. Belland	N3	S4	<B10	P3	M2	ND
himD[f]	Integration host factor subunit; pGC.P14[c]	R. Belland	N4	S9	B2	P12	M1	ND
hsp-63	63-kDa stress protein (*N. meningitidis*); MSP63	58	N1	S12	B1	ND	ND	ND
iga	Immunoglobulin A protease; pVD114	42	N7	S4	<B12	ND	M2	G3
laz	Outer membrane lipid-modified azurin with H.8 epitope; Az-2[g]	89	N2	S3	B6	ND	ND	ND
lbpA	Lactoferrin-binding protein; pUNCH122	G. D. Biswas, P. F. Sparling	N2	S3	B6	ND	M5	ND
lepA[f]	GTP membrane-binding protein (function unknown); pGC.P17[c]	R. Belland	ND	S1	B7	ND	ND	G3
lip	Outer membrane lipoprotein with H.8 epitope; pFLOB861	90	N2	S3	B6	ND	M10	ND
los-1	Glycosyltransferase; —[h]	F. K. Lee, M. Apicella	N4	S7	B2	P4	M1	ND
lpd[f]	Lipoamide dehydrogenase; pSP931[c,i]	R. Belland, S. Porcella	N3	S1	B8 + <B12[j]	P10	M13	ND
lps-1	Lipooligosaccharide antigen; pTME6	56	N1	S6	B10	ND	<M13	G5
mtrR	Increased resistance to antibiotics and hydrophobic compounds; —	57	N6	ND	B4	P8	M8	G4
mutS	Methyl-directed mismatch repair; —	R. Belland	N8	S14	B1	P1	M7	ND
ngoX	Methylase; —	R. Belland	N4	S7	B2	ND	ND	ND
opaA	Opacity OMP; HV1 2100 and HV2 2100[g]	37	N12	S2	B4	ND	ND	ND
opaB	Opacity OMP; HV1 2100 and HV2 2000[g]	37	N16	S5	B4	ND	ND	ND
opaD	Opacity OMP; HV1 2001 and HV2 2000[g]	37	N2	S3	B2	ND	M1	ND
opc	OMP; 896[g]	54	N9	S1	B8	P16	M1	ND
oxiA	Anaerobically repressed OMP; pTME1	22	N3	S1	ND	P3	M2	ND
parC[f]	Topoisomerase activity; pG4[c]	R. Belland	N2	S16	B12	P11	M5	ND
pdh	Pyruvate dehydrogenase complex; 18a	D. Ala'Aldeen	N4	S9	B2	ND	M1	ND
penA	Penicillin-binding-protein 2; pBS134	69	N12	S2	<B12	P7	M6	ND
pep[f]	Prolyl endopeptidase; —	R. Belland	N1	S8	<B12	ND	M4	G9
pgm	Phosphoglucomutase[c]	94	N3	S1	ND	P3	M2	G3
pilC	Pilus assembly (2 chromosomal copies); pABJ01	39	N1	S8	B10	P2	ND	ND
			N8	S14	B1	P1	ND	ND
pilD	Prepilin leader peptidase; p12/7/1[i]	44	N14	S2	B3	ND	<M13	ND
pilF	Pilin assembly; p12/7/1[i]	44	N14	S2	B3	ND	<M13	ND
pilQ (*omc*)	OMP macromolecule complex; S1/pUC19	81	N1	S6	B10	P2	<M13	G5
pil	Pilin locus; Cys1C[g]	91	N8	S14	B1	P1	ND	G2
pilT	Pilus-associated twitching motility and colony morphology; p2a2	44	N8	S10	B1	P1	M7	ND
porA	Outer membrane porin; PorA[g]	6	N2	S3	B2	ND	ND	ND
porB	Outer membrane porin; PorB[g]	13	N1	S11	B1	ND	ND	G7
ppk	Polyphosphate kinase; pBluePPK6001	80	N1	S8	B1	P2	M4	ND
proAB	Proline biosynthesis; pLES8	72	N9	S1	B5	ND	M1	ND
recA	General recombination and DNA repair; pVD308	41	N2	S3	B2	ND	M1	ND
regF	Regulation of *pilE* transcription; pNG200	H. DeReuse, C. Marchal	N1	S12	B1	P2	ND	ND
rfaC	α-1,5 heptosyltransferase; hepT1	93	N8	S14	B1	ND	M7	ND
rfaE	ADP-heptose synthase; DK1	F. K. Lee, M. A. Apicella	N3	S1	B7	ND	ND	ND
rho	Transcription termination factor rho; pNG103	52	N7	S4	<B12	P3	M2	ND
rmp	Reduction-modified OMP; RMP[g]	30	N17	S2	<B12	P6	M6	ND

Continued on following page

TABLE 2—*Continued*

Gene	Gene product or function and probe	Reference or source	Hybridizing fragment					
			*Nhe*I	*Spe*I	*Bgl*II	*Pac*I	*Pme*I	*Sgf*I
rpsR[f]	Ribosomal subunit protein; —[c]	P. Ropp, R. Nicholas	N4	S9	B2	P12	M1	ND
rrnA	rRNA (*N. meningitidis*)[c]	27	N8	S10	B1	P1	ND	ND
rrnB	rRNA (*N. meningitidis*)[c]	27	N14	S13	B3	ND	ND	ND
rrnC	rRNA (*N. meningitidis*)[c]	27	N6	S5	B4	ND	ND	ND
rrnD	rRNA (*N. meningitidis*)[c]	27	N1	S11	B1	P1	ND	ND
sac-4	Serum resistance; pWM3	50	N5	S15	B3	P9	M12	ND
sucA[f]	α-Ketoglutarate dehydrogenase (decarboxylase); pSP931[c,i]	R. Belland, S. Porcella	N3	S1	<B12	ND	ND	ND
tbpA	Transferrin-binding protein TBP-1; pUNCH401	20	N19	S2	B4	P7	ND	ND
tbpB	Transferrin-binding protein TBP-2[c]	5	N19	S2	B4	P7	ND	ND
tufA[f]	Elongation factor (EF-Tu); pSP961[c]	R. Belland, S. Porcella	N13	S10	B11	P18	<M13	ND
tyrS[f]	Tyrosyl-tRNA synthetase; MN32-IC	B. Spratt	N1	S8	B10	ND	ND	G5
uvrA[f]	Excision nuclease (DNA repair); JKD966	J. K. Davies	N20 + N15[j]	S1	ND	ND	M1	G3
uvrC[f]	Excision nuclease (DNA repair); pGC.P16[c]	R. Belland	N4	S9	B2	ND	ND	ND
	29K OMP; pTME2	V. Clark	N4	S9	B2	ND	ND	ND
	61K and 58K OMPs; pTME4	V. Clark	N4	S9	B2	ND	M1	ND
	40K OMP; pTME5	V. Clark	N1	S6	B10	P2	<M13	G5

[a] ND, not done.
[b] <, hybridizing fragment was smaller than the fragment indicated, but its identity was not determined.
[c] Probe was the PCR product.
[d] OMP, outer membrane protein.
[e] Probe provided does not distinguish between *frpA* and *frpC*, which are therefore designated *frpA/C* at both map locations.
[f] The gene designation is based on sequence similarity to a previously described gene from *E. coli* or other bacterial species, as determined by the investigator(s) providing this probe; functional characterization of the gene product has not necessarily been done.
[g] Oligonucleotide probe.
[h] —, no clone designation provided.
[i] Multiple genes were cloned on a single cosmid; gene-specific probes were generated by PCR.
[j] Linking clone.

Several clones that we used as probes contained a recognition site for one of the restriction enzymes used for mapping. When such linking clones are used as probes, they hybridize to two restriction fragments, demonstrating that those fragments are adjacent in the chromosome (67). The linking clones included λ736, containing the *Pac*I site common to P17 and P18; *Nhe*I linking clones uvrA and λM67, containing sites common to N20 and N15 and to N17 and N12, respectively; and *Bgl*II linking clone lpd, containing the site common to B8 and a smaller, unidentified *Bgl*II fragment. Although most of these clones were derived from strains other than Z2491, the restriction sites present in the clones were also conserved in Z2491 DNA.

The circular Z2491 macrorestriction map constructed from these data is shown in Fig. 2. All of the *Sgf*I fragments and all of the *Nhe*I and *Spe*I fragments except N18 (18 kb) and S21 (5 kb) are included on the map, as well as the larger *Bgl*II, *Pac*I, and *Pme*I fragments. All of the probes gave results with each of the different digests that were consistent with this map. Although we do not know where N18 and S21 are located, insertion of either of these small fragments into the appropriate enzyme map cannot change the relative order of the other mapped fragments. Rather than choose arbitrary and potentially incorrect places on the map to depict gaps caused by the absence of N18 and S21, the *Spe*I and *Nhe*I maps have been drawn as complete circles, even though one small fragment is missing in each digest. The alignment of the *Nhe*I, *Spe*I, and *Sgf*I maps was confirmed by determining the sizes of fragments generated in double digests. For example, fragment S18 (20 kb) is cleaved by *Nhe*I into fragments of 8 and 12 kb, which provides a reference point for alignment of the *Spe*I and *Nhe*I maps. Because the *Bgl*II, *Pac*I, and *Pme*I maps lack several of the smaller fragments from each digest, some of the mapped fragments could shift in a clockwise or counterclockwise direction as long as they remain within the constraints of the *Sgf*I, *Nhe*I, and *Spe*I overlaps. Information on the hybridization of probes to fragments in the *Bgl*II, *Pac*I, and *Pme*I digests was important in verifying the arrangement of the *Sgf*I, *Spe*I, and *Nhe*I fragments and in improving the resolution of the map.

By using as many probes as possible that were derived from known neisserial genes, we simultaneously obtained information about the overlaps between different restriction fragments and localized each genetic marker on the completed map. There are 117 genetic markers located on the map; 75 of them represent identified genes, with the remaining markers identified by the anonymous clones of gonococcal or meningococcal DNA. The location of each marker on the chromosome is defined by the region of overlap between the restriction fragments from different digests, with the relative order of the markers within each overlap being unknown. Those overlap regions range from 10 to 143 kb in size.

Comparison of chromosome organization in meningococcal strain Z2491 and gonococcal strain FA1090. Although macrorestriction maps are low-resolution maps, the density of markers on the Z2491 and FA1090 maps is sufficient to allow a comparison of overall chromosome organization in these two strains belonging to closely related species. In general, the arrangements of markers in the two strains over much of the chromosome are similar. However, there are several markers or groups of markers that are not in the same relative locations on the two maps (Fig. 3). These differences are particularly evident in a region of approximately 500 kb that extends from

TABLE 3. Anonymous clones and fragments to which they hybridized

Clone	Hybridizing fragment					
	*Nhe*I	*Spe*I	*Bgl*II	*Pac*I	*Pme*I	*Sgf*I
λ6	N3	S1	B7	ND[a]	M2	ND
λ37	N3	S4	<B12[b]	ND	M2	ND
λ42	N17	S2	<B12	P6	M6	ND
λ130	N5	S15	B3	ND	<M13	ND
λ167	N1	S8	B1	ND	ND	G9
λ351	N6	S5	B4	ND	M8	ND
λ356	N8	S14	B1	P1	<M13	G10
λ371	N3	S1	<B12	ND	ND	ND
λ375	N6	S2	B4	ND	ND	G8
λ379	N6	S5	B4	P11	M11	G4
λ491	N2	S3	B6	P15	M5	ND
λ504	N8	S14	B1	P1	ND	G2
λ511	N1	S11	B1	P1	M3	G7
λ515	N1	S12	B1	P2	ND	G6
λ516	N15	S1	B8	P16	M1	ND
λ522	N6	S5	B4	P7	M8	ND
λ580	N4	S9	B2	P12	ND	ND
λ583	N1	S11	B1	ND	M3	G7
λ588	N4	S9	B2	P12	M1	ND
λ594	N3	S1	B8	P10	M13	G3
λ601	N5	S19	B3	P9	M12	ND
λ608	N4	S9	B2	P13	M1	ND
λ609	N9	S1	B5	P16	ND	ND
λ611	N5	S13	B3	P9	M12	G2
λ644	N10	S6	B9	P14	M2	G3
λ670	N6	S2	B4	P7	ND	G4
λ699	N7	S4	ND	P3	M2	G3
λ701	N2	S3	B2	ND	M1	ND
λ703	N9	S7	B5	ND	ND	ND
λ711	N7	S4	<B12	ND	ND	ND
λ712	N4	S9	B2	P4	M1	G1
λ714	N4	S9	B2	ND	ND	ND
λ728	N2	S16	B12	P11	M5	G4
λ732	N1	S8	B10	ND	M4	G5
λ734	N8	S14	B1	ND	ND	ND
λ736	N13	S18	<B12	P17 + P18[c]	<M13	ND
λ740	N10	S6	B9	P14	M2	G5
sc#1[d]	N17	S2	<B12	P6	M6	ND
sc#5[d]	ND	S9	B2	ND	ND	ND
sc#6[d]	N5	S18	B3	ND	<M13	ND
λM35[e]	N4	S20	B2	P4	M1	ND
λM67[e]	N17 + N12[c]	S2	<B12	P6	M6	ND

[a] ND, not done.
[b] <, hybridizing fragment was smaller than the fragment indicated, but its identity was not determined.
[c] λ736 contains a *Pac*I site, and λM67 contains an *Nhe*I site.
[d] Clones sc#1, sc#5, and sc#6 are anonymous fragments of gonococcal strain FA1090 DNA cloned into pBR322.
[e] Clones λM35 and λM67 are anonymous fragments of Z2491 DNA cloned into λgt11.

6 to 8 o'clock on the circular Z2491 map (Fig. 2). At least three different blocks of markers are translocated and/or inverted in orientation between the two strains; it is impossible to convert one map order to the other without invoking multiple rearrangement events. In addition to the differences within this region of the maps, there are a number of other loci that are located in different positions in the two strains. One such locus is *rrnD*, one of the four *rrn* (rRNA) loci, which is in different positions (nearly 180° across the circular map) in the two strains. The *argJ* locus maps near *rrnD* in both strains. The remaining *rrn* loci are in similar locations in Z2491 and FA1090. Additional loci that are in widely separated map locations in the two species are *lbpA* (encoding the iron-regulated lactoferrin-binding protein) and anonymous markers λ740 and λ644. The comparison of the two maps in Fig. 3 may underrepresent the differences between the two strains. Because the resolution of the gonococcal strain FA1090 map is lower than that of the Z2491 map, there are some regions where we would not detect differences between them in the relative order of certain markers.

For the multicopy *pil* and *opa* genes, the locations in Z2491 match those of *pil*- and *opa*-containing loci in FA1090 (although the gonococcal strain has additional copies of the genes as well). Strain Z2491 produces class 1 pilin, which is structurally and antigenically similar to gonococcal pilin (35, 83). In gonococci, the pilin gene family consists of a single expression locus (*pilE*) and a number of silent (*pilS*) loci containing partial pilin gene copies (51, 64). Antigenic variation of pilin is accomplished by recombination of information from one or more silent copies into the expression locus. In gonococcal strain FA1090, the *pilE* locus and four *pilS* loci are located within a 35-kb region; the remaining *pilS* locus is located approximately 500 kb from the major pilin gene cluster (26, 68). Meningococcal class 1 *pil* genes share extensive homology with the gonococcal *pil* genes, although less is known about the copy number and arrangement of the meningococcal *pil* loci (3, 59, 61). An oligonucleotide specific to a conserved *pil* region hybridized to Z2491 DNA fragments mapping in the region of the chromosome corresponding in location to the major pilin gene cluster of strain FA1090 (Table 1 and Fig. 2). To determine if multiple *pil* loci are located within this region, Southern blots of Z2491 chromosomal DNA restricted with *Cla*I or *Hin*cII and separated by conventional gel electrophoresis were probed with the same oligonucleotide. The probe hybridized to a 7.6-kb *Cla*I fragment and to at least six *Hin*cII fragments (3 and 0.9 kb, a 0.5-kb doublet, and a 0.3-kb doublet; data not shown). A *Hin*cII site is conserved in gonococcal *pil* genes; assuming that the same site is conserved in the Z2491 *pil* loci, at least six *pil* genes are located in a chromosomal region of no more than 7.6 kb in strain Z2491.

The *opa* genes, which encode the antigenically variable Opa outer membrane proteins, share a conserved framework that is nearly identical in gonococci and meningococci, interspersed with a semivariable region and two hypervariable (HV) regions designated HV1 and HV2 (4, 10, 19, 37, 73, 74). Gonococci have 11 or 12 *opa* genes that are distributed around the chromosome (11, 26), whereas meningococci have three or four *opa* genes (4, 37, 74). In a previous study, the three Z2491 *opa* genes were cloned and sequenced (37). To locate the *opa* genes on the macrorestriction map, we used oligonucleotides specific to the HV regions of these genes as probes on Southern blots of restricted Z2491 DNA separated by PFGE (Table 2). The three *opa* loci are widely separated on the Z2491 map, with the location of each of them corresponding to the map location of an *opa* gene in gonococcal strain FA1090.

The genetic markers that we mapped for strain Z2491 included several loci that are present in the meningococcal genome but not in the genome of *N. gonorrhoeae*. These markers were *ctrA* (capsule biosynthesis) (29), *opc* (Opc outer membrane protein) (54, 66), *porA* (class 1 outer membrane porin protein) (6), and *frpA/C* (duplicated loci encoding an iron-regulated, RTX toxin-like protein) (78, 79). These five loci are not located in one contiguous region of the Z2491 map but are distributed throughout the chromosome (Fig. 2).

DISCUSSION

Serogroup A strains of *N. meningitidis* are responsible for many of the epidemics of meningococcal disease that occur in

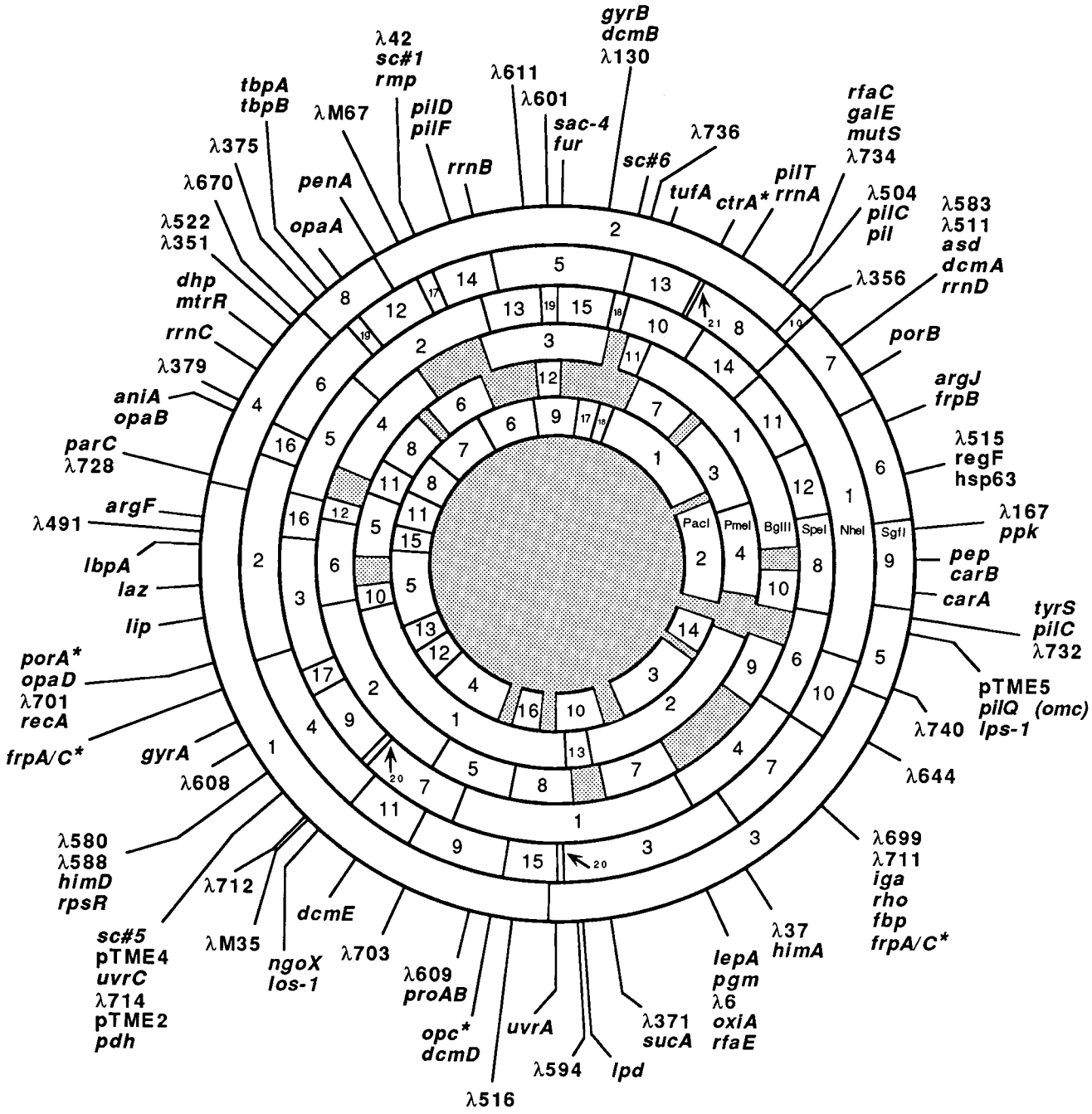

FIG. 2. Macrorestriction map of the strain Z2491 chromosome. Groups of markers that map to the same region of fragment overlap are connected to the map by solid lines. The order of markers within each group cannot be determined. Genetic loci that have been shown to be present in meningococcal DNA but not in gonococcal DNA are indicated (*). The *Sgf*I map is complete. One *Spe*I fragment (S21; 5 kb) and one *Nhe*I fragment (N18; 18 kb) are not included on the maps for those enzymes, because no probes specific for those fragments were obtained. Rather than choose arbitrary and potentially incorrect places on the map to depict gaps caused by the absence of these fragments, the maps have been drawn as complete circles, even though one small fragment is missing in each digest. The relative order of the other fragments in the *Spe*I and *Nhe*I maps cannot be affected by insertion of S21 and N18 into the maps. For enzymes *Bgl*II, *Pac*I, and *Pme*I, only the larger fragments from these digests (B12, P18, M13, and larger) were mapped.

the meningitis belt of Africa (1). There is no standard type strain for each serogroup of *N. meningitidis*; many different strains have been studied by different investigators. However, isolates of subgroup IV-1, of which strain Z2491 is a member, have been subjected to extensive phenotypic and clonal analysis (1, 2, 21). In addition, this strain has been used in studies of the molecular genetics of outer membrane proteins and of the role of specific meningococcal surface components in adherence to and invasion of human cells (37, 53, 54, 66, 84–86). In choosing Z2491 for mapping, we hoped that the resulting information on chromosome organization would both benefit from and contribute to other types of studies using this strain.

The estimates for the sizes of neisserial chromosomes that have been obtained by summing the lengths of restriction frag-

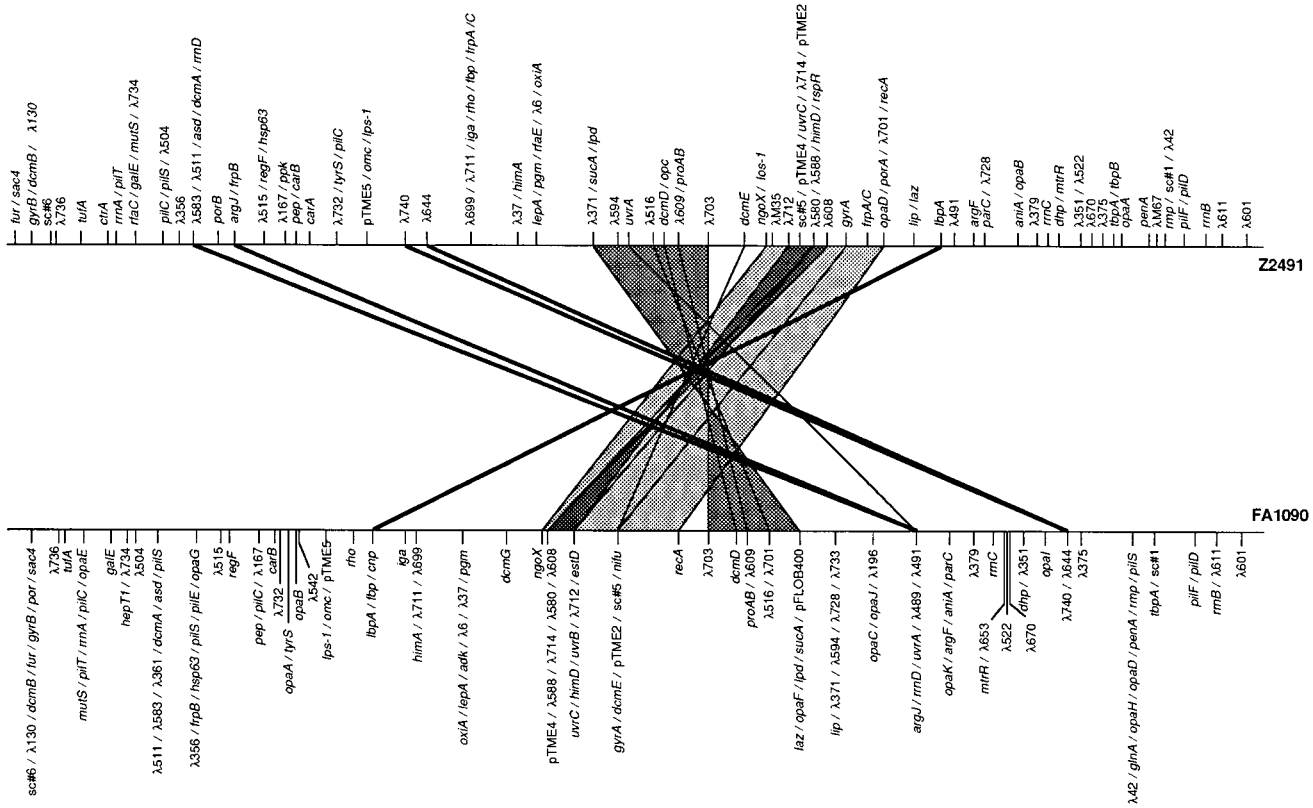

FIG. 3. Comparison of chromosome organization in meningococcal strain Z2491 and gonococcal strain FA1090 (23, 26). Linear representations of the two maps have been aligned so that the maximum number of markers are in the same relative positions, within the limits of resolution of the maps. Lines connect the positions of markers that map to different locations in the two strains; the shaded areas represent groups of markers that are translocated or inverted in position in one strain relative to the other.

ments resolved by PFGE range from 1.8 to 2.3 Mb (7, 11, 14, 26). Although it is possible that genome sizes for different gonococcal or meningococcal strains vary over a range of several hundred kilobases, we believe it is more likely that the genome size determinations were influenced by variation in the specific conditions used for PFGE in the different studies. The apparent molecular masses of DNA fragments separated by PFGE are affected by the base composition of the fragments, the identity of the molecular mass markers, the pulse times used, and the program of ramping pulse time over the course of an electrophoresis run (15, 67, 87). Our studies of meningococcal strain Z2491 and gonococcal strain FA1090 used the same PFGE apparatus, molecular weight standards, and electrophoresis conditions. The resulting estimates of genome size of 2,226 and 2,219 kb (26), respectively, suggest that the genome sizes are essentially the same in the two strains.

We constructed the Z2491 map by using Southern blotting of DNA separated by PFGE to identify overlapping fragments from different restriction digests, walking back and forth between digests along the chromosome. The probes represented different sequences occurring in single copy in the chromosome: cloned gonococcal and meningococcal genes or cloned anonymous fragments of gonococcal or meningococcal DNA. By using specific cloned genes as probes, we simultaneously obtained information about the overlaps between restriction fragments and about the locations of 75 genetic loci on the completed map. The density of markers on the map was increased by using the 42 anonymous clones. Although the specific gene represented by each has not been identified, these clones provided sequence-tagged sites that were useful in constructing the map and in demonstrating differences between Z2491 and FA1090 in chromosome organization.

A comparison of the maps of Z2491 and gonococcal strain FA1090 reveals differences in genome organization. The arrangement of markers is generally conserved over much of the chromosome, within the limits of resolution of the maps. Within this conserved framework, however, a number of markers are in different locations on the two maps. For example, the *rrnD* locus and its neighbor *argJ* are located on opposite sides of the two maps, surrounded by different flanking markers in each case. The most striking differences between the two chromosomal maps, however, are in a region of approximately 500 kb. Within this region, several groups of genes are translocated, and in some cases inverted in orientation, between the two maps. These differences are complex; multiple rearrangement events must be invoked to interconvert the two maps.

At least two types of rearrangements resulting in inversions of chromosomal segments have been described for enteric bacteria. Inversions covering the replication terminus region have been identified in comparisons of different species (45, 46, 62). The origins and termini of replication in the meningococcus or gonococcus have not been mapped. However, if the origins of replication of the Z2491 and FA1090 chromosomes are located near *gyrB* (12 o'clock on the map in Fig. 2), as in the other eubacteria for which replication origins have been mapped (18), then bidirectional replication would probably terminate in the regions of the maps showing extensive rearrangements between Z2491 and FA1090, as in the inversion-

prone region of enteric bacteria. Chromosomal rearrangements, especially inversions, are also generated by homologous recombination among multiple *rrn* operons (46). The differences in marker order between Z2491 and FA1090 could not have arisen by this mechanism, since the rearrangement region does not contain *rrn* loci at its endpoints or within it. However, it seems likely that the abundant repetitive DNA sequences present in the genomes of pathogenic *Neisseria* species could act in a similar fashion as recombination sites for inversions and other chromosomal rearrangements.

We mapped several genetic loci that are present in the genome of *N. meningitidis* but absent in the gonococcal genome. These loci are not located in a single continuous region of the Z2491 genome. Therefore, if this group of meningococcus-specific genes was acquired in a block from another source by horizontal genetic exchange, the genes must have subsequently become separated as a consequence of chromosomal rearrangements. Three of those meningococcus-specific genes (*frpA*/*C*, *porA*, and *opc*) are located in the 500-kb rearrangement region, although the *ctrA* locus, involved in capsular biosynthesis, is elsewhere on the chromosome.

Just as there is no single type strain of the meningococcus or gonococcus, there is probably not a single chromosomal map that is representative of all strains of each of the two species. The interspecies differences in chromosome organization between *N. meningitidis* Z2491 and *N. gonorrhoeae* FA1090 are accompanied by intraspecies differences as well. In preliminary studies, we have found substantial variation in the location of rRNA operons on the chromosomes of different meningococcal strains, even among strains of the same serogroup (24). Although the gonococcal maps for strains FA1090 and MS11 are nearly identical (11, 26), other gonococcal strains differ in the arrangement of rRNA operons on the chromosome (24). The natural competence of both meningococci and gonococci for genetic transformation may contribute to variability in chromosome organization as well as to the formation of mosaic genes containing information from different sources (28, 33, 37, 47, 55, 70, 82, 95). Although we have not yet identified the endpoints of the rearranged segments of gonococcal or meningococcal DNA or the mechanisms by which such rearrangements are generated, the propensity of pathogenic *Neisseria* species to undergo such rearrangements may have important consequences for the evolution and pathogenic potential of these bacteria.

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