CLINICAL MICROBIOLOGY REVIEWS, Apr. 1989, p. S92–S103
0893-8512/89/0S0S92-12$02.00/0

Vol. 2, Suppl.

Genetic Loci and Linkage Associations in *Neisseria gonorrhoeae* and *Neisseria meningitidis*

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The purpose of this review is to present the currently available information on genetic loci that have been identified in *Neisseria gonorrhoeae* and *Neisseria meningitidis*. We will include genes that were identified by mutations that alter the phenotype of the organism, if the gene was transferred into another genetic background, and genes whose products were well characterized, even if the genes were not identified by mutation. We also will include genes that were cloned and identified either by complementation of *Escherichia coli* mutations or by identification of the gene product.

GENETIC NOMENCLATURE

We will attempt to establish genetic nomenclatures for these genes, following the guidelines of Demerec et al. (29), i.e., assigning a three-letter lowercase designation for the gene family, followed by a capital letter, as appropriate. Genes that have been identified by complementation of well-characterized *E. coli* mutants will be given the corresponding genetic symbol, if appropriate. This is not meant to imply that the neisserial gene encodes the same enzyme as the *E. coli* gene, but, rather, that it encodes an enzyme that performs the same function in converting the substrate into the product. For cloned neisserial genes that were identified by the gene products, which are not found in *E. coli*, we will use the previously published nomenclatures, except for genes whose names have been changed by agreement of researchers attending the Sixth International Pathogenic Neisseria Meeting, October 1988. Genes that were identified and named in *N. gonorrhoeae* should be given the same gene symbol if they are subsequently found to occur in *N. meningitidis*, and vice versa.

Since *N. gonorrhoeae* and *N. meningitidis* contain several copies of closely related genes, a special nomenclature has been agreed upon to identify these genes. This consists of giving the genetic symbol following by a subscript notation that identifies the strain containing the gene with a designation of the allele number. For example, the protein IIb gene from *N. gonorrhoeae* FA1090 would be $opa_{FA1090\text{-}2}$. Genetic designations for these multicopy genes, e.g., as *opaA*, are not to be given until the gonococcal chromosome has been genetically or physically mapped and the location of the gene on the map has been determined.

New genetic symbols or proposed revisions of old nomenclature are to be approved by Virginia L. Clark, Department of Microbiology and Immunology, Box 672, School of Medicine and Dentistry, University of Rochester, Rochester, New York. She will also assign allele numbers to designate mutations in specific genes upon written request. It is hoped that this coordination in genetic nomenclature for *N. gonorrhoeae* and *N. meningitidis* will prevent confusion that may arise if different laboratories assign their own genetic designations.

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N. GONORRHOEAE LABORATORY STRAINS

Comparisons of results from different laboratories are sometimes difficult owing to the use of different strains that may vary in their genetic composition. Therefore, we would like to briefly review the laboratory strains that have been used in these studies. The most commonly used laboratory strains are listed in Table 1, along with available information regarding their history and genetic markers.

GENETIC MARKERS IN *N. GONORRHOEAE*

Gonococcal genetic markers identified by transformation, by characterization of the gene product, or by cloning are listed in Table 2, and the limited genetic map is shown in Fig. 1. It should be noted that the genetic map contains a number of ribosomal protein mutations (*str*, *fus*, *tet*, *cap*, and *spc*) and a subunit of ribonucleic acid polymerase (*rif*); ribosomal protein genes have been shown to be clustered in other organisms (6), so it is not surprising to find them clustered in *N. gonorrhoeae*. The other genes that have been shown to be linked in this region are probably involved in peptidoglycan biosynthesis (*ampD*, *ampC*, *ampB*, *ampA*, and *penB*) or outer membrane structures (*por*, *sac-1*, *sac-3*, *mom*, and *tem*). The genes linked to this region that encode enzymes involved in monomer synthesis (*ura*, *argE*, and *hyx*) are found flanking the region, rather than within it.

In addition to the limited genetic map, which represents approximately 3% of the gonococcal chromosome (19), physical maps linking various gonococcal genes have been generated. The largest portion of the gonococcal chromosome that has been physically mapped, using chromosome walking techniques, is 50 kilobase pairs and contains the two pilus expression sites, a pilus silent gene region, and a protein II gene (75, 113).

Little is known regarding genetic organization and control of gene expression in *N. gonorrhoeae*. No repressors or activators have been identified by either classical genetic or gene cloning techniques. However, repression of outer membrane protein synthesis by iron (123) and oxygen (25) suggests that regulatory proteins do exist in *N. gonorrhoeae*. Additionally, two genes, *pilA* and *pilB*, that act in *trans* to regulate the pilin promoter have recently been described (117). The *pilB* gene product represses pilin expression, whereas the *pilA* gene product activates the pilin promoter.

No evidence exists that gonococcal genes are organized into operons, with multiple genes under the control of a single promoter. The cloned *proA* and *proB* loci are contiguous but are under the control of separate promoters (110). It is possible that most gonococcal genes contain their own promoter, analogous to the genetic organization of *Pseudomonas aeruginosa* (54) rather than *E. coli* (6).

TABLE 1. Characteristics of common laboratory strains of *N. gonorrhoeae*

Strain	Genotype and/or relevant phenotypic characteristics[a]	Comments[a]	Reference(s)[b]
ATCC 19424	Met$^-$ Dam$^-$, PIA-1,2	Type strain	119, 121
F62[c]	*proA sac-3* (Sac^s), Lf$^-$ Dam$^-$, PIB-7, antibiotic susceptible	More readily transformed than other gonococcal strains	38, 58, 78, 97, 110, 119, D
FA19[d]	Prototrophic, *sac-1 sac-3* (Sac^r), Lf$^+$, PIA-1, methylation of the DNA sequence GGCC, antibiotic susceptible	Used for much of the genetic mapping of the gonococcal chromosome	78, 93, 97, 105, C, E
FA1090[e]	Pro$^-$ Str^r but susceptible to other antibiotics, Sac^r, PIB	Used for studies of protein II antigenic and phase variation; monoclonal antibodies specific for various protein II types have been made	9, B, C
MS11$_{ms}$[f]	Prototrophic; *pilE1*$^+$ *pilE2*$^+$, PIB-9, Str^r; methylation of DNA sequences PuGCGCPy, CCGCGG, and GCCGGC; restriction endonuclease activity against DNA sequence GCCGGC	Used for studies of pilin expression and antigenic variation	28, 75, A, C, E
P9	Dam$^-$; methylation of DNA sequences PuGCGCPy, GGCC, CCGCGG, GCCGGC, GGNNCC; restriction endonuclease activity against DNA sequences GGCC, CCGCGG	Used for studies of pilin antigenic variation; isogenic variants of P9 have been isolated that produce immunologically distinct pili	28, 65, 84, 114
1342[g]	Arg$^-$, PIB-1	Prototype strain for LPS serotype GC$_1$	1–4, 38
1291[g]	Prototrophic, PIB-1	Prototype strain for LPS serotype GC$_2$	1–4, 38
4505[g]	Prototrophic, PIB-3	Prototype strain for LPS serotype GC$_3$	1–4, 38
8551[h]	Pro$^-$, PIB-4	Prototype strain for LPS serotype GC$_4$	1–4, 38, 66
PID2	Prototrophic, PIB-3	Prototype strain for LPS serotype GC$_5$	3, 4, 38
3893	Prototrophic, PIA-1	Prototype strain for LPS serotype GC$_6$	3, 4, 38
JW31R	Prototrophic, pyocin resistant, serum sensitive	Pyocin-resistant mutant of strain JW31 which has lost the LPS variable and serotype antigens but has retained the common LPS determinants	81

[a] Symbols: *proA*, requirement for proline; *sac*, resistance to the bactericidal activity of normal human serum; Lf, utilization of lactoferrin as a sole source of iron; Dam, methylation of adenine in GATC sequences; PI, protein I serovar (118); Str^r, streptomycin resistance; *pilE*, pilin expression locus; Arg$^-$, Met$^-$, and Pro$^-$, requirement for arginine, methionine, or proline, respectively; LPS, lipopolysaccharide.

[b] Numbers refer to Literature Cited. Letters refer to abstracts or personal communications from the listed individual(s): (A) R. H. Chien, D. C. Stein, H. S. Seifert, K. Floyd, and M. So, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, K41, p. 213; (B) J. G. Cannon; (C) N. Carbonetti; (D) P. F. Sparling; (E) D. C. Stein.

[c] *N. gonorrhoeae* F62 was isolated from the urethra of a female at the Fulton County Health Department, Atlanta, Ga., in 1962. Human volunteers were infected with this strain to demonstrate that virulence was genetically linked to clonal variation (58).

[d] *N. gonorrhoeae* FA19 is a urogenital isolate from the Far East (P. F. Sparling, personal communication). It is strain Ceylon 3 from the collection of A. Reyn and was obtained from her by P. F. Sparling in 1970. It was lyophilized by A. Reyn in 1962. The antibiotic susceptibilities of FA19 are typical of those from strains isolated in the preantibiotic era and are as follows (expressed in micrograms per milliliter): penicillin, 0.007; tetracycline, 0.25; chloramphenicol, 0.5; erythromycin, 0.25; rifampin, 0.12; fusidic acid, 0.12; Triton X-100, 0.5; acridine orange, 100; and crystal violet, 4.0 (93, 94, 105).

[e] *N. gonorrhoeae* FA1090 is a cervical isolate from a patient with disseminated gonococcal infection and was isolated in North Carolina in 1976 (J. G. Cannon, personal communication).

[f] *N. gonorrhoeae* MS11 is from the collection of E. Gotschlich, The Rockefeller University, New York, N.Y., and was initially isolated at the Mount Sinai School of Medicine, New York, N.Y., in 1970. MS11$_{ms}$ was obtained from the collection of E. Gotschlich by M. So and T. F. Meyer (77). *N. gonorrhoeae* MS11$_{mk}$ differs from MS11$_{ms}$ in that the *pilE2* locus is deleted. MS11$_{mk}$ was obtained by J. M. Koomey and J. Swanson from stocks supplied by G. Schoolnik, who serially passaged them from the collection of E. Gotschlich (116).

[g] Isolated in Buffalo, N.Y. (38).

[h] Urogenital isolate from Norway (66).

CLONED *N. GONORRHOEAE* GENES AND CODON USAGE

The ability to clone genes that are readily expressed in *E. coli* has greatly enhanced the identification of genetic loci in *N. gonorrhoeae*. The genes that have been cloned from *N. gonorrhoeae* are listed in Table 3. A variety of vectors, including lambda bacteriophage derivatives, plasmids, cosmids, and phasmids, have been used to clone gonococcal genes. The plasmid pBR322 has been used effectively for cloning genes that are not lethal to *E. coli*, whereas lambda gt11 has proven effective for cloning portions of the protein I (*por*) gene and for cloning the protein III (*rmp*) gene. It appears to be necessary to use restriction-deficient *E. coli* strains (McrB$^-$ HsdR$^-$), in most cases, to obtain high cloning efficiencies (13, 92, 109, 114).

Several of the cloned gonococcal genes have been sequenced, and the *N. gonorrhoeae* codon usage for these genes is listed in Table 4. Codon usage tables are useful for identifying protein-coding regions in a nucleotide sequence (47, 108), locating deoxyribonucleic acid (DNA) sequencing errors (47, 108), and constructing oligonucleotide probes from amino acid sequences. In *E. coli* there is a different pattern of codon usage depending on the level of expression of a particular gene in a cell (46, 48). Even though the 11 gonococcal genes which were examined could all be considered highly expressed genes (i.e., present in more than 10 protein molecules per cell, as opposed to the small number of regulatory protein molecules per cell), the *N. gonorrhoeae* codon usage pattern most resembles that of *E. coli* non-highly-expressed genes, such as the lambda and *lac* repressors. This difference in codon usage cannot be explained by differences in guanine-plus-cytosine content, since the guanine-plus-cytosine content of *N. gonorrhoeae* is similar to that of *E. coli*. The pattern of codon usage in the pilin genes is most different from that in the other gonococcal genes examined, with a much more biased use of cytosine in codon position 3. Codons which are rarely used by *N. gonorrhoeae* are glycine (GGG), arginine (AGG, AGA, and CGA), and leucine (CTA).

TABLE 2. Genetic markers of *N. gonorrhoeae*

Gene symbol	Mnemonic	Linkage association[a]	Alternative gene symbols; phenotypic trait affected	Reference(s)[b]
aniA	Anaerobically induced		Major anaerobically induced outer membrane protein (Pan 1)	25
ampA	Ampicillin	*cap*	Low-level resistance to ampicillin	55
ampB	Ampicillin	*str, fus*	Low-level resistance to ampicillin	55
ampC	Ampicillin	*rif, str*	Low-level resistance to ampicillin	55
ampD	Ampicillin	*rif*	Low-level resistance to ampicillin	55
argA	Arginine		Complementation of *argA* mutation of *E. coli* NK5992 and W3421; acetylglutamate synthetase	23, I
argB	Arginine		Complementation of *argB* mutation of *E. coli* 30SOMA4	I
argE	Arginine	*penB, hyx*	*argJ*; complementation of *argE* mutation of *E. coli* AB1157 and AT2538; ornithine acetyltransferase	5, 22, 23, 98, F, I
argF	Arginine		*argI*; complementation of *argI* mutation of *E. coli* N166 and *argF argI* mutation of *E. coli* N134; ornithine transcarbamylase	22, 98, F, I
argG	Arginine		Complementation of *argG* mutation of *E. coli* UQ27	23, I
cap	Chloramphenicol	*tet, spc*	*cam, chl*; low-level resistance to chloramphenicol	68, 93, 105
carA		*carB*	*car-1*; requirement for ornithine or arginine; complementation of *carA* mutation of *E. coli* FP178; small subunit of carbamoyl-phosphate synthetase	22, 99, I
carB		*carA*	*car-2*; requirement for ornithine or arginine; complementation of *carB* mutation of *E. coli* JEF8; large subunit of carbamoyl-phosphate synthetase	22, 99, I
dcmA[c]	DNA cytosine methylase		DNA methylase M.*Ngo*PI; recognition sequence 5′-PuGCGCPy-3′	28, 114
dcmB[c]	DNA cytosine methylase		DNA methylase M.*Ngo*PII; recognition sequence 5′-GGCC-3′	28, 114, 115
dcmD[c]	DNA cytosine methylase	*dcrD*	DNA methylase M.*Ngo*MI; recognition sequence 5′-GCCGGC-3′	28, D, E
dcmE[c]	DNA cytosine methylase	*dcrE*	DNA methylase M.*Ngo*BIII; recognition sequence 5′-GGNNCC-3′	28, J
dcrD[c]		*dcmD*	Restriction endonuclease R.*Ngo*MI; recognition sequence 5′-GCCGGC-3′	28, D, E
dcrE[c]		*dcmE*	Restriction endonuclease R.*Ngo*BIII; recognition sequence 5′-GGNNCC-3′	28
dud	DNA uptake deficient		Failure to take up DNA into a deoxyribonuclease (DNase)-resistant state; abnormal colony morphology	10a, C
env-1	Envelope	*env-3*	*envA*; nonspecific increased sensitivity to antibiotics, dyes, and detergents; complete phenotypic suppression of *mtr* and *penB*; 40% decrease in cross-linking of peptidoglycan; fivefold reduction in quantity of 52,000 mol wt outer membrane protein	35, 50, 71, 94, 106
env-3	Envelope	*env-1*	*envB*; nonspecific increased susceptibility to antibiotics, dyes, and detergents; 20% decrease in cross-linking of peptidoglycan	35, 50, 71, 94, 106
env-10	Envelope		Increased susceptibility to drugs, dyes, and detergents	96
ery	Erythromycin	*spc*	Low-level resistance to erythromycin	68
fbp	Iron-binding protein		Structural gene for an iron-binding outer membrane protein (37,000 daltons)	80, A
frp	Iron-repressible protein		Family of outer membrane proteins expressed under conditions of iron starvation	85, 123
fhuB	Ferric hydroxamate uptake		Complementation of *fhuB* mutation of *E. coli* BN3307 and BU736	124
fud	Iron uptake deficient		Deficient in iron acquisition	*
fus	Fusidic acid	*str, tet*	Resistance to fusidic acid	104
hsp	Heat shock protein		Family of proteins whose expression increases after a shift up in temperature	59
hyx	Hypoxanthine	*argE*	Requirement for hypoxanthine	5, 72
iga	Immunoglobulin A		Immunoglobulin A protease	61, 90
laz	Lipid-modified azurin		Azurin-related outer membrane protein; recognized by monoclonal antibody H.8	42, 43

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TABLE 2—*Continued*

Gene symbol	Mnemonic	Linkage association[a]	Alternative gene symbols; phenotypic trait affected	Reference(s)[b]
ldhA	Lactate dehydrogenase		Membrane-bound lactate dehydrogenase	B
ldhB	Lactate dehydrogenase		Soluble lactate dehydrogenase	B
lip	Lipoprotein		Outer membrane protein recognized by monoclonal antibody H.8	12, 53, L
lps-1	Lipopolysaccharide synthesis		*los*; produces new lipopolysaccharide band in *E. coli* HB101 which is recognized by antigonococcal antiserum	86
lps-2	Lipopolysaccharide synthesis		*los*; biosynthesis of lipopolysaccharide; complementation of pyocin-resistant phenotype of *N. gonorrhoeae* FA5100	G
lps-3	Lipopolysaccharide synthesis		*los*; biosynthesis of lipopolysaccharide; complementation of pyocin-resistant phenotype of *N. gonorrhoeae* FA5100	G
mom	Modifier of *mtr*	*penB*	Phenotypic suppression of *mtr*; reduction of resistance to benzylpenicillin, oxacillin, erythromycin, and novobiocin mediated by *mtr*	100
mtr	Multiple transformable resistance		*ery-2*; low-level resistance to penicillin, tetracycline, erythromycin, chloramphenicol, rifampin, and fusidic acid and increased resistance to dyes and detergents; fivefold-increased amount of a 52,000-dalton outer membrane protein; 30% increase in cross-linking of peptidoglycan	40, 49, 50, 67–69, 103, 105, 106, 122
metB	Methionine		Complementation of *metB* mutation of *E. coli* χ342	89
nsr[d]	Nonspecific resistance		*mtr*; low-level resistance to antibiotics; does not alter the amounts of a 52,000-dalton membrane protein as does *mtr*	14, 19
ntr-2	Nontransformable		Inability to be transformed by plasmid or chromosomal DNA; normal DNase-resistant DNA uptake; normal colony morphology	10a, C
ntr-5	Nontransformable		Inability to be transformed by chromosomal DNA; normal DNase-resistant DNA uptake	10a, C
omc	Outer membrane complex		Structural gene for the outer membrane protein-macromolecular complex (76,000 daltons)	82, K
opa	Opacity	*pilE1*[e]	Outer membrane protein II structural gene	26, 74, 95, 113
oxiA	Oxygen induced		Oxygen-induced outer membrane protein (28,000 daltons)	25, 36, 37
pem	Penicillin modifier		Modifier gene affecting resistance to penicillin	121
penA	Penicillin		Penicillin-binding protein PBP-2; penicillin-susceptible enzyme involved in peptidoglycan synthesis at cell division; four- to eightfold increased resistance to penicillin without affecting response to other antimicrobial agents	7, 31, 32, 40, 103, 105, 106, 107
penB	Penicillin	*por*, *spc*	Nonspecific low-level resistance to penicillin, tetracycline, and nalidixic acid	24, 27, 40, 100, 103, 105, 106
pilA	Pilin	*pilE*	Gene product activates the *pilE* promoter in *E. coli*	117
pilB	Pilin	*pilE*	Gene product negatively regulates *pilE* promoter; insertional inactivation of *pilB* results in hyperpiliated gonococci	117
pilE	Pilin	*opa*[e]	Expressed locus for pilin structural gene	74, 75, 77, 113
pilS	Pilin	*opa*[e]	Silent (nonexpressed) locus for pilin structural gene	51, 74, 75, 113
por	Porin	*spc*, *penB*	*nmp*; outer membrane protein I structural gene	17, 20, 21, 45
proA	Proline	*proB*	Complementation of *proA* mutation of *E. coli* χ463	110
proB	Proline	*proA*	Complementation of *proB* mutation of *E. coli* χ340	110
proC	Proline		Complementation of *proC* mutation of *E. coli* χ278	H
pyrB	Pyrimidine		Aspartate transcarbamylase	99
pyrE	Pyrimidine		*pyr-2*; orotate phosphoribosyltransferase	99
recA	Recombination		Homologous recombination and DNA repair	63
rif	Rifampin	*ura*, *str*	Resistance to rifampin	5, 93
rmp	Reduction modifiable protein		Outer membrane protein III structural gene	42, 44
sac-1	Serum-antibody-complement	*penB*	Resistance to the bactericidal activity of normal human serum	18
sac-3	Serum-antibody-complement	*penB*	Resistance to the bactericidal activity of normal human serum; alteration in lipopolysaccharide structure	97, 112

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TABLE 2—*Continued*

Gene symbol	Mnemonic	Linkage association[a]	Alternative gene symbols; phenotypic trait affected	Reference(s)[b]
spc	Spectinomycin	*cap*, *penB*	High-level resistance to spectinomycin; alteration of 30S ribosomal subunit	70, 93
str	Streptomycin	*rif*, *fus*	High-level resistance to streptomycin; alteration of 30S ribosomal subunit	68, 70, 93
tem	Tetracycline modifier		Modifier gene affecting resistance to tetracycline and penicillin	122
tet	Tetracycline	*fus*, *cap*	Low-level resistance to tetracycline	68, 93, 105
trpE	Tryptophan		Complementation of *trpE* mutation of *E. coli* K-12 χ478	J
tsg	Temperature-sensitive growth		Family of uncharacterized mutations with the common phenotype of no growth or poor growth at 37°C	111, 125
ura	Uracil	*rif*	Requirement for uracil	5, 72
vel			Hypersusceptibility to vancomycin and erythromycin	60
vnc	Vancomycin		Hypersusceptibility to vancomycin	60

[a] Linkage associations refer to the linkage relationships diagrammed in Fig. 1 or to genes shown to be physically linked.

[b] Numbers refer to Literature Cited. The asterisk refers to a gene which has not yet been shown to exist in *N. gonorrhoeae*; however, researchers at the Sixth International Pathogenic Neisseria Meeting agreed that this nomenclature should be used. Letters refer to abstracts or personal communications from the listed individuals: (A) S. A. Berish, T. A. Mietzner, and S. A. Morse; (B) R. A. Jensen, R. K. Bhatnagar, and A. T. Hendry; (C) G. Biswas, S. Lacks, and P. F. Sparling; (D) R_L Chien, A. Pierkarowicz, and D. Stein; (E) R. H. Chien, D. C. Stein, H. S. Seifert, K. Floyd, and M. So., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, K41, p. 213; (F) P. R. Martin, D. A. Simpson, and M. H. Mulks; (G) E. F. Petricoin and D. C. Stein, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D187, p. 102; (H) F. Picard and J. R. Dillon; (I) F. Picard and J. R. Dillon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D173, p. 100; (J) D. C. Stein; (K) W.-M. Tsai, and C. E. Wilde III; (L) J. P. Woods, S. M. Spinola, S. M. Strobel, and J. G. Cannon.

[c] We have assigned the mnemonic *dcm* to genes which encode a DNA cytosine methyltransferase and the mnemonic *dcr* to corresponding restriction endonuclease genes. The letters *A*, *B*, *C*, *D*, *E*, *G*, and *H* correspond to DNA sequences (methylase specificities) S.*Ngo*I, S.*Ngo*II, S.*Ngo*III, S.*Ngo*IV, S.*Ngo*V, S.*Ngo*VII, and S.*Ngo*VIII, respectively, recognized by a particular cytosine methyltransferase or restriction endonuclease as described by Davies (28).

[d] Cannon and Sparling (19) have designated this locus *nsr* to distinguish it from the phenotypically similar but genetically different locus *mtr*.

[e] In *N. gonorrhoeae* $MS11_{ms}$, three protein II loci, *opaE1*, *opaE2*, and *opaE3*, are adjacent to the unlinked pilin loci, *pilE1*, *pilS5*, and *pilS4*, respectively (74, 113). Schwalbe and Cannon (95) showed by genetic transformation that the six protein II loci in *N. gonorrhoeae* FA1090 are unlinked.

GENETIC MARKERS AND CLONED GENES OF *N. MENINGITIDIS*

Much less work has been done on identification of genetic markers in *N. meningitidis*. Genetic markers of *N. meningitidis* are listed in Table 5. As in *N. gonorrhoeae*, no standard laboratory strain has been used for the majority of these studies. *N. meningitidis* FAM18 (serogroup C, serotype 2A), isolated from the cerebrospinal fluid of a patient with meningococcal septicemia, was used to isolate the gene encoding the H.8 lipoprotein (*lip*) and to demonstrate amino acid and DNA homology between the class 5 outer membrane proteins of *N. meningitidis* and the proteins II of *N. gonorrhoeae* (56); it was also used to identify iron uptake mutants (33).

CONCLUSIONS AND PROSPECTS FOR THE FUTURE

A number of factors have been responsible for the inability to generate a comprehensive genetic map for *N. gonorrhoeae* or *N. meningitidis*. First and foremost is the small number of researchers involved in studies of these organisms as compared with those working with *E. coli*. Second, neither of these neisserial species contains the genetic manipulation systems, conjugation and transduction, that have enabled the interchange of large DNA fragments between strains. The only genetic system available, transformation (102), will demonstrate linkage only if genes are within about 30 to 40 kilobase pairs of each other (19).

A third factor limiting development of a useful genetic map is the difficulty in obtaining mutants. *N. gonorrhoeae* does not contain the error-prone repair systems, found in some other organisms (16), that allow the high-frequency generation of mutants in a mutagenized population. Mutagens such as ultraviolet light or methyl methanesulfonate kill *N. gonorrhoeae* without producing mutants in the surviving population. The effective mutagens, ethyl methanesulfonate and nitrosoguanidine, introduce lesions that result in mispairing and enhance the frequency of gonococcal mutants, but this enhancement occurs at a level significantly below that observed with other bacterial species. In addition to difficulties

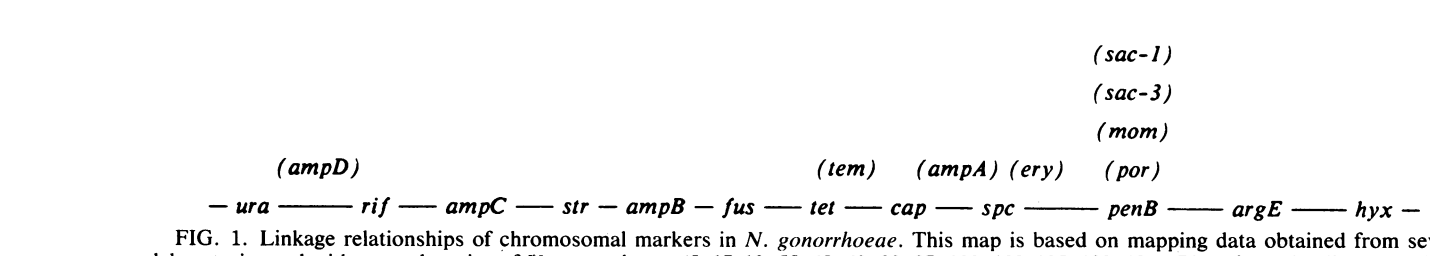

FIG. 1. Linkage relationships of chromosomal markers in *N. gonorrhoeae*. This map is based on mapping data obtained from several laboratories and with several strains of *N. gonorrhoeae* (5, 17–19, 55, 68, 69, 93, 97, 100, 103–105, 122, 126). Therefore, the distances shown do not correlate with actual genetic or physical distances on the gonococcal chromosome. Parentheses around a marker indicate an uncertainty about its position relative to adjacent markers. An explanation of the genetic symbols is given in Table 2.

TABLE 3. Cloned genes from *N. gonorrhoeae*

Gene symbol	Vector	*E. coli* host	*N. gonorrhoeae* parent	Selection or screening method	Reference(s)[a]
argA	λEMBL4	NM539	CH811	Lytic complementation of *argA* mutation of *E. coli* NK5992 and W3421	G
argB	λEMBL4	NM539	CH811	Lytic complementation of *argB* mutation of *E. coli* 30SOMA4	G
argE	λEMBL4	NM539	CH811	Lytic complementation of *argE* mutation of *E. coli* AB1157	G
argE	pUC12	AT2538	CDC50	Complementation of *argE* mutation of *E. coli* AT2538	D
argF	λEMBL4	NM539	CH811	Lytic complementation of *argI* mutation of *E. coli* N166	G
argF	pUC12	N134	CDC50	Complementation of *argF argI* mutation of *E. coli* N134	D
argG	λEMBL4	NM539	CH811	Lytic complementation of *argG* mutation of *E. coli* UQ27	G
carA	λEMBL4	NM539	CH811	Lytic complementation of *carA* mutation of *E. coli* FP178	G
carB	λEMBL4	NM539	CH811	Lytic complementation of *carB* mutation of *E. coli* JEF8	G
dcmA	pBR322	RR1	P9	Resistance of recombinant plasmids to digestion with *Hae*II	114
dcmB	pBR322	RR1	P9	Resistance of recombinant plasmids to digestion with *Hae*III	114
dcmD	pHSS7	GC6	MS11	Resistance of recombinant plasmid to digestion with *Nae*I	C
dcmE	pHC79	HB101	MUG116	Resistance of recombinant plasmids to digestion with *Bam*HI, *Kpn*I, and *Nar*I	B, H
dcrD	pHSS7	GC6	MS11	Resistance of recombinant plasmids to digestion with *Nae*I	C
dcrE	pHC79	HB101	MUG116	Resistance of recombinant plasmids to digestion with *Bam*HI, *Kpn*I, and *Nar*I	B
fbp	pUC13 pUC19	JM105	F62	Hybridization with *fbp*-specific synthetic oligonucleotide probes	A
fhuB	λSE4	RR1	FA19	Complementation of *fhuB* mutation of *E. coli* BU736 and BN3307	124
iga	pREG152	HB101	F62	Cleavage of human immunoglobulin A as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis	61
iga	pBR322	GC1	MS11	Filter assay using radiolabeled human immunoglobulin A antibody bound to nitrocellulose via its secretory component	52
laz	λgt11	Y1089	R10	Immunoblotting with anti-protein I antiserum	42, 43
lip	λsep6	LE392	FA1090	Immunoblotting with monoclonal antibody H.8	12
lps-1	pBR322	HB101	RUN4383	Colony immunoblotting with antigonococcal outer membrane antiserum	86
lps-2	pHC79	HB101	MUG116	Complementation of pyocin-resistant phenotype of *N. gonorrhoeae* FA5100	E
lps-3	pHC79	HB101	MUG116	Complementation of pyocin-resistant phenotype of *N. gonorrhoeae* FA5100	E
metB	pLES2	χ342	JW31	Complementation of *metB* mutation of *E. coli* χ342	89
nal	pHC79	HB101	MUG116	Transformation of cosmid library into *N. gonorrhoeae* FA5100 with selection for nalidixic acid resistance	H, I
omc	λgt11 and λEMBL3	Y1090 and Y1088	2686	Immunoblotting with antigonococcal outer membrane protein-macromolecular complex antiserum	J
opa	pBR322	GC1[b]	$MS11_{ms}$	Colony immunoblotting with anti-MS11 protein II antiserum	113
opa	pBR322	HB101	FA1090	Colony radioimmunoassay with monoclonal antibodies specific for FA1090 protein II variants	26
oxiA	pBR322	HB101	RUN4007	Colony immunoblotting with antigonococcal outer membrane antiserum	36, 37
penA	pBG5131	TG1	CDC77-124615	Transformation of *N. gonorrhoeae* FA19 to increased penicillin resistance (sixfold)	107
penA	pBG5131	TG1	LM306, FA19, CDC84-060418, and CDC84-060384	Hybridization with *penA* gene from CDC77-124615	107
pilA	pBR322	GC1	$MS11_{ms}$	Characterization of previously isolated clones containing *pilE* and *opaE1*	117
pilB	pBR322	GC1	$MS11_{ms}$	Characterization of previously isolated clones containing *pilE* and *opaE1*	117
pilE1[c]	pBR322	GC1	$MS11_{ms}$	Colony immunoblotting with anti-pilin antiserum	77
pilE[c]	pBR322	GC1	P9	Colony immunoblotting with anti-pilin monoclonal antibody SM1	87
por	pGEM-2	HB101	FA19	Hybridization with protein IA-specific synthetic oligonucleotide probes	21
por	pGEM-3 and λgt11	HB101	MS11	Hybridization with protein I-specific synthetic oligonucleotide probes	20
por	λgt11	Y1089	R10	Immunoblotting with anti-R10 protein 1B monoclonal antibodies	45
proA	pLES2	JM83	KH45	Complementation of *proAB* mutation of *E. coli* JM83	110
proB	pLES2	JM83	KH45	Complementation of *proAB* mutation of *E. coli* JM83	110

Continued on following page

TABLE 3—*Continued*

Gene symbol	Vector	*E. coli* host	*N. gonorrhoeae* parent	Selection or screening method	Reference(s)[a]
proC	pGEM-3	χ478	CH811	Complementation of *proC* mutation of *E. coli* χ478	F
rmp	λgt11	Y1089	R10	Immunoblotting with polyclonal anti-protein I antiserum	42
sac-4[d]	pHC79	HB101	JC1	Transformation of *N. gonorrhoeae* F62 to serum resistance	73
recA	pREG153	HB101	$MS11_{mk}$	Complementation of *recA* mutation of *E. coli* HB101	63
rif	pHC79	HB101	MUG116	Transformation of cosmid library into *N. gonorrhoeae* FA5100 with selection for rifampin resistance	H
trpE	pLES2	JM83	JW31	Complementation of *trpE* mutation of *E. coli* K-12 χ478	H

[a] Numbers refer to Literature Cited. Letters refer to abstracts or personal communications from the listed individuals: (A) S. A. Berish, T. A. Mietzner, and S. A. Morse; (B) R. Chien, A. Piekarowicz, and D. Stein; (C) R. H. Chien, D. C. Stein, H. S. Seifert, K. Floyd, and M. So, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, K41, p. 213; (D) P. R. Martin, D. A. Simpson, and M. H. Mulks; (E) E. F. Petricoin and D. C. Stein, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D187, p. 102; (F) F. Picard and J. R. Dillon; (G) F. Picard and J. R. Dillon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D173, p. 100; (H) D. C. Stein; (I) D. C. Stein and T. Cook; (J) W.-M. Tsai and C. E. Wilde III.

[b] *E. coli* GC1 is ($K\text{-}12_{r}^{-}{}_{m}^{+}$) (52, 77).

[c] Additional expressed and silent pilin genes have been cloned from strains MS11 (51, 75) and P9 (83).

[d] We have designated the serum resistance allele of *N. gonorrhoeae* JC1 *sac-4*. This allele may be identical to the previously described *sac-3* allele in *N. gonorrhoeae* F62 (97).

TABLE 4. Codon usage in *N. gonorrhoeae*[a]

Amino acid	Codon[b]	No.[c]	No./1,000[d]	Fraction[e]	Amino acid	Codon	No.	No./1,000	Fraction
Gly	GGG	31	6.34	0.07	Trp	TGG	52	10.63	1.00
Gly	GGA	55	11.24	0.13	End	TGA	10	2.04	0.50
Gly	GGT	103	21.05	0.25	Cys	TGT	8	1.63	0.20
Gly	GGC	228	46.6	0.55	Cys	TGC	32	6.54	0.80
Glu	GAG	40	8.17	0.18	End	TAG	1	0.2	0.05
Glu	GAA	187	38.22	0.82	End	TAA	9	1.84	0.45
Asp	GAT	121	24.73	0.44	Tyr	TAT	87	17.78	0.51
Asp	GAC	152	31.06	0.56	Tyr	TAC	82	16.76	0.49
Val	GTG	54	11.04	0.17	Leu	TTT	106	21.66	0.30
Val	GTA	67	13.69	0.21	Leu	TTA	49	10.01	0.14
Val	GTT	104	21.25	0.33	Phe	TTT	76	15.53	0.45
Val	GTC	93	19.01	0.29	Phe	TTC	94	19.21	0.55
Ala	GCG	82	16.76	0.17	Ser	TCG	33	6.74	0.10
Ala	GCA	131	26.77	0.27	Ser	TCA	54	11.04	0.16
Ala	GCT	64	13.08	0.13	Ser	TCT	85	17.37	0.25
Ala	GCC	205	41.90	0.43	Ser	TCC	76	15.53	0.22
Arg	AGG	23	4.70	0.07	Arg	CGG	49	10.01	0.16
Arg	AGA	28	5.72	0.09	Arg	CGA	29	5.93	0.09
Ser	AGT	21	4.29	0.06	Arg	CGT	67	13.69	0.22
Ser	AGC	73	14.92	0.21	Arg	CGC	112	22.89	0.36
Lys	AAG	58	11.85	0.15	Gln	CAG	78	15.94	0.35
Lys	AAA	331	67.65	0.85	Gln	CAA	146	29.84	0.65
Asn	AAT	106	21.66	0.37	His	CAT	51	10.42	0.45
Asn	AAC	177	36.17	0.63	His	CAC	63	12.88	0.55
Met	ATG	60	12.26	1.00	Leu	CTG	78	15.94	0.22
Ile	ATA	27	5.52	0.15	Leu	CTA	25	5.11	0.07
Ile	ATT	67	13.69	0.36	Leu	CTT	62	12.67	0.17
Ile	ATC	90	18.39	0.49	Leu	CTC	38	7.77	0.11
Thr	ACG	53	10.83	0.20	Pro	CCG	74	15.12	0.37
Thr	ACA	48	9.81	0.18	Pro	CCA	35	7.15	0.17
Thr	ACT	45	9.20	0.17	Pro	CCT	44	8.99	0.22
Thr	ACC	117	23.91	0.44	Pro	CCC	47	9.61	0.23

[a] The program CodonFrequency from the University of Wisconsin Genetics Computer Group (30) was used to generate codon usage tables from each of the *N. gonorrhoeae* genes in the GenBank (release 56, 7/88) and EMBL (release 15, 4/88) data bases (10, 15). A reference codon usage table was generated from the individual codon usage tables for the following genes: *pilE1* and *pilS1* from $MS11_{ms}$ (51, 75); three protein II genes from FA1090 (26); the protein I gene from FA19 (21); *penA* from CDC77-124615 (107); the gene for an azurin-related protein from MS11 (43); the gene for protein III from R10 (44); the immunoglobulin A protease gene from $MS11_{ms}$ (90); and the M.*Ngo*PII DNA methyltransferase gene from P9 (115).

[b] Total number of codons, 4,893.

[c] The number of times a codon occurred in the examined sequences.

[d] The number of times a specific codon would occur per 1,000 codons.

[e] The ratio of the number of occurrences of a specific codon to the number of occurrences of all codons in the same synonymous codon group.

in obtaining mutants, *N. gonorrhoeae* does not contain a variety of genes that could be mutated that are not critical to the growth of the organism, such as the variety of genes involved in catabolism of alternative fuel sources that are found in *E. coli*. Thus, there are fewer mutations available to serve as genetic markers in mapping studies in *N. gonorrhoeae*.

The advent of recombinant DNA techniques has been invaluable to the studies of genes in both *N. gonorrhoeae* and *N. meningitidis*; these techniques have proved to be a useful alternative to classical genetic techniques. We expect that the isolation of neisserial genes will continue and will greatly expand the number of identified genes available for linkage studies. New technologies are continuing to become available, and the one that may prove the most useful in studies of the genetic organization of *N. gonorrhoeae* and *N. meningitidis* is pulsed-field electrophoresis. The discovery of restriction enzymes that recognize 8-base-pair sequences and the ability to separate very large DNA fragments by altering the orientation of the electric field during agarose gel electrophoresis has enabled researchers to physically map the *E. coli* chromosome (101). It should also be possible to generate physical maps of the gonococcal and meningococcal chromosomes by this technique, especially since the neisserial chromosomes are only about 50% of the size of the *E. coli* chromosome (19). Once such maps are developed, the position of a cloned gene on the map could be easily determined by DNA-DNA hybridization.

If physical maps are developed for *N. gonorrhoeae*, we strongly suggest that they be done with one or more of the gonococcal strains that are the most frequently studied (e.g., see Table 1). We also urge investigators to use one of these strains as the source of DNA for their cloning experiments, whenever possible. This would allow mapping of the location of the cloned gene to one or more of the fragments whose position is known on the physical map. Both *N.*

TABLE 5. Genetic markers of *N. meningitidis*

Gene symbol	Mnemonic	Phenotypic trait affected or cloned gene	Cloning or mutagenesis strategy	Reference(s)[a]
cps-1	Capsule	Biosynthesis and assembly of group B polysaccharide capsule	pCOS2EMBL library of *N. meningitidis* B1940 in *E. coli* GC6 screened with monoclonal antibody	76
cps-2	Capsule	5′-Monophospho-*N*-acetyl neuraminic acid synthetase	pACYC184 library of *N. meningitidis* group B was screened by complementation of the corresponding mutation in *E. coli* EV5	D
cps-3	Capsule	*N*-Acetylneuraminic acid synthetase	pACYC184 library of *N. meningitidis* group B was screened by complementation of the corresponding mutation in *E. coli* EV5	D
fbp	Iron-binding protein	Iron-binding outer membrane protein (37,000 daltons); detected by immunoblotting with gonococcal anti-*fbp* antisera		80
frp	Iron-repressible protein	Family of outer membrane proteins expressed under conditions of iron starvation		11, 33, 34
fud-2	Iron uptake deficient	Deficient in iron acquisition from transferrin	Ethyl methanesulfonate mutagenesis of FAM20 (a Nal^r derivative of FAM18) followed by streptonigrin enrichment	33, 120
fud-3	Iron uptake deficient	Deficient in iron acquisition from transferrin and hemoglobin	Ethyl methanesulfonate mutagenesis of FAM20 followed by streptonigrin enrichment	33
fud-4	Iron uptake deficient	Deficient in iron acquisition from ferric dicitrate, transferrin, lactoferrin, hemin, and hemoglobin	Streptonigrin enrichment of log-phase culture of FAM20	33
fud-5	Iron uptake deficient	Deficient in iron acquisition from ferric dicitrate, transferrin, lactoferrin, and hemin	Streptonigrin enrichment of log-phase culture of FAM20	33
hga	Hemoglobin growth alteration	Altered ability to grow with hemoglobin as sole iron source	Streptonigrin enrichment of log-phase culture of FAM20	33, 120
iga	Immunoglobulin A	Immunoglobulin A protease	pBR325 library of *N. meningitidis* 15894 in *E. coli* HB101 screened by hybridization with gonococcal immunoglobulin A protease probe	62
laz	Lipid-modified azurin	Azurin-related outer membrane protein	pBR322 library of *N. meningitidis* FAM18 in *E. coli* HB101 screened by colony immunoblotting with monoclonal antibody H.8	57
omc	Outer membrane complex	Structural gene for *N. gonorrhoeae omc* hybridizes to *N. meningitidis* DNA		E
opa	Opacity-associated protein	Class 5 outer membrane protein; shares homology with *N. gonorrhoeae opa* including pentameric repeat	pBR325 library of *N. meningitidis* FAM18 in *E. coli* HB101 screened by colony blot radioimmunoassay with monoclonal antibodies H.21 and H.22	41, 56
oxiA	Oxygen induced	Structural gene for *N. gonorrhoeae oxiA* hybridizes to *N. meningitidis* DNA and anti-OxiA antiserum reacts with a meningococcal protein of the same size		37
pilE	Pilin	Expressed class I pilin locus	pBR322 library of *N. meningitidis* C311 in *E. coli* GC1 screened by hybridization with oligonucleotide to the coding sequence of the SM1 epitope of gonococcal pilin	91
pilS	Pilin	Silent (nonexpressed) class II pilin locus	pBR322 library of *N. meningitidis* C114 in *E. coli* GC1 screened by hybridization with *pilE* gene of *N. gonorrhoeae* P9-2	88
porA	Porin	Class 1 outer membrane protein structural gene	λgt11 library of *N. meningitidis* MC50 in *E. coli* Y1090 screened by immunoblotting with antiserum against purified outer membranes	8, 41, A
porB	Porin	Class 2 and 3 outer membrane proteins; shares DNA homology with *N. gonorrhoeae por*; antibody to gonococcal protein IB cross-reacts with class 2 proteins	λgt11 library of group B *N. meningitidis* screened with a monoclonal antibody against *N. gonorrhoeae* protein IB and by hybridization with a synthetic oligonucleotide	41, C
rmp	Reduction-modifiable protein	Class 4 outer membrane protein; shares DNA homology with *N. gonorrhoeae rmp*; antibody to gonococcal protein III cross-reacts with class 4 proteins	λgt11 library of *N. meningitidis* BNCV was screened by immunoblotting with a monoclonal antibody to gonococcal protein III	41, B

[a] Numbers refer to Literature Cited. Letters refer to personal communications from the following individuals: (A) A. K. Barlow, J. E. Heckels, and I. N. Clarke; (B) K. P. Klugman and E. C. Gotschlich; (C) K. Murakami and E. C. Gotschlich; (D) C. Reid, S. Ganjuli, T. Wallis, I. Roberts, and G. Boulnois; (E) C. E. Wilde III.

gonorrhoeae and *N. meningitidis* have been shown by restriction length polymorphism studies to have a high degree of sequence variability (39, 64). Thus, it is anticipated that the physical map determined by using pulsed-field electrophoresis of large fragments will be different for every strain. The use of only a few strains in developing these maps and in cloning experiments will allow researchers from different laboratories to compare their data much more readily and thus enable a more rapid analysis of neisserial gene organization. We further suggest that the above-mentioned strains be deposited into the American Type Culture Collection and the National Collection of Type Cultures to make them more readily available to the general research community.

ACKNOWLEDGMENTS

We thank the researchers, too numerous to list, who provided us with unpublished information that was vital to the compilation of genetic markers and cloned genes in *N. gonorrhoeae* and *N. meningitidis*. We specifically thank P. F. Sparling for his helpful suggestions during the preparation of this review.

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