CLINICAL MICROBIOLOGY REVIEWS, Apr. 1989, p. S78–S82
0893-8512/89/020S78-05$02.00/0

Vol. 2, No. 2, Suppl.

DNA Restriction and Modification Systems in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae appears to be a genetically isolated species. There are no known bacteriophages capable of infecting this organism, and although most gonococcal strains carry plasmids, the variety of those plasmids is amazingly small. Only two plasmid species might be described as native to the gonococcus (1). The only other deoxyribonucleic acid (DNA) molecules that have ever been shown to enter this species are the penicillinase-producing plasmids (which probably originated in a *Haemophilus* species) (1), and the *tetM* determinant (which probably entered via *Mycoplasma* or *Ureaplasma* species) (10).

Despite this rigid barrier against the entry of foreign DNA, gonococci are highly competent for transformation (1), and it has been suggested that transformation may be the genetic system that results in the antigenic variation of several gonococcal surface components (9, 12). Thus, gonococci appear to have evolved a system that rigidly excludes foreign DNA, but still allows the free exchange of genetic information within the species. Exclusion of foreign DNA could be achieved by an efficient restriction barrier. Restriction barriers result from the production of restriction endonucleases which bind to, and cleave, a specific DNA sequence (17). Most restriction endonucleases will cleave unmodified DNA sequences only, and a cell that is producing a restriction enzyme will protect its own DNA from degradation by simultaneously producing a modification enzyme that binds to, and methylates, the DNA sequence recognized by the restriction enzyme (11).

Any variation, within the species, in these restriction modification systems might be expected to limit genetic exchange during mixed infections and perhaps some of the sequence diversity generated during antigenic variation (9). One such barrier to intraspecies genetic exchange has been directly demonstrated and seems to act specifically on DNA transferred by transformation, rather than conjugation (19, 20). This reflects the nature of the incoming DNA during these two genetic exchange processes. DNA is single stranded as it enters the recipient cell during conjugation and is therefore resistant to the action of most restriction enzymes. On the other hand, DNA fragments are taken up as double-stranded molecules during transformation of the gonococcus (1).

The true extent to which such barriers limit genetic exchange within this species was unknown, and the result has been an increased interest in characterizing both the number and the distribution of the gonococcal restriction modification systems.

REVISION OF NOMENCLATURE

Recently, an agreement was reached among the various laboratories involved in isolating restriction enzymes and methylases from *Neisseria gonorrhoeae* to revise the names of the gonococcal enzymes so that the nomenclature system properly reflects that used for all other bacterial species (17).

Restriction Endonucleases

The revised names for the previously characterized gonococcal restriction enzymes that had been misnamed are shown in Table 1. The new names contain a reference to the particular strain from which a restriction enzyme was isolated. An enzyme activity denoted R.*Ngo*X (4; M. K. Duff, M.Sc. thesis, Monash University, Clayton, Australia, 1986), has since been shown to consist of a mixture of two restriction enzymes, isoschizomers of R.*Hae*II and R.*Hph*I (4a).

Methylases

Korch et al., in an analysis of the DNA sequence of the gonococcal cryptic plasmid pJD1 from strain 82409/55, were able to obtain evidence that specific bases were methylated (5–7). The presence of these bases in palindromic sequences was used to suggest the presence of seven different type II methylases (6). A single incidence of a methylated base in a nonpalindromic sequence was suggested to be the result of the action of a type III methylase (7). Each of these putative methylases was given a specific name (6, 7); this has raised several nomenclature problems. No methylases have actually been characterized in this strain, and cases of single methylase enzymes with multiple sequence specificities have been reported (24). Again, no reference to the particular strain was made in the methylase names. The revised nomenclature (Table 2) has given names to the methylated DNA sequences; for example, the sequence methylated by the putative methylase M.*Ngo*I has been named S.*Ngo*I, where the S designates a methylase specificity, thus avoiding the issue of whether the enzyme that methylates this particular sequence can also methylate other sequences. The names for the putative methylases in strain 82409/55 have been discarded, and as gonococcal methylases (from this or any other strain) are characterized, they will be given names that indicate the strains from which they were isolated.

RESTRICTION ENZYMES

A number of restriction enzymes have been partially purified from *N. gonorrhoeae* and are listed in Table 3. Thus far, five different specificities have been identified, including a methylation-dependent endonuclease (4, 4a; Duff, M.Sc. thesis). All of the restriction enzymes so far identified are isoschizomers of characterized enzymes from other species (17).

METHYLASES

When DNA is directly extracted from a particular gonococcal strain, it is commonly resistant to cleavage by a variety of restriction endonucleases (4, 5, 13–15, 20, 22, 23, 25). Such cleavage resistance is undoubtedly the result of methylation of restriction enzyme recognition sites, but it is

TABLE 1. Revised nomenclature for gonococcal restriction enzymes

Previous name	Recognition sequence	Isochizomer	Strain	Revised name	Reference(s)
R.*Ngo*I	PuGCGCPy	R.*Hae*II	?[a]	R.*Ngo*WI	17
R.*Ngo*II	GGCC	R.*Hae*III	CDC66	R.*Ngo*CI	2, 3
R.*Ngo*III	CCGCGG	R.*Sac*II	KH7764-45	R.*Ngo*KI	13, 14
			JKD109	R.*Ngo*JI	4; Duff, M.Sc. thesis
			JKD211	R.*Ngo*DI	4; Duff, M.Sc. thesis
R.*Ngo*IX	G^{me}ATC[b]	R.*Dpn*I	JKD211	R.*Ngo*DIII	4, 4a; Duff, M.Sc. thesis
R.*Ngo*X	PuGCGCPy + GGTGA	R.*Hae*II + R.*Hph*I	JKD109	R.*Ngo*JII + R.*Ngo*JIII	4, 4a; Duff, M.Sc. thesis
			JKD211	R.*Ngo*DII + R.*Ngo*DIV	4, 4a; Duff, M.Sc. thesis

[a] Producing strain unknown.
[b] ^{me}A, N^6-Methyladenine.

not possible to use such data to directly infer that a particular strain is producing a methylase with the same specificity as the restriction enzyme used in the assay. The sequence recognized by the methylase may merely be contained within, or overlap, the recognition site for the restriction enzyme. For example, DNA from most gonococcal strains is resistant to cleavage by the restriction enzyme *Not*I, which recognizes the sequence 5′-GCGGCCGC-3′ (17; J. K. Davies, unpublished data). Such cleavage resistance is almost certainly due to the presence of a methylase that recognizes part of the *Not*I sequence, i.e., 5′-GGCC-3′ (see below). Only when a particular strain has been demonstrated to produce an isoschizomer of the restriction enzyme used in the assay is it reasonably safe to infer that it is also producing a methylase with the same specificity. Thus, it is reasonable to assume that the strains listed in Table 3 are also producing methylases with specificities identical to those of the listed restriction enzymes.

Evidence for Methylase Activity

There have been direct demonstrations of methylase activity in extracts from gonococcal cells (16; Duff, M.Sc. thesis). Such extracts have been shown to possess both adenine and cytosine methylase activity (16).

Purified Methylase Activities

Only in a few cases has a methylase been purified to such an extent that a particular sequence specificity can be assigned to an individual enzyme. The enzymes that have been purified, and the sequences that they recognize, are listed in Table 4. Among these is a cytosine methylase, M.*Ngo*BI, with the specificity that Korch suggested might be the target for a type III methylase (7). M.*Ngo*BI, however, shows all the characteristics of a type II enzyme (15) and is part of one of the small group of type II restriction modification systems that recognize nonpalindromic sequences (17). The same strain also produces appreciable amounts of another methylase, M.*Ngo*BII, that methylates an interrupted nonpalindromic sequence (15). The existence of this methylase was entirely unexpected, since the strain concerned does not produce appreciable amounts of a restriction enzyme with the same recognition sequence, nor is there an available isoschizomer that might have been used in an assay for cleavage resistance.

CLONED GENES ENCODING RESTRICTION MODIFICATION SYSTEMS

The other way in which the presence of a restriction modification system can be directly demonstrated is through the cloning of the genes for the enzymes. One advantage of this direct approach (see below) has been the demonstration that such systems can be isolated from strains that do not produce appreciable amounts of the relevant methylase or restriction enzyme.

Cloning Procedures

The procedure used to clone the gonococcal genes is based on a general method that has been used to clone genes from a variety of restriction modification systems. The procedure aims to isolate a methylase gene, but because the relevant restriction enzyme gene is often closely linked, the genes for the entire restriction modification system are often found on the cloned DNA fragment. The method relies on the availability of an isoschizomer for the restriction enzyme concerned and the presence of at least one recognition site for that enzyme in the plasmid cloning vector used. A genomic library is constructed in the cloning vector in *Escherichia coli*, and this construction is followed by a bulk extraction of the recombinant plasmids. This mixture of recombinant plasmids is then digested with the isoschizomeric restriction enzyme and retransformed into *E. coli*. The only recombinant plasmids that should be resistant to cleavage, and therefore able to replicate in *E. coli*, are those that carry the relevant methylase gene.

Cloned Methylase and Restriction Enzyme Genes

The gonococcal restriction enzyme and methylase genes that have been cloned thus far are listed in Table 5. As might be expected, in one case the entire restriction modification system was cloned. It is also worth noting that this approach has been successful with strains that do not produce the relevant methylase in amounts sufficient to allow its purifi-

TABLE 2. Revised nomenclature for gonococcal methylase specificities

Putative methylase name[a]	Methylated sequence	Restriction enzyme acting at same site	Name of methylase specificity
M.*Ngo*I	PuGCGCPy	R.*Hae*II	S.*Ngo*I
M.*Ngo*II	GGCC	R.*Hae*III	S.*Ngo*II
M.*Ngo*III	CCGCGG	R.*Sac*II	S.*Ngo*III
M.*Ngo*IV	GCCGGC	R.*Nae*I	S.*Ngo*IV
M.*Ngo*V	GGNNCC	R.*Nla*IV	S.*Ngo*V
M.*Ngo*VI	GATC	R.*Mbo*I	S.*Ngo*VI
M.*Ngo*VII	GC(G/C)GC	None	S.*Ngo*VII
M.*Ngo*VIII	GGTGA	R.*Hph*I	S.*Ngo*VIII

[a] These names have now been discarded.

TABLE 3. Restriction enzymes isolated from *N. gonorrhoeae*

Specificity	Recognition sequence	Isoschizomer	Strain	Name	Reference(s)
S.*Ngo*I	PuGCGCPy	R.*Hae*II	?[a]	R.*Ngo*WI	17
			JKD109	R.*Ngo*JII	4, 4a; Duff, M.Sc. thesis
			JKD211	R.*Ngo*DII	4, 4a; Duff, M.Sc. thesis
S.*Ngo*II	GGCC	R.*Hae*III	CDC66	R.*Ngo*CI	2, 3
			P9	R.*Ngo*PII	21–23
			Pgh3-2	R.*Ngo*SI	19, 20
S.*Ngo*III	CCGCGG	R.*Sac*II	KH7764-45	R.*Ngo*KI	13, 14
			P9	R.*Ngo*PIII	21–23
			JKD109	R.*Ngo*JI	4; Duff, M.Sc. thesis
			JKD211	R.*Ngo*DI	4; Duff, M.Sc. thesis
S.*Ngo*VIII	GGTGA	R.*Hph*I	JKD109	R.*Ngo*JIII	4, 4a; Duff, M.Sc. thesis
			JKD211	R.*Ngo*DIV	4, 4a; Duff, M.Sc. thesis
			WR302	R.*Ngo*BI	15a, 19
—[b]	G^{me}ATC[c]	R.*Dpn*I	JKD211	R.*Ngo*DIII	4, 4a; Duff, M.Sc. thesis

[a] Producing strain unknown.
[b] —, No specificity name.
[c] ^{me}A = N^6-methyladenine.

cation (R. Chien, A. Piekarowicz, and D. Stein, unpublished data) and that a restriction enzyme gene can be cloned from strains that do not produce identifiable quantities of the enzyme (R. H. Chien, D. C. Stein, K. Floyd, M. So, and H. S. Seifert, unpublished data).

EVIDENCE FOR ADDITIONAL METHYLASE ACTIVITIES

There is evidence to suggest that gonococci possess additional, as yet uncharacterized methylases. First, Stein and co-workers have cloned the gene for a methylase with a specificity different from that of any of the known gonococcal enzymes (D. C. Stein, personal communication). Second, it has been known for some time that some strains of *N. gonorrhoeae* produce an adenine methylase (16; Duff, M.Sc. thesis). As yet, the gene for this methylase has not been cloned, nor has the methylase itself been purified. There is some evidence, although no direct proof, that the adenine methylase activity in these strains is the result of a single enzyme that recognizes the sequence 5′-GATC-3′ (4, 4a, 13–15, 20, 25). There is also no report of a restriction enzyme that recognizes this sequence when it is unmethylated (an isoschizomer of R.*Mbo*I), although a restriction enzyme that recognizes the methylated sequence has been found in a strain that lacks adenine methylase activity (Table 3), i.e., an isoschizomer of R.*Dpn*I.

CHARACTERIZED RESTRICTION MODIFICATION SYSTEMS

A consolidated list of all the restriction enzymes and methylases that have been purified or cloned from *N. gonorrhoeae* is shown in Table 6. The list includes enzymes with eight different specificities and six of the eight specificities postulated by Korch et al. (6, 7). If M.*Ngo*BII (for which no restriction enzyme counterpart is known) is put to one side, the gonococci possess at least seven restriction modification systems.

TABLE 4. Purified gonococcal methylases

Specificity	Recognition sequence	Strain	Purified methylase	Reference(s)
S.*Ngo*II	GGCC	WR220	M.*Ngo*AI	15, 15a
S.*Ngo*VIII	GGTGA	MUG116	M.*Ngo*BI	15
—[a]	GTAN$_5$CTC	MUG116	M.*Ngo*BII	15

[a] —, No specificity name.

DISTRIBUTION OF RESTRICTION MODIFICATION SYSTEMS

Although the data in Table 6 indicate the number of restriction modification systems so far identified in the species, they do not indicate how many of these systems are usually possessed by an individual strain, and that is the information needed to ascertain whether restriction barriers might hinder the free exchange of genetic information within the species. The results cited above seem to indicate that when a gonococcal strain modifies its DNA so that it is resistant to cleavage by an isoschizomer of a characterized gonococcal restriction enzyme, that strain carries the methylase gene whether or not the methylase is being produced in amounts sufficient to be purified. In addition, it seems that such a strain also carries the gene encoding the corresponding restriction enzyme, whether or not the restriction enzyme is also being produced in amounts sufficient to be characterized. It has been suggested that this low level of production of some enzymes may be related to the growth phase of the cells (Chien, et al., unpublished). Some indication of the distribution of the known gonococcal restriction modification systems might therefore be gathered by looking at how often the DNA of individual strains is resistant to cleavage by isoschizomers of the known gonococcal restriction enzymes.

Incidence of Restriction Modification Systems

From the above criteria, and on the basis of our own surveys of different strains (M. K. Duff and J. K. Davies, unpublished data) and any published information, it seems that most gonococcal strains possess at least six of the restriction modification systems listed in Table 6. A small proportion of strains (perhaps 5%) seem to lack the S.*Ngo*II system, and an even smaller fraction lack the S.*Ngo*III system. The major variation, however, seems to be in the ability to produce the adenine methylase referred to above. We estimate that perhaps 30% of gonococcal strains have the ability to methylate the sequence 5′-GATC-3′.

TABLE 5. Cloned gonococcal restriction enzyme and methylase genes

Specificity	Recognition sequence	DNA resistant to cleavage with:	Strain	Enzyme(s) encoded on cloned DNA fragment	Reference(s)
S.*Ngo*I	PuGCGCPy	R.*Hae*II	P9	M.*Ngo*PI	23
S.*Ngo*II	GGCC	R.*Hae*III	P9	M.*Ngo*PII	23, 23
S.*Ngo*IV	GCCGGC	R.*Nae*I	MS11	R.*Ngo*MI, M.*Ngo*MI	R. H. Chien et al., unpublished
S.*Ngo*V	GGNNCC	R.*Nla*IV	MUG116	M.*Ngo*BIII	R. Chien et al., unpublished

Potential Barriers to Genetic Exchange

It would seem that the DNA from the few strains that lack the S.*Ngo*II and S.*Ngo*III systems would not be successfully transformed into the majority of gonococcal strains. Indeed, one of these restriction barriers has been directly demonstrated (19, 20). The main barrier to free genetic exchange within the species, however, seems to involve enzymes that act on the sequence 5'-GATC-3'.

ADENINE METHYLATION

As mentioned previously, many gonococcal strains appear to produce an adenine methylase that recognizes the sequence 5'-GATC-3' (4, 4a, 13–15, 20, 25). Since no restriction enzyme recognizing the same sequence has ever been isolated from gonococci, it has been suggested that this methylase may be the equivalent of the *dam* methylase of *E. coli* (13, 14, 18, 25). The *E. coli* enzyme is not part of a restriction modification system but appears to have a variety of roles within the cell, including strand discrimination during mismatch repair (18). These characteristics do not seem to apply to the gonococcal enzyme. First, strains lacking this methylase do not appear to be hypermutable, as do the *dam* mutants of *E. coli* (13, 14). Second, a strain that lacks this methylase has been shown to be producing a restriction enzyme that cleaves the methylated sequence (4, 4a; Duff, M.Sc. thesis). This situation seems reminiscent of that found in *Streptococcus pneumoniae* (8).

Some strains of *S. pneumoniae* produce R.*Dpn*I, which cleaves the methylated sequence 5'-G^{me}ATC-3', while others contain the complementary enzyme R.*Dpn*II, which cleaves only the unmethylated sequence 5'-GATC-3' (8). Cells that produce R.*Dpn*I have no need to protect their DNA against this enzyme, whereas cells that produce R.*Dpn*II must produce a methylase to modify this site. It has been shown that the genes responsible for one restriction phenotype are not present in cells of the opposite phenotype but that each strain does share sequence homology on either side of the regions of the chromosome encoding the restriction enzymes (8). Therefore, the complementary restriction systems are found in nonhomologous and mutually exclusive cassettes, which are apparently inserted into a specific position in the chromosome. Whether or not such a system exists in the gonococcus remains to be demonstrated.

CONCLUSIONS

N. gonorrhoeae can efficiently exclude foreign DNA because of its multiple restriction barriers. This plethora of restriction modification systems does not limit the exchange of genetic information within the species, because most strains protect their DNA against cleavage by all of the gonococcal restriction enzymes. The only barrier to this free flow of genetic information seems to be the existence of a mutually exclusive set of restriction systems that recognize methylated or unmethylated versions of the sequence 5'-GATC-3'.

TABLE 6. Characterized gonococcal restriction modification systems

Specificity	Recognition sequence	Isoschizomer	Restriction enzyme	Methylase
S.*Ngo*I	PuGCGCPy	R.*Hae*II	R.*Ngo*WI R.*Ngo*JII R.*Ngo*DII	M.*Ngo*PI
S.*Ngo*II	GGCC	R.*Hae*III	R.*Ngo*CI R.*Ngo*PII R.*Ngo*SI	M.*Ngo*PII M.*Ngo*AI
S.*Ngo*III	CCGCGG	R.*Sac*II	R.*Ngo*KI R.*Ngo*PIII R.*Ngo*JI R.*Ngo*DI	Inferred, none characterized
S.*Ngo*IV	GCCGGC	R.*Nae*I	R.*Ngo*MI	M.*Ngo*MI
S.*Ngo*V	GGNNCC	R.*Nla*IV	None known	M.*Ngo*BIII
S.*Ngo*VIII	GGTGA	R.*Hph*I	R.*Ngo*JIII R.*Ngo*DIV R.*Ngo*BI	M.*Ngo*BI
—[a]	G^{me}ATC[b]	R.*Dpn*I	R.*Ngo*DIII	None
—[a]	GTAN$_5$CTC	None known	None known	M.*Ngo*BII

[a] —, No specificity name.
[b] ^{me}A = N^6-methyladenine.

There remains one important, and unanswered question. If the purpose of a restriction system is to exclude foreign DNA, this can be achieved by producing large amounts of a single restriction enzyme recognizing a 4-base-pair sequence. Why, then, have the gonococci maintained the ability to produce at least six restriction barriers? This is even more puzzling, considering that the gonococcal transformation system apparently has the ability to discriminate between gonococcal DNA and DNA from other sources, and to preferentially take up DNA from its own species (1). Why, then, the need for any restriction barrier?

ACKNOWLEDGMENTS

I thank D. Stein, A. Piekarowicz, R. J. Roberts, and J. Saunders for their contributions of unpublished results and preprints of articles in press.

The work in my laboratory was supported by the Australian National Health and Medical Research Council.

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